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Trans. 481
(In full)
By:
A. Antik

Voitchishina, O. N.

Formirovanie ustoichivosti k zzhavchine u gibridov ozimoi pshenitsy

[[Formation of resistance to rust in winter wheat hybrids].

Selektsiiá i Semenovodstvó, vol. 20, no. 5, pp: 31-33. May 1953. 61.9 Se5

(In Russian)

# FORMATION OF RESISTANCE TO RUST IN WINTER WHEAT HYBRIDS

Creation of rust resistant varieties of winter wheat is one of the basic control measures against this disease. Therefore the development of means furthering the formation in hybrids of rust resistance and its subsequent retention in a variety when the latter is used for production, are problems of great practical importance.

The first results of our research were published in 1951 [1]. In 1950 our observations showed that rust resistance in winter wheat hybrids can be increased drastically by providing plants during the growing period with additional feeding outside the roots with salts of calcium, phosphorus and potassium; by cultivating hybrid plants when rust contamination is absent (this was achieved by dusting the plants with sulfur which is a fungicide in regard to rust); by spraying the plants with a sodium chloride solution. According to data in the literature NaCl acts as a fungicide which kills rust spores; but besides that, the presence of sodium stimulates intake and greater accumulation of potassium in plants. And potassium plays an important role in wheat's resistance to rust. The 1950 research was continued in the same direction in 1951 on 19 hybrids of the North-Osetiia state selection station.

1. Inter-varietal hybrids of free pollination (with selected male parents): Osetinskaia 3 x (Iubileinaia Osetin + Osetinskaia 4); Osetinskaia 3 x (Iubileinaia

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1. Voitchishina, O. N. Zhurnal "Selektsiia i semenovodstvo" No. 10, 1951

Table 1 (p.32)

Effect of additional feeding outside the roots on rust resistance in winter wheat hybrids ( in %).

| Hybrids | Variant of the test | Intensiveness of affection w. brown rust | Affected plants | Yield from plots (in relation?) to control |
|---|---|---|---|---|
| Osetinskaia 3 x Eritrospermum 15 | 2-year addit. feeding outside roots | 0 | 0 | 132,0 |
| | Results of 1-yr addit. feeding | 3,0 | 10,0 | 117,0 |
| | 2-yr spraying with NaCl | 0,2 | 0,1 | 116,0 |
| | Results of spraying with NaCl of 1st year | 3,7 | 12,0 | 108,0 |
| | 2-year dusting with sulfur | 9,5 | 25,0 | 184,0 |
| | Results of sulfur dusting 1st yr. | 9,7 | 30,0 | 145,0 |
| | Control | 43,0 | 65,0 | 100,0 |
| Zemka x (Iubileinaia Osetin + Osetinskaia 3 + + Kubanskaia 133 + Eritrospermum G-569 | 2-year addit.feeding outside roots | 43,6 | 50,0 | 167,0 |
| | Results of 1-yr addit.feeding | 45,0 | 50,0 | 103,0 |
| | 2-year spraying with NaCl | 42,0 | 55,0 | 150,0 |
| | Results of spraying with NaCl of 1st year. | 40,0 | 55,0 | 102,0 |
| | 2-year dusting with sulfur | 39,5 | 65,0 | 98,0 |
| | Results of sulfur dusting 1st yr. | 68,9 | 75,0 | 95,0 |
| | Control | 70,0 | 100,0 | 100,0 |

Use of these methods, even for one year, considerably decreases the intensity of disease and the percentage of plants affected by rust in the following generation of hybrids as compared with the control; it also increases the yield of grain by 10-16%.

Artificial inoculation with brown rust of hybrid seedlings of the following generation demonstrated that additional external root feeding and spraying of plants with a NaCl solution for two years increase the resistance of hybrids to brown rust to a higher degree than when these methods are applied for one year. (Table 2).

Phenological observations indicated that plants of hybrids which were exposed for two years to additional external root feeding and to spraying with a sodium chloride solution, proceed 3-4 days faster through growing stages as compared with the control. Seeds of hybrids which were exposed to a two-year

additional external root feeding and to spraying with a NaCl solution increased the germination energy as compared with control. (Table 3).

Table 2 (p.33)

Results of artificial inoculation of hybrids with brown rust (in %).

| Hybrids | 2-year additional feeding outside roots | | | 2-year spraying with NaCl | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|
| | Intensiveness of affection | Type of infection | Affected plants | Intensiveness of affection | Type of infection | Affected plants | Intensiveness of infection | Type of infection | Affected plants |
| Zemka x (Iubileinaia Osetin + Osetinskaia 3 + Kubanskaia 133 + Eritrospermum G-569 | 10-25 | 1;2 | 45,0 | 5-25 | 1;3 | 45,0 | 25-65 | 3;4 | 100,0 |
| Osetinskaia 3 x (Osetinskaia 4 + Iubileinaia Osetin + Kubanskaia 133 + Eritrospermum G-569 | 5-10 | 1;2 | 40,0 | 5-10 | 1;2 | 50,0 | 10-25 | 3;4 | 100,0 |

Osetin + Osetinskaia 4 Kubanskaia 133 + Eritrospermum G-569); Eritrospermum G-19 x (Osetinskaia 4 + Kubanskaia 133 + Eritrospermum G-569 + Zemka x (Iubileinaia Osetin + Osetinskaia 3 + Kubanskaia 133 + Eritrospremum G-569).

2. Of limited-free pollination: Iubileinaia Osetin x branching wheat; Osetinskaia 3x branching wheat; Osetinskaia 3 x Eritrospermum 15.

3. Inter-varietal crossings of free pollination: Iubileinaia Osetin of free pollination; Zemka of free pollination.

4. Paired crossings [?]: Iubileinaia Osetin x Zemka; Iubileinaia Osetin x Eritrospermum G-720; Iubileinaia Osetin x Kubanskaia 133.

5. Paired crossings: Zamka x Iubileinaia Osetin; Ardito x Zemka; Zamka x Ardito; Iubileinaia Osetin x Voroshilovskaia; Voroshilovskaia x Iubileinia Osetin; Eritrospermum G-720 x Zemka; Zemka x Eritrospermum G-720.

For external root feeding of hybrids was used a solution of a combination of salts: Ca $(NO_3)_2$ 1 part + $KH_2PO_4$ 1.5 part per 1 L. of water. For spraying hybrids with sodium chloride was used a 2% solution.

The expenditure of salt solutions for each spraying of 10 square m. plots was 3-3.5 L. The spraying was carried out during the following stages of plant growth: sprouting, tillering, booting and heading.

The hybrids were dusted with sulfur 6 times during the growth period. The first dusting took place after the appearance of the first pustules of brown rust on Krasnodarka, a variety susceptible to it. The sulfur expenditure for each dusting was 100-120 g. per a 10 square m. plot. The test was conducted in triplicate.

In 1951 the effect was studied of a two-year use of additional external root feeding, of spraying [begin p. 32] with a NaCl solution and of dusting with sulfur. Besides that was being clarified the effectiveness of the results of the use of the mentioned processes for the increase of resistance among hybrids of the next generation.

Field observations of brown rust and records of yields according to variants of the experiment, showed that hybrids of almost all the combinations when fed additionally outside the roots, sprayed with sodium chloride solution, and dusted with ground sulfur decrease the affection with brown rust and increase the yield (table 1).

It is seen from the data of table 1, that the use of additional feeding outside the roots with a combination of Ca $(NO_3)_2$ + $KH_2PO_4$ during two years as well as spraying with a NaCl solution and dusting with sulfur, decreases the damage from brown rust and lowers the percentage of rust-diseased plants. The yield from a plot in some cases increases by 30-60% as compared with the control.

Table 3 (p.33)

Energy of germination of hybrid seeds.

| Hybrids | Speeding up of growing stages (in days) | | | Energy of germination of seeds (in % to control |
|---|---|---|---|---|
| | Booting | Heading | Flowering | |
| Osetinskaia 3 x (Iubileinaia Osetin + Osetinskaia 4)..... | 5 | 4 | 3 | 117,0 |
| Iubileinaia Osetin x spreading wheat..... | 5 | 4 | 3 | 106,0 |
| Zemka of free pollination..... | 5 | 3 | 3 | 112,0 |

The research of 1951 justifies the following conclusions.

1. Additional external root feeding of hybrid plants with a mixture of Ca, P, and K salts and spraying with sodium chloride solution, increase their resistance to brown rust.

2. The character of resistance is stable and is passed on to the following generation of hybrids. Increase in rust resistance in hybrids suggests that these methods form resistance to rust in the hybrid material of winter wheat.

3. Additional external root feeding and spraying with a NaCl solution increase the yield capacity of winter wheat hybrids.

4. Use of these methods increases the germination energy of seeds of hybrid plants.

All this permits us to speak of a future in the use of the mentioned procedures for selecting wheat in regard to resistance to rust.

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Trans. 482
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Ryzhkov, V. L., and Loidina, G. I.

Vzaimsdeistvie virusa mozaichnoi bolezni tabaka s miozinom i aktinom

[Interaction between the virus of the mosaic disease of tobacco and myosin and actin]

Dok. Akad. Nauk, vol. 92, no. 4, 1953, p. 851-853
511 P444A
(In Russian)

There are data in the literature on interaction between the virus of the mosaic disease of tobacco (TMV) and protamine, some protein enzymes and serum proteins (1-3). It is obvious from these data that the behavior of TMV is no different from that of other proteins and that between the iso-electric points of the TMV and its interacting protein formation of an insoluble complex can be observed most of the time.

Muscle proteins differ considerably from proteins the interaction of which with TMV was studied before. A. V. Engel'gard's and M. N. Liubimova's discovery of fermentative activity of myosin (4) and the peculiarity of interactions between myosin and actin makes the study of interactions between these proteins and TMV especially interesting.

We used in all the experiments a myosin solution of 0.5 M KCl, which contained about 1.3% of myosin. The purified TMV preparation was used in a 0.2% aqueous solution. In tests in which the effect of interaction between myosin and TMV was determined per titer of TMV, the titer was determined according to the number of lesions on the leaves of N. glutinosa, and as a solution of virus protein to which a KCl solution was added instead of myosin served as a control. The concentration of this salt in the experimental and control solutions was, of course, maintained strictly equal. The actin solution was prepared in 0.1 M KCl by dissolving 1 g. of actin in 20 ml. of liquid. For polymerization of actin 0.001 M $MgCl_2$ was also added.

In the first experiment 0.5 ml of myosin were combined with 0.5 ml of TMV and water was added gradually until a precipitate was formed. This precipitate was obtained with the 0.178 M KCl concentration. In the second experiment 0.4 ml. of myosin were combined with 0.1 ml. of TMV, after which water was added and the sediment was formed at 0.2 M KCl. Pure myosin is separated out of the solution only at 0.05 M KCl (5). The result obtained provided a basis to assume that myosin formed a complex with TMV. The precipitates formed were centrifuged out and then suspended in 0.5 ml of water. In the sediment and the supernatant fluid, was determined the titer of the virus (Table 1). This determination of titer confirmed our hypothesis that part of the virus passed into the sediment.

An attempt was made to obtain threads from the myosin + TMV complex. For this purpose a mixture of solutions of the mentioned proteins was blown into water through a Pasteur pipette. The formed thread broke down rapidly. It was not possible to obtain durable threads similar to the actomyosin ones. Under the influence of the adenosintriphosphorus acid (ATP) the TMV and myosin complex was contracted (?) similar to the actomyosin.

Further on, experiments were conducted in order to find out how actin affects the interaction between myosin and TMV; for this purpose all three components were combined (begin p. 852) in equal ratio, but in one test TMV was added to myosin before the actin and in another - actin was added first. In these experiments actin F was used (polymerized actin). To 0.3 ml of the mixture 0.7 ml of water was added and thus a precipitate was formed which separated out and the titer of the virus was determined only in the supernatant fluid. Besides these mixtures that of 0.1 ml TMV + 0.1 ml of actin + 0.8 ml of water was used. It can be seen from table 2 that the presence of actin did not hinder the depression of the virus titer. Furthermore, the actin itself appeared to be able to depress the virus considerably.

Table 1 (p. 852)

Interaction between TMV and myosin (the titer of virus is expressed in the number of lesions on 10 half leaves of N. glutinosa)

| No. of preparations | Fraction being titrated | Titer of virus: test | Titer of virus: control | % of retained activity of the virus |
|---|---|---|---|---|
| 1. | Sediment . . | 80 | 213 | 37.5 |
| | Fluid . . . | 123 | 231 | 53.2 |
| 2. | Sediment . . | 55 | 226 | 24.3 |
| | Fluid . . . | 41 | 91 | 45. |

Table 2 (p. 852)

Interaction between TMV and myosin and actin (the titer of virus is expressed by the amount of necroses on 10 half leaves of N. glutinosa)

| Mixture | Titer of virus: test | Titer of virus: control | % of retained activity of the virus |
|---|---|---|---|
| Myosin + TMV + actin | 40 | 101 | 39.6 |
| Myosin + actin + TMV | 34 | 83 | 40.9 |
| TMV + actin | 17 | 107 | 15.8 |

The problem of interaction between actin and TMV was studied more in detail. It was established that the non-polymerized actin (actin G) depresses the virus similarly to actin F. Thus in one test with actin we obtained 52 necroses while there were 185 necroses in the control, i.e. retention of activity was 6.6%. Further tests were conducted with actin.

When there was interaction between TMV and this protein in distilled water (pH about 6.2), the activity of the virus in each of several tests remained 19.6%, 20.0%, 22.1%, 23.9%. In order to clarify how pH affects the depression of the virus by actin, experiments were conducted with a glycocal buffer; with pH 7 we found in various tests the following retention of activity: 8.5%, 16.5%, 27.1%, while with pH 4.6 - correspondingly 37.1%, 25.4%, 62%. Thus the virus is depressed by the actin most strongly when the media has a neutral reaction, and least strongly at pH 4.6. The latter pH is very close to the isoelectric point of the actin and at this point a cloudiness of the solution could be observed.

In order to find out how a KCl concentration affects the reaction which we are studying, an experiment was conducted with various salt concentrations in the solution and while in 0.1 M KCl the retained activity was 21.8%, at 0.25 M it was 49.3% and, finally, in 0.5 M - 80.5%. Thus higher concentration of KCl leads to a decomposition of the actin and TMV complex.

Experiments were also conducted on the influence of ATP on the complex. The results of these experiments are shown in table 3 from which it is seen that ATP itself depresses the activity of the virus negligibly, but at the same time it decreases the effect of the actin since it is apparently capable of breaking down its complex with the TMV.

Viscosimetric studies were also conducted and it appeared that the combination of actin F with TMV does not produce an increase of viscosity. As to the mixture of myosin with TMV, the obtained result was not sufficiently definite. In some tests we observed a quite considerable increase in viscosity, in others it was entirely lacking or was very slight. In cases where the viscosity was noted it depended greatly (begin p. 853) on the ratio between myosin and TMV and it reached a maximum when the ratio of myosin to TMV was 5:4. In one test with this ratio the speed of running-off of the fluid in the viscosimeter was 2 min. 20 seconds while for the TMV it was 49.2 sec., for water - 44.2 sec. and for myosin - 1 min. 44 sec.

Table 3 (p.853)

Interaction between the TMV and actin and adenosintriphosphorus acid (the titer of the virus is expressed by the amount of necroses on 10 half leaves).

| Combination (mixture?) | Titer of virus | | % of retained activity of virus |
|---|---|---|---|
| | Test | Control | |
| VTM + actin | 23 | 115 | 20.0 |
| VTM + 0.05% ATF | 57 | 77 | 74.0 |
| VTM + actin + 0.05% ATF | 26 | 79 | 32.0 |
| VTM + actin | 79 | 334 | 23.9 |
| VTM + 0.0125% ATF | 112 | 180 | 62.2 |
| VTM + actin + 0.0125% ATF | 121 | 328 | 36.9 |
| VTM + 0.025% ATF | 158 | 256 | 61.7 |
| VTM + actin + 0.025% ATF | 140 | 265 | 52.8 |

The obtained results differ from what has been known before on interaction between TMV and various proteins in that in our tests it was possible to observe the formation of a complex at pH located above iso-electric points of interacting components and not in the interval between these points. It is known that the interaction between myosin and actin is observed also at pH levels above the iso-electric points of these proteins, which is related to their selective capacity to adsorb ions of potassium as well as other not yet sufficiently studied properties. It is possible that due to varying capacity to connect ions of potassium in interacting proteins a difference is created in their charge which leads to the formation of complex (5). That the interaction between TMV and actin, at least in part, is similar to the interaction between myosin and this protein is seen from the fact that the complexes of TMV with actin depend on the concentration of KCl and are broken down by ATP as are the complexes of myosin with actin.

We do not know yet the substrata in the plant's protoplasm with which the TMV connects, however it has been demonstrated that the development of a virus infection can be suppressed by certain concentrations of potassium and magnesium salts (6 & 7). It is possible that the TMV forms a complex with structural proteins of the plant's protoplasm, which (complex) can be broken down by high concentrations of electrolytes. However, the solving of this problem requires further research on plant proteins sensitive to TMV.

In conclusion the authors consider it their pleasant duty to thank M.N. Liubimova for providing myosin and actin preparations as well as for her valuable consultation.

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Ryzhkov, V. L.

Elektronnaia mikroskopiia
dokletochnykh form zhizni

[Electron microscopy of pre-cell forms
of life]

Priroda, v. 40, no. 9, pp. 48-52,
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(In Russian)

# ELECTRON MICROSCOPY OF PRE-CELL FORMS OF LIFE

The electron microscope made it possible to conduct direct observations on objects which, due to their size, are beyond the range accessible to light optics. It refers first of all to the minute biological individuals the mere existence of which we could judge before only by their activity. Thus it became accessible to study the causing agents of the most severe illnesses.

The first objects of electron-microscopical observations were the filterable viruses, but recently other minute agents of illnesses are being studied as well as filterable saprophyte microbes which in their size are close to viruses.

The data of electron microscopy indicate that the world of pre-cell life forms is extremely diversified in sizes, forms and structures.

Rickettsia, representative of which is the causal agent of spotted typhus [fever], belongs to minute organisms. Unlike the viruses they disclose respiration also outside the cells in which they parasitize. It was possible to see through an electron microscope that the Rickettsia are surrounded with a quite extensive mucous membrane. No other group of pathogenic agents from the ultra-microbe world has a similar membrane.

Very peculiar are the "tsistotsety" [L-forms]. To their number belong the causal agents of pneumonia of cattle, of "agalaktiia" of goats, as well as agents of very little studied diseases which are manifest in malignant rheumatism and other afflictions. To the same group belong also the saprophyte forms which it was possible to isolate from sewage. The "tsistotsety"

[L-forms] are very sensitive to streptomycin which cannot be said about other groups being examined here. They look like very tender small bubbles lacking any kind of wall. Sizes and forms of one and the same species of "tsistotset" vary, which indicates the complex development cycle through which they pass.

There are only single electron-microscopic data on "bartonelia"[Bartonella]. They are causal agents of pernicious anemia and affect the red blood corpuscles. In a regular microscope they look like minute rods, and with the help of an electron microscope it was possible to disclose that these rods are not individuals but represent aggregates of smaller single particles which, in size, are close to viruses. The position of "bartonelia" in living Nature is very unclear.

Among the viruses proper the largest are the representatives of the psittacosis group (fig. 1). Psittacosis is a disease of parrots, its virus can cause severe pneumonia in humans. To the same group belong viruses causing pneumonia in cats, mice, and also the so-called "fourth venereal disease" in humans (infectious limphogranulomatosis). Particles of these viruses are apparently very rich in water, and since with the help of electron microscope only dried out preparations can be studied, the particles of these viruses have to be examined when they are quite out of shape which does not happen to other viruses. Virus particles of the psittacosis groups vary considerable in size and form. The pneumonia virus of cats is 350-770 mm in diameter. As far as it can be judged according to electron-microscopic observations, the large particles at certain development stages break up into smaller ones. The viruses of the psittacosis group are similar to microbes in that they are sensitive to sulfanilamides, penicillin, aureomycetin and chloromycetin. Viruses examined further on do not disclose any sensitiveness to either of these substances.

Fig. 1 (p. 49). Filterable viruses according to data of electron microscopy:

Pneumonia of cats
Pneumonia of mice
Psittacosis of parrots
Limphogranuloma (fourth venereal disease)
Ornitosis of pigeons
Meningo-pneumonia of mice
Atypical pneumonia of humans
Variola (small pox) of birds
" " " of humans
Contagion mollusk of humans
"Ektromeliia" of mice
"Muksoma" of rabbits
Zoster of humans
Chicken pox of humans
Herpes of humans
Mumps of humans
Pseudo bird pest
Bird pest
Grippe B of humans
Grippe A. of humans
Bacteriophage of intestinal bacillus $T_2$
Encephalomyelitis of horses
Bacteriophage of intestinal bacillus $T_7$
Papilloma of rabbits
Mosaic of beans, pumpkin, of tillering of tomatoes
Poliomyelitis Lansing
Mosaic of turnip
Grippe of pigs

Fig. 1 (cont'd)

Mosaic of tobacco
X-virus of potato
Curliness of tobacco
Yellow jaundice of the silk worm
Granulose of insects

Fig. 2 (p. 50)
Virus of small pox vaccine under electron microscope. The preparation is dusted with metal which increases the sharpness of picture. Magnified about 22 thousand times.

Fig. 3 (p. 50)
Virus of grippe under electron microscope.

A quite homogeneous group is composed of viruses, as representatives of which could be considered the causal agents of small pox. The elementary corpuscles of these viruses have been long ago and in detail studied with the help of light optics. Seen through a regular microscope they look like minute spheres. Electron-microscopic observations showed that in reality they are not globular but rectangular. (Fig. 2).

Very peculiar is the causal agent of the pseudo pest of birds. Placed into a table salt solution it forms a long branch and being transferred into distilled water the virus particles become rounded. It is possible to repeat the transition from one form into the other several times, but if the virus with the branch is killed with vapors of [sic] osmic (formic?) acid, then when transferred to water it will not become rounded any more. Both virus forms are infectious. In all these viruses the diameter of the particle is above 100 mn in diameter. Another virus group has 100 mn (microns) and less in diameter. The most studied representative of this group is the grippe virus. Its various strains are 78-103 mn in diameter (Fig. 3).

The particle of the encephalomyelitis of horses is even smaller, it hardly reaches 50-51 mn [microns]. Of similar size is the virus which causes papilloma of rabbits, though in other respects it has no likeness with the causal agent of encephalomyelitis.

A large number of viruses which affect plants are 25-30 mn in diameter. This size is inherent to viruses of the southern mosaic of beans, of tillering of tomatoes, of pumpkin mosaic and tobacco necrosis. Causal agents of turnips and of streaking of beans are still smaller, they are 17-19 mn [micron] in diameter. To the viruses affecting animals and humans belongs also the agent of the hoof and mouth disease which is 20-30 mn in diameter. Similar sizes are indicated for some species of the poliomyelitis (viruses), however it is not yet achieved to obtain these viruses, which affect animals and humans in form of

sufficiently purified and concentrated preparations and the electron-microscopic data pertaining to them is rather disputable.

Among viruses which have rod-shaped particles, deserving attention are first of all the agents of the polyhedral diseases of insects. Called polyhedra are the crystal-like corpuscles, which form in the gut of caterpillars sick with yellow jaundice. It appears that these bodies have a very complex structure. Besides the albumen substance with particles which are 10 mn (micron) in diameter and were first taken for the virus itself, polyhedra have also virus particles which are quite thick rods. The virus of the polyhedral disease of the mulberry silk worm is 88 mn (micron) in diameter and 350 mn (micron) long.

Virus particles of the yellow dwarfiness of potato are also rod-shaped and their size is 200 x 50 mn (micron).

Follows a large number of viruses which have the shape of thin rods 15 mn (micron) in diameter. The length of the rods is 150-300 mn and these rods not infrequently join each other at the ends forming threads which might reach several thousands of mn(micron). To viruses having such structure belong the viruses of the mosaic disease of tobacco, cucumbers, cabbage, potato, orchid, streakiness of peas (pea streak), etc. Similar structure was for some time attributed to some species of the poliomyelitis virus, but it has not been confirmed.

Thus the forms and sizes of viruses vary greatly. We have all gradations of sizes beginning with the giant forms of the virus of cat pneumonia which reaches the size of the smallest microbes and finishing with such as the virus of the mouth and foot disease and mosaic of turnips the particle volume of which is about 40,000 smaller than the volume of an elementary corpuscle of largest viruses. Viruses vary not only in form and size, but in their physiological peculiarities as well, which becomes evident in their relation to antibiotics. Besides that they differ also in their chemical composition. Viruses which contain lipoids besides "neukleo proteidy" (?nuclear proteins?), are larger in size than viruses lacking lipoids. The virus of the encephalomyelitis and all the larger viruses contain various lipoids, among them phospholipids(?). In smaller viruses the presence of a small amount of lipoids is known with certitude only in the virus papilloma. Absence of lipoids in smaller viruses distinguish them essentially from protoplasts, because each protoplasm, except albumen, contains lipoids. Bacteria and higher organized beings contain two nucleinic acids--thymo-nucleinic and ribose(?) - nueleinic acids. The majority of viruses have only one of these nucleinic acids, and, as a rule, the highest viruses, i.e. the larger ones, contain the thymo-nucleinic acid. In the grippe virus two nucleinic acids were discovered, in the virus of encephalomyelitis-only the ribose(?) - nucleinic acid, same as in all the known to us viruses affecting plants. It can be said that a certain simplicity of chemical composition corresponds with greater simplicity in virus organization.

Electron microscopy contributed very much to the study of bacteriophage. It became apparent that it is characteristic for the

"phage" to have an appendix (?) but there are "phages" without it as well. Such typical, or rather more widely spread particle of the "phage" consists of a head, the diameter of which varies from 45-100 mn, and an appendix which reaches 150-200 mn in length. This appendix ends frequently with a widening. Earlier the appendix was called quite wrongly-the tail and was looked upon as being an organ of motion. The electron microscopy made it possible to observe how the "phage" in many cases attaches itself with its appendix to the bacterial cell and it is quite probable that the "phage" is (at least partly) an ectoparasite of the bacteria. But then the world of the "phages" is also extremely diversified. The intestinal bacillus is lysed by a large number of "phages" which differ from each other in their antigenic properties, chemical composition and structure. Along with the "phages" of the intestinal bacillus, which have an appendix (fig.4) there are known such which lack it. Three different "phages" of typhoid fever are described which, according to data of ultra-filtration, are very different in size. This corresponds with data of electron microscopy (see table).

The electron microscopy makes it possible to observe various specific reactions of viruses. Thus an agglutination of virus particles with specific anti-serums can be seen. Some viruses are adsorbed by red blood corpuscles. To such viruses belong those of grippe, mumps and others. Similarly, through the electron microscope can be seen the attachment of "phage" particles to sensitive bacteria. They attach themselves not only to living but to the destroyed bacteria as well, but this attachment is always strictly specific, i.e., it takes place only in regard to bacteria which are able to be lysed by the given "phage".

Fig. 4. (p. 51) Bacteriophage of the intestinal rod (bacillus) (To) through an electron microscope magnified about 36 thousand times.

Table (p. 51)

| "phage" | Data of ultrafiltration (in mn) | Data of electron microscopy (in mn) |
|---|---|---|
| No. 117 | 70-110 | Head 40-60, Shoot (appendix) 60-120 |
| No. 128 | 20-30 | 30-40. No shoot (appendix) |
| No. 114 | 8-12 | Not disclosed |

Even though the electrons penetrate inadequately through virus particles, it was possible to obtain most valuable data on the structure of many viruses. Elemental corpuscles of smallpox have a more solid formation in the central part and four smaller corpuscles at the sides. It was recently proved accurately that the head of the "phage"(?) has a wall. If the "phage"(?) is placed in a strong solution of some salt and then the salt concentration is rapidly decreased, then the "phage" becomes inactivated; the electron microscope makes it possible to establish visually the causes of the inactivation. It appears that the heads are destroyed by such treatment and it can be seen not infrequently that only their walls are preserved with the appendixes attached to them. Thus the "phage" heads react to sharp changes in osmotic pressure as would any protoplast. In the light of these data any attempts to defend the enzyme nature of the "phage", seem very archaic.

The biological individuals which we are examining are so minute that they cannot but be affected by various forces of purely physical attraction and repulsion which play such an important role in the world of molecules. The electron microscope allows to observe the aggregration processes of virus particles. Many photographs are obtained of preparations of concentrated solutions of the tobacco mosaic virus, from which it is seen how the rod-shaped virus particles arrange themselves in regular rows forming complicated lines (fig.5). These virus aggregates look similar to various kinds of fibers of biological origin from connecting and other tissues which are seen through an electron microscope. In particularly favorable cases the structure of virus crystals of individual virus corpuscles can be seen through an elctron microscope. It should be pointed out in general that even viruses not obtained as crystals, arrange themselves very frequently in electron-microscopic preparations in quite regular rows. The latter was observed also in regard to "phage".

Fig. 5 (p.52) Concentrated solution of virus of tobacco mosaic under (through?) the electron microscope. "Nucleoproteid" forms complex aggregates. Seen are also single isolated virus particles.

One of the hardest problems is that of reproduction of viruses. Only quite recently it became possible, not without success, to approach this problem with the help of the electron microscope. Observations indicated, that virus particles of tomato tillering are sometimes about twice the size of the regular ones and have a constriction in the middle; in this case they remind greatly of particles which are in a state of reproduction by division. Similar observations exist on the encephalomyelitis virus. In observing the lysis of a bacterial cell under the influence of the bacteriophage it was possible to see in the decomposition products of this cell, the individual small colonies of the "phage", which later broke down into single "phage"-particles. So far these observations have a rather fragmental character and the method itself connected with the study of dry preparations is such that it deprives them of absolute conclusiveness, however - they deserve further research.

Particularly interesting are the thread-shaped virus forms. They were first found in the grippe virus. They represent rather long rods with a diameter inherent to the grippe virus. Sometimes it is possible to observe how at the end of such a rod a cord is formed (?) by typical virus particles of grippe. There was an attempt to explain these forms as an artifact which originates at the expense of the wall of a red blood corpuscle; however, against it speaks the circumstance that similar thread-like forms are obtained also in the grippe virus which is not adsorbed on blood corpuscles. Very similar forms are obtained also among bacteriophages and here it can sometimes be seen how from the rod-shaped form separate "phage" particles with an appendix. Such rod-shaped "phage" forms originate usually under conditions unfavorable for the "phage" 's reproduction, for example, at an abnormally low temperature. It is possible that they appear due to inhibition in reproduction when the growth does not cease. If such an assumption is correct, then rod-shaped "phage" forms are analoguous with elongated bacteria forms, which also originate when their reproduction is suppressed.

It would be a bad mistake to judge the nature of viruses on the basis of only morphological or chemical data, away from the complex conditions under which the viruses circulate in the Nature and enter inter-relations with higher organized beings. For the judgment of the nature of viruses the electron microscopy provides very valuable but only extremely individual data. These data confirm our notions on great variety in the world of pre-cell life forms, on the existence in it of more complex and more simple formations. There is no doubt that the electron-microscopic method will further on find its application also in solving of such difficult and exciting problems as that of filterable microbe forms, their genesis and structure as well as inter-relations between microbes and viruses.

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By:
A. Antik

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Grushko, Ia. M.

Khrom kak bioelement

[Chromium as a bioelement]

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(In Russian)

# CHROMIUM AS A BIOELEMENT

Vernadskii and Vinogradov refer chromium as well to the number of micro-elements (trace elements) which are contained in the bio-sphere. Pointing out the existence of a cycle rotation of chemical elements in the nature, Vernadskii considers its probability for chromium as well. Data in literature on existence and content of chromium in organisms are very contradictory. Some authors consider that normally there is no chromium in the human body or that it is found in living substance accidentally, entering it with the food. Other researchers discovered chromium as traces in various organs, in endocrine glands, in gall stones, in milk. Vinogradov indicates that chromium is disclosed in animal and plant tissues and that a living substance contains it in a $10^{-4}$--$10^{-3}$ % concentration in ratio to body weight.

In order to determine the existence of chromium as a micro-element in a living organism, we carried out determination of chromium in organs and tissues of humans (corpse material), of some animals (rabbit, sheep); determination of chromium was carried out also in various food products and in drinking water.

The determination was conducted with the help of spectral analysis which is of exceptional value for determination of micro-elements in a biological material. The advantages of the spectral analysis of biological material as compared with other methods are: its high sensitivity (in ashes of burned organs and tissues, up to 0.0002% of the material being studied is determined), possibility of using for research of very small amounts of material (weighted portion 10-20 mg), specific character for each element being determined, simultaneous determination in a test object of several elements without a preliminary treatment of the test object, rapidity of determination (during 10-15 minutes).

For carrying out of the spectral analysis we had at our disposal a

large quartz Khil'ger spectrograph. As a source of excitation during analysis an arc of direct current (220V, 10A) was used because this method produces best results for our purposes. Use of a condensed spark would require undesired chemical preparation which could distort the picture of the real content of chromium in the samples.

The principle of the emitting spectral analysis which we applied consists in the following: in burning various substances in a volt arc, a spectrum is formed form a series of lines characteristic for each element. The intensiveness of each line is in proportion to the concentration of the element in the sample. The spectrum of the substance being analysed is compared with standard samples, which are photographed on the same film. For greater precision we applied the "method of inner standard" when the intensity of the spectrum being analysed is compared with the resembling spectrum curve of another element which is introduced into the test object with a constant concentration. We used cobalt as such a standard.

The technique of spectrographic analysis consisted of the following stages: 1) enriching of sample, 2) sealing in of the test object into a graphite anode crater, 3) spectrographic reading, 4) quantitative determination of chromium.

Enriching of samples was carried out by their calcination. The samples in amounts of 20-50g were dried out at $120^0$ during 12 hours, then they were burned in a muffle furnace for 8 hours with gradual increase of temperature in the furnace up to $500^0$. The results of the study were calculated in milligramm-percents of chromium in the raw substance.

The sealing in of the samples was carried out in graphite anode crater 2.5mm in diameter drilled in an electrode 4 mm in diameter. Spectrographing was done with a 2.5-3.0 minutes exposure; the 0.02 mm wide slit in the spectrograph was evenly lighted with the help of an additional system of condensers and intermediate stops.

The quantitative determination of chromium was carried out in the following manner. On the film were photographed: 1) a series of samples being analysed, 2) mixtures (compounds) with certain chromium concentrations (standard), 3) graduated scale. In order to obtain a characteristic curve and a subsequent determination of intensiveness of lines, a concentration curve was built according to which the chromium concentration was determined. Photometrics of films was done with a projection micro-photometer; the obtained results were determined as percentage of chromium content in ashes, after which they were recalculated to the content of chromium in milligramm-percents in raw material.

We determined the content of chromium in organs and tissues of two bodies of people who died as a result of different illnesses.

Table 1 (p. 125)

Content of chromium in organs and tissues of a human being.

| Name of organs and tissues | Chromium, mg% of raw weight | | Name of organs and tissues | Chromium, mg% of raw weight | |
|---|---|---|---|---|---|
| | I | II | | I | II |
| Hair | 0,2 | ---- | Stomach wall | 0,007 | ---- |
| Nails | 0,12 | ---- | Cartilage | 0,001 | ---- |
| Gall | 0,08 | ---- | Hypophysis | 0,0006 | ---- |
| Salivary gland | 0,04 | ---- | Spleen | 0,0005 | 0,01 |
| Kidney | 0,028 | 0,027 | Muscle | 0,0002 | ---- |
| Diaphragm | 0,016 | ---- | Suprarenal gland | | |
| Wall of large intestine | 0,012 | 0,063 | ("nadpochechnik") | 0,0005 | ---- |
| | | | Pancreas | 0,0002 | ---- |
| Heart | 0,01 | ---- | Wall sm. intestine | 0,0006 | ---- |
| Liver | 0,001 | 0,013 | Lung | 0,0007 | ---- |
| Brain | 0,002 | 0,002 | Blood | 0,0035 | 0,012 |
| Thyroid gland | 0,005 | 0,005 | Bone | ------ | 0,085 |

It is seen from table 1. that chromium has been discovered in many organs and tissues; in large amounts-in exterior skin covers (hair, nails) as well as in gall. The least amount of it was found in muscles, pancreas, suprarenal gland, thyroid gland, hypophysis, spleen and liver. For the purpose of comparison we conducted determination of chromium in tissues of a rabbit and a sheep. The obtained data are in table 2.

Table 2 (page 125.)

Content of chromium in organs and tissues of a rabbit and a sheep

| Names of organs and tissues | Chromium, mg% of raw weight | | Names of organs and tissues | Chromium, mg% of raw weight | |
|---|---|---|---|---|---|
| | Rabbit | Sheep | | Rabbit | Sheep |
| Contents of stomach | 0,108 | --- | Lung | 0,011 | --- |
| Contents lge. intestine | 0,16 | --- | Liver | 0,002 | --- |
| Wall, stomach | 0,07 | --- | Suprarenal gland | --- | 0,01 |
| Wall, sm. intestine | 0,013 | --- | Spleen | 0,01 | --- |
| Wall, lg. intestine | 0,09 | --- | Kidney | 0,02 | 0,012 |
| Claws | 0,10 | --- | Muscle | 0,03 | --- |
| Bone | 0,09 | 0,12 | Brain | --- | 0,043 |
| Cartilage | 0,04 | 0,15 | Wool | --- | 1,0 |
| Heart | 0,043 | --- | Hoof | --- | 0,2 |

It is seen from the data in Tables 1 and 2 that we discovered chromium in the majority of examined organs and tissues of humans and animals in slightly larger amounts in exterior covers (epithelium) and in the digestive tract.

Table 3 (P. 126)

Content of chromium in various food products.

| Name of product | Chromium mg% | Name of product | Chromium mg% |
|---|---|---|---|
| Flour | 0,185 | Beet | 0,025 |
| Bread | 0,087 | Carrot | 0,011 |
| Cream of wheat | 0,065 | Egg powder | 0,003 |
| Buckwheat (cereal grain) | 0,028 | Noodles | 0,005 |
| Cabbage | 0,031 | Fish ("khariuz") | 0,002 |

It is seen from table 3, that chromium is contained also in various food products. This is quite understandable since according to data of our research its contents in the soil was 0.0016%, in the sub-soil ground water — from 0.0009 to 0.0020 mg/liter, and in river water — from 0.0011 (Angara) to 0.0017 (Irkut) mg/liter.

## Conclusions

It has been established by spectral analysis, that chromium is contained in organs and tissues of humans and animals, in plant products as well as in the soil and the water. Its contents in the human body reaches up to hundredths and even tenths of a milligramm-per cent.

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# ANIMAL NEUROVIRUS DISEASES SIMILAR TO HUMAN POLIOMYELITIS

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The absence of a satisfactory laboratory model of poliomyelitis makes the experimental study of this disease extremely difficult.

At present, the only method of detecting the poliomyelitis virus is the experimental infection of monkeys or apes (obyez'yany) by means of virus-containing material obtained from the brains of dead human beings or the excrement of afflicted persons. The higher apes (such as chimpanzees) seem to be the most receptive to this method, followed by Javanese macaques, rhesus monkeys, hamadryas baboons, and green guenons, in that order. Increased interest in research pertaining to the study of the possibility of adapting the poliomyelitis virus to other species of animals is, therefore, understandable.

As yet, only a few strains of the poliomyelitis virus have been successfully adapted to white mice and cotton rats (Sigmodon hispidus). This refers to Lansing "SK," "K", "MM," Levkovich "113," and others.

In recent years a great deal of attention has been given to neuroinfections of animals which are similar to human poliomyelitis. At present, their epidemiological significance in the dissemination of poliomyelitis has not yet been established. These poliomyelitis-like infections of animals, however, are of undoubted scientific and practical interest to the medical world. Encephalomyelitis of mice and enzootic paralysis of swine (the Teschen disease) are two such infections.

The virus of encephalomyelitis of mice was isolated in the Soviet Union by I. A. Shifrin, L. Ya. Yablonovskaya, and others.

The particles of the virus which causes the disease in mice have extremely small dimensions, approximating the dimensions of the virus of human poliomyelitis. The mouse virus, it has been found, affects primarily the cells of the spinal cord. It is very stable, and can be preserved for several weeks or even months. Seriological investigations of mice to detect antibodies did not give accurate results. Contradictory data likewise were obtained in experiments attempting to determine the presence of specific immunity in mice after they had been infected intracerabrally. These characteristics of the virus of encephalomyelitis of mice in many ways closely resemble the properties of the virus of human poliomyelitis.

Dispite the similarity of these viruses, however, there is no basis whatsoever for assuming that mice or any other animals act as a reservoir of the poliomyelitis virus.

To clarify this question, it will be necessary to carry out investigations, since information concerning the spread of the virus of mouse encephalomyelitis is very meager and the relationships of this virus to the virus of human poliomyelitis so far have not been adequately studied.

A number of authors have pointed out the great similarity of human poliomyelitis to the Teschen disease of swine. This disease was first described by Trefni (1930-1931) in the Teschen district of Czechoslavakia, from which it received its name. It next became widespread in the Sudeten region, Bohemia, and Moravia. It was recorded in the border regions of Bavaria, Austria, and Central Germany (Leipzig). Spreading further, the disease affected the northeastern districts of Slovakia and then penetrated into Hungary, Yugoslavia, Switzerland, and Italy.

Comparatively recently, in 1950, Lepine and Apanasiou isolated on Madagascar a virus causing encephalomyelitis of swine. This virus turned out to be identical with the Czechoslavakian strain of the virus of Teschen disease. It is pathogenic exclusively for domesticated and wild swine. Monkeys and apes, hamsters, mice, guinea pigs, and rabbits are not susceptible to intracerebral infection with this virus. Blood serum of recovered animals neutralizes both the Madagascar and Czechoslovakian strains to a certain degree. At the same time, the virus of the Teschen disease does not prevent mice from becoming ill when they are infected with a Lansing-type strain of poliomyelitis.

According to the data of Klobouk, Fortner and others, the virus (of Teschen disease) passes through a Berkefeld N filter. Consequently, the dimensions of its particles are approximately the same as those of the poliomyelitis virus. It is very stable, can be preserved for 20 or more months in glycerine, does not lose its virulence for 3 months when stored at a temperature of minus 15°, withstands drying in the sun for 23 days, and does not perish in a brine solution at minus 6° to minus 10° or in putrescent material for about 6 days. The virus is inactivated in 30 minutes at a temperature of plus 70°.

The virus cannot be cultured in chicken embryos and does not infect chicks which have just hatched (Gallia, 1949). Under natural conditions, outside a living organism, it preserves its virulence for a long time.

The virus is detected in large quantities in the central nervous system at the start of the illness, especially during the first few days after the appearance of paresis and paralysis. Less regularly, it appears in the various secretions and excretions of the mucous membrane of the nasopharynx. A clinical case of the disease cannot be induced by artificial infection with blood or a suspension made from organs that contain blood.

Only domesticated and wild swine, and then primarily young animals, have been successfully infected. The basic sources of the infection are, evidently, sick animals with either a manifest or hidden form of the disease and animals which have recovered but still remain carriers and transmitters of the virus for a long time after their recovery. Under natural conditions, infection occurs mainly through the alimentary tract and the nasal passage. Under specific external conditions (severe changes in the weather, dampness, cold weather, or weakness brought about by a vaccination or surgical operation) susceptibility to the disease increases significantly.

Young pigs who have lived through the natural disease become immune, but some cases have been recorded in which the animals were reinfected or even died. Some authors look upon this as proof that several types of the virus of the Teschen disease exist. By subcutaneous and intravenous injections of the living virus an active immunity can be developed. Individual animals can be made to incur a clinical form of encephalitis by feeding them on infected matter.

The use of vaccines for prophylactic purposes is limited in effectiveness, since the immunity develpps approximately 30 or 40 days after inoculation and then lasts for no more than 2 to 4 months.

The cited characteristics of the properties of the virus which causes the Teschen disease in young pigs are similar in many ways to the characteristics of the virus of human poliomyelitis. The immunological behavior of the two viruses is different, however.

Comparisons of clinical poliomyelitis-like animal diseases with human poliomyelitis are of some interest. Gard in 1943 and Kaplan and Merentze in 1948 pointed out the unusual similarities of the Teschen disease of swine and encephalomyelitis of mice to human poliomyelitis.

Both in poliomyelitis and the Teschen disease the preparalytic stage of the illness is characterized by fever and meningeal phenomena (there is no information concerning encephalomyelitis of mice). The paralytic stage is much the same in all three diseases: flaccid paralysis is observed, primarily of the extremities, and cerebral forms appear, accompanied by ataxia and spastic symptoms, or tonic and clonic spasms.

Nonparalytic forms of all three infections have been recorded. The incubation period in an experimental infection lasts from 10 to 14 days. The disease can occur in an abortive form, resulting in full recovery without the development of paralysis. Some similarity can also be detected in the pathological changes, but there is an essential difference, wiich is especially apparent when a study is made of the dynamics of the pathohistological changes.

All three of these disease-causing agents are distinguished, as is shown above, by a typical species characteristic. Furthermore, they primarily affect young individuals. The diseases also are characteristically seasonal; the greatest incidence of the disease is recorded in the spring. On the other hand, each of the diseases being compared has its own peculiarities and variations, which establish a basis for considering the Teschen disease and encephalomyelitis of mice as independent diseases not related to human poliomyelitis.

Reports recently have appeared concerning the possibility of using antigens obtained from the virus of the Teschen disease for the laboratory diagnosis of poliomyelitis. Semenits and Rush in 1950, basing their work on the similarity of the Teschen disease of mice (this should possibly be of swine) to human poliomyelitis, prepared in a corresponding manner an antigen from the brain matter of young pigs which had died of the Teschen disease. They then used this antigen to set up a reaction of complement fixation in spinal fluid obtained from children ill with poliomyelitis.

Semenits and Rush confirmed a diagnosis of poliomyelitis in 57 children. In 23 children and adults suffering from serous meningitis, the complement-fixation reaction was negative. Negative results were also obtained when the reaction was set up with 60 control samples of spinal fluid from persons suffering from tuberculous meningitis, spinal atrophy, meningococcal infection, etc.

There are references in literature to the existence of poliomyelitis-like diseases in other domestic animals. Thus, for example, Frauhiger, in 1938, reported such a disease affecting calves during a poliomyelitis epidemic in Switzerland. The illness of the calves resembled Landry's ascending spinal paralysis with the affliction first in the rear and then in the front extremities. Changes detected in the spinal chord of the animals were similar to those recorded in human poliomyelitis. These changes were most evident in the lumbar and sacroiliac regions. They consisted of a perivascular infiltration by leukocytes and plasmocytes, especially into the forward vertebral processes, and of the destruction of ganglionic cells accompanied by phenomena of neuronophagy.

In 1938, Frauhiger and Hofman succeeded in experimentally infecting three calves with the poliomyelitis virus. They did this by simultaneously injecting into their brains, noses, and abdominal cavities of the calves a suspension containing the infectious agent. The animals incurred paralysis in their extremities.

Ferlicus observed a poliomyelitis-like disease in dogs in 1950.

Proceeding from these data, and considering poliomyelitis from the evolutionary-ecological point of view, several authors have expressed the hypotheses that in nature there exists a whole group of poliomyelitis-like viruses, which may have one common ancestor. It remains unclear, however, what the first host organism for the poliomyelitis virus was -- human or animal.

An assumption was also made that the poliomyelitis virus developed as an intestinal parasite of rodents, without perhaps causing a clinical infection in them. However, the virus gradually evolved and at some point became pathogenic for human beings. The causes for this change in its pathogenic properties remain unclear. Several authors, for instance, Barnet, suggested that the origination of new strains with increased virulence might be explained by mere chance.

However, "Science is the enemy of chance. Science can only really be called science, when it discovers, the laws governing development" (T. D. Lysenko, according to M. B. Mitin, For a Materialistic Biological Science (Za Materialisticheskuyu Biologicheskuyu Nauku), p 103, Moscow-Leningrad, 1949). Doubtlessly, the solution of this complicated problem is only possible on the basis of Michurin's biology, which regards the mutability of microbes and viruses as a result of changes in environmental conditions.

Proceeding from the similarity between the Teschen disease and human poliomyelitis, we attempted, in 1950, to infect young pigs with several strains of poliomyelitis. To 12 young pigs was administered a massive dose (1 ml into the brain, 20 ml into the abdominal cavity, and 3 ml into the nose) of six strains of poliomyelitis virus: Kvade, strain "113," KRF-1,

MV, 1-16, and Shevk. Two young pigs were used for each strain. The animals remained healthy. In some of them there was an insignificant rise in temperature, but this was not accompanied by any clinical manifestation of the disease whatsoever. One of the young pigs which had been infected with the 1-16 (Lansing) strain and had had no clinical manifestation of the disease was killed 13 days after the attempted infection. A suspension of brain tissue from this pig was used to infect a monkey and two other young pigs. None of them became diseased.

Thus, attempts to adapt human poliomyelitis to young pigs were fruitless. An attempt to cause a monkey to become ill by infection with a massive dose (1 ml into the brain, 10 ml into the abdominal cavity, and 3 ml into the nose) of the Czechoslovakian strain of the Teschen disease was likewise unsuccessful, although the simultaneous infection of a young pig with this virus caused it to fall ill, and, subsequently, to die on the 10th day after the infection.

After successful preliminary passages of a strain of the virus of the Teschen disease, we set up a titration experiment with the virus. Ten young pigs were injected with diluted solutions of a brain suspension obtained from animals which had died. The dilution ranged from 1:10 to 1:10,000. Titration of the virus showed that intracerebral infection of the young pigs with a 0.5 ml dose was possible with dilutions as high as 1:1,000. One out of two of the young pigs became ill, and at the same time, the incubation period for the disease was extended to 14 days.

A comparison of various methods of infecting young pigs with the Teschen disease is of undoubted interest. For this purpose we produced infection by injection into the brain, the nose, the tonsils, and also intracutaneously. In the last method, 0.05-ml doses of the virus were injected at ten points on the hind feet. Intracutaneous infection was repeated every week, just as we did in the intracutaneous infection of monkeys and apes with poliomyelitis virus (Rodin, Itselis, 1951).

All the young pigs infected by injection into the brain became ill in 5-9 days. We succeeded in causing the disease in one of the two young pigs infected by injecting the virus into the tonsils. Intracutaneous infection caused the disease in one of the two young pigs injected. On the 5th day after the second intracutaneous injection of the virus, the pig's temperature increased sharply and the animal soon developed a flaccid paralysis of the legs, particularly the front ones.

The infection of two young pigs by injection into the nose, 1.5 ml in each nostril, was likewise successful.

The clinical pattern of the disease following experimental infection of young pigs with the Teschen virus, despite differences in the individual neurological symptoms, is generally quite uniform. It comprises nystagmus,

convulsive movements, trembling of the whole body (especially the head), opisthotonus, ataxia, and weakness of all the legs, usually without paralysis. Flaccid paralysis of the extremities developed in only two of the young pigs, one infected through the nose and the other intracutaneously.

Simultaneously with the infection of the young pigs with the Teschen virus, white mice, cotton rats, and guinea pigs were repeatedly, but unsuccessfully, injected with the virus.

Our investigations show that the Teschen disease of swine is characterized by the duration of the incubation period, which almost never varies in length subsequently to intracerebral experimental infection. The length of the incubation period changes somewhat depending on the means of infection and the degree of dilution of the virus. According to our data, the majority of young pigs infected by injection into the brain become ill in 6-7 days, whereas those infected by injection into the nose, the tonsils, or intracutaneously exhibit an incubation period of 11-12 days.

This constancy of the length of the incubation period with the same method of infection, and the constancy of the clinical symptoms, which are clearly pronounced, afford us the possibility of using our observations on the Teschen disease for the solution of some of the problems of pathogenesis and immunity in neuroinfections.

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Trans. 486

VSESOIUZNAIA AKADEMIIA SEL'SKOKHOZIAISTVENNYKH NAUK

imeni V. I. Lenina

VSESOIUZNYI INSTITUT ZASHCHITY RASTENII

# COLLECTION OF WORKS ON NEMATODES OF AGRICULTURAL PLANTS

Ed. by E. S. Kir'ianova

SEL'KHOZGIZ

GOSUDARSTVENNOE IZDATEL'STVO
KOLKHOZNOI I SOVKHOZNOI LITERATURY
Moskva 1939 Leningrad

III. Our tasks in the domain of investigating nematode diseases of plants.

1. Question of terminology.

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II. Spreading of the root nematode in the USSR according to the 1935 inspection data.

III. Distribution and area of injury of the root nematode.

IV. Certain ecological requirements of the root nematode.

V. Host plants of the nematode.

VI. Biological observations of the nematode.

VII. Control measures for the root nematode.

VIII. Sanitary and quarantine measures.

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I. Material.

II. Inspected regions.

III. Method.

1. Preparation of compounds.

IV. General evaluation of the fauna of tomato nematodes found in the inspected localities.

V. Nematode fauna of tomatoes in the Crimea.

VI. Nematode fauna of tomatoes in the Krasnodar Territory (Sochi Region).

VII. Nematode fauna of tomatoes of Abkhazia.

VIII. Nematode fauna of tomatoes of Adzharia.

IX. Nematode fauna of tomatoes from Gruziya [Georgia].

X. Systematic situation and description of the separate representatives of the nematode fauna of tomatoes and the surrounding soil.

XI. Conclusions.

References:
6 Soviet, 12 foreign.

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Nematode diseases of the rubber plant tau-saghyz and the problem of their control. pp. 208-222.

1. Method.

References:
12 Soviet, 5 foreign.

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Method of study and technics of gathering injurious plant nematode material. pp. 223-235.

I. Method of study of injurious nematodes.

a) Systematic part

b) Measurements of nematodes

II. Methods of study of the injuriousness of nematodes.

III. Methods of study of the biology of injurious nematodes.

IV. Technics for gathering injurious plant nematode material.

a) Analysis of plants.

b) Analysis of soil.

V. Technics for processing nematode material.

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3 Soviet, 14 foreign.

English summary on p. 243.

THE LENIN ACADEMY OF AGRICULTURAL SCIENCES, INSTITUTE FOR PLANT PROTECTION

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A COLLECTED WORKS ON NEMATODES OF AGRICULTURAL CROPS

# A STUDY OF NEMATODE DISEASES OF PLANTS IN THE U. S. S. R.

E. S. Kirjanova

## Summary

The first attempts to study the nematode diseases of plants in our country were made by I. K. Tarnani in 1895-1898, who found the sugar-beet nematode Heterodera schachtii Schmidt in the territory of the modern Poland. In 1895 I. K. Tarnani investigated the sugar-beet plantations in the former province of Kiev, Podolia, Volynia and Kharkov, but H. schachtii was not found there. The first indication in literature on the occurrence of the sugar-beet nematode on the territory of the U.S.S.R. is to be found in an article by K. I. Shishkin (1923), he states that H. schachtii was discovered in the Ukraine by I. I. Korab and V. N. Shevchenko.

In 1898 I. K. Tarnani discovered the root-gall nematode Heterodera marioni Cornu (I. K. Tarnani, 1898), in some greenhouses in Kiev. Later it was found on vine roots in the Caucasus (N. N. Speshnev, 1899 and I. A. Staroselsky, 1899) and recently by A. A. Ustinov and other authors.

In 1907 S. A. Mokrgetsky found the wheat nematode, Anguillulina tritici Steinbuch, in Crimea. Further information concerning this nematode have appeared only 17 years later in the following articles: A. Egorova (1924), V. Zdravomyslov (1925), N. M. Kulagin (1928) and A. A. Meyer (see this volume p. 160).

During the last 5—7 years a nematode disease of potatoes caused by the stem nematode, Anguillulina dipsaci Kühn, was found (E. S. Kirjanova, 1935; O. D. Belova, see this volume, p. 142).

In 1933—1936 there has been observed in Moscow a nematode disease of chrysanthemums caused by Aphelenchoides ritzema-bosi Schwartz (Z. V. Lomakina).

Injurious nematodes have been found as well on cotton, cucumbers, onion, cyclamens', Dalmatian daisy (E. S. Kirjanova), on tomatoes (A. T. Tulaganov), on strawberries (A. N. Khakhulina), Scorzonera tau-saghyz (N. M. Sveshnikova and T. S. Skarbilovich) and on many other plants. The study of injurious nematodes has been started in many research institutions of our country.

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# THE ROOT-KNOT NEMATODE Heterodera marioni (Cornu) IN THE U. S. S. R.

(Results of the Plant-Quarantine Administration work in the U.S.S.R.)

A. A. Ustinov

## Summary

Root-knot nematode — Heterodera marioni (Cornu) is widely distributed in the southern republics of the U.S.S.R. (Fig. 1).

Biological observations on the nematode have been carried out in Western Georgia. In that country the nematode gives birth to 4—5 generations. The number of eggs laid by one female amounts to 1800. Oviposition lasts up to 61 days.

In summer the development from the stage of an invading larva to an oviparous female takes 24 days, while the emergence of adult males takes place in 17 days. Active movements of nematode larva within the podzol soils of Georgia proves to be very slow. The larva advances not more than 25 cm during the summer season, while the nematode larva penetrate deep into the soil and may be found at a depth of 50—80 cm from the surface.

Investigations and experiments carried out for the purpose of checking the immunity of plants to the root-knot nematode corroborated the resistance of: Arachis hypogaea, Stizolobium, Crotalaria sp., Vigna sinensis (varieties: Braham and Iron), Sweet potatoes (varieties — Southern Queen and Jersey) and cereals.

Fumigating the soil with big doses of chlorpicrine and carbon-bisulphide greatly reduces the number of nematodes infesting the soil. Applying 100 gr. of chlorpicrine to the square metre the infestation of plants with the nematode decreases to 80—95% in comparison with the check.

Sodium cyanide and formalin have not proved effective when used against the nematode.

Hot water treatment used to kill the root-knot nematode in different plants showed that the pest died within the roots of herbaceous plants after being dipped into hot water 51°C. for 10 minutes or 53°C. for 5 minutes.

An application of calcium cyanomide as a fertilizer at the rate of 7 tons to a hectare decreases the rate of infection by two-thirds. Other fertilizers do not reduce nematode infestation but greatly increase the yield of the infested plants.

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## RESULTS OF INVESTIGATING THE ROOT-GALL NEMATODE ON *Lavandula vera* IN CRIMEA

T. A. Ikhtinskaya and M. N. Arkhangelskaya

### Summary

1. The root-gall nematode *Heterodera marioni* (Cornu) has been found in the U.S.S.R. on the following essential oil plants: iris, lavender, rosemary, tuberose, thyme and geranium. The nematode has not been found on such essential oil cultures as: *Andropogon citratus*, *Lippia citriodora*, Kazanlyk rose and lemon verbena.

2. Under experimental conditions the nematode was more active at a soil moisture of 70—80%. Decreased soil moisture (30%) retards the development of the pest while the soil moisture amounting up to 100% has a depressive effect on the pest as well as on the plant.

3. The method of disinfecting the plants with warm-water treatment may be not recommended for *Lavandula vera*, because the minimum temperature and length of exposition (50°C. for 10 minutes), which killed the nematode, prove to be destructive for the plants.

4. No positive result has been obtained with application of cyanomide of calcium dosed 0.5 to 5 gr. to a kilogram of the soil. Minor doses have a favorable effect on the development of the plants and the pest.

5. The root-gall nematode cannot be considered as a serious pest of lavender in Crimea owing to the drought climate of the country.

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## THE ROOT-GALL NEMATODE IN KAZAKH S.S.R.

N. F. Litvinova

### Summary

In this paper are given the results of an examination of several regions in Kazakhstan as to the presence or absence of the root-gall nematode.

The root-gall nematode was found in Alma-Ata and in the vicinity of Tashkent. It attacks many vegetables, some leguminous plants and the rubber-plant kok-saghyz. It is interesting to note that on two farms in the Alma-Ata District on fields where water-melons and musk-melons were grown together,

the latter were severely attacked by root-gall nematodes while the former were quite free from infection.

This shows that these nematodes are selective as regards choice of hosts, a characteristic, already noted by other investigators (Tischler and others).

In the vicinity of Mirzoyan, Merke, Tyulkubas, Chimkent and Burnoye no root-gall nematodes were found.

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## RESULTS OF THE STUDY AND CONTROL OF THE SUGAR-BEET NEMATODE, Heterodera schachtii Schmidt

I. I. Korab and A. P. Butovsky

### Summary

In this work the authors have summarized the results of practical studies of the sugar-beet nematode Heterodera schachtii Schmidt, carried out in various places of the U.S.S.R. during 1926—1936. The main nidus of soil infestation by nematodes, are concentrated in the oldest regions of the main sugar beet growing zone (Fig. 2).

Notwithstanding the considerable spread of this most dangerous sugar-beet pest, the intensity of the soil infestation caused by it seldom reaches the limits beyond which its effect acquires economical importance (except single farms). This paper presents the results of experimental work carried out by the writers at the Nizovsky sugar-beet collective farm where the infestation of the ground with sugar-beet nematode was very intense.

Among the methods of controlling the nematode the greatest attention should be paid to crop-rotation (Chapter IV), it reduces considerably the actual infestation of the soil by nematodes.

The following plants cultivated in the field prove to have a most negative effect upon the sugar-beet nematode, they have been tested in heavily infested soil: succory, rye, maize, wheat, timothy, oats, barley and among the leguminous — lucerne, clover, vetch, esparcet, and lupine.

Among measures securing high and lasting yields of sugar-beet in fields heavily infested with nematodes the method of overfertilizing such fields should be mentioned as one tested under field conditions. Using double, three-fold and even four-fold norms of mineral fertilizers may prove profitable, owing to a considerable increase of yield.

The chemical method of controlling the nematode with chlorpicrine is one

of the most effective measures of control (Tables 22, 23). It is rather expensive for use on large areas, but it may be applied to comparatively small nidi badly infested by nematodes.

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# THE STEM NEMATODE AS A PEST OF AGRICULTURAL CROPS IN THE U.S.S.R.

E. S. Kirjanova

## Summary

In the U.S.S.R. the following plants have been registered as hosts of A. dipsaci.

| Family | Common name | Host plant |
|---|---|---|
| Gramineae | Rye | Secale cereale L. |
| " | Wheat | Triticum sativum L a m. |
| " | Oats | Avena sativa L. |
| " | Barley | Hordeum sativum J e s s. |
| " | Millet | Panicum miliaceum L. |
| Compositae | Daisy | Pyrethrum cinerariaefolium T r e v. |
| " | Succory | Cychorium intibus L. |
| " | Tau-saghyz | Scorzonera tau-saghyz Lipscher and Besse' |
| Rosaceae | Strawberry | Fragaria sp. |
| Solanaceae | Potato | Solanum tuberosum L. |
| " | Tomato | Lycopersicum esulentum Mill. |
| " | Tobacco | Nicotiana sp. |
| " | Pepper | Capsicum annuum L. |
| Chenopodiaceae | Beets | Beta vulgaris L. |
| Malvaceae | Cotton | Gossypium sp. |
| Cucurbitaceae | Cucumber | Cucumis sativus L. |
| Linaceae | Flax | Linum usitatissimum L. |
| Vitaceae | Vine | Vitis vinifera L. |
| Primulaceae | Cyclamen | Cyclamen latifolium cult. |
| Leguminosae | Clover | Trifolium arvense L. |
| | Alfalfa | Medicago sativa L. |
| | Kidney Bean | Phaseolus vulgaris L. |
| Liliaceae | Onion | Allium cepa L. |
| Cannabineae | Hemp | Cannabis sativa L. |
| Cruciferae | Rape | Brassica napus L., var. esculenta D.C. |
| Labiatae | Lavender | Lavandula vera D.C. |

In addition, the nematode has been found in virgin soils in the vicinity of Orsk, Orenburg Region.

The damage caused by the nematode to the host plants has been studied as yet very little. It is known that A. dipsaci attacks potatoes in the Ukraine and in the Smolensk Region, Pyrethrum cinerariaefolium Trev. in the Crimea, probably oats in Siberia and some other plants.

The potato strain of this nematode differs in body dimensions, length of larval stage, and size of eggs from the onion strain (Tabl. 2, 3, 4).

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## RESULTS OF OBSERVATIONS AND FIELD EXPERIMENTS MADE WITH THE STEM NEMATODE ON POTATOES

O. D. Belova

### Summary

1. The stem nematode Anguillulina dipsaci Kuhn affecting potatoes is widely distributed in the Ukraine.

Usually the percentage of infestation in single farms does not prove to be high (2%, 3%), but in some instances it amounts up to 23%.

2. The potato plants are infested by the nematode through the soil and tubers.

3. Potato varieties differ in their resistance to nematode attacks. Of the 7 varieties tested the most resistant proved to be Voltman and Parnassia; Smyslovsky, Deodora and Minecrop were partly resistant, while the Early Rose and Epicure were mostly subjected.

4. The measure to be recommended for controlling the disease is the use of resistant varieties, as well as healthy tubers for planting.

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# NEMATODES INJURING WINTER WHEAT IN THE NORTHERN PART OF THE OREL REGION

N. I. Koroleva

## Summary

1. The destruction of wheat in the northern part of the Orel Region during the winter and spring seasons is some years very considerable. It is caused not only by unfavorable meteorological conditions, during the over-wintering period, but is also the result of damage caused by pests and diseases comprising round worms — nematodes.

2. In plants taken out of snow the nematodes have been found in a benumbed state in the leaf axils. At the temperature of 12—14°C. they became active and multiplied rapidly.

3. Meteorological conditions effect greatly the development of nematodes. Moist and long autumn creates conditions favoring the development of nematodes. On the contrary, a droughty spring may considerably reduce the injurious effect of the pest.

4. The nematode invades winter wheat in autumn causing by then a partial destruction which increased in early spring.

5. The sowing term of winter wheat is in great connection with the infestation by nematodes. Wheat sown in early August is particularly affected by the pest.

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# A STUDY OF NEMATODES ON CEREALS

M. I. Frolova

## Summary

During the years 1933—1936 the writer carried out observations on the destruction occuring in wheat at the Stony Steppe Experiment Station and at the Ternovsky Sugar-Beet State farm of the Voronezh Region. This destruction of winter wheat does not invariably depend on unfavorable meteorological conditions, as it is usually considered, but to a considerable extent is the effect of the parasitic activity of nematodes. In every affected plant nematodes have been found in great numbers. Besides invading winter corn, wheat and rye, they infest spring wheat, oats and barley. In the diseased

plants there were found the following nematodes: Anguillulina dipsaci Kühn, Paraphelenchus pseudoparietinus Micoletzky, Cephalobus elongatus de Man, Aphelenchus avenae Bastian and some other species.

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## THE WHEAT NEMATODE, Anguillulina tritici, IN THE CRIMEA

A. A. Meyer

### Summary

In the autumn and winter of the year 1929 the Crimean Plant Protection Station carried out analysis of 2215 samples of Crimean winter wheat sent from various regions of the Crimea.

230 samples from the vicinity of Evpatoria were affected by wheat nematode galls while from all the other parts of the Crimea only 20 samples proved to be affected by this pest. The ratio of the affected samples to the entire amount of samples of Crimean wheat tested was 10.69%.

It should be mentioned that the wheat nematode was discovered only in samples of ordinary wheats and was not found in the varieties Novokrymka and Cooperatorka.

*********

## NEMATODE FAUNA IN TOMATO PLANTS (Lycopersicum esculentum Mill.) AND IN THE SOIL SURROUNDING THEIR ROOTS

A. T. Tulaganov

### Summary

1. The list of nematodes inhabiting the tomato plant, its root system and the surrounding soil includes up to 50 species belonging to 23 genera (Table 9).

2. Those mentioned below are to be considered injurious: Heterodera marioni Cornu, Aphelenchoides kuhni Fischer, Anguillulina pratensis de Man.

3. Among other nematodes in the soil surrounding the roots of tomato plants there was found Procriconema membranifera Micoletzky. It is necessary to notice that P. membranifera described first in 1925 was never found since.

It is interesting to note that two new species of the genus Londigorus were found in the soil surrounding tomato roots in Georgia (Tulaganov, 1937); this genus proves to be poor as to the number of species composing it.

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## THE NEMATODE DISEASE OF THE RUBBER-BEARING PLANT Scorzonera tau-saghyz Lipsch. and Bossé AND THE PROBLEM OF ITS CONTROL

N. M. Sveshnikova

### Summary

During field investigations made in 1933—1935 there were observed cases of active intrusion of the nematode Tylenchus multicinctus Cobb into the roots of the seedlings Scorzonera tau-saghyz. The anterior part of the body of this pest penetrating into the tissues of the root, whilst the posterior one remained outside (Fig. 1). The plant appeared quite healthy, only brown dots being noticeable on the root. The infection of seedlings under field conditions attained 63%.

The experimental infection of tau-saghyz seedlings by Tylenchus multicinctus carried out by the investigators (N. M. Sveshnikova and T. S. Skarbilovich) gave an infestation up to 82% of the experimental seedlings (Tabl. 3 and 4); the intrusion of the nematodes beginning on the 4th day, and a mass destruction of the plants results on the 12th, 15th day.

A microbiological investigation of fresh cases of nematode intrusion into the roots of the seedlings, made by the method of cultivating upon a nutritive medium, enabled the microbiologist Kalinenko to see the development of the microflora causing root maceration. These data establish the fact that the nematodes introduce a microflora into the healthy root, and that the seedlings, in spite of their outward healthy appearance, are in the initial stage of the maceration process.

The biological observations made by T. S. Skarbilovich, establish definitely that the nematode Tylenchus multicinctus and T. pratensis deposit

their eggs in the tissues of the root of S. tau-saghyz.

An investigation of the soils in Middle Asia and in the Ukraine at the depth of 50 cm, surrounding the roots of infested plants, showed that the quantity of the nematodes varied according to the degree of soil humidity. In irrigated soils the number of nematodes is ten times as great as in dry ones.

Fertilization with CaO in doses of 420 and 1680 kg. per hectare did not decrease the number of nematodes in the soil.

Fumigation of the soil with chlorpicrine in doses of 200, 400 and 800 kg. per hectare or with calcium cyanamide (CaCH) in doses of 800 and 1300 kg per hectare was tried. Neither of these poisons had any destructive effect upon Tylenchus multicinctus and T. pratensis, perhaps, owing to the great compactness of the soils in Middle Asia.

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## METHODS AND TECHNIQUE USED IN THE STUDY OF PLANT PARASITIC NEMATODES

T. S. Skarbilovich

### Summary

The work includes indications on some methods used for studying the biology, systematic and pathology of plant parasitic nematodes. Besides considerable attention is given to the technique of sampling.

Some experimental breading of nematodes Anguillulina multicincta and Anguillulina pratensis on a medium recommended by Byars was unsuccessful as the nematodes, on being introduced into this medium, perished without having penetrated into the roots of the rubber-plant S. tau-saghyz offered as host. Another medium, Hellriegel's mixture on 1% agar, was used, but the nematodes perished in it as well.

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# COLLECTION OF WORKS ON NEMATODES OF AGRICULTURAL PLANTS. II.

PAVLOVSKII, E. N. and KIR'IANOVA, E. S. -

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References:
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A list of references on phytopathogenic, soil, fresh water and marine nematodes of the USSR, as well as on parasites of insects; compiled to supplement a list published earlier (Kir'ianova 1939). pp. 368—377.

a) Phytopathogenic and soil nematodes
143 Russian (1903-1949)

b) Freshwater and marine nematodes
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1. Races in garden beet nematodes
2. Races in stem nematodes
3. Origin of biological races
4. Conclusions

References:
17 Soviet, 45 foreign.

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I. Morphology and development
   a) Embryonic and postembryonic development of the gall nematode.
   b) Comparative sketch of the structure of the gall nematode at different stages of its development.
   c) Metamorphosis. Development of males and females.
   d) Morpho-physiological adaptation of the gall nematode to parasitism.

II. Ecology of the gall nematode
   a) Ecology of the pre-parasitic larva.
   b) Life span and fertility of females. Condition of development of males.
   c) Climatic factors. Influence of temperature and moisture.
   d) Role of predators and parasites.

III. Interrelation between the gall nematode and the plant.

a) Conduct of the nematode and reactions of the plant.

b) Quantitative counts of the gall nematode in plants.

c) Innoculation of microbes.

d) Pathogenesis of the gall nematode.

e) Plant immunity to the gall nematode.

IV. Host plants of the gall nematode.

a) Quantity and systematic position of the hosts.

b) Susceptible and resistant plants.

c) The biological forms of gall nematodes.

V. Control measures for the gall nematode

a) Physical control measures.

b) Chemical control measures.

c) Agrotechnical control measures.

d) Biological control measures.

e) Systems of control measures against gall nematode.

References:
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1. Symptoms and causes of the disease.
2. Description of the parasite.
3. Biology.
    a. Host plant
    b. Varietal resistance of Chrysanthemum to the disease
4. Control measures.
5. Conclusions.

References:
5 Soviet, 12 foreign.

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1. Working method.
2. Composition of the nematode-fauna of onions - Allium cepa L.
3. History of the study of the onion nematode - Ditylenchus allii (Beijerinck).
4. Morphology.
5. Development and mode of life.
6. Influence of the onion nematode upon the plant organism.
7. Host specificity.
8. Control measures for the onion nematode.
9. Comparison of the populations of onion nematodes growing on different plants.
10. Conclusions.

References:
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3. Analysis of soil for nematodes.
4. Conclusions.

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Nematode fauna of sugar beet shoots. (Vsesoiuznyi Institut Gel'mintologii im. Akad. K. I. Skriabina). pp. 597-601.

I. Characteristics of the nematodes found.
   1. Stem nematode - Ditylenchus dipsaci (Kuehn, 1858)
   2. Rotylenchus multicinctus (Cobb, 1893)
   3. Pratylenchus pratensis (de Man, 1881)
   4. Tylenchus filiformis (Buetschli, 1873)
   5. Aphelenchus avenae, Bastian, 1865.
   6. Aphelenchoides parietinus (Bastian, 1965).
   7. Paratylenchus bukowinensis, Micoletzky, 1922.
   8. Eucephalobus elongatus, de Man, 1880.
   9. Genus TAcrobeles, von Linstow, 1877.
   10. Genus Rhabditis, Dujardin, 1845.
   11. Genus Dorylaimus, Dujardin, 1845.

II. Conclusions.

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   a) Subfamily Demaniellainae nov. subf.

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Izmenchivost' u Rastenieiadnykh nematod pod vliianiem ikh kormovoi spetsializatsii

[Variability in phytopathogenic Nematodes caused by host specificity]

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"The principal matter here is that each progress in organic development is at the same time a regress because it secures a one-sided development and excludes the possibility of development in many other directions. But this is a basic law". (F. Engels. Dialetics of Nature. 1949. Gospolitizdat. p. 249)

Development of the problem of species is one of the basic tasks of the Soviet biologists. Of particularly great merit in this field are the immortal works by I. V. Michurin and by his outstanding contemporary T. D. Lysenko who continued his work. Academician T. D. Lysenko and his numerous disciples and followers proved that under the influence of certain conditions of development which continue through several generations, it is possible to achieve a transformation of one species into another (change of hard wheat into soft one) and to obtain new species of plants of another genus (formation of rye grains in wheat ears). Even earlier, by way of hybridization, I. V. Michurin brilliantly solved the problem of the possibility of obtaining experimentally not only new species (bergamot rennet), but also new plant genera ("tserapadus" [?]).

These works are guiding stars for all the Soviet biologists and, in particular, for the Soviet nematologists who draw from them invaluable information of practical as well as methodological order. The work being published has the purpose of dealing with problems connected with formations of species in phytopathogenic nematodes in the light of Michurin's biology.

In phytopathogenic nematodes the adaptive variability has a tendency towards steadily greater adaptability of these worms to their host plants. And two leading tendencies of variability are outlined.

1. One of them is outlined towards an increasing adaptability of development cycles of Nematodes to development cycles of their host-plants. There are a series of examples which show how close-

development of the host plant. Let us concentrate on the best known example — wheat nematode Anguina tritici (Steinbuch). This nematode is usually spread with seed grain, with which it became universally distributed.

The nematodes in amounts of several tens of thousands of larvae are inside the galls which are admixed with the wheat grain. The exterior form of the galls is similar [begin p. 379] to the original grains. Entering the soil the wall of the gall becomes soft due to the moisture and the nematode larvae leave the gall in order to find their host-plant. Having found a young wheat plant the larvae make their way to the point of stem growth or into "pazukhi" [sheaths] of leaves and with the formation of the spike they migrate inside the embryonic parts of future flowers. The affected spike does not bloom and instead of flowers, galls of wheat nematode are formed which appear to be completely formed at the moment of wheat blossoming. At that time the gall is shiny green. Inside of it there takes place the copulation of males and females of wheat nematode which have changed into mature worms. By the time of the milky maturity stage of wheat inside the galls nematode eggs are already deposited from which after a short time interval hatch larvae of the first generation which at the moment of ripening of wheat grains are transformed into larvae of second generation and which enter the state of anabiosis in a mature gall. The maturity of the latter begins simultaneously or even a little earlier than that of wheat grains. During the harvest the gall carried with the threshed grain into the granaries and then into the fields where the larvae of wheat nematode continue their development. It is interesting that in a dry place inside galls, the nematodes can be preserved more than 10 years, and according to some data — even up to 27 years, without losing their viability. Variability of this species is observed mainly in the change of form and size of galls depending on the species or variety of wheat which is infected (fig. 1).

Fig. 1 [p. 379] Different forms and sizes of galls of wheat nematode - Anguina tritici (Steinbuch), grown on various wheats (Fig. by J. V. Grigor'ev, magnif.). (According to Kirianova, 1941). A-galls grown on spikes of regular hard wheat, from the village Kusary, Gil'skii raion, Azerbaidzhan; b - galls grown on spikes of regular soft wheat, from the village Sakobo, Signakhskii raion, Georgian SSR; B - galls on spikes of winter wheat, of the "Ukrainka" variety, from the village Khrebtov, Krasnodar krai.

The second example is the smartweed nematode Anguina pieridis (Kirjanova, 1944), discovered by the author in 1942-1944 in the Gissarskaia valley of Tadshikistan, in different points of the Rokhatinskii, Vrdzhonikidzeabadskii and Varzobskii raions, as

well as in the vicinity of Stalinabad, exclusively on smartweed [?] — Acroptilon pieris C.A.M. This nematode [fig. 2] produces galls on the stem, leaves and the crown of smartweed, which can be very numerous. At times the affected plants are very ugly due to considerable deformation of the main and the side stems, which causes a complete or a partial under-development of their generative organs. As a rule the latter was observed in specimens with a large number of galls in the lower part of the stem [begin p. 380] and especially near the crown. The amount of galls was sometimes so large that they were distributed in clusters around the main stem or formed continuous growths on its side branches which in such cases were bearing exclusively galls (fig. 3).

Fig. 2 [p. 380]. Smartweed [?] nematode -- Anguina picridis (Kirjanova, 1944). (Fig. by V. N. Lototskaia, orig.)
1 - head end of male body;
2 - Tail of male, seen from belly side;
3 - same, seen from the side;
4 - spicules of the male;
5 - general view of female body.
6 - head and
7 - rear end of female body
8 - eggs.

Fig. 3 [p. 380]. Smartweed [?] — Acroption picris C.A.M., seriously affected by smartweed [?] nematode. Instead of side stems and leaves on the main stem of the plant developed only galls of the smartweed [?] nematode. (Fig. by V. N. Lototskaid, orig.)

But most frequently the diseased plants developed almost normally, but carried in some spots single galls (fig. 4 and 5) or had deformed stem sections and a considerably reduced number of flower-bearing shoots as compared with healthy specimens. In normal smartweed plants the leaves and stems are usually covered with thin long white hairs, which form a dense fluff of a felt type, which gives it a grayish-green color. And the stem is noticeably more fluffy than the leaves. On the surface of the galls the felt cover is developed very strongly, so that the galls look grayish-white and therefore stand out distinctly among the healthy parts of plants. With age the fluff of the gall [begin p. 381] can become dirty-gray due to the dust. The walls of the young galls are green, but in the course of time they darken considerably and become almost black. In old galls the epidermis together with the fluff separates frequently from the bark; the latter becomes exposed and then the galls acquire, partly or completely, a dark color. The galls have quite thick and durable walls and a vast cavity inside. The latter is filled with tens of thousands of microscopically minute nematodes in all stages of development (eggs, larvae, mature ones) but usually with some one predominant stage. At the end of the growing period, together with the drying-out of the affected plant-host, the smartweed

nematode enters an anabiotic state and hibernates inside the galls, which are preserved for a long time on stems of dried-out diseased plants continuing to remain in the fields until their destruction by the activity of the wind, rain or trampling cattle. When the remains of diseased plants come to the surface of the soil and the wall of the gall is destroyed, the nematodes crawl out into the soil and attack the young sprouts of smartweed which by that time begin to appear and the life cycle of the parasite, inseparable from the life cycle of the plant-host starts from the beginning.

Fig. 4 - [p. 381]
Gall of the smartweed nematode at the tip of the leaf of the smartweed -- Acroptilon picris C. [?S?] A. M.
(Fig. by V. N. Lototskaid, orig.)

Fig. 5 - [p. 382]
Gall of the smartweed nematode on a stem at the base of smartweed leaves.
(Fig. by V. N. Lototskaia, orig.).

2. - In other cases such complete coinciding of the development cycles of the parasite with the development cycle of the plant-host is not observed. On the contrary, it frequently happens that in one host-plant develop several and even many nematode generations. In these cases the tendency of the adaptive variability will apparently be expressed in formation of specific characteristics in the metabolism between the parasite and the host, which are frequently the cause of a start of various new formations and degeneration of tissues in the plant-host. The development of the parasite usually takes place in the latter.

As an example can be taken the gall nematode Heterodera maroni (Cornu) which affects a very large number of various plants (over 1500 species). Larvae of second generation of the gall nematode, while in the soil, penetrate into young roots of the host-plant and develop there into a mature phase. Under the influence of the nematode's life activity, apparently in connection with the secretion of its salivary glands, inside the central cylinder of the root, peculiar gigantic cells are formed which feed the parasite (fig. 6). The gigantic cells become surrounded with specific vascular cells which serve as their nutrition and in the points where these new formations originate the fibrovascular bundle degenerates. All the basic plant juices go for the nutrition of the gigantic cells which, in turn, go to feed the parasite. The parenchyma of the root expands abnormally and forms a gall around the body of the nematode (fig. 7). Inside the gall takes place the copulation of the nematodes, depositing of eggs and further development. At the same time part of the eggs escape outside with drops of the slime discarged by females through the sexual opening [vaginal outlet]; part of them can continue developing inside the gall. The slime corrodes the bark tissues which eases the escape of larvae into the soil [begin p. 382] in order to find a new host-plant and at that point a deep wound forms in the bark (fig. 8).

Variability in the gall nematode is apparent in the different sizes of populations taken from different host-plants, which have their own variation curve. Very characteristic is also the reaction of various plants to infection by the gall nematode which form galls of peculiar size and form (fig. 8 and 9). Plants with grass roots (cucumbers, melon, pumpkin) usually form large galls with many nests; in plants with a solid root (fig. pomegranate), particularly if large trees are being infected, the galls are negligible and when the nematodes deposit the eggs the bark tissues are destroyed rather rapidly in small sections near the vulva of the nematode, allowing their slimy egg sac to protrude freely outside.

Both tendencies of variability cannot be considered as sharply individual. On the contrary, in the first as in the second case the decisive [begin p. 383] significance belongs to the metabolism between the parasite and the host which reflects on the entire development of both. I wanted only to emphasize the specific peculiarities of this metabolism.

The two above-mentioned tendencies of variability are relatively little related to the morphological anatomical characteristics of organization of the nematodes which change very little. As an example, the nematodes of the genus Aphelenchoides can be pointed out, namely: strawberry nematode — A. fragariae (Ritzema-Bos), chrysanthemum — A. ritzemabosi (Schwartz), current — A. ribes (Taylor) and A. olesistus (Ritzema-Bos) (fig. 11). All these nematodes differ from each other very slightly, mainly by relative sizes and the relationship between individual organs and parts of the body, as well as by the tail ends of the males being twisted more or less towards the ventral side. However the plants' reaction to infection by these species of nematoda is very unique and characteristic for each species (fig. 10-13). The strawberry nematode causes dwarfness and an abnormal growth of all the plant tissues not only in strawberries (fig. 10), but in chrysanthemums as well (fig. 12). The chrysanthemum nematode never causes dwarfness in any of the plants which it affects: chrysanthemum (see fig. on p. 480), aster, dahlia, begonia, rudbeckia, etc. The currant nematode causes a characteristic bushiness on tops of black currant shoots (fig. 13), where an enormous accumulation of nematodes can be observed. Peculiar is also the reaction of plants to infection with other nematodes of the genus Aphelenchoides.

There are also very slight morphological-anatomical differences in some nematodes of the genus Heterodera, namely: beet nematode — H. schachtii (Schmidt, 1871) potato — H. rostochiensis (Wollenweber, 1923), oats — H. avenae Filipjev, 1934 and other related species. The males of all these species [begin p. 384] are almost indistinguishable — so closely similar are they to each other (fig. 14); the females differ in sizes, in the form of the posterior end of the body and structure of cuticle, but these differences are very weakly expressed. The mentioned nematodes of the genus Heterodera are characterized by great specificity in the choice of host-plants and do not cross over from potatoes to beets or oats and vice versa. In the West-European literature the potato, beet and oats nematodes are frequently considered as one species.

Fig. 6. [p. 383]
Longitudinal section through the root of tau-sagyzh infected by the gall nematode — Heterodera marioni (Cornu) visible are poly-nuclear gigantic cells surrounded with vascular cells. Head ends of two nematode females represented in the drawing are directed towards the gigantic cells and come close to them. Inside the nematode bodies are seen numerous eggs. (Fig. by I. V. Grigor'ev from a microphot.) (Kir'ianova, 1950)

Fig. 7. [p. 384]
Cross section through the root of a tau-sagyzh infected by the gall nematode. The central cylinder is pushed aside and gigantic cells (1) are formed inside it; the body of the nematode (2) is surrounded with overgrowing cells of parenchyma of the bark. (Fig. by I. V. Grigor'ev from microphot.) (Kir'ianova, 1950).

Fig. 8. [p. 385]
Part of the side roots of the fig tree infected by the gall nematode. After the depositing of eggs by the nematode females, in the points of their localization on the roots round wounds were formed. Some of the wounds became united into continuous cracks. (Fig. by Iu. A. Podlesnov). (Kir'ianova, 1950).

There are still slighter differences among various stem nematodes which for a long time were known as one species Tylenchus dipsaci (Kühn) = Anguillulina dipsaci (Kühn) = Ditylenchus dipsaci (Kühn) Filipjev, in the host-plant index of which there were up to 300 species of plants. The majority of authors have continued to hold to this point of view up to the present time. On the basis of my research I pointed out already in 1939, that the stem nematode is a collective species which [bogin p. 385] should be divided into a series of species. There are several stem nematodes in the USSR (fig. 17 and 18) of which relatively well studied is only the stem nematode of onions — Ditylenchus allii (Beijerinck, 1883); two species are very little studied: the stem nematode of potatoes — Ditylenchus destructor Thorne, 1945 and the stem nematode of phlox — Ditylenchus phloxidis nom. nov. and one species has been studied even less — the stem nematode of strawberries — Ditylenchus fragariae nom. nov.

The stem nematode of onion affects different varieties of onion and garlic in the USSR. In young plants it causes dwarfness, strongest growth [expansion] of tissues and cracking of the scales from the bottom towards the crown; in onions [? Allium cera ?] it causes friability and cracking of bulbs, in the vicinity of the bottom and the neck and lengthwise from the bottom to the neck. In storage the bulbs deteriorate quite

rapidly and dry out so that only outside scales remain which look like a whole bulb. By that time the nematodes settle in masses on the surface of the bulb in points of cracking and enter the anabiotic state looking like a yellowish- or dirty-cream colored film consisting of many tens and hundreds of thousands of worms. This capacity of the nematodes to settle on the surface of the bulbs and to enter a prolonged anabiotic state while preserving their viability up to 3 years, represents a remarkable adaptation which furthers the survival of the species at the advent of unfavorable conditions [begin p. 386].

The stem nematode of potatoes infects all the potato varieties but more so the early varieties than the late ones (fig. 15). Under usual agrotechnical conditions for these crops there is no migration of it from potato to onion and back to potato. The nematode does not cause a typical dwarfness in infected plants of potatoes, the stems of which are only somewhat thicker, the inter-nodes are somewhat shortened and the leaves are smaller with curved edges. Apparently a settling of nematodes on the surface of the deteriorated tubers, described in the stem nematode of onions, does not take place here. Morphological characteristics of the stem nematode of potato consist in the fact that this is a thicker and shorter nematode as compared with the stem nematode of onion and its vulva is pushed further back. In males of the potato stem nematode (fig. 17) the spicules are provided with characteristic off-shoots which are lacking in the onion stem nematode.

Fig. 9.
Melon root infected by the gall nematode. (A. A. Ustinov, 1938).

Fig. 10.
Part of a strawberry bush intensely infected with strawberry nematode — Aphelenchoides fragariae (Ritzema-Bos) The bush presents a deformed, abnormally grown mass of various organs and has not even a remote similarity with a healthy strawberry bush. (Phot. from a sample brought from Germany).

Fig. 11. [p. 387]
Head and tail ends of the body of various Aphelenchoides. (Gudoi, 1928, reprinted from Filip'ev, 1934). 1.- Strawberry nematode — Aphelenchoides fragariae (Ritzema-Bos); 2.- Chrysanthemum nematode — A. ritzemabosi (Schwartz); 3.- Currant nematode — A. ribes (Taylor); 4- A. olesistus (Ritzema-Bos).

The stem nematode of phlox causes a pronounced bushiness of stems (fig. 16) and deformities in structure of flowers and leaves which are not registered in [begin p. 387] the potato stem nematode. Its vulva

is pushed still farther back than in the potato stem nematode to which it is similar in the thickness of the body. No one has conducted experiments for determining the possibility of transition of the phlox stem nematode over to other plants, but such transition is hardly possible for onions, potatoes and strawberries.

The stem nematode of strawberries causes dwarfness in affected plants, crimping and twisting of leaves, swelling and twisting of stems and a general under-development and deformation of the entire bush (Danilov, 1949). This is the shortest of the mentioned stem nematodes; its size does not exceed 1 mm or slightly longer. This nematode is one-third smaller than the stem nematodes of onion and phlox. At the same time it is known that it can cross over to onions and tomatoes.

Inasmuch as at the present-day state of systematics of nematodes almost only the morphological-anatomical properties of their organization were usually taken into account as a basis of species differences among them, — the peculiarities in the mode of life, cycle of development, specificity of inter-relations between parasitic nematodes and their host-plants were considered unimportant, or rather, they were little considered as characteristics of the species. It is not difficult to see, as it will be demonstrated further on, that such a position was created as a result of an anti-dialectical, formal approach to taxonomy, based to a considerable degree on the Mendel-Morganist opinions of many foreign scientists, which due to insufficiently critical attitude to their work were partly transplanted to the soviet soil. For the same reason the generally known phenomenon of the so-called "biological races" among various representatives of the class nematodes, received an anti-Darwinistic, non-scientific explanation in writings of many foreign nematologists. Into the concept of biological races usually there is introduced a notion that in Nature inside the species exist specific groups which differ from each other by definite biological or physiological peculiarities. It is considered that in individual cases the biological races can have also some morphological peculiarities to which however it is not usual to ascribe any essential significance in diagnostics. To the problem of biological races [begin p. 388] among protozoa, sponges, coelenterata, echinoderms, mollusks, worms, arthropoda and other animals is devoted quite extensive literature to which I shall refer only in relation to round worms. Among nematodes biological races are known in representatives of various orders. Thus among parasitic nematodes of the Ascaridata order well known is the example of human and swine Ascaridea, which according to the opinion of a number of authors are two biological races of one species — Ascaris lumbricoides L. (Goodey, 1931). According to the opinion of Soviet scientists (Pavlovskii, 1934 and 1948; Skriabin, 1931) the human — A. lumbricoides L. and swine A. suum Goetz [Ascaridea] are closely related species even though morphological differences between them were not found. In size, general morphology and even in number and form of chromosomes and their complexes, the human and hog Ascaridea do not differ from each other. From the eggs of a swine Ascarid swallowed by a human being hatch larvae which are able to migrate in his organism and even cause certain damage. The same happens when eggs of human Ascarid are

swallowed by a pig. But works by many authors proved that the human Ascarid is not capable of developing up to the state of sexual maturity in a pig, or the hog Ascarid in humans. Therefore the infection of pigs with a human Ascarid or of humans with the swine Ascarid is not of an epidemic character.

Fig. 12.
Dwarf chrysanthemums seriously infected with the strawberry nematode. (According to Steiner and Burer, 1933).

Fig. 12.
End parts of branches of a currant bush seriously infected with the currant nematode. (According to Taylor, 1917)

In intestinal Anguillulidae - Strongyloides stercoralis (Bavay) of the Strongylata order also two biological races are recorded: one parasitizes humans, causing tropical dysentery, the other — dogs. Both are considered indistinguishable morphologically, but experiments with cross inoculation indicated that the intestinal Anguillulidae from the humans are not contagious for dogs [begin p. 389] nor those from dogs — for humans (Pavlovskii, 1934 and 1948). There are reports on the existence of biological races also among fresh water nematodes of the order Enoplata.

There are reports on the existence of heat-tolerant and cold-tolerant races among the widely spread nematode Dorylaimus stagnalis Dujardin. Many authors recorded also the presence of numerous races adapted to parasitism on a few host-plants and rarely — polyphagus races, among plant nematodes of the genera Heterodera and Ditylenchus which belong to the Order Anguillulata. At the present time, on the basis of Thorne's work (1936), the Dorylaimus stagnalis is separated into several species, also the beet nematode — Heterodera schachtii, among which the presence of the largest number of races was recorded, is divided into several separate species in works by I. N. Filip'ev, both in an independent writing (1934) as well as in cooperation with Shuurmans-Stekkhoven (1941). At the same time a certain number of authors still hold to old notions on the systematic position of the beet and stem nematodes, which will be discussed further on. It should only be pointed out, that the stem nematode of potato — Ditylenchus destructor was isolated by Thorne in 1945 into a separate species.

## ON THE ORIGIN OF BIOLOGICAL RACES

There are several points of view on the origin and nature of biological races. It is not without interest to report on the deliberations in regard to this, which took place at the meeting of the Association of Economic Biologists in England in 1931 when the problem of biological races and their significance in evolution was discussed specifically. The majority of participants were inclined towards the anti-Darwinistic non-scientific explanation of the problem as it can be seen from a short resumé of opinions of individuals which is given further on. [begin p. 396] Imms (1931) maintained that the reson for the origination of biological races is the formation of specific "conditional reflexes", which cause the origination of a nutrient specialization but which has no hereditary character. In regard to this statement by Imms it has to be pointed out that it is absolutely incomprehensible, how the "conditional reflexes" can be carried over from larvae to grown insects which undergo several transformations in the process of their development, particularly to those which undergo a complete metamorphosis. And the originated food specialization is, of course, hereditary as it is testified by the study of numerous examples among the highly-specialized species of nematodes. Thorpe (1931) assumed that differences in feeding habits and in other peculiarities characterizing individual biological races depend on a specific "larval memory" due to which a grown animal "has a tendency to find for egg depositing a plant of the same variety as the one on which it fed during the larval stage". It is not difficult to see that the first involved explanation by Imms and the second, clearer one, by Thorpe start out from idealistic positions and essentially do not explain anything. The matter here is, of course, not only in the unsuitable term of "larval memory" but also in the content which is put into this notion. It is apparent from Thorpe's further deliberations that under the notion of "larval memory" he does not imply the origination within the given group of organisms of changes of physiological and chemical order as a result of using certain food. According to his understanding "larval memory" and "conditional reflexes" of Imms' terminology, can only in some cases enable the formation of physiological barrier, which, in turn, could further the origination of a new species.

Analogous to the preceding was the address of Goodey (1931) who declared that, for an explanation of causes of the origin of biological races he finds the greates satisfaction in the "food memory hypothesis" which consists of the following: to the descendants is passed on a "factor X" which bears in it some imprint of properties of the host-plant, due to which larvae become predisposed to find this host. "Factor X" has to be in harmony with the organs of sense and has to possess a chemotoxic stimulus for the finding of the corresponding host-plant. It can be completely localized in the somatic cells and does not cause substantial changes in reproducing cells. Goodey stated that the "food memory hypothesis" is not yet sufficiently substantiated and that therefore a possibility is not excluded that later on more facts will occur in favor of the "theory of selection of genotypes" brought forward by Ouboter (1930) of the theory of adaptation brought forward by Steiner (1925). Ouboter assumes that biological races originate as a result of a combination of genotypically

different material as well as by way of mutations. Not having the possibility of becoming acquainted with this work in the original, I have to quote it from Goodey (1931) who gives the following example as an elucidation of this theory. In experiments of Ritzem-Boz there was a case where buckwheat transplanted into a field affected seriously with the rye race of stem nematode, was free of disease the first year, the second year diseased plants appeared, the third year — the infection was observed on many plants. From the point of view of Ritzem-Boz and Steiner this case is to be explained by the adaptation of the rye race to buckwheat. And accordingly to Ouboter's opinion a crossing of two nematode populations took place here, as a result there took place [begin p. 397] a mixing of homozygotes which can live only on rye with heterozygotes able to live on buckwheat as well as on rye. The latter will be found in small numbers, because in a case when only rye is present the homozygotes for buckwheat always die out. However when buckwheat appears, homozygotes are formed anew by way of segregation and they continue to live on it" (Goodey, 1931, p. 418). In course of time a nematode population with genes for buckwheat increases greatly and causes in buckwheat a flair-up of the disease. Goodey's above-mentioned experiments on varying susceptibility of clovers and beans to affection with the stem nematode is explained by Ouboter by the fact that apparently in his experiments was present a population containing many specimens with a gene for red clover, not many — with a gene for the Swedish clover, quite few — with a gene for white clovers and not one specimen with genes for alfalfa and red clover. Finally Hodson's experiments made her come to the conclusion that with prolonged life on one plant a formation of homozygote races takes place. The above deliberations are absolutely not competent. The transmitting to the progeny in somatic cells of "factor X", as Goodey assumes, or of the "gene for a certain host-plant" as Ouboter thinks, cannot explain the originating of biological races, because they are trying to explain one unknown phenomenon by another, which besides has not been proved and is but little understandable. These theories are not compatible with Ch. Darwin's theory of evolution which became the property of the universal science even in 1859. Both authors attribute the formation of biological races to unchangeable particles of living substance in form of "factor X" or "gene for a definite host-plant" which enters the organism from outside and without which the organism would remain unchanged. They apparently do not propose that in nature there is characteristic self movement and that there is no stable and unchanged substance. Organisms develop continuously and improve in their development, but the tendency of this process depends in each concrete case, and to a considerable degree, on surrounding conditions of the environment. The role of the environment, the role of expert education is proved very graphically by works of the great transformer of Nature, I. V. Michurin and his talented follower Acad. T. D. Lysenko as well as by their numerous students and disciples who develop successfully Darwin's ingenious doctrine. Environment is the factor which selects organisms best adjusted to it. Conditions of development are the basic reason for obtaining from similar wheat seeds of winter and spring forms, cold-and draught-resisting varieties or vice versa (Lysenko, 1939), as well as for transformation of one plant species into another. Creating certain conditions for the development of an organism it is possible to obtain soft wheat from a hard variety and to change wheat into rye (Lysenko, 1948 and 1949). In the light of these ingenious discoveries, the complete incompetence of the

above mentioned "theories" about causes of origin of biological races and their significance in the evolution of organisms begins to be very obvious.

In Nature, under prolonged exposure to factors of the environment, a gradual accumulation of definite quantitative changes which lead to radical, rapid qualitative changes takes place. Appearance of new species takes place in leaps. Natural selection of specimens best adjusted to the concrete conditions of the environment of the artificial selection by the humans of needed forms, lead to common results — establishment of new species of plants and animals. And this process, as Engels said, "is accomplished through constant [begin p. 398] fighting between heredity and adjustment" (Engels, 1949, p. 166). Development of organisms is unthinkable without their constant inter-relation with the environment, because life itself is a method of existence of albumin bodies, the essential moment of which is a constant exchange of substances with environment surrounding them; with the ceasing of this exchange of substances life stops, which leads to deterioration of albumin" (Engels, 1949, p. 244). Not to consider important or to deny the role of the condition of development, the role of the environment — means not to see or to deny the contemporary achievements of natural science.

Fig. 18 - Head ends of female bodies of various species of stem nematodes. (Fig. by T. A. Gavrilova according to preparations by E. S. Kir'ianova).
1. - 2-stem nematode of potato — Ditylenchus destructor Thorne. (1-according to Thorne, 1945;
2. - according to material from Irsha, Ukrain. SSR);
3. - of onion — D. allii (Beljerinck);
4. - of phlox — D. phloxidis nom. nov.

In the case being discussed of origin of biological races which differ sharply from each other by host-plants, the principal role is apparently played by the composition of the food being used. Under the influence of feeding on new plants, profound physiological and chemical changes can originate in animals, which will gradually reflect [begin p. 399] on all their functions and influence their morphological peculiarities. As an example the facts cited by K. A. Timiriazev (1939) conserve to illustrate the influence on the organism of the chemical and physiological processes which take place in it. The garden primrose — Primula sinensis has two variants: colored and white of which the first one is predominant. It has been proved experimentally, contrary to the "teaching" of mendelists, that the color of the primroses depends not on the presence or absence in the organism of genes of coloration or of colorlessness, but on a much more complicated process" in which at least four bodies participate: chromogen (may be also with its protochromogen), peroxidase, oxygenase, and an inhibitor which determine the coloring and two different cases of colorlessness of a flower" (Timiriazev, 1939, p. 482). It was possible during the experiments to change white flowers into colored ones under the influence of high temperature. At another point Timiriazev mentions (1939 p. 133-134)

the results of Guker's researh which established that in the Bengalian valley in India the same species of plants in one locality possess medicinal properties which they lack in another place.

Many such examples of the role of the physiological and chemical changes in the organism which originate under the influence of the environment already accumulated on it. Formal genetics which sees in the unchanging genes the only bearers and creators of hereditary nature, lost entirely the ground under their feet; this has been expressed in following words by Acad. Lysenko: "The hereditary cha ractoristic is inherent only to the living. And any living by way of assimilation or dissimilation builds itself from food, and from surrounding conditions. More than that the initial living [matter] was developed some time in the past from non-living [matter]. But if the initial living [matter] developed from non-living [matter] and any growing organism builds its body from non-living [matter], from food, then naturally, a hypothesis originates that also all the properties of a living body are inherent in it, among them the property of requiring specific conditions for development, i.e. the property of inheritance is developed, built, and changed simultaneously and continuously with the development of the body of the organism.

We have already at our disposition much factual material, experimental and practical, which indicates that not only the body of the organism, but also its hereditary nature is built in the development process, i.e. in the process of absorption and assimilation of conditions surrounding the organism" (Lysenko, 1940, p. 117).

Coming back to hypotheses on the origin of biological races with the help of the "factor X" (Goodey), of " a separate gene for each host-plant" (Ouboter), of "larval memory" (Tori) or of "conditional reflexes (Imms), their complete incompetence should be emphasized once more. The solving of the problem has to be looked for in the specific effect on the organism of the changes which took place in its metabolism in connection with its assimilation of substances from new host-plants; the latter, apparently, have a decisive significance in the process of natural selection. And this creative role of selection is the cause of originating of biological races among phytopathogenic nematodes and these races should be considered as various stages in the origination of new species. In favor of this hypothesis speaks the unequal degree of differentiation of races in biological and morphological respects. Part of them, for example, races of the best nematode of the genus Heterodera and highly specialized races of the Ditylenchus dipsaci, withdrew so far from each other that there cannot be any doubts that at the present time they represent clearly expressed species. [begin p. 400] The problem has to be solved just as K. A. Timiriasev says (1939, p. 96) in one of his works dedicated to Darwin's teaching: "And thus the differences presented by variants can be sometimes so considerable that we are being compelled to recognize them as independent species". In regard to the genus Heterodera it has already been accepted in our country's literature in which the representatives of the genus have been long ago separated into a series of independent species, beginning with I. N. Filip'ev's work (1934). We see a different situation in the writings

of a number of the above-mentioned foreign authors who attempt to smoothe over the existing differences between closely related species of the genus Heterodera, due to incorrect methodological positions which they occupy in the problem of formation of species. Their basic error consists in misunderstanding and underestimating the role of the environment in the formation of organisms and therefore they try not to notice the profound differences which characterize individual biological races (species — in our understanding), because to explain their origin is possible only in a case when the leading significance of the environment in this problem will be admitted. Therefore a rather large group of foreign scientists considers that the biological races have no significance in evolution, because the changes connected with their formation cannot have a hereditary character. In other words, this group of scientists tries to go against facts which are obvious for all or they invent for their explanation unprovable "factors X", genes and other non-existing reasons. In practice it leads to extreme confusion and hampers the control of harmful nematodes, inasmuch as into one species are united forms which have a series of essential differences of morphological and biological order. Also in describing new races due attention is not given to their morphology on the premise that these changes cannot have a hereditary character. It is not hard to see that such a situation greatly damages the methods of control of harmful species whose morphological differences are weakly expressed and the appearance of which due attention is not paid. Further research should be directed towards more intensive study of differences between related species in order to establish more precise diagnostical border lines between them which are so important for practical purposes. Serious attention should be paid in particular to the study of epidermis in female nematodes of the genus Heterodera which have obvious differences in the structure of the cuticle as it is apparent from Franklin's work (1939), as well as from the author's personal research.

Fig. 19 - Dwarfed plants of wheat, from the vicinity of Krasnoufimsk, infected by the oats nematode — Heterodera avenae Filipjev, 1934. (According to Kir'ianova, 1941).

[begin p. 401] A somewhat different position is occupied by the group of races of the stem nematode — Ditylenchus dipsaci (Kühn, 1858). An enormous number of races is found among the stem nematode and a considerable part of them is characterized by high specialization and clear biological and morphological peculiarities which allow one to separate them into a series of related independent species. To their number should be referred the following species: 1) Ditylenchus tobaensis (W. Schneider, 1937), which causes galls in aqueous plants: pond weed — Potamogeton malayanus and [urut'] — Myriophyllum spicatum in lake Toba in South Sumatra and in the vicinity of Vienna; 2) Ditylenchus amsinckiae Steiner et Scott, 1934, which causes galls in the field flower Amsinckia intermedia in America; 3) D. allocotus Steiner, 1934, found in the soil in America and which is characterized by a series of morphological peculiarities; 4) D. destructor Thorne, 1945, which parasitized potatoes; 5) D. allii (Beijerinck, 1883)

which affects onion and garlic; 6) D. dipsaci (Kühn, 1858) parasitizing fuller's teazel; 7) D. phloxidis nom. nov. parasitizing phlox and D. fragariae nom. nov. which affects strawberries — in the Krasnodar krai. This list probably does not exhaust the number of related species of stem nematodes existing in Nature, but they have been little studied and their biology is not at all or only little known. But it should be pointed out that in the USSR infection by stem nematodes of clover, alfalfa, Dalmatian daisy and some other crops was registered.

The polyphagous stem nematode, on which Kwan'o in Holland worked in 1927 and later Wilson (1929), belongs to the species which is in that stage of development when an accumulation of unnoticeable quantitative changes occurs, not yet developed into profound qualitative changes and the species consists of such variants which "represent a ladder — a whole series of finest shades of changes starting from negligible individual characteristics of isolated, indivisibles even abrupt species characteristics". (Timiriazev, 1939, p. 97). These variants almost do not differ from each other or differ very slightly and the index of their host-plants is sufficiently diversified. But if one of these variants turns to feeding on a definite plant and continues feeding on it for a long time, then this change in nutritional regime has a deep influence on the entire subsequent history of the species development, enabling the appearance of more and more sharply expressed variants, which can transform suddenly into a new species already narrowly adjusted to feeding on the given host-plant. Analogous to this the highly specialized onion nematode in changing the host-plant (in populations which passed from onion over to tomatoes and peas) acquire smaller size and a thicker body (p. 549). Populations of the gall nematode developed on clover, roses and cucumbers had their own variantal diagram. All this speaks in favor of the fact, that the tendency of the adjusting changeability in phytopathogenic nematodes is determined first of all by their feeding specialization. Changes negligible at first can bring about more substantial changes in the future if a rapid return to initial conditions will not occur. It can be assumed that the role of physiological and chemical phenomena accompanying and causing this process is exceptionally great. Probably an important role in these changes also involves the nervous system, the leading significance of which in all the processes of life activity of higher animals and humans has been proved by the great physiologist [begin p. 402] of our country Academician I. P. Pavlov. Unfortunately the role of the nervous system in the life of lower organisms, among them nematodes, has been studied very slightly and in phytopathogenic nematodes even the structure of the nervous system is not sufficiently known. Other effects of the environment on formation of species in phytopathogenic nematodes have been studied even less, but they are probably of secondary importance.

## CONCLUSIONS

1. The tendency of the adjusting changeability in phytopathogenic nematodes is determined by their host specialization. Change in nutritional regime is of profound influence on the entire subsequent developmental history of the species, furthering an accumulation inside it of unnoticeable quantitative changes which lead suddenly

the new species. And at that this process is directed towards steadily greater adjustment of nematodes to host plants. The latter takes place in two leading directions: towards an increasing adjustment of development cycles of nematodes to the development cycles of their host-plants and the originating of specific peculiarities in the exchange of substances between the parasite and the host.

2. Opinions of some foreign scientists (Imms, Goodey, Thorpe, Oyboter, etc.) who assume that biological races originate as a result of "conditional reflexes", "larval memory", "factor X" or "gene for a definite host-plant", are anti-Darwinistic and unscientific. In practice it leads to a series of misunderstandings and erroneous conclusions, in particular in the matter of organizing anti-nematode crop rotations in fields infested with nematodes, inasmuch as into one species are united various species which have a different assortment of host-plants.

3. The highly specialized "races" of the genus Heterodera should be considered in reality as a series of closely related species, which has been long ago generally accepted in the Soviet literature. To these species belong: potato nematode — Heterodera rostochiensis (Wollenweber), beet-nematode — H. schachtii (Schmidt), oats nematode — H. avenae Filipjev, 1934, alfalfa nematode — H. goettingiana (Liebscher) and other species.

4. In the same manner should be approached also the highly specialized races of the genus Ditylenchus which represent a not less numerous group of related species. To their number belong: stem nematode of onion — D. allii (Beijerinck), stem nematode of potato — D. destructor Thorne, stem nematode of phlox — D. phloxidis nom, nov., stem nematode of strawberry D. fragariae nom. nov. and a number of other species.

5. Little specialized polyphagous races of the genus Ditylenchus are also specific species, but not yet adjusted to feeding on only certain plant-hosts.

"Novoe v urhenii o gallovoi nematode"

[NEW IN THE STUDY OF THE GALL NEMATODE]

A. A. Ustinov

pp. 446-452.

## CONTROL MEASURES AGAINST THE ROOT-KNOT NEMATODE

There hardly exists another plant parasite against which so many control measures were tested as against the root-knot nematode. In foreign countries the bourgeois regime does not allow expansion of state measures and therefore the invasion of the fields by the nematode steadily increases. For example in Florida and California the cultivated [begin p. 447] fields are so severely infested by the root-knot nematode that only cultivation of plants resistant to it or ripening early is possible.

In the USSR a series of effective control measures are applied against the root-knot nematode.

### Physical control measures

Among physical control measures the thermal ones are developed, first of all — partial sterilization of the soil with steam. The technical requirement is a sufficiently deep heating of the soil up to 60-65°C. This method is used in the U.S.A. for control of the root-knot nematode in greenhouse soil, but in other countries it has not spread; in open ground on a somewhat extensive scale it is not adapted anywhere. Various temperatures were suggested for heating of the soil; the most widely used for this purpose are sets of perforated pipes through which super-heated steam is led under high pressure from a steam boiler. Attempts were made to use boiling water instead of steam for the heating of the soil, but this is a less effective measure and requires an enormous amount of hot water — more than 800 liters per cubic meter of soil.

Hot water was suggested more than once for "dehelminthization" of living plants; in the Soviet Union corresponding experiments were conducted by Ikhtinskaia and Arkhangel'skaia (1939) for treatments of "levanda" [lavender ?], by Selivonchik (1938) and Ustinov (1938). Warm baths were arranged in a barrel into which a pail was dropped. Into both containers was poured water of equal temperature and during the work very little hot water had to be added into the inner container in order to maintain needed

temperature. Before dehelminthization the plants were kept for 30 - 40 minutes in warm water at 42°C, than they were immersed in the bath and after the bath immediately cooled in cold water. A 50° temperature was effective when the exposure was considerable (at least 15 minutes). According to our experiments the most suitable method is the immersion of roots in a bath for 5 - 10 minutes at a 51 - 53° temperature; but not all the plants can stand this treatment.

Heat dehelminthization of plants in the control of the gall nematode cannot be standardized in the same measure as was possible in the control of the stem nematode in hyacinth bulbs. The assortment of plants affected by the root-knot nematode is very large and their tolerance of heat varies, and many of them can endure it considerably less well than the hyacinth bulbs. It is very difficult to adjust the temperature such that the nematodes would be killed and the plants would not be harmed. Therefore this measure did not become part of established practice either as means of quarantine or treatment.

## Chemical control measures

An enormous number of chemical substances were tested against the root-knot nematode. Attempts were made to introduce "OV" [according to Callaham: abbrev. "otravliaiushchie veshchestva" — toxic agents, i.e. fumigants] into the soil for treatment of infected plants, but they were not successful; all the fumigants are poisonous for plants and can be applied only for the treatment of the soil before planting. Soil fumigation presents considerable difficulties. The interaction which develops between the fumigants and the soil changes the effectiveness [begin p. 448] of fumigation in various soils (Chigarev, 1936). A considerable portion of the chemicals are not available for fumigation, being adsorbed by soil particles, and some of the fumigant evaporates; therefore the amount of poison used for soil fumigation should be hundreds of times larger than for surface treatment. Carrying out of fumigation is more successful in warm (but not hot) weather (about 20°C); the soil has to be well cultivated and relatively dry, because the greater part of the fumigant does not penetrate moist soil. The poison should be introduced into the soil in large amounts at 25 cm intervals; in small areas the work can be carried out with the help of an injector or under a shovel; for the treatment of large areas apparatuses are constructed which are attached to plows. When powdered substances are used, it is better to distribute them all over the plot [continuously] cultivating the earth so that the poison will be beneath a layer of earth. For successful fumigation, immediately after introducing the chemicals the soil has to be covered — this is a necessary condition for the effectiveness of fumigation. A certain substitute of covering can be achieved by watering the soil from the surface, which also inhibits evaporation of fumigant. The surplus of chemical substances is toxic for plants; therefore after removing the cover, and 10 - 15 days are required for airing of the soil before planting. The majority of fumigants have a favorable effect on the growth of plants increasing the

amount of nitrogen substances in the soil.

As a rule no substance exterminates completely the nematodes in the soil, but only reduces the population to an economically unnoticeable level. A negative characteristic of fumigation is that it destroys also natural enemies of nematodes. It is pointed out that a valuable characteristic of fumigants is that the majority of them are not only poisonous for animals but are also fungicidal. And this means that they kill not only the devouring pre-parasitic larvae of predators but also the fungi parasitizing nematodes and thus considerably reduce the number of their enemies. It is understandable that this makes it easier for the surviving specimens to rebuild the population. The results of experiments with the control of root-knot nematode conducted in the USSR were already published (Ustinov, 1934, 1939). After the publication of these works, the treatment of soil with "tsianplav" [? cyan-fusion ?] was tested. At a dose of 100 g. per 1 $m^3$ ( 1 ton per 1 hectare) in a sandy soil the "tsianplav" [?] produced a noticeable nematocidal effect by reducing the contamination of plants in cultivated plots on the average 1-1/2 times as compared with control plots; nevertheless about 30% of the plants in treated plots were infested by the nematode.

In order to compare the effectiveness of various control measures it is necessary to give a numerical evaluation of the degree of infestation of the field plot with root-knot nematode; and for that purpose the infestation index of the given plot has to be calculated. We determined the index in the following manner: the plants in the plot were pulled out and examined thoroughly; if the plot was small — all the plants were examined, in case of a larger field section — every fifth or tenth plant. The most precise method for the evaluation of the infestation of individual plants is the tabulation of all the galls on their roots. But even this method cannot produce entirely precise results because the galls can be of very different sizes; in order somewhat to reduce these differences it has been accepted to count each 2 mm (lengthwise) of large galls for one gall. However when the thickness of the gall is considerable, in 2 mm of its length there are many nematodes so that such a section equals not one but many individual galls. It is necessary to limit field work to an approximate evaluation of the degree of damage [begin p. 449] of the roots expressing it in units. Adding the units and dividing the sum by the number of plants checked, the mean level of infection of one plant in the plot is obtained. Multiplying this by the percent of affected plants and dividing the product obtained by 100, the index of infestation of the plot is calculated. Comparison of indexes of various plots made it possible to express the effectiveness of measure in percentage. The most effective of the now known nematocides is chloropicrin. In comparing the results which we obtained from the use of this fumigant with data from literature, it can be seen that for the heavy and moist soils of Abkhaziia larger dosages of chloropicrin are required than those recommended in the U.S.A. There, 25 to 50 g. per 1 $m^2$ dosages are usual while in our experiments the 25 g. dosage produced a weak effect; a 50 g. dosage (which equals 500 kg. per hectare) reduced 4 times the infestation in comparison with the control

and freed the plants for one year from a serious invasion. Only very high dosages of 100 g. per 1 $m^2$ produce a considerable effect reducing the infestation more than 20 times.

Ethylene-dichloride and methyl-bromide were suggested as fumigants which are cheaper than chloropicrin. Both substances are toxic to humans and the former is, besides, inflammable and explosive. American publications recommend for ethylene-dichloride a dosage of 15 $cm^3$ and for methyl-bromide — 40 g. per 1 $m^2$ of soil. There are no reports yet on tests in the USSR of these substances against the root-knot nematode. Apparently the ester of dimethyl-dithio-carbamic acids synthesized in our Scientific Institute for Fertilizers and Insecto-fungicides represent nematocides with greater promise; they are produced as a dust (with kaolin) and are powder-like substances which do not require the use of a gas mask; according to preliminary experiments in 1948 they cause a considerable decrease in soil infestation. DDT and hexachlorane appeared to be ineffective in the control of the root-knot nematode.

Progress in chemistry promises discovery of new fumigants which will make the cultivation of soil a less expensive measure; nevertheless it is hard to see how soil fumigation can be so attainable that it will be possible to use it on a large scale in the field. So far the chemical method is being used only in a covered [enclosed ?] ground. Soil treatment with chloropicrin was successfully applied in the control of the root-knot nematode in 1935 in vegetable hothouses of the sovkhoz "Chervoni Zori" in Khar'kov.

## Agrotechnical control measures

The difficulty of control of the root-knot nematode by the chemical method compelled already the first researchers to emphasize control with the help of resistant plants. Resistant plants in a suitable crop rotation are the most practicable and often the only possible method of root-knot nematode control in the field in relatively large areas. Other agrotechnical measures such as fertilizers, dates of planting, clean fallow, as well as "lovchie"[?] plantings and flooding or drying out of the soil, are of lesser importance and in majority of cases are only auxiliary measures.

Data on plant resistance to root-knot nematode are scattered in very many works published in most diverse publications. In Russian language most of the material is gathered [begin p. 450] in the collection published by the All-Union Institute of Plant Protection "Collection of articles on nematodes of agricultural plants", edited by E. S. Kir'ianova (Sel'khozgiz, 1939).

The problem of the cause of resistance was brought up only rarely (Klechetov, 1947). Foreign papers, mostly short notes frequently in form of letters, are limited to simple statements of instances of resistance,

without efforts at their explanation and theoretical basis. And also the foreign authors quite incorrectly consider the plant resistance to root-knot nematode as an unchanging static property of the given variety which does not depend on the plant's growth conditions, stage of its development and state. Therefore the data from different authors on resistance and susceptibility of plants frequently contradict each other.

The most important task is the further study of immunity and the establishing which resistance factors — anti-bodies or a rapid root-development — are of greatest significance. Under unfavorable conditions of development the resistance is lower and sometimes highly resistant plants are affected by the nematode as, for example, the peanut, a considerable infestation of which was observed in the Kazakhstan and Uzbekistan; even more frequently corn loses its resistance. It is true that not all the observations of infestation of resistant plants are reliable, because susceptibility is sometimes mistakenly registered according to the presence in the roots of only larval stages of the nematode and we know, that larvae penetrate resistant plants and begin to develop there, causing thickening of roots.

Anti-nematode crop rotations are built chiefly of resistant species and varieties of legumes and of cereals as well as grasses. Susceptible plants should be planted in contaminated soil after a 2-3 year interval. The influence of a change of crops on the root-knot nematode was followed up at the Abkhaziia zonal tobacco experimental station. Records taken in experiments preceded with tobacco showed that even a one-year cultivation of resistant plants greatly reduces the damage to plants (Ustinov, 1934). Study of soil infestation with invading larvae confirmed it: on plant-indicators planted in 900 $cm^3$ of soil from the tobacco field 976 galls developed and from the adjoining corn field — only 5 galls. The Department of Field Industry of the station developed for Abkhariia's tobacco farms a six-field crop rotation with the following order of crops:

1st year - Wheat and perennial grasses (red clover and rye grass);
2nd " - perennial grasses of second year of use;
3rd " - tobacco over a layer of perennial grasses, in the fall white lupine over green fertilizer;
4th " - corn over green fertilizer, in the fall vetch-oats mixture for hay;
5th " - tobacco over stubble, in the fall peas with rye for hay;
6th " - corn over stubble, in the fall sowing of wheat with perennial grasses.

The experimental crop rotation was conducted in a plot infested with root-knot nematode and with its introduction the root-knot nematode ceased to multiply in mass and did not cause severe reduction of tobacco yield.

In another plot of the same experimental station in 1935 71 - 95% of tobacco was affected with root-knot nematode and damage to the plants was

so severe that a further planting of tobacco had to be stopped. The plot was occupied partly with cabbage and sugar sorghum and remained partly unused. By 1938 the soil infestation was so much reduced [begin p. 451] that of the plant-indicators planted here only a few were slightly affected by the root-knot nematode. The grass field crop rotation which are being introduced now due to the resolution of the Party and the government, have to be effective both prophylactically as well as a treatment against the root-knot nematode, especially when cereal grasses and not legumes are introduced. The vegetables are the ones which suffer mainly from the root-knot nematode in the Soviet Union; on vegetable farms it is also possible to control nematodes by way of crop rotation. On large farms crop rotation with introduction of perennial grasses is possible; in small workers' and home gardens the earth has to be planted every year with vegetables, but it is necessary to alternate the planting of more susceptible plants with resistant ones. For the latter can be used onion, garlic, corn, cabbage and other members of the mustard family, spinach, sorel, Jerusalem artichoke (very resistant plant), in the South — resistant varieties of sweet potatoes.

In arid and hot areas nematocidal effect is produced by clean fallow under the condition of several additional repeat plowings of the soil during the hottest period; Brodskii and Zemlianskaia (1946) checked the effectiveness of this measure in the vicinity of Tashkent. Mineral and manure fertilizers reduce harvest losses caused by root-knot nematode, but they are not a control measure, with the exception of calcium cyanamide, large dosages of which (7 t. per 1 h.) decrease soil infestation. Green fertilizers are more effective than are the mineral ones, because they are a biological method of nematode control.

## Biological control measures

Practice long ago demonstrated the effectiveness of biological control measures against the root-knot nematode. It is frequently recommended in order to reduce the harmfulness of the root-knot nematode to introduce into the soil around the trees fertilizers of straw, grass or other plant materials. The nematocidal action of this measure was explained by an improvement of conditions of tree growth or by the separation, during the decomposition of the introduced substance, of poisonous gases which destroy the nematodes. It has been only relatively recently found out that the effectiveness of plant residue is conditioned by the fact that they create favorable conditions for the development of a large number of parasites of the root-knot nematode. Korab and Butkovskii (1939)point out the harmfulness for the beet nematode of plowing under of sweet clover as a green fertilizer, explaining it by the fact that the rotten mass of sweet clover is harmful for the nematode. But the significance of sweet clover is hardly specific and probably the matter here is also in the development of nematode's enemies in abundant deteriorating organic matter. Our observations in the Abkhaziia tobacco experimental station demonstrated the favorable effect of "sideration" [enriching land by planting legume crop] on decontamination of plots infested with root-knot nematode. Particularly marked was the decontamination in the plot where the tobacco suffered also from soil erosion and was so damaged that it produced almost no yield. After a completed "sideration"

(mainly with lupine) the tobacco yield was satisfactory and its infestation with nematode was very limited.

## Outlines of control measures against the root-knot nematode

In greenhouses the control measures come down to removing and destroying roots of diseased plants and to destroying larvae of root-knot nematodes in the soil. This can be done by partial sterilization of the soil with steam, its treatment [begin p. 452] with fumigants and, in an extreme case, by replacing the contaminated earth with non-contaminated and by thorough cleaning and coating with lime of the walls, corners and bottom of the frames.

Of great importance are prophylactic measures against introduction of root-knot nematodes into the hothouses. The law of the Five-Year Plan specifies the development of hothouse farms in the vicinity of large cities which makes the problem of their protection against the nematode very real; foci of the latter were found in many points of the Soviet Union and probably by no means all of them were disclosed because the damage caused by the nematode under field conditions further north is negligible. But when brought into hothouses the nematode can become a most dangerous parasite and its control will be very costly.

The leading measures under field conditions are agrotechnical. The decisive factor for annual plants in the control of the root-knot nematode is the introduction of crop rotations preferably in combination with other measures: green fertilizers, introduction of inexpensive fumigants (for example calcium cyanamide) in arid areas-drying of the soil by way of additional re-plowings in the summer. In order to reduce the state of invasion of the soil, the roots of severely affected plants have to be removed from the field after harvesting and destroyed; it is particularly important and easily accomplished in vegetable gardens.

Methods for destruction of the nematode in growing perennial plants are not yet known. Higher resistance of particularly susceptible species and varieties can be achieved by way of their vegetative hybridization with more resistant varieties. Improved feeding of diseased plants by way of introducing fertilizers decreases the harmful effect of the nematode; planting between the trees of cover crops, resistant to the root-knot nematode or keeping the soil as a clean fallow reduces the state of invasion of the soil.

A radical decontaminating measure is the planting, in a seriously infested soil, of perennial plants resistant to Heterodera infestation. Such action was taken in a sovkhoz near Sukhumi: lavander which was in the process of destruction from "heteroderosis" was liquidated and the plot was occupied with Aurantiaceae. After a few years we could not find here any nematodes at all even though a small amount of them probably remained on weeds.

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ISPYTANIE NOVYKH ORGANICHESKIKH PREPARATOV V BOR'BE S'GALLOVOI NEMATODOI

[TESTING OF NEW ORGANIC COMPOUNDS FOR THE CONTROL OF THE GALL NEMATODE]

pp. 460-461

Ustinov, A. A. and Mitrofanov, P. I.

The Scientific-research Institute for Fertilizers and Insecto-fungicides suggested to the author of this article to conduct tests of new compounds, synthesized by the Institute, for the control of the gall nematode. The work was carried out in the summer of 1948 in Sukhumi by P. I. Mitrofanov with consultations with A. A. Ustinov.

Index of new preparations tested for tho control of the gall nematode

| Number of Preparations | Number of the Preparation in the index of NIUIF | Composition of the preparation | Concentration (in %) |
|---|---|---|---|
| 1 | | "Cystogon" (active agent — methyl ester of dimethyl-dithiocarbamic acid) | |
| 2 | 23 | Ethyl ester of dimethyl-dithiocarbamic acid | 20 |
| | | Kaolin (Glukhovetskii elutriated [purified]) | 80 |
| 3 | 24 | Propyl ester of dimethyl-dithiocarbamic acid | 20 |
| | | Kaolin | 80 |
| 4 | 25 | Butyl ester of dimethyl-dithiocarbamic acid | 20 |
| | | Kaolin | 80 |
| 5 | 26 | Isoamyl ester of dimethyl-dithiocarbamic a cid | 20 |
| | | Kaolin | 80 |
| 6 | 27 | Dinitrophenyl of dimethyl-dithiocarbamic acid | 20 |
| | | Kaolin | 80 |

[cont. next page]

| No. of preparations | No. of the preparation in the index of NIUIF | Composition of the preparation | Concentration (in %) |
|---|---|---|---|
| 7 | 28 | Ethyl ester of diethyl-dithiocarbamic acid<br>Kaolin | 20<br>80 |
| 8 | 29 | Propyl ester of diethyl-dithiocarbamic acid<br>Kaolin | 20<br>80 |
| 9 | 30 | Butyl ester of diethyl-dithiocarbamic acid<br>Kaolin | 20<br>80 |
| 10 | 31 | Ethylene-bis-dimethyl-dithiocarbamate<br>Kaolin | 20<br>80 |

10 compounds were tested which represent mainly various esters of dimethyl-dithiocarbamic acid. [begin p. 461]

Compounds no. 23, 24, 25, 26, 28, 29, 30 and 31 are white powders, compound no. 27 - is yellow; "cystogon" is dark brown. Most of the compounds have a strong disagreeable odor. There are indications in the literature about the harmful effect of "cystogon" on skin, therefore in working with this preparation it is necessary to protect the hands and the face.

## Work Method

The small amounts of preparations obtained made it necessary to limit the conduction of experiments to flower pots — 8 l. in cubic capacity and parallel to this — to small flower beds — 30 x 50 cm (0.15 $m^2$) in size. 6 g. of the compound were introduced into each flower pot and 15 g. into each flower bed, i.e. 1, 2, and 3 g. of active substance.

For the tests in flower pots the measured soil was previously mixed on a sheet of cardboard with the alloted amount of the compound and then placed into the container; in flower beds the compound was scattered on the surface after which the soil was mixed. The test was carried out in a plot of sandy soil highly infested with gall nematode. Prior to filling the test pots with the soil it was thoroughly mixed with a shovel which furthered a more or less even distribution of larvae in it.

The effectiveness of the compounds was determined according to the quantity of galls on the roots of the indicator plants, which were tomato

and tobacco plants. Three days (from 24th to 27th of April) passed between the treatment of the soil and planting of plants in the pots and 10 days between June 3 - 13th in flower beds. Removing of plants and examining of roots was carried out after 84 (in pots) and 96 (in flower beds) days.

## Results of Experiments

The "cystogon" and preparations no. 23, 24, 25, and 26 appeared to be very effective nematocides; after the soil was treated with them, no galls were found on the indicator plants in the flower pots and in flower beds the plants were only slightly infected, while the control plants were very heavily infected with the nematode.

Preparations no. 27 and 31 had no effect either in the field test or in flower pots and compounds no. 28, 29 and 30 were effective in flower pot tests but not in tests conducted in flower beds.

## Effect on Plants

Unfortunately all the effective compounds, with the exception of "cystogon", harmed the plants more or less considerably. The "cystogon" had no hardful influence on plant development; compounds no. 23 and 24 caused less serious harm than others, but compound no. 25 killed some of the plants completely.

## Conclusions

The results of the preliminary tests demonstrate that "cystogon" and compounds no. 23-26, i.e. the methyl, ethyl, propyl, butyl and isoamyl ester of dimethyl-dithiocarbamic acid are effective anti-nematode agents, at least in sandy soil.

Further work with these compounds should be considered as expedient and having a future. Transition should be made from laboratory tests to field production experiments. In order to reduce the harmful effect of the compounds on the plants, the intervals between soil treatment and planting should be lengthened to, at least, 2 weeks (for "cystogon" it is not needed). Tests have to be conducted on dosages of the agents.

Test of hexachlorane

Simultaneously with the described experiments, and by similar methods, the nematocidal action of hexachlorane obtained also from the NIUIF was tested. Hexachlorane was introduced at the ratio of 60 or 30 g. per 1 $m^2$ of 2.5 and 1.25 g. per flower pot. The hexachlorane produced no nematocidal effect.

Quarantine laboratory of Abkhaziia

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pp. 505-506

Nematodovaia bolezn' khrizantemy i bor'ba protiv nee

[Chrysanthemum nematode disease and its control]

Kir'ianova, E. S.

CONCLUSIONS

1. The chrysanthemum nematode — Aphelenchoides ritzemabosi (Schwartz) was first discovered in 1935 in Moscow, appeared to have spread to a number of other large cities in the USSR, namely: Leningrad, Pavlovsk, Pushkino, Minsk, Krasnodar, Sukhumi, Baku, Ashkhabad, Tashkent and Samarkand.

2. Under conditions favorable for the nematode's development it can cause devastating damage to chrysanthemums, asters and other flowers resulting in a loss of 10 - 75% of plants affected by it. [begin p. 506]

3. The chrysanthemum nematode attacks exclusively the above ground parts of plants of chrysanthemums as well as other flowers or weed flora, never being found in their root system and very rarely in the upper-most layer of soil at the depth of 0.10 cm.

4. In order to obtain healthy planting material from the infected mother plants, the best results are obtained with wet thermal treatment of plants at a 55°C temperature for 3-5 min. or 5-10 m. at 50°C, which [treatment] destroys nematodes in plant tissues and gives an opportunity

to obtain adequate shoots for grafting.

5. Wet thermal treatment of high temperatures is adaptable also for sterilization of rooted young chrysanthemum plants as, for example, of the "Serebrianyi dozhd'" [silver rain] and "Griunval'd" [Grünwald ?] which after treatment developed more rapidly and produced better flowers as compared with the control plants.

6. Good results for obtaining full-value flowers for cutting are produced by using a warm bath: 15-20 m. at a 40°C temperature, 20-30 min. at 35°, 30-40 min. at 30°. It is used for dehelminthization of noticeably affected plants.

7. Of most essential importance in the control of the chrysanthemum nematode is the system of agro-technical measures directed towards prevention of nematode infection of chrysanthemum plants during the entire growing period. Carrying out this system in highly infested farms meets with certain difficulties, but nevertheless this is the only method of getting rid of the nematode disease or of reducing it to a little noticeable volume.

One of such measures is a thorough removal from the fields of all the remains of over-ground parts of chrysanthemum plants and weeds; this removal has to take place not less than twice before the planting (fall and spring cleaning of the ground) and a certain number of times after planting. This measure alone will drastically decrease the supplies of the pest in Nature and therefore greatly weaken the flair-up of the nematode disease.

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pp. 508-511

# NOVYI PARAZIT RISA — Aphelenchoides oryzae YAKOO

# [NEW PARASITE OF RICE — Aphelenchoides oryzae YAKOO]

Sveshnikova, N. M.

At the end of 1940 there was sent from the Krasnodar quarantine inspection to the Central laboratory for plant quarantine a panicle of rice with distinct symptoms of disease. The panicle was sparse, with shriveled brown grains and a dried out stem. Analysis disclosed in the panicle small nematodes of the genus Aphelenchoides, related to A. parietinus Bast. However the insufficient amount of material and of information sent on it, as well as the necessity to exclude the presence here of pathogenic agents of fungal and bacterial order compelled us to stop the research until additional data would be obtained.

The war which followed in 1941-1945 made it impossible to study this material. Only in 1946 did reports come in again on this disease of rice which became economically perceptible and diseased rice panicles were sent in again for study.

In the investigated dry rice panicles of the "Kendzo" and "no. 18" varieties, several hundreds of nematodes were observed per panicle. The infected panicles are small in size, brown with whitish tops, the heads are not filled out and there are a large number of empty and light-weight grains; frequently the panicles remain in the leaf sheaf and do not produce any yield.

In 1913 Butler described analogous symptoms of nematode disease of

rice "ufra". The disease was observed in the delta of Ganges river in India and in Malayian Islands and it was caused by the nematode Ditylenchus angustus (Butler, 1913) Filip'ev, 1934, which inhabits rice plants ecto-parasitically. The symptoms of the rice disease "ufra" are as follows: the tops of shoots become white, the stem becomes crooked and thin and later on dried out. "The spike does not grow out of the sheaf, the grains do not develop; sometimes the spike frees itself from the leaf but it does not develop grains. The yield is lost. The nematodes are on the outside of the plant, under the sheaf of the leaves and under the scales of the spikes" (Filip'ev 1934). The drawing of the diseased panicle in Butler's work reminds us of diseased rice plants which were at our disposal. However the nematodes which we separated from spike scales of rice are typical representatives of the genus Aphelenchoides and not Ditylenchus which were described by Butler. Cralley (1949) reports on rice disease "white tops" so called because of this principal characteristic symptom appearing on leaves. [Begin p. 509] The disease was of great importance in the U.S.A. in 1949 and was observed in Japan in 1947. It was caused in both cases by the nematode Aphelenchoides oryzae Yakoo. Here is the description of this parasite.

## Aphelenchoides oryzae Yakoo.

Small, thin nematode, $\frac{\text{alpha}}{l} = 44 - 60$. The length of the male, 0.54 — 0.60 mm, of the female 0.64 — 0.79 mm. The tail of both sexes is pointed. At its tip there is a clearly distinguishable small spine, characteristic for many representatives of the genus Aphelenchoides. On the sides of the body from the head and to the tail there are side ridges. The head is sharply separated from the body. The mouth capsule is provided with a stilet 9 microns long with three swellings at the base; the front part of the stilet is conical, the rear part — cylindrical. After the stilet follows the esophagus with the typical Aphelenchoides bulb after which the nerve ring and the esophagus glands are seen. The excretory opening is at the level of the rear border of the bulb (fig. 1, A). The vulva of the female is located at a distance of 70 — 75% of the body length. The vagina is on the slant, there is a rudiment of a rear womb. It was not possible to observe eggs. The tail of the male (fig. 1, b) is without a "bursa" sac, it is curved toward the ventral side. There are three pairs of papilla: one in the rear, another slightly below the middle of the tail and the third at the very tip of the tail, near the tail thorn [?]. The spicules are 9 microns long in a shape typical for the genus Aphelenchoides. There is no rudder.

Fig. 1, [p. 509] -
Aphelenchoides oryzae Yakoo
A)- head end; b)- tail end of the male;
B)- tract of the vulva. (Orig.)

In size the new species being studied is related to the Aphelenchoides parietinus (Bast), which is seen from the given table; A. oryzae is also closely related to A. fragariae from which it differs by location of the excretory

opening and smaller size of spikules A. oryzae does not cause the dwarf disease in rice plants, which it damages, as much as is observed in strawberries, chrysanthemums and other crops which are affected by the A. fragariae. Besides that the aqueous environment in which rice grows is not natural for the strawberry nematode — A. fragariae.

As to A. parietinus, the indexes of our species are very close to those of this species with the exception of the size alpha; besides that our species [begin p. 511] has a slightly shorter tail and spicules of different size. A. oryzae Yakoo belongs to the group of very slim nematodes (alpha = 44 — 65), while A. parietinus has a relatively thick body. According to Guidei (1933) the alpha of females of A. parietinus is 28 — 38 and of males — 25 — 30; according to Micoletzky (1921) the alpha of A. parietinus fluctuates within the limits of 23 — 43. However according to data of various authors the sizes of A. parietinus vary considerably. As to the biology of A. parietinus — it is an extremely widely spread species described as an inhabitant of fresh waters, soil, rotting organic substances. It has been found also on healthy plants and on plants with distinct symptoms of the disease: in roots of the coffee tree in Java, in roots of the lily of the valley in Germany and North America, in turnips in Switzerland, on turnips, clover and strawberries in Holland, in diseased bulbs of narcissus, in potato tubers and in cotton seedlings of South Carolina. In the USSR this species was recorded many times on various plants and inside them and in the soil (Kir'ianova, 1935, 1939; Tulaganov, 1941, Sveshnikova and Skarbilovich, 1935, and others), without presence in them of definite disease symptoms. In practice the Central laboratory for quarantine of plants found this species in onions, strawberries, Dalmatian daisy and in large roots of gentian.

Fig.2 - [p. 511]
Panicle of rice infected by
Aphelenchoides oryzae Yakoo
(Fig. by T. A. Gavrilova)

The rice disease being described is recorded for the first time in the USSR. At the present time in the Soviet Union there are being conducted studies of resistant varieties of rice, those indicated in literature are: "Nira", "Fortuna" and "Blue Bonnet". It is recommended for the control of the nematode disease to disinfect the rice seeds with hot water at 52 - 53°C. for up to 15 minutes. The effectiveness of the treatment was apparent in a reduced infection of plants.

Central laboratory for
Quarantine of Agricultural
Plants of the Ministry of
Agriculture, USSR

Basic differences between three representatives of the genus Aphelenchoides (p. 510)

| Name of the species | Sex | Length of body (in mm) | Size | | | Length of stilet (in u) | Shape of bulb | Excretory opening | Size of spicules (in u) | | Vulva in % to the total body length |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | alpha | beta | gamma | | | | back side | belly side | |
| A. parietinus according to Gudei, 1933) | ♀♀ | 0.6-0.9 | 28-38 | 9-11 | 14-15 | - | slightly square | rear border of the bulbus | | | 70 |
| | ♂♂ | 0.57-0.75 | 25-30 | 9-11 | 14-15 | | | | 22 (according to Hoffart, 1930) | | |
| A. oryzae Yakoo (According to the author | ♀♀ | 0.60-0.79 | 44-65 | 11-12 | 16-20 | 9 | oval-egg-shaped | same | | | 70-75 |
| | ♂♂ | 0.55-0.60 | 54-61 | 11-12 | 16 | 9 | | | 18-19 | 8-9 | |
| A. fragariae (According to Gudei, 1933 | ♀♀ | 0.57-0.92 | 44-60 | 11-15 | 15-20 | 8-9 | oval | near the nerve circle | | | 70 |
| | ♂♂ | 0.59-0.85 | 45-57 | 11-12 | 18-19 | 8-9 | | | 21-23 | 10 | |

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[p. 551]

# Nematoda luka — Ditylenchus allii

# [Onion nematode — Ditylenchus allii]

E. S. Kir'ianova

## CONCLUSIONS

1. The onion nematode — Ditylenchus allii (Beijerink, 1883) is a fairly widely spread pest of onion crop in the USSR.

2. The onion nematode is characterized by its large size (on the average 1400-1500 u); alpha=45 in female and 55-60 in males which indicates a considerable slimness of body in these worms; the vulva is located at 81% distance from the head; the bursa [sac] is rather short and does not reach the end of the tail by 22-23 u. The host plants of the onion nematode are a very limited range of crops: onions, tomatoes, peas and possibly garlic. It does not harm oats, wheat, rye, millet, potatoes, egg-plant, tobacco, beets, flax, clover, alfalfa, carrots, cabbage, cucumbers, horse-radish, dill, lettuce and spinach. The latter may be infected by other related species of stem nematodes.

3. The basic points of onion nematode localization in the onion are: the bulb, scales, "strela" [leaves]; in the root system and in seeds the nematodes are found considerably less frequently and in small numbers.

4. Onion plants are infected throughout the entire growing period, beginning with seed germination.

5. When the infected bulbs are drying under storage conditions, the worms migrate in masses to the surface, most frequently to the area of cracks or to other places where the exterior covers are not very hard. Gathering on the surface of the bulbs in form of lumps or a mossy cream-colored incrustation, the nematodes (♀, ♂, larvae) enter an anabiotic state, rapidly becoming alive again under favorable conditions of humidity. The cream-colored incrustation of worms can easily fall off [crumble] and scatter and thus become a source of infection for new bulbs.

6. Under field conditions the place of onion nematode accumulation is in various remains of infected plants. In the soil itself the supply of worms is relatively small, therefore the role of soil as a reservoir of the pest is of secondary significance.

7. The author recommends for onion nematode control: 1) application of crop rotation with a selection of types plants which are not susceptible;

2) thorough cleaning of the fields from post-harvest remains of onion plants; 3) thorough selecting of bulbs for the seed fund during the harvest, because at that time it is easiest to separate healthy bulbs; 4) disinfection, drying and cleaning of storage spaces immediately after they have been vacated from onions which were stored there; 5) Storing of bulbs at low (1-3°C) or high (above 14°C.) temperatures which are the least favorable for the development of the onion nematode; 6) wet thermal treatment of seed-onions carried out before sowing at following temperatures and exposures:

| | | |
|---|---|---|
| 45 - 46°C | during | 15 - 10 min. |
| 50 - 52°C | " | 10 - 5 " |
| 55 - 57°C | " | 5 - 3 " |

7) fertilization of the soil for onion fields to be carried out with well rotted manure, in order to avoid an introduction of onion nematode together with dry scales, skins and other remains of diseased plants.

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[p. 571]

## GARLIC FORM OF THE STEM NEMATODE - Ditylenchus

A. A. Paramonov

### CONCLUSIONS

1. In studying the stem nematode of garlic the thermal method of controlling nematodes was applied.

2. Ditylenchus of garlic is characterized by a number of symptoms both interior as well as by typical infection of parenchyma.

3. The temperature plays an important role in the increase of intensiveness and extensiveness of invasion.

4. The garlic form of the stem nematode differs in a number of symptoms from the onion nematode.

5. Under the conditions of Moscow and vicinity Ditylenchus of garlic reaches its highest development in August.

6. At a temperature of about 15°C. the bulbs infected by Ditylenchus perish rapidly; keeping them at a temperature of about 5-8° inhibits the development of the disease.

7. Fall plantings should be preferred to the spring ones because in case of Ditylenchus of garlic the spring plantings can lead to a higher percentage of plant destruction.

8. With Ditylenchus of garlic the soil has to be disinfested. It is possible that "forbiat" is applicable.

9. Onions cannot be sowed in the same soil after garlic infected by Ditylenchus.

10. Potatoes can be planted, however, disinfestation of soil is desirable.

11. Treatment of garlic bulbs by the thermal method is possible if the heating is carried out in a water bath and not by a dry process; duration of exposure -- about 2 hours and in a case when the disease is apparently localized in the bottom and the exterior scales.

12. Further work is necessary on treatment of the bulbs by this and other methods.

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[pp. 583-591]

# Kizuchenii kartofel'noi nematody— Heterodera rostochiensis Woll v SSSR

# Study of the potato nematode — Heterodera rostochiensis Woll. in the USSR.

H. M. Sveshnikova

The potato nematode — Heterodera rostochiensis Woll., 1923 has long been known in foreign countries. It was discovered in England in 1905, in Germany in 1913, in Sweden in 1920, in Denmark in 1928, in the U.S.A. only in 1941, in the USSR in 1948.

The potato nematode as a distinct species was described in 1923 by Wollenweber who gave it the species name Rostochiensis according to the name of the city of Rostok where it was discovered on potato roots. This species is the causal agent of one of the economically most important potato diseases (fig. 1). Its foci were not detected during the first several years of infestation but only after it had accumulated so much in the soil that it caused drastic drops in yield capacity of potatoes. Thus, according to data of Kenner (1929), in nematode infested sections in Sweden in 1929 only 6 kg. of 10 kg. of planted potatoes were harvested. In Germany in 1931 in analogous sections the yield reached on the average only 147 g. per bush (Goffart, 1941). In the U.S.A. on Long Island, the decrease in potato yields started in 1930. Up to 1938 the farmers could not establish the causes of reduced yields and demanded a soil analysis be conducted. Only by 1941, when the potato nematode was discovered on potato roots from infested fields, was the cause of the disease established.

Due to the great damage caused by the potato nematode, it is an object of foreign trade quarantine which forbids the import of potatoes from countries where an infestation by this parasite exists. Therefore, according to published data, the distribution of potato nematode was limited to some localities in Germany, England, Scotland, Ireland, Sweden. The potato nematode was imported during the war of 1914 to Denmark. However, as has been found out at the present time, the distribution of the potato nematode is considerably greater than has been published. In Great Britain it spread everywhere potatoes are cultivated, and in Germany, according to data of 1945, it spread out widely, moving far to the West and the East from the point of its initial discovery. [begin p. 584]

Due to strictly enforced quarantine regulations, up to recent times there has been no potato nematode in the USSR territory, which has been confirmed by numerous investigations starting in 1932 and later (Filip'iev, 1934; Kir'ianova, 1935; Sveshnikova, 1948).

Fig. 1 - [p. 584]
General view of a plot infested by the potato nematode: in the front — severely infected bushes, in the rear — bushes grown in soil disinfested with a preparation of dithiocarbamic acid. (Orig.)

Potato nematode is more readily discovered in gardens with inadequate agro-technique and insufficient fertilization, in most cases in sandy soil. A general view of a plot with a typical aspect of the disease is presented in fig. 1. The picture was taken in the middle of August 1949. In comparison with a normal bush, a bush infected by potato nematode looks like a dwarf (fig. 2). It was easy to show on roots of diseased bushes with the naked eye, female-cysts of potato nematode, red or brown in color, which penetrated with the head end in the roots (fig. 3). The density of infestation reached as much as 20 female specimens per 1 cm. of root. We registered the following susceptible potato varieties: "Alma belaia [white]", "Alma rozovaia [pink]", "Skorospelka", Vol'tman, "Vale". Roots of other garden plants were also examined but the potato nematode was discovered only on tomatoes (Solanum lycopersicum L.) and deadly night shade (Solanum nigrum L.).

## The potato nematode

The potato nematode is a worm, microscopic in size, which has a sexual dimorphism. The cyst (old female) [begin p. 585] has a regular globular shape, but its head end retains a worm-like form to the extent of a double length of the esophagus, which lends the cyst the shape of a round flask (fig. 4).

Fig. 2 - [p. 585]
Potato bushes (Orig.)
On the left— healthy; on the right— infected by potato nematode.

Fig. 3 - [P. 585]
Potato root infected by females of potato nematode. (Microphot. orig.)

The cyst is enclosed with a thick brown wall. On the outside the wall has an ornament which is similar to the curves on a walnut shell, but smaller and distributed in a more dense net. The size of the root cyst varies, depending on its age, from 0.376 to 0.825 mm. in length and from 0.191 to 0.710 mm. in width. The head, worm-like end ("neck") can reach in length 1/4 of the lengthwise diameter of the cyst body. Later cysts are smaller and have not always a regular globular shape as seen in fig. 4. Thickening and browning of the wall of the female starts soon after the appearance of

the female on the outside of the root, according to our observations — beginning in July. Apparently the oxidizing reaction of the air plays a role in the browning. The white color of the females changes first to lemon-yellow, towards the middle of July — red and brownish and by the end of July the majority of females are brown. The wall at the head end is thin with a distinguishable transverse stripes. The mouth spear has 3 swellings at the base which are not always easily distinguishable. The esophagus varies in length depending on the stretch of the head end. The bulbus is well manifest and is mostly in the center of the "neck". The head end sinks [begin p. 586] into the potato root up to the level of the esophagus bulbus from which starts the drastic thickening of the wall of the globular body of the cyst. The cyst is packed with eggs which sometimes enter even into the "neck" section of the body. According to our data, the number of eggs varies, depending on the age and size of the cyst, from 45 to 830. We conducted the counting of eggs by way of crushing the cysts and counting the eggs with the help of a glass cytometer. Females containing 150-300 eggs (mean 271) in July — August most frequently are found; specimens with a larger and smaller number of eggs are less frequently observed (Table 1).

Table [p. 586]

Approximate quantity of eggs in cysts of potato nematode in July - August 1949

| No. of cysts in order | Quantity of eggs | No. of cysts in order | Quantity of eggs. |
|---|---|---|---|
| 1 - 7 | From 49 to 99 | 47 | 503 |
| 8 - 19 | " 131 " 199 | 48 | 624 |
| 20 - 35 | " 203 " 297 | 49 | 743 |
| 36 - 42 | " 315 " 395 | 50 | 830 |
| 43 - 46 | " 412 " 496 | | |

## Sexually mature young female

A sexually mature young female is white, lemon-shaped 0.408 mm long and 0.158 mm wide. The mouth spear 0.019 mm long is easily seen in the head end. The esophagus has a strong muscular bulbus; its length is 0.076 mm; the esophagus glands are very evident (fig. 7). The excretory opening is located below the bulbus, at a distance of 0.089 mm from the front end of the body. The sex tubes are in pairs; the vulva opens at the rear end of the body and the anal opening is slightly displaced towards the back side.

## The male

The male (fig. 6) is worm-like, transparent, with a body bent in a semi-circle to the ventral side, apparently in conformity with the globularly

swollen body of the female. The length of the male is about 0.97 mm, the width — 0.03 mm, (alpha = 25-27). The cuticle has sharp transverse lines and cuticular ridges leading from the head end to the tail. At the head end there is a "little hat", i.e. the head is sharply separated with a clear chitinous skeleton. The length of the spear is 0.016 mm with swellings at the base; the esophagus (0.07 mm in length) and bulbus are developed weaker than in the female; esophagus glands are not noticeable . Beta = 12. "Amfidy" which open at the base of the "little hat" at the sides of the head are easily seen. The sex sinus is at the very end of the body. Gamma = 323. Two spicules: 0.028 mm long and 0.008 mm wide are near the head. A rudder in the shape of a small trowel is seen only on rare occasions when the male is examined from the ventral side.

## The egg

The egg (fig. 5) is oval with flattened poles: in the fall larvae are being formed. The length is 0.09 - 0.1 mm, width — 0.04 mm.

## The larva

The larva in the egg (fig. 5) is rather large, 0.33 mm long and 0.015 mm wide, with a sharp tail. The head is separated with a very evident chitinous skeleton and vigorous spear, 0.016 mm long, with swellings at the base. The esophagus and bulbus are well developed; the [begin p. 587] intestines and the anus are not distinguishable. In the egg the larva is prepared for molting which is indicated by the scaling of the wall at the rear end of the body. The larva from the potato root by the end of the first ten days of potato's growing period is thickened, 0.396 mm long and 0.019 thick (fig. 7). The head is typical; seen are: excretory opening and the esophagus gland. Besides the spear (0.016 long), esophagus and bulbous there is an embryo of sex organs, in about the middle of the ventral side of the body. The larvae molts quickly throwing off its case which can be easily seen. The tail is rounded.

Fig. 4 [p. 587] -
Female cysts of potato nematode.
Left -- white, center and right — brown.

Fig. 5 [p. 588] -
Eggs and larva of the potato nematode

Fig. 6 [p. 588] -
Male of potato nematode.
A - head end
b - tail end of the body

The larva from the root during the 10 — 20 days of the potato growth does not differ from the preceding stage (0.398 mm) but is considerably more swollen in width (0.039 — 0.046 mm). The esophagus and the mouth spear do not differ

from those of the preceding stage. Clearly distinguishable is the excretory opening located somewhat below the rear border of the bulbus. The embryo of the sex glands is considerably larger in comparison with the preceding stage. On the wall of the larva, especially behind the rounded tail end is seen the remainder of the cast-off wall — the case (fig. 7). Apparently at this stage the differentiation of larvae into females and males begins.

## The biology

The biology of the potato Heterodera was studied in recent years by Chitwood and Buhrer (1946) in the U.S.A., their data coincide in general lines with our data. In test plots where our observations were conducted on May 24, 1949, potatoes of the "Vol'tman" variety were planted. On the 10th day of growth inside the roots close to their surface, were larvae with thickened bodies, with embryos of sex organs and with rounded tail ends; they were located above the zone of growth parallel to vascular system. The body of the larvae was covered with a case which it was shedding. (fig. 7). On the 20th day of growth even thicker larvae than those in the preceding stage were observed, with traces of cast-off [begin 588] walls, differentiated into females and males. Their head ends had frequently a flat front surface adjoining the vascular system of the root. On the 30th and 35th day females and males were observed from the moment of their /female appearance on the outside of the root (fig. 10). A young sexually mature / ready for fecundation has a lemon-shaped body which in thickening during its development in the root tears its [the root's] tender tissue. Sticking out the female remains in the root to the end of its life, attached with its head end. The wall of the female soon begins to thicken and to become hellow. We had a chance to observe young females which were slightly sticking out from the root with their posterior end and in this small protruding section the wall was 4-5 times thicker in comparison with the part which was inside the root. The ornamented wall begins to develop very early. In our experiment by July 1, it was possible to observe on females a tender ornamented wall which fell to pieces under the weight of the cover glass. Small brown cysts are the late females. The completely formed young male lies curled up 2-3 times in the larval shell due to which the root also swells and bursts; the male tears the larval cover (fig. 8) and crawls out into the soil. On the 48th day of plant growth females with several eggs were observed. Thus the development of one generation of potato nematode which we observed from the egg to the stage of formation of new eggs took place during 40-50 days.

## Distribution

Distribution of the potato nematode basically takes place through soil which contains cysts that stick with the earth to all the objects which are in contact with infested soil [begin p. 589] during the harvest (tubers, shovels, potato digging apparatus and other tools, bags, wheels, feet of humans and animals), with the help of which the cysts can be transferred from an infested plot to a non-infested one. Besides that, the tubers transfer the cysts not only mechanically, but as it has been established, are the

usual overwintering place of the potato nematode; we found females which developed inside the tuber and appeared on its surface by way of tearing the skin of the tubers. Thus the biological connection was established between the nematode and the tubers, where the females can develop as in the roots. There is no doubt that rain water and strong wind can also transfer the cysts.

Fig. 7 [ p. 589 ] -
Development stages of the potato nematode. (Orig.)

Fig. 8 [p. 589] -
Appearance of female (right) and male (left) of the potato nematode on the surface of the root. (Orig.)

## The disease picture

The disease picture observed by the author was as follows: the tubers sprout slowly, develop weakly with 1-2 stems, by July they noticeably lag behind in growth and development. Thus on July 21 the potato bushes in infested plots reached 10-20 cm, maximum — 30 cm. in height. The bushes did not blossom or only single flowers were observed. In table (2) are presented figures of measurements of 15 potato bushes from each of the plots — infested and free from the potato nematode — which were taken on July 21, 1949.

In infected plants the lower leaves begin to wilt in June and the bushes look altogether exhausted, dwarfed, attracting attention by their bad looks (fig. 1). Each bush bears 1-2 tubers not exceeding chicken eggs in size, or the tubers are entirely lacking. The yield decreases in relation to the degree of soil contamination and [begin p. 590] age of the disease center. Part of the bushes perish before completing growth. According to my calculations, by the 15th of September, i.e. by harvest time, the yield from infested plots reached 10-27 g. per bush.

Table (2) [p. 590]

Height of potato bushes grown in experimental plots, infested and free from infestation with the potato nematode

| | Nos. of bushes in order | | | | | | | | | | | | | | | Total | Mean height of bush (in cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | |
| Height of bushes (in cm) infested with the potato nematode | 30 | 21 | 20 | 16 | 14 | 10 | 5 | 20 | 13 | 10 | 10 | 8 | 15 | 15 | 14 | 221 | 14 |
| Height of bushes (in cm) free from the | 60 | 50 | 50 | 52 | 46 | 53 | 45 | 46 | 49 | 38 | 67 | 63 | 45 | 53 | 62 | 779 | 51 |

## The Harmfulness

The harmfulness of the potato nematode is manifest in destruction of potato roots. It is seen in preparation [prepared specimens] that around the penetrated larvae in the root begins a necrosis of its cells and since the state of invasion of the root reaches up to 10 larvae per 1 linear centimeter, the damage caused the plant by the potato nematode is very great. The roots die and the feeding of the plants is disturbed, especially in inadequately fertilized, poor and sandy soils with a low water supply. Triffitt (1931) describes the formation of gigantic cells clogging the root vessels and hindering the movement of liquids. Many larvae perish together with the roots but since there is a large amount of larvae in the soil and their hatching from the cysts takes place gradually, as the roots develop, the plant undergoes numerous and multitudinous assaults by larvae and becomes exhausted wasting material on formation of new roots to replace the perishing ones. In seriously infected potatoes the potato nematode causes exhaustion during the growth period, the leaves of the bushes are stunted and dry, therefore tubers are not formed.

## Control measures against the potato nematode

In order to control the potato nematode in a number of countries (Germany, England, U.S.A.) a series of chemicals were tested which in most cases did not produce satisfactory results. Thus in Franklin's (1939) work she searched for control measures for disinfection of infected potato planting material, phenol, corrosive sublimate, iodine, formalin and hot water were tested. Franklin found that only a 5% solution of formalin when tubers are immersed into it for 6 hours, kills the cysts of the potato nematode which are sticking to the roots with the soil.

In order to use infested plots for potato crops, in Germany potatoes and all the potato family members are excluded from growing in them for 6 years and longer and in cases of adapted crop rotation for atleast 3 years. [begin p. 591]

As a radical measure directed towards the extermination of cysts in the soil, recently a compound "cystogon" was offered: Methyl ether of dithiocarbamic acid (Sveshnikova, 1949). The author tested "cystogon" as well as a series of other analogous compounds of Soviet manufacture for control of the potato and other netodes (Sveshnikova, 1949) and obtained good results. The essence of this work and the methods are discussed in a special article in the given collection [Trudy Zool. Inst. AN SSSR, V. IX, no. 2, 1951].

In the U.S.A. for the control of the potato nematode a mixture of D-D(dichlorprogene-dichlorpropylene) as an independent compound and in combination with chloropicrin is used. Recently a report (Schmitt, 1948) was noted on a series of bromine preparations of which the dibromide and Iscobrom D. produced good results.

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"K Voprosu o raspredelenii nematode v pochve, kornevoi i nadzemnoi chastiak rastenii"

[On the Problem of distribution of nematodes in the Soil, Root and Above-ground parts of Plants]

pp. 613-623

K. V. Beliaeva

The widespread occurrance of nematodes populating the soil and plant tissues has been confirmed by numerous writings of Soviet as well as foreign authors. However most of the research in this field pertains either to the study of soil nematodes having no connection with plants, or of plant nematodes and, mainly, pests of cultivated plants. Literature dealing with this latter problem is particularly voluminous. At the same time there are still very few works concerned with study of worms populating the soil which penetrate from the soil into the plant; basically there are the works by E. S. Kir'ianova (1931-1947), A. T. Tulaganov on nematodes of tomatoes (1939) as well as on nematodes of soils and plants in the Zeravshanskaia Valley in the Uzbekistan (1938-1949). But the simultaneous study of worms and plants and of the soil surrounding the root system will make it possible to find out the regularities in [facts concerning] the distribution of nematode species in these bio-types and to establish the inter-relation of their biological cycle. The density of nematode population in the soil, which has been mentioned repeatedly in the literature, the infestation by them of the root system of many plants, allows one to raise the question of the role of the round worms in the entire complex of the soil bio-coenosis, of their inter-relations with other co-members of the rhizo-sphere — fungi, bacteria, protozoa.

The present report deals with the study of the nematode population of plants which, on the outside, do not show any disease symptoms. For the work were used materials obtained durine the simultaneous study of species which inhabit the near-root parts of the soil and species which penetrate from the soil into the plant and become localized in its root and above-ground parts. The combined analysis of obtained materials makes it possible to draw inferences on more or less typical distribution of worms in areas varying in their agro-technical condition. We used the results of research on nematodes of alfalfa (an unplowed crop), tomatoes (crop which undergoes the effects of various agro-measures — plowing, periodical hilling) and of the field edges which are covered with a wild vegetation (basically Cynodon dactylon and in the spring Hordeum sp.) and called by term "mezha" [boundary line between field sections, i.e. "fence rows"]. Collections were made in 1940 and partly supplemented on the "mezha" in 1944. [begin p. 614] Places of collecting: 1) kolkhozes in the vicinity of Tashkent and fields of the All-Union Institute of Plant Industry (VIR) at the All-Union Academy of Agricultural sciences imeni V. I. Lenin, near Tashkent — biennial alfalfa, tomatoes, "mezhi"; 2) Iangi-inl'skii raion, Tashkent oblast' — alfalfa, tomatoes, "mezhi" as well as "mezhi" of the fields of the Botanical garden near Tashkent. Altogether 20 samples were taken from alfalfa (counting as a

sample simultaneously taken soil, roots and above-ground parts of plants), 8 samples — from tomatoes and "mezhi". In all instances for analysis was taken a square of soil with the root system down to 18 - 20 cm. and part of the above-ground stems and leaves. The soil and the plants were analyzed by methods accepted in phyto-helminthology. Due to the fact that in analyzing the root and the above-ground parts of the plants it is very difficult to determine the monotypicalness [i.e. distinguish species], there was no point in a precise calculation of the found worms, therefore we indicated the number of disclosed nematodes conditionally: "single", "many", "mass".

Nematodes were discovered in the soil and the roots of all the samples examined (100%), and in the above-ground part the worms were absent 4 times in alfalfa, once in the plants of the "mezha", 5 times in tomatoes. Results of the work conducted on species make-up of the disclosed worms and presented in the table in which is shown the distribution of the species composition of nematodes and plants on the basis of frequency of their occurrence expressed in percentage to the number of examined samples from each section. Of course the calculated percentage is not significant as an indicator of absolute occurrence of the species, but it allows to judge the relative predominance of one or another worm species in the soil, roots or the above-ground part of the plant. Altogether during the research period, in the soil and plants of virgin ground, alfalfa and tomatoes were found 28 species of nematodes, some of them have not been determined down to the species and could be heterogeneous (Mononchus sp. sp., Acrobeles sp. sp., Cephalobus sp. sp.)

Characterizing the plots being examined, according to the number of species of nematodes, it could be noted that the "mezha" and the alfalfa are close in the number of species (24 and 23 species) while the tomatoes are considerably poorer (14 species). It is seen from the presented summary that 27 nematodes i.e. almost all of the disclosed species can be found in the soil, 19 species are connected both with the soil and the plant. However the frequencey of occurrences of the species which can be found only in the soil and both in the soil and the plant are far from being similar. Using the indicators of frequency of occurrences of species which we expressed in percentage to the number of samples taken in each section, all the disclosed worms can be divided into two categories: 1) species predominant only in the soil, 2) species penetrating into plants.

Let us concentrate on the analysis of these nematode groups. Into the first group are separated free-living species which can be referred to "geobiont" because these forms are found mostly in the soil, less frequently in fresh water. According to the character of nutrition, belonging here are either species which feed at the expense of the microscopic population of the soil (bacteria, fungi, algae) or predatory species like Mononchus which attack larger prey. Altogether we discovered only 9 species of "geobionts" within the range of the rhizosphere and then the specific importance of this category is not large, the mean percentage of occurrence does not exceed 25-37 for the most widely distributed forms — Tylenchus davainei Bast., Mononchus sp. — and goes down to 2-5% for less frequent forms. From among the latter it is interesting to point out the occurrence of Diptherophora communis de Man - a species which we found in soils near Tashkent but which was not mentioned within the limits of the USSR by other authors. It is

interesting that species predominant in the soil were found in the soils of a "mezha" (9 species) and in soils under alfalfa (5 species) while [begin p. 165] under tomatoes these nematodes were not found. The absence on tomatoes of nematodes inhabiting only the soil begins to be comprehensible when we consider that the soils under tomatoes undergo plowing, periodical hilling after watering, i.e. measures which are not applied in 2-3 year cultivation of alfalfa. We mentioned analagous decrease of nematodes in the soil of thoroughly plowed crops in the previous work (1942). It is quite understandable that in "mezhi" where the root system of plants becomes interwoven, or on alfalfa, the root system of which is also vigorously developed, a biocoenosis is created all the members of which enter to some degree or other the zone of prolonged influence of the rhizosphere and become connected with it mostly by complicated bonds of nutrition. Disturbance in these complicated inter-relations in thoroughly plowed soils leads to cessation of a number of forms. Of course the nematode — "geobiont" fauna can be more diversified and the limited number of discovered species can be explained by the fact that we could not make a record of nematodes which live outside the zone of the rhizosphere or below the plowing horizon.

Into the second category are separated out nematode species which penetrate plants. They in turn, can be sub-divided into forms: a) predominating in the soil, b) predominating in the plant (chiefly in the root system). As it is seen from the index of the percentage of occurrence frequency, the species separated into category "a" are still clearly predominant in the soil as compared with those which are found in roots of plants and very rarely ascend to their stem parts. Thus the index of occurrence in the soil for the most widely spread forms — Dorylaimus obtusicaudatus Bast., Rotylenchus multicinetus Cobb, Acrobeles sp., Tylenchus filiformis Bütschli — is within 43-75%, while in the roots — 16-34%. Of 8 species which we separated into this category, 7 were found in virgin ground, 8 — in connection with alfalfa, and only 4 forms were discovered also in a tomato field. The fact should be emphasized that 4 species found in the soil under tomatoes are the most usual ones in virgin ground and in alfalfa. Therefore, in the thoroughly plowed soils an impoverishment of nematodes is observed and remaining are only the species which are most widely spread in other bio-types.

Let us concentrate on the characteristics of species which penetrate into a plant but predominate in the soil. The first place in this category is occupied by the Dorylaimus obtusicaudatus Bast., one of the most widely spread nematode species, universal, frequently found in the soil, in humid moss and roots of various plants. Kir'ianova (1935) discovered D. obtusicaudatus in roots of eleven plants out of twenty-one crops which she examined. Tulaganov — in the soil, roots of tomatoes (1939) and of alfalfa (1941), Sveshnikova — in roots of rubber-bearing plants (1939). In our investigations the D. obtusicaudatus is found in soils of the "mezha" — 100%, in soils of alfalfa — 55%, of tomatoes — 72.5%, while in the root system of plants in the same plots — 20-37%.

Similar to the Dorylaimus obtusicaudatus, other species — Tylenchus filiformis Bütschli, Dorylaimus monhystera de Man, Tylenchorhynchus dubius (Bütschli) — are predominantly soil inhabitants which relatively seldom

penetrate a plant, and in insignificant quantities.

Rhabditis filiformis Bütschli was found twice in the soil near alfalfa and once each in roots of alfalfa and tomatoes. This is a typical saprozoic nematode and its adaptation to one or another substrate is determined by the presence of deteriorating organic substances. In particular, the Rhabditis is always found in the root system in cases when sections of destroyed and rotten root are in the sample. [begin p. 616].

A special place is occupied by Rotylenchus multicinctus (Cobb). The role of this species is not yet sufficiently defined, but in recent years data appeared which indicate the harmvulness of R. multicinctus as a form which can feed at the expense of living plant tissues. This species is widely spread, it is mentioned equally for different soils and roots of many plants, southern-banana, sugar cane, rubber-bearing plants, rice, as well as northern — turnip, potato, clover, etc. Finding out experimentally which nematode species are attracted by living roots and cuts of roots, Linford (1939) observed that R. multicinctus actively seeks living and, especially, freshly injured roots and penetrates into them from the side near the root cap. The faculty to gather in masses at points of penetration into the roots leads frequently, according to the author's observations, to formations of large wounds. Skarbilovich (1938) relates the R. multicinctus together with the Pratylenchus pratensis to parasitic nematodes on the basis of the damage caused by these species to young shoots of rubber-bearing plants. However the prevalance of adult females of R. multicinctum in the soil in all the cases of our observation (mean: soil 54%, roots 37%), allows us to assume that according to the character of nutrition this species is polyphagous and can use for food not only plant tissues but also organisms in the soil. Presence of a well developed spear and chitinized reinforcements of the head end, relates the R. multicinctus to forms well adapted to puncturing of even hard covers, while in nematodes which already have changed to intra-tissue feeding, a considerable reduction in the puncturing apparatus is observed (females of Heterodera, Anguina tritica).

Let us proceed to the analysis of nematode species separated into the category of "predominating in the root system". Already at the first look at the table it is possible to note, that the first five species placed in order of frequency of their occurrence are equally predominant in the root system of plants in the virgin soil, of alfalfa and tomatoes and frequently penetrate into their above-ground part. The index of occurrence frequency in the roots, of the order of 80-87% for Eucephalobus elongatus de Man, 70-80% for Aphelanchus avenae Bast., 50-62% for Ditylenchus intermedius (de Man), 37-50% for Sephalobus sp. sp. and Aphelenchoides parietinus (Bast.) — speak suffieiently for themselves and allow one to assume that these species represent a basic selection of nematode population of plants. Data of literature confirm very wide distribution of these species and their permanent connection with the majority of examined plants; they are also always found in soils of various types.

The first place in latitude of distribution, on the basis of literary sources as well as according to our data, is occupied by Eucephalobus elongatus de Man. According to our observations the E. elongatus occurs in the soil less frequently than in plants where the frequency of its occurrence is within

the limits of 80-87%, equally for cultivated (alfalfa, tomato) as well as for wild-growing plants. In practice it can be considered that this species can be always found in roots and stems especially if there are damaged or destroyed parts. In the latter case the worms accumulate in masses and it is almost impossible to count them; usually the number of specimens of the E. elongatus exceeds that of other nematode species. In all the works concerning the study of plant nematodes, E. elongatus occupies one of the first places in regard to frequency of occurrence as well as to the number of worms being found. In examining 21 crops of agricultural plants Kir'ianova (1935) detected this species [begin p. 617] in 17 different plant species and in all the 16 areas of investigation. She also indicates it for other crops, like cotton (1931), potato, grain cereals (1939). Tulaganov (1939) found E. elongatus everywhere when he inspected tomatoes in the Crimea and the Caucasus, as well as alfalfa in the kolkhozes of Uzbekistan, in amounts sharply predominant in comparison with other species. Examining wild and cultivated plants of Abkhaziia and of the Black sea coast, M. M. Levashov (1935) found predominant everywhere representatives of the sub-family Cephalobinae, in particular Eucephalobus elongatus; Sveshnikova (1939) and Skarbilovich (1938) mention it also as an inhabitant of rubber-bearing plants. However in spite of constancy and abundance of the E. elongatus in various [biotops] bio-types, the harmfulness of this species has not been established. Martsinovskaia's (1909) attempts to obtain disease by way of inoculation of healthy plants with Eucephalobus elongatus were not successful. Experiments conducted by American researchers Arndt and Christie (1937) for clarification of the role of the saprozoic nematodes, among them Eucephaobus elongatus, in the etiology of rotting of cotton seedlings, did not produce clear results either. The authors come to the conclusion that sometimes a large number of worms in the soil can cause an inhibition in the development, but in comparison with other accompanying species like Aphelenchus avenae. Aphelenchoides, the Eucephalobus elongatus occupies the last place decreasing the germination by 14%. The majority of authors come to the conclusion that this species is a typical saprozoic organism and it can have importance then, only as a secondary disease agent which accumulates in masses at points of preliminary damage; its settling in healthy tissues does not cause destruction. Mass accumulation in points of abundant food or moisture, be it soil or plant, is characteristic for this species. The same trait is at the basis of the method of bait-traps recommended for catching in the soil of saprozoic species of nematodes (Brodskii, 1937), and at that, the "baits" can vary greatly. Thus, for example, I discovered Eucephalobus elongatus in soil layers [deposits] of the Komstok's [Comstock] scale insects which were placed, by members of the Department of Lower Plants at the Middle-Asiatic State University, into nutrient media with the purpose of checking on micro-flora which accompanies the deposits. In Petri dishes with nutrient media, where deposits taken out of soil were placed, the Eucephalobus elongatus developed in such an amount that frequently the entire agar was completely pierced with worms in various stages of development and colonies of bacteria which developed in the culture disappeared completely at the time of mass development of worms.

According to our observations, the second place in frequency of occurrence in plants is occupied by Aphelenchus avenae Bast. spread universally similarly to the Eucephalobus elongatus, and found in the USSR, as well as in other countries. In examining plants and soils we found A. avenae in alfalfa roots in 90% of cases and in roots of wild plants and tomatoes in 70-72%. In soils

of cultivated fields the A. avenae is found considerably less frequently, it is quite usual (60%) only in virgin soils. We found indications of the presence of this species in connection with investigations of plants, among all the authors who participated in such investigations. Kir'ianova found it in cotton plants in Uzbekistan and in 20 out of 21 species of agricultural plants in western oblasts of the USSR, Levashov — in all the crops of the Black sea coast, Skarbilovich and Sveshnikova — in roots of rubber-bearing plants. Tulaganov has the A. avenae in second place after the Eucephalobus elongatus according to the number of specimens found in tomato roots. [begin p. 618]. In equal degree it is common in northern, temperate and southern latitudes and various "bio-tops" [bio-types] — water, soil and, predominantly, plants. Similar to the majority of species of the family Tylenchidae, the A. avenae has a piercing stylet; however many researchers are inclined to relate A. avenae, according to the character of nutrition, to saprozoic species. Interesting observations on nutrition of A. avenae and Aphelenchoides parietinus were conducted under experimental conditions by Christie and Arndt in 1936. Cultivating these species on agar plates together with Neosporia sitopila the authors observed that A. avenae gathered at the periphery of the colony, the heads of the worms touched the hyphae of the fungi; then followed the piercing of the hypha walls with the spear and sucking in of the content of the cells by way of a pressure contraction of the bulbus of the esophagus. Of course the fungi are not the only nutrient substrate of this species. It has been found many times on damaged points of under-ground parts of plants, in particular, of some cotton seedlings. Linford (1937) clarifying experimentally the relation of some nematodes to their potential host-plants observed that A. avenae practically do not gather around healthy roots and do not react to freshly destroyed tissues which attract stylet species of such nematodes as Pratylenchus pratensis and Rotylenchus multicinctus. However sometimes, while migrating into damaged sections of plant tissues, the nematodes penetrate into the cortical part and can use for nutrition the juices of living tissues. For example Skarbilovich (1938) indicates that accumulation of A. avenae in roots of seedlings of rubber-bearing plants led to formation of typical browning, she also observed worms and their eggs in the healthy tissue of kok-saghyz. Nevertheless this species was found in predominant numbers in macerated sections of roots of rubber-bearing plants, which allowed Kalinenko (1934) to draw a conclusion on the important role of A. avenae in maceration of the root of rubber-bearing plants together with other saprozoic organisms. Direct experiments conducted by the above-mentioned researchers (Arndt and Christie, 1937) for the purpose of clarification of the role of nematodes in the inoculation of the fungal flora, which cause rotting of cotton seedlings, indicate that only in some cases the presence of A. avenae can reduce the germination of seeds by 38%. As it is seen from the presented review of literature, the A. avenae can be referred to as species predominantly saprozoic but which can sometimes feed on juices of living, healthy tissues. The fact that this species never gathers on bait-traps in the soil (Brodskii, 1937) and is never disclosed in soils away from plants in places where deteriorating remains are accumulated, distinguishes it from typical saprozoic organisms.

Ditylenchus intermedius (de Man) is morphologically very similar to the stem nematode D. dipsaci (Kühn), but unlike the latter, it is harmless. In

the literature D. intermedius is mentioned as a regular inhabitant of soils near plant roots. Kir'ianova (1935) discovered this species in a series of agricultural crops, Tulāganov (1941) found it in alfalfa in kolkhozes of the Zeravshanskaia valley. The author discovered D. intermedius in all the fields investigated, in the soil as well as in plants. In the soil the frequency of occurrence of this species is within 25-37%, but usually only a few specimens of worms are found in samples. Most frequently this nematode has been found in plant roots of virgin ground (62%) and in alfalfa roots (50%), more seldom in tomato roots (25%). However in all the cases of occurrence of it in roots, the degree of infection by this species is very slight. The worms are never found in masses, usually their number does not exceed 8-10 specimens per sample. D. intermedius was found predominantly in the above-ground part of Cynodon dactylon (37%); [begin 619] in alfalfa stems and especially on tomatoes; its occurrence is limited to 12-15% with single specimens in a stem section which was examined.

Aphelenchoides parietinus (Bastian) is related in its biology to the Aphelenchus avenae; it is a species very rich on synonyms and "varietet" [varieties] which indicates that its species unity has not been defined more accurately. This is one of the most widely spread species found in various media — in water, soil, even in intestines of mollusks. It is mentioned for very many plants. Among diseases connected with its presence I. N. Filip'ev (1934) points out "dwaftness" of oats observed in Siberia. In our research A. parietinus is found chiefly in alfalfa's root system (40%), in wild plants of virgin land (37%) and somewhat less frequently in tomatoes (25%); in the soil it is also quite common, mean percent is 27. We discovered A. parietinus in the above-ground part of alfalfa in 30% of investigations, while in other plants it has not been found at all. This species has never been discovered in mass quantities, but in a number of specimens it is always predominant in roots and not in the soil. All the just enumerated species of nematodes are apparently basic components of the nematode fauna of the rhizosphere and usually do not specifically harm the plants, but episodically accumulate in masses, due to some reason or other, they can change to a status of pests or facultative parasites.

Let us turn to other forms of our index of nematodes predominant in plants. As it is seen from index of frequency of occurrence, the remaining 6 species: Rhabditis sp., Plectus sp., Wilsonema otophorus (de Man), Paratylenchus bukowinensis Mic., Heterodera marioni (Cornu.), Pratylenchus pratensis (de Man) are limited in their distribution and concentrated mostly in the root system, they rarely penetrate or do not rise at all to the stem part of the plant. Among them there are saprozic forms — like the species of the genera Rhabditis, Plectus and Wilsonema, and typical parasites — like the gall nematode — H. marioni and the shoot [seedling] nematode — P. pratensis. Rare, isolated occurrence of parasitic species on the outside of healthy plants which were taken for analysis, are, of course, understandable.

The gall nematode was found on tomato roots and in the soil (larvae of the male) as well as on roots of alfalfa and of a series of other cultivated and wild plants. Its adaptability to one or another crop is of local significance.

The second form of P. pratensis (de Man) which is considered to be a

serious pest, according to our data has no wide expansion within the boundaries of the Tashkent oasis[1]. We discovered it once in the soil of virgin ground and in the root system of Cynodon dactylon and once in the root system of a 3-year old alfalfa, each time in a limited number of specimens. In our previous explorations of nematodes in soils of the Tashkent oasis (1942) P. pratensis was found in virgin soils but very unevenly. Namely, being absent in the majority of cases this species occurred sometimes in amounts of 40, 80 or even 180 specimens per 100 $cm^3$ of soil. In examining alfalfa in 5 raions of the Zeravshanskaia valley. Tulaganov discovered only 4 specimens of this species. [begin p. 622] Parasitizing a large number of plants in the root system of which takes place the entire cycle of its development, P. pratensis can apparently be the cause of destruction of the roots and of penetration into them of bacterial infection.

There remain to be said a few words on nematodes discovered in above-ground parts of the plants. Altogether of the total number of 28 nematode species, in the stems and leaves were found 11, and in each individual crop 5.8 species were registered. In each case into the above-ground part of plants ascend the same forms which are predominant in the root system. No specific fauna was observed in the stems and leaves (according to our research). Most common in stems and leaves are Eucephalobus elongatus, Aphelenchus avenae, Ditylenchus intermedius, Aphelenchoides parietinus, Cephalobus sp. Singly found are also other representatives, as for example: Rotylenchus multicinectus, Tylenchus filiformis, Tylenchorhynchus dubius, but their presence can be considered as accidental because the number of occurrences and the number of worm specimens are very small. A similar picture was observed also by other authors who conducted examinations of plants. Of the 28 species of alfalfa nematodes, Tulaganov found in the Zeravshanskaia valley 12 species in above-ground parts and 7 of them had only one specimen each which speaks of the chance character of the findings, or, at least, of a rare penetration of worms into the above-ground part. In the tomatoes of Crimea and Caucasus this author discovered 21 species of nematodes and of the 9 species found in the above-ground part only Eucephalobus elongatus was found in 97 specimens and Aphelenchus avenae — in 8 specimens, only one specimen each were found among the rest of the species, which again indicates the non-typicalness of presence of these species in above-ground parts of plants. Of 35 species discovered by Kir'ianova (1935) in crops of the western zone of the USSR, 21 species are mentioned for above-ground parts, but 10 of these nematode species were found only once, 5 — 2-3 times and only 8 forms — more or less constantly, and these are the same species which occurred in our research as well. Therefore it can be assumed, that the above-ground parts of plants are populated

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1. - P. pratensis is a parasite, therefore it can be found in large numbers in stunted plants and not in those looking healthy, which were investigated by the author. [Editors note].

— with the exception of specific parasites — by nematodes which are permanently connected in their biology with the root system and the penetration of which into the stem and leaves is a result of a, by far not obligatory, further migration of worms. As migratory routes serve the vascular or parenchymatous tissues on — as some authors indicate — nematodes can rise along the surface of the stems and penetrate inside the leaves through the stoma.

## CONCLUSIONS

1. Nematode population of soils is composed of typical soil forms which usually do not penetrate into plants and of species which penetrate into plants more or less permanently.

2. The diversity of species and the amount of worms populating the soil depend on agrotechnical soil cultivation and density of the root system. Soils not being cultivated ("mezhi", field edges), which have a continuous plant cover and soils under perennial crops (alfalfa), are richer in nematodes than soils which are cultivated every year.

3. The fauna of nematodes which populate the soil, but penetrate into plants are composed of species:
   a) predominant in the soil
   b) predominant in plants [begin p. 623]

4. Nematodes predominant in the soil penetrate into plants, but usually they are represented by species which are not harmful to plants. However some of these species, when reproduced in masses in plants can become pests, as for example, the Rotylenchus multicinetus (Cobb).

5. The basic make-up of the fauna of nematodes predominant in plants is represented by universal species of a wide range of potential host-plants. To those in most cases belong: Eucephalobus elongatus de Man, Aphelenchus avenae, Bast., Ditylenchus intermedius (de Man), Aphelenchoides parietinus (Bast), Sephalobus sp. which are frequently found when various plants are examined. Usually their presence has no harmful effect on plants, but when they reproduce intensely they can accumulate in sections of damaged tissue and become a cause of bacterial infections.

6. Usually into above-ground parts of plants — with the exception of specific stem species — penetrate species predominant in the root system.

7. "Mezhi" [and field edges] are sources for distribution of nematodes into cultivated fields.

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Frequency of occurrence of Nematode in the soil, in the root and above-ground parts of plants in (percentage to the number of samples taken) [pp. 620-621]

| No. of Preparations | NAME OF FORMS | Virgin Ground | | | Alfalfa | | | Tomatoes | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Soil | Root | Above-ground part | Soil | Root | Above-ground part | Soil | Root | Above Ground Part |
| | I. Species predominant in the soil - "geobionty" [?] | | | | | | | | | |
| 1 | Monhystera sp. | 25.0 | -- | -- | 35.0 | -- | -- | -- | -- | -- |
| 2 | Mononchus sp. sp. | 25.0 | -- | -- | 25.0 | -- | -- | -- | -- | -- |
| 3 | Diphtherophora communis de Man | 12.5 | -- | -- | 20.0 | -- | -- | -- | -- | -- |
| 4 | Criconema sp. | 37.0 | -- | -- | 10.0 | -- | -- | -- | -- | -- |
| 5 | Tylenchus davainei Bast. | 37.0 | -- | -- | -- | -- | -- | -- | -- | -- |
| 6 | Alaimus primitivous de Man | 12.0 | -- | -- | 15.0 | -- | -- | -- | -- | -- |
| 7 | Dorylaimus leicarii Bütschli | 25.0 | -- | -- | -- | -- | -- | -- | -- | -- |
| 8 | Anguillulina sp. | 12.5 | -- | -- | -- | -- | -- | -- | -- | -- |
| 9 | Diplogaster sp. | 12.5 | -- | -- | -- | -- | -- | -- | -- | -- |
| | Total of samples | 9 | -- | -- | 5 | -- | -- | -- | -- | -- |

Frequency of occurrence of Nematodes in the soil, in the root and above-ground parts of plants in (in percentage to the number of samples taken)
pp. 620-621

| No. of Preparations | Name of forms | Virgin Ground | | | Alfalfa | | | Tomatoes | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Soil | Root | Above-ground part | Soil | Root | Above-ground part | Soil | Root | Above-ground part |
| | II. Species penetrating into the plants. a) Predominant in the soil | | | | | | | | | |
| 10 | Dorylaimus obtusicaudatus. Bast. | 100.0 | 37.0 | -- | 55.0 | 20.0 | -- | 72.5 | 37.5 | -- |
| 11 | Rotylenchus multicincus (Cobb) | 50.0 | 12.5 | -- | 60.0 | 40.0 | 15.0 | 50.0 | 37.0 | -- |
| 12 | Acrobeles sp. sp. | 75.0 | 75.0 | 12.0 | 35.0 | 25.0 | -- | 50.0 | 25.0 | -- |
| 13 | Ditylenchus filiformis (Bütschli) | 72.0 | 12.5 | -- | 45.0 | 20.0 | -- | 25.0 | 12.5 | 12.5 |
| 14 | Dorylaimus sp. | 25.0 | 37.0 | -- | 50.5 | 5.0 | -- | -- | -- | -- |
| 15 | Tylenchorhynchus dubius (Bütschli) | 62.0 | 25.0 | 12.0 | 10.0 | -- | -- | -- | -- | -- |
| 16 | Dorylaimus monhystera de Man | 12.0 | -- | -- | 5.5 | 5.5 | -- | -- | -- | -- |
| 17 | Rhabditis filioformis Bütschli | -- | -- | -- | 10.5 | 5.0 | -- | -- | 12.0 | -- |
| | Total of samples | 7 | 6 | 2 | 8 | 7 | 1 | 4 | 5 | -- |

Frequency of occurrence of Nematodes in the soil, in the root and above-ground parts of plants in (percentage to the mumber of samples taken)
pp: 620-621

| No. of Preparations | Name of forms | Virgin ground | | | Alfalfa | | | Tomatoes | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Soil | Root | Above-ground part | Soil | Root | Above-ground part | Soil | Root | Above-ground part |
| | II. Species penetrating into the plants. b) predominant in the root system | | | | | | | | | |
| 18 | Eucephalobus elongatus (de Man) | 62.0 | 87.5 | 87.5 | 20.0 | 80.0 | 60.0 | 50.0 | 87.0 | 12.0 |
| 19 | Aphelenchus avenae Bast. | 62.0 | 75.0 | 50.0 | 45.0 | 90.0 | 30.0 | 25.0 | 72.0 | 12.5 |
| 20 | Ditylenchus intermedius (de Man) | 37.0 | 62.0 | 37.0 | 30.0 | 50.0 | 15.0 | 25.0 | 25.0 | 12.5 |
| 21 | Cephalobus sp. sp. | 50.0 | 75.0 | 25.0 | 35.0 | 50.0 | 20.0 | 12.5 | 50.0 | 12.5 |
| 22 | Aphelenchoides parietinus (Bast.) | 25.0 | 37.0 | -- | 35.0 | 40.0 | 30.0 | 12.5 | 25.0 | -- |
| 23 | Rhabditis sp. | -- | 12.0 | -- | -- | 20.0 | 10.0 | 12.0 | 25.0 | -- |
| 24 | Plectus sp. | 25.0 | 37.0 | 12.0 | 5.0 | 5.0 | -- | -- | -- | -- |
| 25 | Heterodera marioni (Cornu) | -- | -- | -- | -- | -- | -- | 12.0 | 50.0 | -- |
| 26 | Paratylenchus bukowinesis Micoletz | -- | -- | -- | 5.0 | 5.0 | -- | -- | 12.5 | -- |
| 27 | Pratylenchus pratensis (de Man) | 12.5 | 12.5 | -- | -- | 5.0 | -- | -- | -- | -- |
| 28 | Wilsonema otophorus (de Man) | -- | -- | -- | -- | 5.0 | -- | -- | 12.5 | -- |
| | Total of Samples | 23 | 14 | 7 | 20 | 17 | 8 | 11 | 14 | 5 |

Drobkin, B. S., [Action of benzoic aldehyde upon certain invertebrates]. Doklady Akademii Nauk SSSR 89(4):705-707. April 1, 1953.

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We have cited evidence favoring the hypothesis that the phytoncidal action of the common birdcherry (Padus racemosa Lam.) is due to the presence of cyanogenic glucosides in its tissues, and that one of the components of the volatile fractions of birdcherry phytoncides, obviously, is prussic acid split off during hydrolysis of the above mentioned glucosides (1). This, however, does not mean that the volatile substances -- bearers of phytoncidal properties in birdcherry -- are being exhausted by prussic acid.

There are indications that a series of protozoa are comparatively insensitive to cyanides (2). Neither are enteric nematodes, which are capable of anaerobiotic metabolism, characterized by a high sensitivity to cyanides (3). Yet, we have become convinced that the action of volatile phytoncides of birdcherry exert on these organisms is no less strong than the action they exert on rainworms which are anybiosa [obligate anaerobes].

These observations encourage the hypothesis that the volatile fractions of birdcherry phytoncides represent a complex of substances which includes other components beside prussic acid. The direct relation observed between the phytoncidal action of birdberry on organisms comparatively resistant to cyanides and the isolation of prussic acid from birdberry may, possibly, be attributed to the circumstance that the formation of free hcn is accompanied by an equivalent isolation of another toxic element. In connection with this, other aglucones released in hydrolysis of cyanogenic glucosides deserve consideration.

It is known that glucosides of the amygdaline type prevalent in many representatives of the rose family and, particularly, in birdcherry become decomposed in the process of hydrolysis with the splitting off of two aglucones; prussic acid and benzoic aldehyde. As this occurs, a direct quantitative relationship exists between their formation.

To arrive at a solution of the question concerning the role of benzoic aldehyde which forms in the phytoncides of birdcherry, it was necessary to ascertain just how benzoic aldehyde affects the organisms of interest to us.

Literary data concerning the biological action of benzoic aldehyde are few and relate only to vertebrates, toxicity with respect to these was studied in conjunction with the use of benzoic aldehyde in the perfumery and food industries. There are indications that benzoic aldehyde is not poisonous if administered per os. V. I. Shvortsov (4) asserts that toxicity of pure benzaldehyde is minimal.

Data concerning the action of benzoic aldehyde on invertebrates we did not find. This fact prompted us to investigate the action of benzoic aldehyde upon some forms of the lower animals serving as objects of our experiments with phytoncides of birdcherry. The results obtained constitute the subject of the present report.

The work was conducted with a chemically pure benzoic aldehyde representing a liquid with the odor of bitter almond strongly reminiscent of the odor of crushed tissues of birdcherry.

A study was made of the action of benzaldehyde on Paramecium caudatum, Euglena viridis, nematodes (Commoscera sp.) which act as parasites in the intestines of toads, on earthworms (Lumbricus sp.) and on flies (Musca domestica).

In the first series of experiments, the action of benzoic aldehyde fumes was investigated.

The action of benzaldehyde fumes upon Paramecium and Euglena was produced as follows. A certain amount of benzaldehyde (0.1, 0.2, and 0.3 ml [milliliter]) was placed at the bottom of a Petri dish. Over it, at a distance of 0.5 cm, was placed on cork supports, a slide with a drop of medium containing 15-20 Protista turned upside-down [obrashchennoi vniz]. Observations were conducted under a microscope through the lid of the Petri dish.

The action of benzoic aldehyde fumes upon nematodes was also studied, however, a drop of a physiological solution of sodium chloride containing two nematodes from the rectum of a toad was applied on the slide.

Experiments with rainworms were conducted in cans [biuky] of a 75 $cm^3$ holding capacity. Benzoic aldehyde was put on the bottom of the can and two rainworms were placed on a cardboard lattice resting on cork supports.

In the experiments with flies, a piece of cotton soaked in a fixed amount of benzaldehyde was put in a test tube, then 3-4 flies were quickly let into it, and thereupon the test tube was closed with a cork.

Control animals were placed under the same conditions as experimental ones, however, without benzoic aldehyde. The results obtained are cited on table 1.

Table 1.

Action of benzoic aldehyde fumes upon some invertebrates (period of destruction since inception of experiments; average of 10 experiments)

| Benzaldehyde entered in ml | Paramecia | Euglena | Nematodes | Rainworms | Flies |
|---|---|---|---|---|---|
| 0.1 | 67 sec. | 63 sec. | 4.5 min. | 11.5 min. | -- |
| 0.2 | 52 sec. | -- | 3.1 min. | 9 min. | -- |
| 0.4 | 42 sec. | -- | 3 min. | 8 min. | 9 min. |
| Control | Alive | Alive | Alive | Alive | Alive |

In the second series of experiments, the action of the solutions of benzoic aldehyde was studied. The solubility of benzaldehyde in water equals approximately 1:300. And so we used in the experiments benzoic aldehyde in a dilute of 1:300 or more. The aldehyde was diluted in a physiological solution of sodium chloride.

The experimental method used was the following: To a drop of medium containing 15-20 Paramecia or Euglena placed on the slide, was added a drop of a solution of benzoic aldehyde of a fixed concentration. The preparations were placed in damp compartments - Petri dishes lined on the inside with moistened filter paper.

In experiments with nematodes, two nematodes were placed in a drop of a physiological solution, then a drop of benzoic aldehyde solution was added. During the experiment the preparations were kept in a moist compartment. In control cases a drop of a physiological solution was added. In studying the action of benzoic aldehyde solutions on rainworms, the latter were put in a can containing 10 ml. of benzoic aldehyde solution of an appropriate concentration (see table 2). Control worms were placed in a can with an equal amount of physiological solution.

Table 2.

Action of benzoic aldehyde solutions upon some invertebrates (period of destruction since inception of experiments; average of 10 experiments)

| Initial dilute of benzoic aldehyde added | Paramecia | Euglena | Nematodes | Rainworms |
|---|---|---|---|---|
| 1:300 | 30 sec. | 8.7 min. | 59 min. | 52 sec. |
| 1:600 | 5.3 min. | 17 min. | 112 min. | 10.7 min. |
| 1:1200 | 18.2 min. | 24 min. | Alive 3 hrs later | 56 min. |
| Control | Alive | Alive | Alive | Alive |

From the data on table 1 and 2 it is apparent that the solutions as well as the fumes of benzoic aldehyde exert a sharply toxic action upon the invertebrates which served as objects in our experiments.

The results obtained justify the hypothesis that benzoic aldehyde which forms in hydrolysis of certain glucosides may become a composite part of the phytoncide complex.

In the light of such a hypothesis one can visualize the biological importance of glucosides of the amygdalin type which accumulate in the tissues of a number of representatives of the rose family and, in particular, of the almond subfamily.

Being biologically neutral, these substances, upon injury of the plant, easily hydrolyse and form two biologically very active agluconas; prussic acid and benzoic aldehyde capable of performing an essential role in the regulation of interspecific reciprocal relations and, particularly, in accomplishing protective functions.

Apparently, it is not only in the birdcherry, but also in other representatives of the rose family, particularly in such powerful phytoncids producers as cherry laurel (Laurocerasus officinalis), dwarf almond (Amygdalus-nana), the formation of phytoncides is associated with glucosides of the amygdalin type found in the tissues of these plants.

Of late, there has accumulated data (5) verifying the fact that other glucosides likewise have a share in the protective media of plants. Possibly this comprises one of the functions of glucosides whose physiological role in the plant organism cannot, until recently be considered as conclusively established.

Trans. 488
(In full)
By:
A. Antik

Kameraz, A. Ia.

Novye fitoftoreuctoichivye i
rakoustoichivye Sorta Kartofelia

[New Phytophthora - and wart-resistant
varieties of potatoes].

Sad i Ogorod 1:45-48. Jan. 1954
80 Sa13

(In Russian)

488

## New Phytophthora - and wart-resistant varieties of potatoes

The September Plenum of the TSK KPSS (Central Committee of Communist Party of Soviet Union) set a goal - to bring, during the coming two - three years, the production of potatoes and vegetables up to a volume which would satisfy completely not only the needs of the city population and industry but also the potatoes needed by the livestock industry. Of great significance in the complex of agro-measures necessary for the completion of this most important task is the correct selection of a variety and good quality of seed. The resolution of the Council of Ministers of the USSR and of the Central Committee of the KPSS "On measures for increase of production and on store of potatoes and vegetables in Kolkhozs and Sovkhozs during 1953-1955" obliges the Ministry of Agriculture of the USSR, Ministry of Sovkhozs of the USSR, the soviet and agricultural organizations to take measures for improvement of production of varietal potato seeds, for a speeding up of reproduction and penetration into Kolkhoz and Sovkhoz production of best-yielding varieties distributed according to areas, particularly of early potatoes, in order to make, by 1957, a complete switch to varietal potato plantings in all the Kolkhozs and Sovkhozs.

Of particular importance is the adoption in potato production of varieties resistant to the most dangerous diseases -- canker and Phytophthora. At the present time our country possesses canker-resistant potato varieties for various economic purposes and of various ripening dates.

Up to the recent time in the world's potato assortment there were no varieties resistant to the most dangerous disease - the Phytophthora (late blight). It is not a quarantined disease; against it the usual quarantine measures are worthless. The fungus causing the disease appears everywhere where it encounters conditions favorable for its development. Almost everywhere in the basic areas of

potato industry the moist and warm latter part of the summer creates conditions favorable for intense spreading of Phytophthora. The fungus affects the leaves and the tubers which causes a drastic decrease in potato yields. The sooner the Phytophthora appears the greater can be the decrease in potato yields.

In some areas, for example in the Sakhalin oblast, the considerably reduced yields of potatoes due to damage by Phytophthora has a systematic character (regularly occurs).

Of quite particular importance is the disclosure in the vast collection gathered by the All-Union Institute of Plant Industry, of wild potato species resistant to Phytophthora.

Disclosure of such species made realistic the task of creating full-value potato varieties more resistant to Phytophthora than all the previously existing selections. For the first time in the world, potato varieties with higher resistance to Phytophthora were created in the Soviet Union. At the present time selection work for creation of Phytophthora-resistant varieties on the basis of the initial material from the collection of the All-Union Institute of Plant Industry (VIR) is being developed in numerous scientific institutions of the USSR.

Observations in various geographical areas indicate that Phytophthora-resistant potato varieties which were not damaged in the given area by Phytophthora through a number of years, in some years, when the conditions were especially favorable for the fungus, showed at the end of the growing period some degree of symptoms of Phytophthora infection. This is due (begin P. 46) to a number of causes. There is no doubt that the degree of aggressiveness of the fungus can change depending on different conditions. In certain localities, under favorable conditions, the fungus can gradually become adjusted to the host which was absolutely unsuitable before. Thus a more aggressive biotype of Phytophthora can be developed.

This process of appearance of more aggressive fungus forms is furthered by the growing of potato collections which include diverse interspecies hybrids having various degrees of resistance to Phytophthora. When Phytophthora appears early and the following conditions in regard to temperature and humidity are favorable, the fungus can transfer from less resistant forms (hybrids) to better resistant ones, gradually acquiring greater resistance. Phytophthora acquires the capacity to attack the more resistant hybrids at the end of the growing season when changes due to age begin to take place in the plant and make it less resistant.

Observations show that the more aggressive bio-types of the fungus disappear the next year under conditions unfavorable for Phytophthora development, particularly when specific control measures are carried out against the disease.

In order to prevent the appearance of more aggressive bio-types it is necessary in (variety) selecting institutions which maintain potato collections to spray the plantings with Bordeaux mixture or dust with the compound AB. It is necessary to select carefully and to destroy the tubers which are to some degree infected with Phytophthora and in no case should their planting be permitted. All the potatoes set aside for sowing must be treated (chemically) in the fall.

Under production conditions, two or more inter-species hybrids originating from crossing with Phytophthora-resistant wild species and having a varying degree of resistance - should not be grown on one farm. Our observations in the Leningrad oblast show that in growing one Phytophthora-resistant variety it is considerably easier to avoid the appearance of a more aggressive bio-type of the fungus even in years particularly favorable for the development of Phytophthora.

It follows that it is not enough to create a Phytophthora-resistant variety, that a system of measures is necessary which would permit retaining for many years the most valuable property-resistance to Phytophthora.

In 1953, which was an exceptionally favorable year for the development of Phytophthora, at the Belorussia state selection station, the plantings of almost all the varieties, which were resistant to this disease before, were damaged to some degree. In the Leningrad oblast also some of the hybrids which in previous years had no symptoms of the disease were partly diseased.

However, a great difference was observed in the Leningrad oblast between the incidence of Phytophthora in plantings of some hybrids and of regular varieties. In 1953, in certain areas of the oblast

Phytophthora appeared exceptionally early. The first disease symptoms in the fields of early varieties (begin p. 47) were recorded in some

cases by July 5, and in others - in the middle or in the third ten days of July. By the middle of August on many farms Phytophthora on many farms completely destroyed the leaves in the fields not only of the early but also of the medium-late regular varieties distributed for the oblast. By this time numerous Phytophthora-resistant hybrids had no symptoms of disease. By the end of August - beginning of September the first symptoms of Phytophthora infection appeared on less resistant hybrids. On many hybrids the first symptoms of Phytophthora were disclosed by the middle or the end of September.

It follows that even in years most favorable for the development of the fungus, the hybrids to some degree resistant to Phytophthora have an enormous advantage as compared with regular varieties. If the disease even appears on them, it occurs considerably later and, therefore, has no practical importance and hardly influences the yield.

At the present time, in the All-Union Institute of Plant Industry, a study is being conducted on methods for creating varieties more resistant to aggressive bio-types of Phytophthora than the ones already existing. However, on the basis of data obtained under varying conditions of the Leningrad and adjoining oblasts, the conclusion can be drawn that the varieties which are more Phytophthora-resistant represent a considerable value and have to be introduced into production as soon as possible.

The more widely distributed Phytophthora-resistant variety in the Leningrad oblast is the "Kameraz", selected by the All-Union Institute of Plant Industry which is recommended for the Leningrad, Pskov, Novgorod, Velikie-Luki, Kalinin, and Molotov oblasts. The variety combines resistance to Phytophthora with that to canker

(black wart). It is obtained by way of repeated crossings of the wild Mexican variety Solanum demissum with the varieties: "Granat" (twice), "Narodnyi", "Lichingen". The variety was grown from seedlings in 1937, was turned over in 1947 for the state varietal testing; was for the first time distributed to areas in 1951.

The tubers are round, white with a flat top, slightly indented "stolonnyi sled" (stem end). The skin is netted. The eyes are few, small, sometimes deeper at the top, not colored. The flesh is white, does not darken when cut.

The corolla (petals) is medium large, white, sometimes light violet on the outside. Formation of berries (fruit) is abundant. Medium-ripening variety, for table consumption; productive. Tubers of good taste, store well. As a shortcoming should be considered lack of resistance to the fungul disease--macro-sporiosis. When agrotechnique is inadequate and a systematic seed industry is lacking, then sometimes symptoms of degeneration can be observed which are manifest mainly in the form of spottiness. Testing at the station resulted in an average yield of 433 centners per 1 hectare from 1945 to 1950; starch content on the average 14.5 - 15.0%.

In tests in state varietal plots this variety usually occupied one of the top places in regard to yield capacity, producing, for example, in the Leningrad varietal plot 109 - 133%, in the Luzhsk - 108 - 120%, in the Gatchina plot - 114-125% in ratio to the standard variety "Berlichingen". The starch of the "Kameraz" variety is always higher than that of "Berlichingen". Thus in the Leningrad (begin p.48)

varietal plot the mean starch content of tubers of the "Kameraz" variety during 1947 - 1950 was 14.72%, almost 1% higher than that of "Berlichingen". In regard to the supply of starch on the average for 1948-1950 the potatoes of the "Kameraz" variety presented 123% of the standard and according to the mean weight of the commercial (?) tubers - 132%.

Especially significant was the behavior of the variety in 1953 in the Leningrad oblast when the Phytophthora was spread intensely.

At the experimental base of the VIR "Krasnyi Parkhar'" near Leningrad in production fields when square-hill planting of the standard variety "Berlichingen" produced a yield of 160 centners per 1 hectare the "Kameras" variety produced 270 c. per 1 h. In the Kolkhoz imeni Lenin, Gatchina raion, the potato yield of the "Kameraz" variety in a 48 hectare area was 160 to 350 c. per 1 h., in the Kolkhoz imeni Ihdanov, Volosovskii raion, - 185 c. per 1 h. (and "Berlichingen" variety 117 centners), in the sovkhoz "Krasnaia zaria" in large production areas the yield reached 300 c. per 1 h. A considerable surpassing of yield capacity in comparison with varieties non-resistant to Phytophthora is recorded also in other Kolkhozs and Sovkhozs of the Leningrad and adjoining oblasts.

According to data of the Sakhalin branch of the AN (Academy of Science) SSSR, the mean yields for 3 years in the Sakhalin oblast of the variety most widely distributed here "Mestnyialyi" -- was 200 centners per 1 hectar and of the variety "Kameraz" - 330 c. Total waste of varieties was: "Berlichingen" - 15%, local - 6.6% and of the "Kameraz" - 1%.

In the same oblast, according to the material of the Aleksandrovskii state varietal plot, of the fifteen varieties being tested in 1952, the "Kameraz" variety stood out in its yield which was 327 c. per 1 h.

Besides the medium - late Phytophthora - and canker-resistant varieties, needed for the Leningrad and the adjoining oblasts are also productive early and medium-early canker-resistant varieties more resistant to Phytophthora than the already existing varieties. The standard early variety "Cobbler" is to a high degree damaged by Phytophthora which hinders its expansion. Other early varieties are also seriously damaged by Phytophthora.

At the present time in a series of Kolkhozs and Sovkhozs good results were obtained from tests of new varieties grown by the All-Union Institute of Plant Industry: Pushkinskii (U8-236) and Detskosel'skii (R8-168). The first is an early variety, the second -- medium-early. According to preliminary data both varieties are canker-

resistant (the test is not yet completed) and more resistant to Phytophthora (especially the Pushkinskii variety) than the already existing early and medium-early varieties.

Penetration into production of varieties more resistant to Phytophthora is of tremendous importance for the increase in potato yields in the zone of extensive incidence of this disease. We have at the present time varieties more resistant to Phytophthora and ripening at different dates. It is necessary to reproduce the seeding material rapidly and to test the varieties mentioned. At the same time systematic seed-growing is a must, in order to sustain, on a high level, the seed properties of new varieties. It should also be noted, that these varieties produce good results under conditions of sufficient fertilization and moisture (without excess water). Therefore, in cultivating the Phytophthora-resistant varieties it is obligatory to apply the entire complex of methods of progressive agrotechnique.

Expansive entrance into production of more Phytophthora-resistant potato varieties together with many other measures will further a most rapid transition into life of the historical resolution of the September Plenum of TSK KPSS in regard to the field of potato industry.

Trans. 489
(Summary)
By:
A. Antik

Zadina, J.

Die biotypen der kartoffelkrautfäule (Phytophthora infestans) und die resistenzzüchtung gegen die kartoffelkrautfäule.

Ceskoslov. Akad. Zemedel. Sborn.
26:569-574. Dec. 1953.
19.5 C332C

German Summary

(In Czeck)

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15 variants of phytophthora isolated in various locations of Czechoslovakia are discussed in this article. By way of applying these local variants to an assortment of 4 potato varieties (Aquilla, Falke, Fridolin, Roswitha) which were resistant to phytophtora on tubers, they were divided into 6 bio-types. The aggressiveness of these bio-types was compared with that of the Polish bio-type which affects tubers of all the selected potato varieties resistant to phytophthora. So far this aggressive bio-type was not found in Czechoslovakia's territory.

Further on the article discusses the problem of originating of new bio-types in the territory of the Czechoslovakian Republic (CSR) and of the possibility of using wild potato varieties in breeding varieties resistant to highly aggressive phytophthora bio-types. According to tests carried out up to now, the Polish bio-type affects 60% of hybrids of the wild variety Sol. demissum and cultivated varieties, while the bio-type from Velke Karlovice affects only 20% of these hybrids.

Trans. 490
(In full)
By:
A. Antik

Afanas'ev, P. V.

O mekhanizme i kinetike fermentativnogo sinteza

[The mechanism and kinetics of fermentative synthesis].

Biokhimiia 14(5):424-431.
Sept./Oct. 1949. 385 B523

(In Russian)

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# THE MECHANISM AND KINETICS OF FERMENTATIVE SYNTHESIS

Enzymes possessing only a synthetic action are so far not known, therefore it should be assumed that the same biological catalyzers produce reactions of decomposition as well as of synthesis. This corresponds with requirements of thermodynamics, because in an opposite case the enzymes could disturb the chemical equilibrium.. Tendency of the process towards decomposition or synthesis depends only on the question-in what case will the process lead to a decrease of free energy of the system.

According to Oparin's (1) presentations the tendency of enzymatic processes in the living cell is determined by adsorptive factors. In some combinations the adsorbtive factors can create conditions under which synthetic processes can proceed with a decrease in free energy of the system, though individual components change with an increase of free energy. A detailed presentation of the mechanism and a concrete notion of conditions under which such processes take place in the living cell, cannot be given yet because of extremely limited information and an exceptionally complex system.

Simple cases of conversion (?) of enzymatic decomposition were realized by many researchers. These cases have the peculiarity that they take place with relatively negligible changes of free energy; these changes can be compensated easily (and even change their symbol (?sign?) ) with the help of experimentally accessible changes of concentrations of reacting components. In a case of high levels of changes of free energy it is not possible to realize the conversion with simple means. And the majority of the most important biochemical processes belong to the latter type.

One of the methods of conversion of the enzymatic hydrolysis - which is biologically one of the most important ones - is experimentally realized and theoretically substantiated conclusively by Bresler and his co-workers (2), who demonstrated that in a case when the enzymatic process takes place with an increase in the volume of the system, it is possible, with the help of external pressure, not only to compensate but also to convert the symbol of change of the free energy of the process. This permitted conversion of the enzymatic hydrolysis of polypeptides and polysaccharides, i.e. to realize synthetic processes. Theoretical deliberations and experimental results obtained by Bresler are of great significance in principle for comprehension of biochemical processes and they open a wide perspective for experimental and theoretical study of enzymatic reactions.

The purpose of the present work is to clarify the mechanism of action of enzymes in a synthetic process, to make theoretical conclusions of kinetic relations and to test experimentally these conclusions.

Our research was conducted on the example of hydrolysis with trypsin of polypeptides under atmospheric pressure and re-synthesis of hydrolysates under high pressure.

In the preceding report (3) - on the basis of notions, which we developed, on the nature of enzymatic activity - an equation was given of the relation between the speed of enzymatic hydrolysis of polypeptides and the depth of hydrolysis

$$V = K_3 \frac{FS - K_1 K_2 S^2}{K_3 K_1 + 2S}, \qquad (1)$$

where V - is the speed of the process; F - concentration of the enzyme, S - concentration of the substrate (depth of hydrolysis); $K_1$ $K_2$ and $K_3$ - constants of speeds of intermediate reactions. We demonstrated that kinetic peculiarities of enzymatic hydrolysis of polypeptides are well described by the suggested equation. This equation (1) was derived on the basis of the hypothesis that enzymatic hydrolysis proceeds according to the scheme

1. $F + S \xrightarrow{K_1} FS;$

2. $FS + F \xrightarrow{K_2} F_2S;$

3. $F_2S \xrightarrow{K_3} 2F + 2S\frac{1}{2},$

where F is - concentration of the enzyme; S - concentration of the substrate; $K_1K_2K_3$ - constants of speeds of intermediate reactions. The active link which produces the reaction is the complex $F_2S$. In this complex takes place the activation of the substrate which eases its hydrolytic decomposition. It follows from equation (1), that the

hydrolytic process has to proceed with increasing inhibition by the substrate and the reacting system gets finally into a kinetic dead-end.[1] In this dead-end the reaction cannot take place. Due to the fact that this dead-end is reached long before a complete breakdown of the polypeptide, it becomes understandable, why the experimental depth of the ultimate hydrolysis is never great. On the basis of equation (1) the condition of the kinetic dead-end is expressed by the relation:

$$V = 0, \text{ when } F = \frac{K_1}{K_2} S$$

In a case when the system, for example trypsin -- polypeptide, is under conditions when thermo-dynamically advantageous is the process of synthesis of peptide relations and not the hydrolysis---the enzyme will catalyze the synthetic process to a higher degree. It is impossible to suppose that in reversing the process of hydrolysis, all the intermediate stages are fully and completely reversed. For that we would have to assume that the synthesis is realized in the act:

$$FS\tfrac{1}{2} + F_2S\tfrac{1}{2} \longrightarrow F_2S$$

But this is impossible because the complex $FS\frac{1}{2}$ is inactive and therefore activation of the substrate cannot take place there. In previous reports we expressed considerations about the reason why the complex $FS\frac{1}{2}$ is inactive. Therefore the intermediate stage, where the act of synthesis of peptide linkage takes place, should be a stage which includes the active complex $F_2S\frac{1}{2}$. In this active complex takes place an activation of the substrate which makes it capable of reacting with water in case of hydrolyses or with another molecule of the substrate--in case of synthesis. Therefore it is necessary to assume that the act of synthesis of peptide linkage is realized in the following intermediate reaction:

$$F_2S\tfrac{1}{2} + S\tfrac{1}{2} \longrightarrow F_2S$$

Accepting this hypothesis the scheme of ferment action with a synthetic process can be presented in the following manner:

1. $F + S\frac{1}{2} \xrightarrow{K_1} FS\frac{1}{2}$
2. $FS\frac{1}{2} + F \xrightarrow{K_2} F_2S\frac{1}{2}$
3. $F_2S\frac{1}{2} + S\frac{1}{2} \xrightarrow{K_3} 2F + S$

[1] Since during a hydrolytic dissociation of the substrate (polypeptide) products are generated which are also a substrate, the actual concentration of the substrate, as a result, increases.

Applying the principle of quasi-stationary property of centrations of of intermediate products and expressing them through concentrations of enzyme and substrate, we shall obtain an equation for the speed of reaction:

$$V = \frac{dS}{dt} = \frac{K_1}{1+2\frac{K_1}{K_3}} \left(FS - \frac{K_1}{K_2} S^2\right) \qquad (2)$$

Obtained are a mechanism and equation of kinetics identical with those which we proposed in the preceding report for catalase.

It follows from equation (2) that: $V = 0$ either when $S = 0$ or when $F - \frac{K_1}{K_2} S = 0$; i.e. when $F = \frac{K_1}{K_2} S$ or $S_o$ (not clear) $= \frac{K_2}{K_1} F$.

Differentiating equation (2) we shall obtain:

$$\frac{dv}{ds} = \frac{K_1}{1+2\frac{K_1}{K_3}} \left(F - 2\frac{K_1}{K_2} S\right), \qquad (3)$$

from where $\frac{dv}{ds} = 0$ when $F - 2\frac{K_1}{K_2} S = 0$, i.e.

$$F = 2\frac{K_1}{K_2} S \text{ or } S_M = \frac{K_2}{K_1}, \frac{F}{2}$$

Therefore the relation between the speed of reaction and the concentration of substrate, equation (2), has a maximum at $S_M = \frac{So}{2}$, i.e. the function is symmetrical ($S_M$ — maximum concentration of substrate at which the speed of reaction is completely suppressed).

It is necessary to point out that equation (1) has an unsymmetrical aspect and the maximum speed of reaction is realized at relatively small substrate concentrations.

From equations (1) and (2) follows that kinetics curves (S, T,) where S is the substrate concentration and T---the(time) for hydrolysis and synthesis have to be quite different in form. Under the usually applied conditions for carrying out of enzymatic hydrolysis of polypeptides, the curve (S,T) is close in form to the normal type, i.e., at first the reaction speed is a maximum one and then it steadily drops to zero. The kinetics curve (S,T) of the synthesis has to have an unusual S - like (symmetrical) aspect.

## Experimental Part

In order to test the exposed theoretical deliberations and conclusions, we conducted following experiments.

Kinetics of hydrolysis with trypsin of egg slbumin (crystallized) was measured: 1% egg albumin in borate buffer (0.2M) at pH 9.15 (before experimenting the albumin was denatured by heating in a boiling water bath for 10 min.), trypsin 1/25 part of the albumin weight at 38°. Antiseptic-thymol. Determination of amine nitrogen was conducted according to the Van-Styke method. The results of measurings of hydrolysis are given in fig. 1.

N mg/ml

N mg/ml

(p. 426)

Fig. 1. Hydrolysis of 1% egg albumin in the borate buffer (0.2M); pH 9.15 with trypsin being 1/25 part of albumin weight. Temperature 38°, antiseptic-thymol.

Fig. 2. (p.426) Re synthesis of hydrolysate of egg albumin under the pressure of 6,000 atmospheres at a 38° temperature.

For a resynthesis under a 6,000 atmosphere pressure at 38° was taken hydrolysate 22 hours after the beginning of hydrolysis and then several samples of hydrolysate were placed under pressure for varying time periods. At the end of the required period the apparatus was cooled to room temperature, the pressure was removed and the samples were immediately analysed by the Van Slyke method for content of amine nitrogen. The technique for conducting the experiments under pressure is analogous to that described in Bresler's works. The results of the experiments on synthesis under pressure are presented graphically in fig. 2.

In comparing fig. 1 and 2 it is apparent that the character of kinetic curves (S,T) corresponds completely with those expected. It is necessary to point out that the kinetics of resynthesis experimentally studied by Bresler and Glikina on the system gelatin of trypsin, is not fully known. Our detailed measuring of kinetics of resynthesis on the system of egg albumin---trypsin is in complete accordance with observations by Bresler and Glikina.

In differentiating the curves (fig. 1 and 2), are obtained curves (V,S) of dependence of speed on substrate concentration which correspond to equations (1) and (2); they are presented in fig. 3 and 4. It can be seen that the curve (V,S) of fig. 3 for hydrolysis is indeed unsymmetrical. The curve (V,S) of fig. 4 for synthesis presents a symmetrical curve. These results correspond fully with the requirements of the above-developed theory. Equation (3) requires a direct-line relation between $\frac{dv}{ds}$ and S.

In fig. 5 are presented the results of differentiation of the experimental curve in corresponding coordinate axes. The direct-line character of relation of experimental values coincides completely with the requirements of the theory.

N mg/ml. hour

(p.427)

Fig. 3. Relation between the speed of enzymatic hydrolysis and the depth of hydrolysis (concentration of substrate).

Fig. 4. Relation between the speed of enzymatic synthesis under pressure and the depth of hydrolysis (concentration of substrate).

Fig. 5. Relation between derived speed of enzymatic synthesis of substrate concentration and the concentration of substrate.

Bresler and Glikina indicate that the possibility and depth of resynthesis depend sharply on the depth of the substrate hydrolysis. In an ultimate case when the substrate hydrolysis reaches a maximum depth it is not possible to acheive a resynthesis. They gave no exhaustive explanation of this fact. In the preceding work we explained the reason conditioning the limitations of the depth of enzymatic hydrolysis when the substrate is highly polymeric. Turning to equation (2) it is easy to show that the same reason, conditions the impossibility of resynthesis with a maximum depth of hydrolysis. It is remarkable that the decrease in speed of enzymatic processes down to a zero value is carried out in both cases at equal concentrations of substrate. It follows from the fact that $V = 0$ under the condition that $F = \frac{K_1}{K_2} S = 0$ and this derives from equation (1) as well as from equation (2). Thus the upper limit of substrate concentration is the same for hydrolytic and synthetic processes.

If our hypotheses on mechanism and kinetics of enzymatic synthesis are correct, then methods can be sought for leading the reaction system out of the kinetic dead-end.

The kinetic dead-end (upper limit) takes place when the equation $F = \frac{K_1}{K_2} S$ is realized. When this equation is disturbed, the kinetic dead-end is removed. There are different ways of disturbing this equation. The simplest method is to add a supplementary amount of enzyme. Bresler and Glikina demonstrated experimentally

that in this manner it is possible to realize resynthesis under pressure. Another method for leading the system out of the dead-end is to decrease the substrate concentrations, for example, by way of removing part of the substrate with dialysis through semi-penetrable membrane. This method has not yet been accomplished experimentally. And, finally, the third method for leading the system out of the kinetic dead-end can consist of procedures which decrease the coefficient $\frac{K_1}{K_2}$. The decrease of this coefficient will bring about that the condition of the dead-end will be realized at a greater value of substrate concentration, i.e., at a greater depth of hydrolysis.

In the preceding work we indicated that catalytical properties of enzymes are not constant and vary depending on the composition of the media. The constants $K_1$ and $K_2$ characterise the catalytical properties of the enzyme and the latter depend considerably on the state of the enzyme. Therefore, in changing the state of the enzyme it is possible to disturb the equation $F = \frac{K_1}{K_2} S$, i.e., to lead the system out of the

kinetic dead-end.

According to opinions of D. L. Talmud and co-workers (4), globular albumins undergo structural transformations when the compositions of the solvent changes. Structural transformations of globular albumins is accompanied by changes in physical-chemical and bio-chemical properties. We think that structural transformations have place among enzymes as well. Since according to our notions the enzymatic activity is a special case of manifestation of structural transformations, (therefore) the factors which cause structural transformations change the catalytical properties of enzymes.

In order to check these notions experimentally we conducted experiments on resynthesis under pressure, applying enzymatic hydrolisates with a maximum depth of hydrolysis. In order to change the catalytical properties of the enzyme we applied two procedures: 1) change in the pH system which brings the enzyme closer to the iso-electric state, and 2) adding of caprylic acid which, as it is known, considerably improves the symmetry of globular albumins. And improvement of the symmetry of the enzyme molecules facilitates the formation of the complex $F_2S$, i.e. increases the constant $K_2$. Our experiments produced following results:

Obtained is hydrolysate from a 4% gelatin in "barat" buffer 0.2M; pH 9.15; trypsin-1/50 part of the gelatin weight; temperature - 38°. Determination of amine-nitrogen according to Van-Slyke gave:

| Time in hours | 0 | 24 | 48 |
|---|---|---|---|
| N mg/ml | 0.174 | 0.343 | 0.340 |

Three samples from the obtained hydrolysate were placed under a 6000 atmosph. pressure, at 38° and were analyzed after 18 hours. Following results were obtained: Sample I - control (hydrolysate was taken as such): 0.340 N mg/ml; Sample II - prior to the test the hydrolysate was acidified 1N HCl (5 drops per 20 ml.): 0.306 N mg/ml; SampleIII - to the hydrolysate was added caprylic acid, up to about 0.02 M: 0.280 N mg/ml.

From the given data can be seen that we succeeded in leading the system out of the kinetic dead-end and to realize resynthesis. The control test under similar conditions produced no resynthesis.

In order to check that the resynthesis was possible because adding of caprylic acid results in increase of the upper limit of substrate concentration (depth of hydrolysis), we studied the kinetics of hydrolysis. We measured for purposes of comparison the kinetics of hydrolysis with and without added caprylic acid. The results of the experiments are presented in fig. 6, from which it is seen that in presence of 0.02M of caprylic acid the depth of hydrolysis is 37% higher than without caprylic acid. To hydrolysate obtained without caprylic acid, we added after 19 hours of hydrolysis (i.e. when hydrolysate did not change anymore) caprylic acid up to 0.02M. After 6 hours an increase in the depth of hydrolysis was established up to the same value as it was in the hydrolysis with caprylic acid added at the beginning of the test. The obtained results indicate that resynthesis of an equivalent weight of hydrolysate in presence of caprylic acid results from the leading of the system out the kinetic dead-end at the expense of the increase of the depth of hydrolysis.

N mg/ml

Fig. 6. (p.429) Hydrolysis of 40% gelatin in "barat" (?) buffer (0.2M) pH 9.15 with trypsin 1/50 part of albumin weight. 1- without additions;

2- added caprylic acid up to 0.02 M/e; 3- added caprylic acid to 1- after 19 hours.

We came across the fact that with realtively high concentrations of trypsin, the depth of hydrolysis does not depend practically on concentration of the enzyme. And, according to our notions of the nature of enzymatic activity, the depth of hydrolysis on the upper limit have to be in proportion to the concentration of the enzyme. It is characteristic that with high concentrations of the enzyme the hydrolysis proceeds with a sharper increase of inhibition. This inhibition is particularly intensely manifest during the second part of the process and leads practically to the same depth of hydrolysis as with a lower concentration of the enzyme. This is shown in fig. 7 where the curves of kinetics of hydrolysis are presented which we obtained with different enzyme concentrations (in the ratio of 2:1).

N mg/ml

N mg/ml

(p. 430)

Fig. 7 Hydrolysis of 4% gelatin in borate buffer (0.2M), pH 0.15, with trypsin.
1-trypsin 1/50 of the albumin weight;
2-trypsin 1/25 of the albumin weight.

Fig. 8 Hydrolysis of 4% gelatin in borate buffer (0.2 M); pH 8.15, with trypsin.

1-without additions; 2-with added 0.02 M/1 glycocol , 3-with added 0.02 N/1 aspartic acid.

We supposed that the reason for it can be the appearance as a result of hydrolysis of such products as aspartic and glutamic acids. These amino-acids are dibasic and they block trypsin and thus decrease its active concentration. And the decrease in active enzyme concentration leads to decrease of the depth of hydrolysis. Amino-acids which contain one amine and one carboxyl group should not have an effect on

the depth of hydrolysis. In order to check this hypothesis, we measured the kinetics of hydrolysis in the presence of glycocol and aspartic acid in concentrations close to those actually possible in hydrolysates. The results of the experiments are shown in fig. 8, and it is seen that the experimental results confirm our hypothesis concerning the depressing effect of aspartic acid.

In conclusion I express my appreciation to N. A. Selezneva for participation in our experiments.

## Conclusions.

As a result of theoretical study a mechanism of enzymatic synthesis was suggested, the basic link of which is the interaction of an active complex $\{(\text{enzyme})_2 \text{ substrate}\}$ with a substrate molecule. Given is a kinetic equation of the synthetic process, which presents a symmetrical function distinguishable from an unsymmetrical function for a hydrolytical process. It is demonstrated that the upper limit of substrate concentration (maximum depth of hydrolysis) corresponds with a complete cessation of processes of hydrolysis and synthesis. With synthetic enzymatic processes it is not possible to use substrate concentrations above the upper limit.

Experimentally demonstrated on the example of the egg albumin-trypsin system is the symmetrical character of relation between the speed of reaction and the substrate concentration with synthetic process and the unsymmetrical character of relation with hydrolytic process. Confirmed is the impossibility of a synthetic process in a enzymatic hydrolysate under high pressure in a case when hydrolysate is brought to a maximum depth of hydrolysis.

Discussed are the possibilities of realization of synthesis in systems with a maximum depth of hydrolysis. Experimentally realized are synthetic reactions under pressure with hydrolysates with a maximum depth of hydrolysis. It was demonstrated that synthetic reactions become possible by changing pH in the direction towards, iso-elective point of the enzyme, or by adding caprylic acid which improves the symmetry of the molecules of the enzyme.

It was experimentally demonstrated that in presence of caprylic acid increases the depth of the gelatin hydrolysis with trypsin. Adding of caprylic acid to the enzymatic hydrolysate with a maximum depth of hydrolysis causes a process which increases the depth of hydrolysis to the same limit as in hydrolysis in presence of caprylic acid from the very beginning of hydrolysis.

Aspartic acid decreases sharply the maximum depth of the enzymatic hydrolysis. Glycocol has no noticeable effect on the depth of hydrolysis.

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Gidrogenaza izolirovannykh khloroplastov

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# HYDROGENASE OF ISOLATED CHLOROPLASTS

Hydrogenase is an enzyme with the help of which the molecular hydrogen is used in living cells. It acts as a catalyzer establishing a balance between the molecular hydrogen and various intra-cellular water-transferring systems. This balance is quite reversible:

$$H_2 \rightleftarrows 2H + 2e$$

and is the most negative oxidation-reduction system in living cells (1).

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Hydrogenases were discovered first of all among various anaezobic and facultatively anaezobic bacteria, then among aerobic nitrogen fixing agents. In 1940 Gaffron succeeded in demonstrating that the green normally photosynthesizing algae "are also able to use molecular hydrogen in their metabolism" (2). Being placed under anaerobic conditions, in the darkness, they reduce carbonic acid with two equivalents of hydrogen oxidizing at the same time the hydrogen into the water according to the so called "oxihydrogenic reaction".

$$CO_2 + 6H_2 + 2O_2 \longrightarrow (CH_2O) + 5H_2O$$

Thus was proved the presence in the same plant cells of photosynthesis, photochemical reduction of carbonic acid of photo-reduction type and its chemical reduction in the dark. On the basis of numereous experiments with specific poisons, Gaffron discovered a direct connection between the action of hydrogenase and the function of the assimilation system. It developed that the green cells can not only use the molecular hydrogen for reduction of carbonic acid, but in its absence can also isolate hydrogen from intracellular "donators" (3). Among the various organic substances which were tested glucose appeared to be such a "donator". Isolation of hydrogen from it took place in the dark at a $QH_2 = \frac{MM^3H_2}{mg \times hour} \leq 1$ speed.

However it was not possible to determine the exact proportion between the expenditure of glucose and the products of it decomposition-hydrogen, carbonic acid, organic acids. In the reviewing work of 1944 Gaffron gives

a new scheme of individual reactions of photo-synthesis, according to which the beginning itself of the process is determined by a different state of activity of the hydrogenase system (4).

It is clear from the above account, of what importance would be the isolation of hydrogenase from the green cell for reproduction of at least a part of assimilation reactions of carbonic acid in vitro.

Isolation of hydrogenase from bacterial cells was achieved only in some cases, in cells dried by acetone or after a 16-day autolysis. Preparations retained only one third of activity. Further, the hydrogenase preparations as well as some dehydrogenase preparations with a low oxidation - reduction potential are active only in combination with a hard residue from cellular fragments. In a case of hydrogenase of green plants the work of which is connected with their photochemical activity, it was natural to suppose its localization on chloroplasts. Actually the author discovered the capacity of chloroplasts, isolated from a cell, to form in the dark hydrogen from glucose and fructose at a speed of $QH_2 = 1-5$ (5). The present work is a continuation and development of these experiments.

## METHODS

For isolation of chloroplasts, the leaves of white clover were cut with scissors in a 0.5M solution of saccharose. Other plants were also taken instead of clover as, for example, aspidistra, but the yield of whole chloroplasts was in these cases much lower due to breaking down into parts. The obtained green solution was pressed out through linen against (to remove) undestroyed cells and was further filtered through a paper filter in Buchner's funnel with a weak suction of a water-jet pump. The chloroplast fragments pass through the filter, forming a solution of so - called "natural chlorophyll", according to Luibimenko. But the major part of them, particularly from white clover, does not break down under the conditions of the experiment and remains on the filter as a dark-green, almost black film. Such films were thoroughly washed free of traces of saccharose, were dried in the air with the help of a water - jet pump and weighed. In part of them the moisture content was determined, it fluctuated from 5 to 8%.

For further testing of presence of enzymes, the films were placed either in Tunberg's test tubes with corresponding "donators" or into Einhorn's eudiometers. The calculation of obtained data was conducted in relation to the dry weight of introduced chloroplasts. Usually 10 to 30 mg. were used. In working with Einhorn's test tubes acid was introduced at the end of the experiments for isolation of dissolved carbonic acid. Then the entire carbonic acid was absorbed by alkali. After testing with alkali pyrogallol for absence of oxygen, the remaining gas was absorbed by palladium black and was thus considered hydrogen (6).

For explanation of the entire process of gas - formation and /for/ calculation of the $QH_2$ coefficients, the experiments were conducted in Barkroft's differential manometers. The measuring was conducted by the "direct method" according to Dixon in two double manometers, in one of which the carbonic acid was absorbed by alkali (7).

Determination of glucose were carried out by the Issekutts method of reduction of potassium ferricyanide on alkali solution. Ascorbic acid was determined with "dichlorphenolindophenol" /?/ in an acid media according to Harris and Oliver. Decomposition of forminate /?/ formate of sodium was calculated by titration with hydrochloric acid.

## EXPERIMENTAL PART

Chloroplast films isolated from a plant and the solution which passed through a paper filter were tested for catalase, glucose - dehydrase and hydrogenase.

The activity of glucose-dehydrase was calculated according to the time of change in color of the dye-indicator Janus green from blue into pink (EH= -- 0.035). This change of color is clearer than the discoloration with methylene blue (EH= --0.005). In the given experiment the separation of chloroplasts was conducted not in sacharose but in mannite because the presence of invertase in the filtrate could change the concentration of the "donator" being introduced--glucose.

Table I

Comparative distribution of enzymes

| Enzyme | Films with chloroplasts in % | Solution in %. | Method |
|---|---|---|---|
| Catalase | 85 | 15 | According to breaking down /decomposition?/ of $H_2O_2$ in Einhorn's test tube. |
| Glucose-dehydrase | 5 | 95 | According to discoloration of dyes/colors?/ in Tunberg's test tubs. |
| Hydrogenase | 100 | 0 | According to isolation of hydrogen in Barkroft's manometers. |

It is seen from the figures of Table I, that the hydrogenase is concentrated wholly in chloroplasts, 85% of catalase is also here according to data in literature. And the dehydrase is almost entirely in the soluble part. This separation of dehydrase caused great complication in subsequent work. If the chloroplast preparations were not sufficiently washed off, a high per cent of

dehydrase was still preserved in them. In dehydrogenising the glucose and thus lowering the oxidizing - restoring potential of the media, it prepared conditions for the action of hydrogenase. And with more through cleaning of chloroplast preparations and separation of dehydrogenase, a disturbance of oxidizing - restoring system existing in living cells took place. The beginning of hydrogen isolation was inhibited and its total amount was negligeable in comparison with preparations containing dehydrase.

It is known from the literature that such inactivation of enzyme due to disturbance of the natural oxidtion - reduction system can sometimes be compensated for by adding of dyes from the "redox" indicator group (comp. for example, (8) [p. 156].

It is seen from data in table 2, that introduction of dye in a case of formate increases the isolation of hydrogen 4 times, in a case of glucose - 8 times.

TABLE 2

Importance of adding dye for hydrogenase activity
30 mg of chloroplasts; exposure at 35° for 20 hours

| Concentration of dye | "Donator" | Isolation of $H_2$ in ml. | Mean values of $QH_2$ |
|---|---|---|---|
| Without dye | Na formate 0.5% | 1,1 | 1,8 |
| Janus green | | 4,4 | 7,3 |
| Without dye | Glucose | 0,5 | 0,8 |

According to Graffon, the value of $QH_2$ for living cells does not exceed 1 (3). According to the weight the chloroplasts constitute about 16% of the entire leaf, i,e. 1/6 - 1/7 part of it. In presence of the entire hydrogenase in the chloroplasts its activity should increase 6-7 times when isolated from the leaf. Just such values of $QH_2$ are obtained by adding dyes. Still greater values could be obtained only by separating the enzyme from chloroplasts, however such complete separation of cellular particles has not been achieved so far in regard to other hydrogenases either. For many bacterial hydrogenases the mean values of $QH_2$ = 30 or higher. Thus the hydrogenase of chloroplasts is relatively weak, though on its participation depends the most powerful reduction in the bio - sphere. This becomes understandable in remembering that its significance is in preparation for the process of photo - synthesis. Moreover, presence of hydrogenase with high activity would inhibit the process in the photo-reduction stage and would retard the transition to a normal photo-synthesis. There is a kind of gradual decrease of activity, from bactorial hydrogenases described by

Stephenson and other authors (9,10,11) to bacteria - photoreducer's of the type studied by Nakamura - Rhodobacillus (12) and, finally, to hydrogenases of green photosynthesizing plants.

On the basis of Gaffron's data we used glucose first of all as a hydrogen "donator".

It is seen from table 3, that without adding donators there was no hydrogen isolation in any case in films free of sugar traces. This indicates that chlorophyll itself could hardly be such a "donator". Its amount remained altered at the end of the experiment. From ascorbic acid hydrogen was not at all formed either, though at pH = 7.5 it [ascorbic acid] oxidized during the test. From fructose hydrogen was obtained even faster than from glucose. As in the case of hydrogenases of bacteria, glucose could also be replaced by Na-formate. Formaldehyde could not serve as a hydrogen "donator".

TABLE 3

Various hydrogen "donators" for hydrogenase
30 mg. of chloroplasts; experiment at 35°, 10 hours

| "Donator" + 0.5% $CaCO_3$ + Janus green 1: 100000 | Final concentration of "donator" in % | Isolation of $H_2$ in ml. |
|---|---|---|
| Without "donator" | --- | 0 |
| Ascorbic acid | 0,05 | 0 |
| Fructose | 0,3 | 2,0 |
| Glucose | 0,3 | 1,4 |
| Na-formate | 0,1 | 2,5 |
| Formaldehyde | --- | 0 |

TABLE 4

Effect of temperature on the work of hydrogenase
30 mg of chloroplasts: duration of test 10 hrs.
Everywhere 0.5% glucose + 0.5% $CaCO_3$ +
1: 100 000 of Janus green

| Temperature °C | Isolated $H_2$ in ml. | Decrease of glucose in mg. | Decrease of glucose in mg. per 1 ml. of isolated $H_2$ |
|---|---|---|---|
| 20 | 0,1 | 8,60 | 86,0 |
| 25 | 0,25 | 11,35 | 45,4 |
| 30 | 0,4 | 12,30 | 30,7 |
| 35 | 0,3 | 22,50 | 17,3 |
| 40 | 1,1 | 11,35 | 10,3 |
| 45 | 0 | 2,80 | - - |

TABLE 5

Concentration of "donator" and the work of hydrogenase
30 mg of chloroplasts: duration of test 10 hours. at 35°.
Everywhere 0.5% $CaCO_3$ and 1: 100 000 of Janus green

| Glucose in % | Isolated $H_2$ in ml. | Decrease of glucose per 1 ml.$^3$ of isolated $H_2$ | % of use of glucose |
|---|---|---|---|
| 0,03 | 0 | - - | - - |
| 0,06 | 0,45 | 17,5 | 84,8 |
| 0,12 | 0,75 | 12,8 | 51,2 |
| 0,25 | 1,1 | 10,9 | 32,1 |
| 0,50 | 2,0 | 9,5 | 25,3 |
| 1,00 | 1,5 | 8,6 | 8,6 |

TABLE 6

Relation between activity of hydrogenase and pH
30 mg. of chloroplasts: duration of test 10 hours at 35°
Everywhere 0.5% of glucose, 1: 100 000 of Janus green;
buffer: Ca-acetate + acetic acid

| pH | Isolated $H_2$ in ml. | Isolated $CO_2$ in ml. | Ratio $H_2$: $CO_2$ |
|---|---|---|---|
| 6,0 | 0 | 0 | - - |
| 6,5 | 0 | 0 | - - |
| 7,0 | 1,0 | 0,8 | 1,25 |
| 7,5 | 1,8 | 0,85 | 2,1 |
| 8,0 | 1,55 | 1,5 | 1,03 |

TABLE 7

Relation of hydrogen being formed to carbonic acid
30 mg. of chloroplasts; 0.5% Na-formate; 1: 100 000
Janus green

| Time in hours | Isolated $H_2$ in ml. | Ratio $H_2$:$CO_2$ | Decrease of $CO_2$ in mg. |
|---|---|---|---|
| 4 | 0,7 | 1,0 | - - |
| 6 | 1,1 | 2,2 | 1,2 |
| 8 | 1,5 | 2,5 | 1,8 |
| 10 | 2,1 | 3,5 | 3,0 |
| 20 | 3,4 | 3,8 | 5,0 |
| 30 | 4,4 | 3,7 | 6,4 |

It is seen from table 4, that even though decomposition of glucose is observed below 30°, isolation of hydrogen from it is very weak. Amount of decomposed sugar, for formation of 1 ml. of hydrogen, fluctuated between 30 - 86 ml. Greatest yield of hydrogen is obtainable at 35°. Though at about 40° the process is more rapid - it stops earlier, and at 45° there was no isolation of hydrogen. Temperature limits of hydrogenase of chloroplasts are close to bacterial hydrogenases.
Thus the optimum temperature for hydrogenase of Azotobacter, according to Wilson, was 40°, for Clostridium, according to Woods, 37°, but in both cases the enzymes became more rapidly inactivated that at 33 - 35° (13, 14).

Though at low glucose concentrations less hydrogen is isolated, the total percentage of sugar utilization is higher. With the increase of concentration up to 0.5%, the isolation of hydrogen increases, the percent of utilization decreases. Though at still greater concentrations the isolation is speedier, it then stops soon, as is observed in a case of high temperatures. The same refers to Na-formate concentrations: at a concentration of about 1% the isolation of hydrogen stops entirely. Similar decrease in utilisation percentage of Na-formate with increase of its concentration was pointed out by Gale for dehydrase of formic acid (8).

Calculating of formation of carbonic acid was carried out in all the experiments, however in carbonate solutions this isolation corresponded with the general acidification including, besides carbonic acid, also the originating organic acids. For direct calculating of formation of carbonic acid from glucose, was taken a media not with carbonate of calcium, but with its acetate. However in this case pH moved during the test strongly in the direction of acid and after a certain time the process stopped. Even though at the beginning the isolation of hydrogen from glucose proceeds better at pH = 7 than at pH = 7.5, greatest yields are achieved in the second case, since at about pH = 7 it is difficult to avoid a certain acidification which completely stops the isolation of hydrogen. With Na - formate isolation of hydrogen proceeds from the very beginning best at about pH = 7.5; the optimum here is slightly more alkaline, because already at about 7 - isolation almost stops.

For hydrogenase of B.Coli the optimum for formation of hydrogen from glucose is at pH = 6, from formate - at pH = 7. Among various anaerobic Clostridiums the best pH for the work of hydrogenases is = 7. For the study of hydrogenase participation in bacterial photosynthesis of Rhodobacillus, Nakamura took pH = 7.2. Wilson gives pH = 7.3-7.8 values for aerobic Azotobacter.

The relation between the isolated hydrogen and the simultaneously generated carbonic acid fluctuated considerably in experiments

conducted by Gaffron and other authors. Woods explains it by the possibility of more than one decomposition method. The most frequent ratio for glucose in hydrogenase of chloroplasts was 1:1 and 2:1. In hydrogenase of Rhodobacillus it was 10:1, on the basis of which Nakamura gives the following equation:

$$C_6H_{12}O_6 \longrightarrow CO_2 + 10H_2 + X,$$

where X means other fermentation products, mainly organic acids. In case of Na-formate this ratio was 1:1 or $HCOOH \longrightarrow CO_2 + H_2$.

According to the above Nakamura's equation, formate breaks down completely into hydrogen and carbonic acid and the ratio 1:1, unlike glucose, is quite stable. Neither does a formation of other organic acids take place here, which is seen also from the increasing alkalinity of the media. A definite deficit in carbonic acid is observed in the tests and it increases with time. Decrease in amounts of carbonic acid, which generates from formate, progresses parallel to the increase of the reducing properties of the media. Further experiments are necessary in order to determine the products of its reduction.

## Conclusions.

1. From leaves of white clover and other plants were isolated preparations of chloroplasts which contain the enzyme hydrogenase. The activity of the enzyme expressed by the coefficient $QH_2$, exceeds 6-7 times Gaffron's data for living cells.

2. For normal activity of hydrogenase, adding of dyes of the type of methylene blue or Ianus green is necessary, which compensate the oxidation-reduction system disturbed by removal of dehydrases. Under these conditions the isolation of gaseous hydrogen continued for many hours reaching 4-5 ml.

3. Influence was studied on the activity of hydrogenase of various hydrogen "donators", various temperatures, "donator" concentrations and pH. Relations between the expenditure of "donator", isolation of hydrogen and carbonic acid was determined.

4. In experiments with decomposition of Na-formate with hydrogenase, a decrease in carbonic acid amounts without a corresponding appearance of other organic acids was disclosed. Products of reaction are being studied.

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# THE INFLUENCE OF ULTRA-VIOLET RAYS ON THE ABSORPTION SPECTRA OF AMINO ACIDS

The essence of biological action of ultra-violet rays is up to the present time not sufficiently clarified. Since the albumin substances are the principal components of a living matter, the essence of the biological action of ultra-violet rays should be apparently connected first of all with just this component of tissues and cells (1). By way of a systematic study of spectra of absorption of amino-acids in ultra-violet rays, we can obtain results which will bring us close to the understanding of the essence of the biological action of ultra-violet rays. The offered work has as its goal to demonstrate the changes in the absorption spectrum of a series of pure amino-acids under the influence of exposure to ultra-violet rays.

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## Methods

We examined agueous solution of the following amino-acids: glycocol, alanine, serine, cysteine, cystine, taurine, l-leucine, d-leucine, arginine, lysine, asparagine, tyrosine, tryptophane and histidine.

The indicated amino-acids were examined in 0.001 to 10% concentrations when they were not exposed to light and also after they were exposed to ultra-violet rays during half an hour to 125 hours. The exposure took place in special containers made of quartz glass, at a 10 cm. distance from the source of light (mercury lamp 120 V, 2.3 A). Between the light source and the test tubes was placed a quartz cylinder with a constant flow of cold water. The absorption was measured in the ultra-violet part of the spectrum by the photo-electric measuring method (the thickness of the layer being measured was 0.8 cm.). Absorption curves were drawn on the basis of reading of the double quartz "monochromator" [1] Measurements were conducted within the area of -230-365m..

## Experimental data

Glycocol. When glycocol was exposed to rays in the solution appeared a new substance which changed the absorption curve characteristic for glycocol. It is seem from fig. 1-A, that with an increase in time of exposure of glycocol solutions to ultra-violet rays, the absorption curve indicates a gradually ap-

pearing new maximum at = 297m., particularly sharply manifest after a 30-hour exposure. Even at prolonged exposures to ultra-violet rays the solutions remained clear and colorless.

Alanine. After 5 and 10 hour exposure the alanine colutions change little their absorption spectrum, but after a 20 and 30 hour exposure the solutions show a new absorption maximum at = 297m in addition to the already existing at = 230m . (fig. 1- ). The solutions remained clear and colorless.

Serine. Serine solution (fig. 1-B) shows already after a 5 hour exposure a difference as compared with the unexposed control, disclosing a maximum at 280-289 m . Already after a 5 hour exposure the serine solutions produce a slightly yellowish-golden coloring, however the solutions remain absolutely clear. After a 30 hour exposure the solutions become again colorless as before the test.

Cysteine. The most suitable concentration for experiments was the 0.2% solution of cysteine. It is seen from fig. 2, that with the lengthening of exposure time the type of the absorption curve changes. Particularly intensive was the absorption with cysteine solutions which were exposed for 15 hours. In prolonging the exposure time (45, 95, and 125 hours) the content of absorbing substance decreases gradually. It can be assumed, that at a still longer exposure the absorbing substanee would not be disclosed anymore.

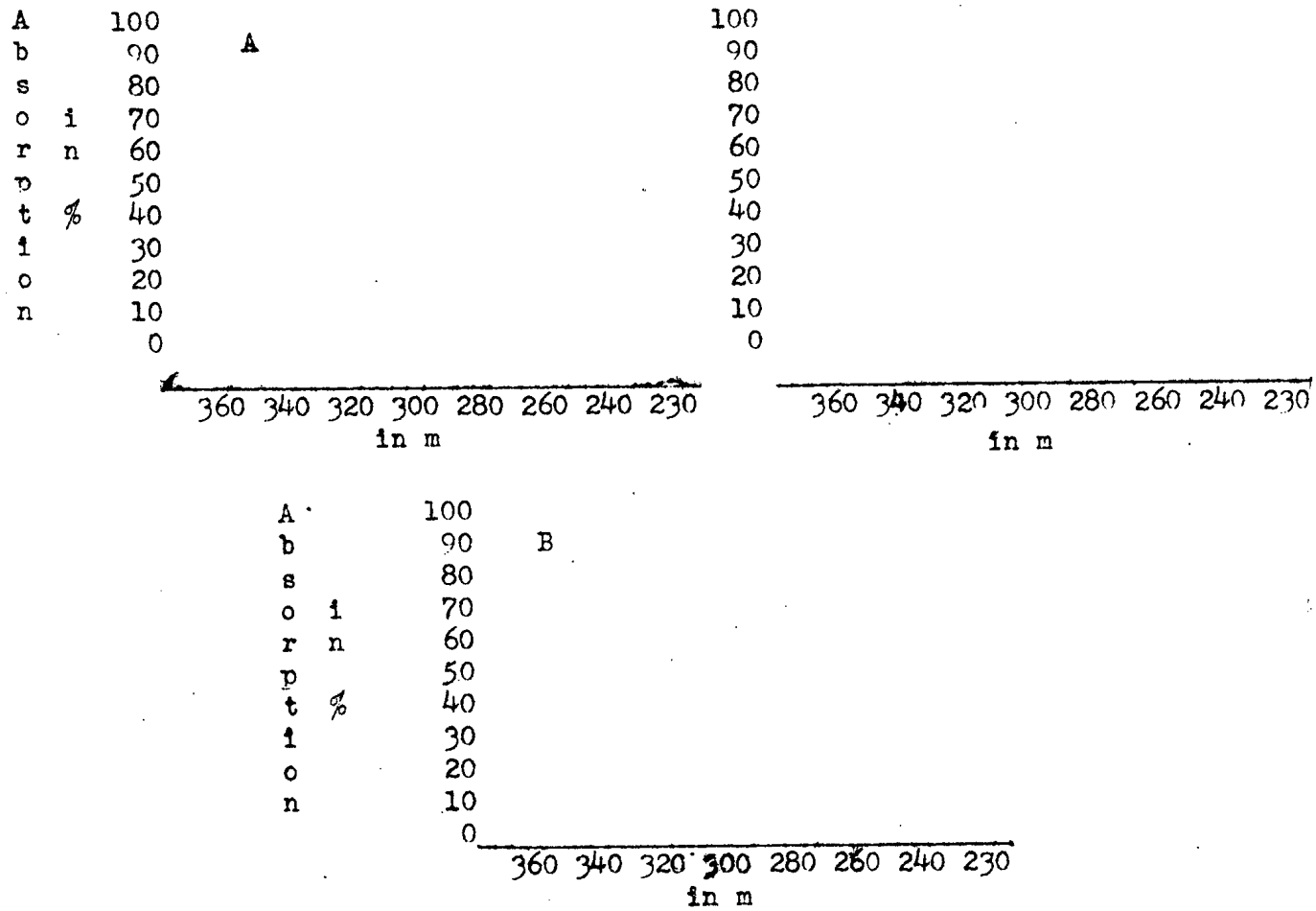



Fig. 1. [p.83] Absorption curves when exposure with ultra-violet rays of amino-acids of various concentrations took place.
A-5% glycocol; -2% alanine; B-1% serine. I-control; II-5 hours; III-10 hours; IV-20 hours; V-30 hours.

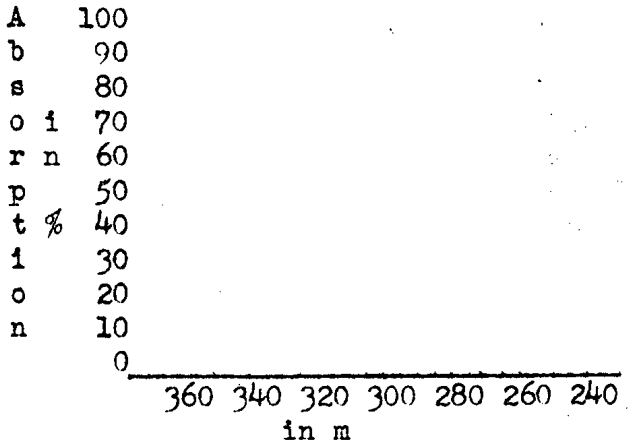



Fig. 2. /p. 84/ Absorption curves when 0.2% concentration cysteine was exposed. I-control; II-1 hour; III-2 hours; IV-6 hours; V-9 hours; VI-15 hours; VII-45 hours; VIII-95 hours; IX-125 hours.

Table 1 /p. 84/

Per Cent Content of Destroyed Cysteine after Exposure to Ultra-violet Rays

| Cysteine solution in % | Duration of exposure of cysteine in hours | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ½ | 1 | 1½ | 2 | 2½ | 3 | 6 | 9 | 12 | 24 |
| 9.01 | 0.8 | 1.6 | 3.2 | 6.1 | 6.7 | 7.6 | 9.6 | 6.5 | 6.5 | $H_2S$ absent |
| 0.02 | 0.8 | 1.6 | 1.6 | 5.4 | 8.2 | 13.8 | 4.8 | 3.2 | 1.0 | Same |
| 0.03 | 0.5 | 1.8 | 5.1 | 6.4 | 7.2 | 9.2 | 2.2 | 1.8 | 2.2 | " |
| 0.04 | 0.82 | 2.0 | 3.4 | 5.4 | 6.9 | – | – | – | – | " |
| 0.05 | 0.33 | 2.1 | 3.8 | 5.4 | 6.6 | – | – | – | – | " |
| 0.06 | 0.44 | 0.9 | 1.7 | 2.3 | 3.7 | – | – | – | | " |
| 0.07 | 0.47 | 1.5 | 2.3 | 2.6 | 3.9 | – | – | – | – | " |
| 0.08 | 0.47 | 1.8 | 2.3 | 2.7 | 3.5 | – | – | – | – | " |
| 0.09 | 0.72 | 1.2 | 2.2 | 2.1 | 3.5 | – | – | – | – | " |
| 0.1 | 0.65 | 1.1 | 2.1 | 2.4 | 3.3 | – | – | – | – | " |

Already during the first hours of exposure of cysteine solutions to ultra-violet rays their yellowing was noted as well as the appearance of a loose flocculent brown sediment which could be easily centrifuged out. All the spectro-photo-metric measurements were carried out after a many-hour centrifugation. Besides that the fact of formation of hydrogen sulfide in exposed cysteine solutions was established.

Presence of hydrogen sulfide in the air layer of the quartz test tube was determined by the smell as well as with a piece of paper moistened with a 5% solution of lead acetate. According to Lehmann (2) such a paper produces a slight yellow-brown coloring already at a volume content in the air of 0.0014 mg% of hydrogen sulfide. We used this author's tables for its quantita-

tive determination in the air layer of the test tube. Quantitiative determination of hydrogen sulfide in the solution was carried out by Winkler's (3) colorimetric method.

On the basis of the quantitative determination of hydrogen sulfide in a series of experiments, we calculated the per cent content of the destroyed cysteine. These data were given in table 1, which demonstrates the relation between the per cent of the destroyed cysteine and the concentration and the duration of exposure. It should be pointed out that weaker cysteine concentrations do not show presence of hydrogen sulfide after a 24 hour exposure. Apparently part of it was diffused into the atmosphere (quartz test tubes were covered with cork stoppers); a certain part was destroyed under the influence of rays with isolation of sulfur. It is known that under the influence of light and air hydrogen sulfide changes very rapidly into sulfur.

In the following experiment the 0.06% solution ofcysteine was exposed to ultra-violet rays for different time periods up to a complete cessation (after 125 hours) of separation of sediment and hydrogen sulfide. The obtained results were as follows:

| Time of exposure in hours | Weight of sediment in g | Percentage of sedi- in relation to the initial amount |
|---|---|---|
| 40 | 0.0066 | 11 |
| 80 | 0.0072 | 12 |
| 95 | 0.0036 | 6 |
| 125 | 0.0038 | 6.3 |
| Total | 0.0212 | 35.3 |

It is seen from the above data that 0.06 g. of cysteine after a 125 hour exposure gave 0.0212 g. of sediment, or 35.3% of the initial material. After this duration of exposure the solution became absolutely colorless and clear.

Cystine. Results of absorption measurings after various exposure durations of a 0.05% cystine solution are shown in fig. 3. Already

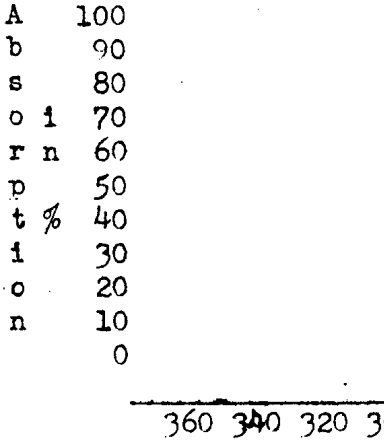



Fig. 3. [p. 85] Absorption curves when 0.05% concentration cystine was exposed to rays. I-control; II-1 hour; III-3 hours; IV-9 hours; V-24 hours; VI-54 hours; VII-63 hours; VIII-86 hours; IX-118 hours.

after a one-hour exposure to ultra-violet rays a very thin cloudiness is formed which does not yield to sedimentation after many hours of centrifugation up to 4000 revolutions. Besides that the solutions become yellow colored. A maximum absorption was observed after a 54 hour exposure. With continued exposure there occurs a clearing and discoloration of the solution with a hardly noticeable formation of a sediment. After a 118 hour exposure the cystine solutions become clear and colorless and show almost no absorption.

In exposing cystine solutions the formation of hydrogen sulfide was also established. Its presence could be determined a few minutes after the exposure by the above described methods.

The percentage of the destroyed cystine in relation to concentration and duration of exposure of solutions is shown in table 2.

Table 2 /p. 86/

Per Cent Content of Destroyed Cystine after Exposure to Ultra-violet Rays

| Concentration in % | Duration of exposure in hours | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ½ | 1 | 1½ | 2 | 2½ | 3 | 6 | 9 | 12 | 24 |
| 0.01 | 5.4 | 19.2 | 33.1 | 43.4 | 51.9 | 22.2 | 21.5 | 21.5 | 26.1 | $H_2S$ absent |
| 0.02 | 3.3 | 13.1 | 16.5 | 19.2 | 19.2 | 11.1 | 7.3 | 27.7 | 12.7 | 5.4 |
| 0.03 | 2.4 | 9.5 | 14.6 | 12.8 | 12.8 | - | - | - | - | - |

In this case a similar regularity was observed as when cysteine solutions were exposed, namely — small concentrations showed a high per cent of cystine destruction, weaker concentrations, after a 24 hour exposure, do not indicate a presence of hydrogen sulfide which apparently became destroyed during this time.

It is necessary to point out, that equal concentrations of cystine and cysteine, at an equal duration of exposure, show an entirely different destruction per cent. Thus, for example, 0.01% solutions after a 150 min. exposure produce only 6.7% of destruction for cysteine and 51.9%-for cystine.

Taurine. We obtained a characteristic absorption curve of taurine only when its concentration was high. 10% solutions of taurine were exposed to ultra-violet rays during 30 hours. It is seen from fig. 4-A, that no formation of substances which change taurine's characteristic curve is observed. Established is only a gradual decrease in absorption which after a 30 hour exposure drops down to zero. Which means that the taurine molecule has so changed that it does not absorb ultra-violet rays anymore. Before as after the exposure, the taurine solutions remained colorless and clear.

Leucine. l-leucine and d-leucine were exposed to a prolonged action of ultra-violet rays. With stronger (from 0.1 to 1%) solutions of l-leucine, generation of yellow coloring and a drastic cloudiness were noted, the latter did not produce a sediment even after many hours of centrifugation at 4000

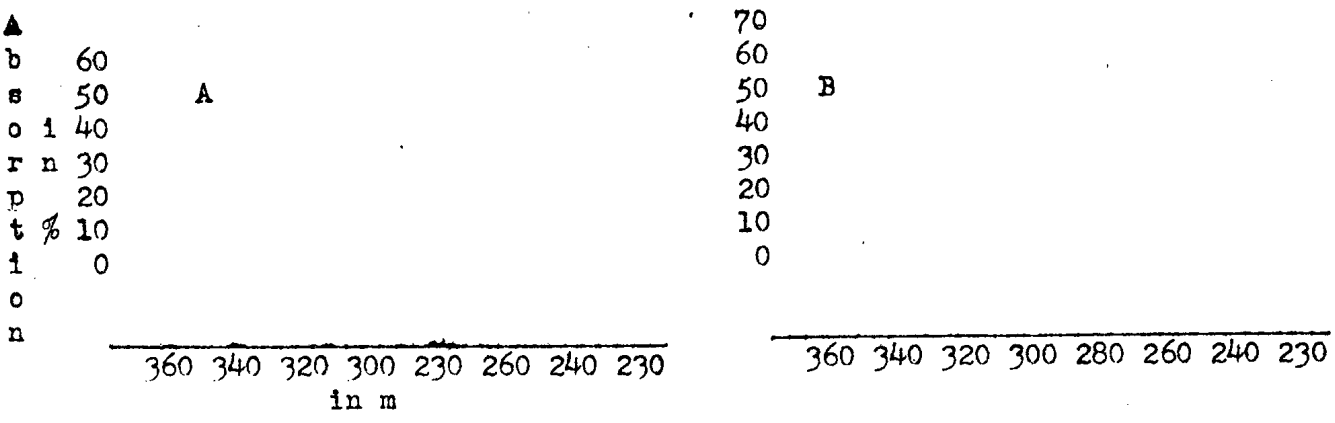
revolutions. The exposed solutions had a peculiar rotten odor and formed, when shaken, a foam which did not disappear for a long time, while the non-exposed solution did not produce such foam. Due to a persistent cloudiness, measuring of the absorption was hampered. In measuring the absorption of exposed solutions of 0.1-1% concentration (duration 5-30 hours), it gave 100% all the time. Prolonged exposure of 0.01% l-leucine showed a gradual destruction of initial substance under the influence of ultra-violet rays.

We observed an entirely different picture in exposing d-leucine solutions for similar time intervals the 1% solutions of this amino-acid being exposed, remained all the time colorless and clear. After 20-30 hours of exposure, the solutions emitted a sharp acid odor. We see from fig. 4- , that already after a 5 hour exposure a new substance is formed in the solution with an absorption maximum at = 270 m . With further exposure the absorption decreases gradually.

Lysine. Almost the same picture is observed also after prolonged exposures of lysine solutions (fig. 4-B). If for the control 1% lysine solution the steep rise of the absorption curve begins, as for arginine, at = 254 m , then after a 30 hour exposure this point moves to - 365 m . Unlike the arginine, a prolonged exposure of lysine solutions above 20 hours shows a tendency to decrease the newly formed substance. The lysine solutions which were exposed to rays remained all the time colorless and absolutely clear.

Asparagine. Exposed asparagine solutions remained colorless and clear. After exposure the character of the absorption curve changed considerably (fig. 4- ). At = 280 m appeared a second maximum which remained persistently even after a 30 hour exposure. A prolonged exposure of asparagine solutions (up to 30 hours) did not show a decrease in this substance.

Arginine. The exposed 1% solutions of arginine remained clear and colorless. In prolonging the duration of exposure, the beginning of the steep rise of the absorption curve (fig. 5-A) moves gradually towards longer waves. Thus, for example, for a 1% control solution of arginine the steep rise of the curve starts at = 254 m , for the same solution after a 30 hour exposure at =313 m . It should be noted, that even after a prolonged exposure of arginine to rays the newly generated substance does not show a tendency for decrease.



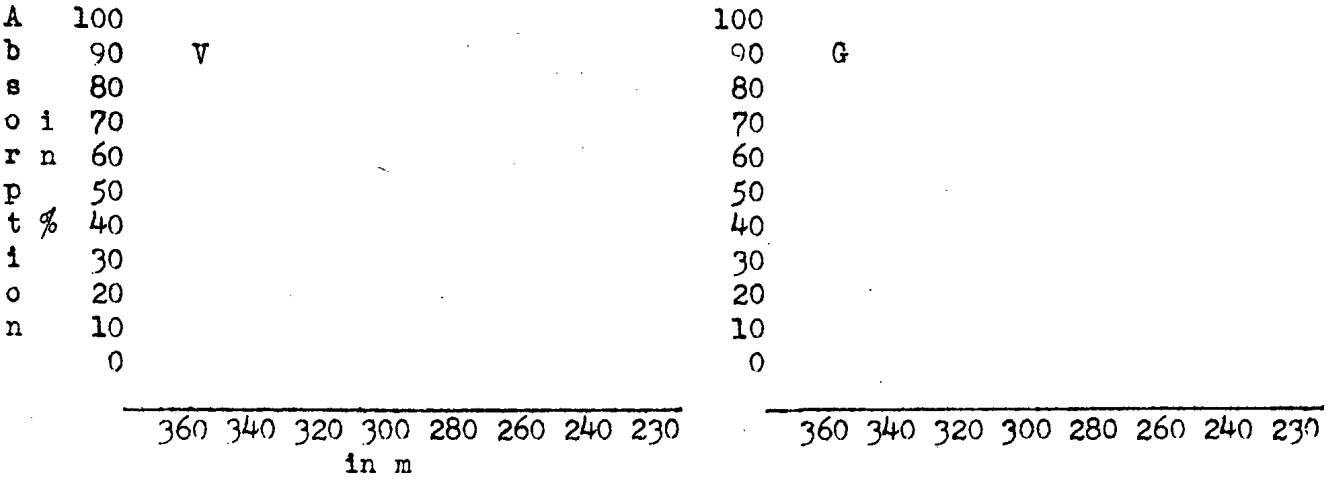
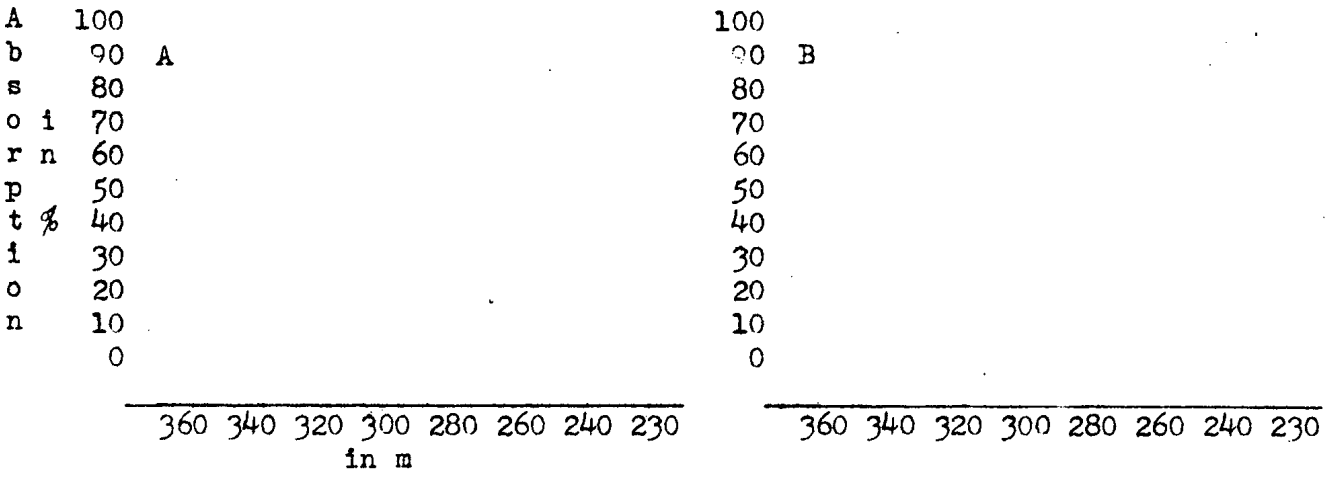



Fig. 4. [p. 87] Absorption curves with exposure of amino-acids of various concentrations to ultra-violet rays.
A- 100% taurine; B-1% d-leucine; V-1% lysine; G-1% asparagine. I-control; II-5 hours; III-10 hours; IV-20 hours; V-30 hours.



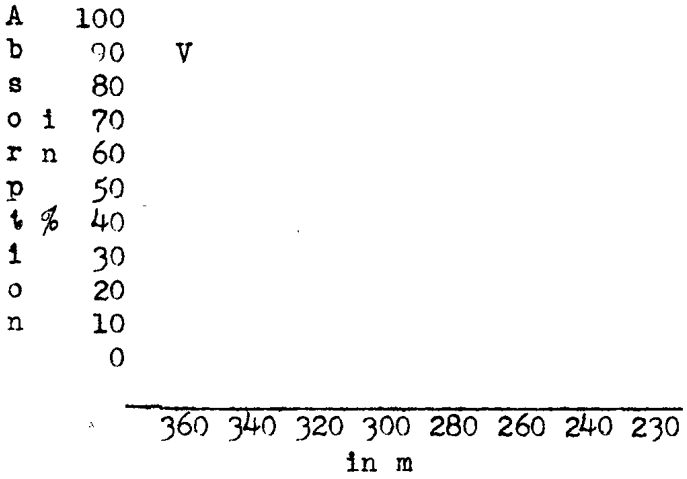




Fig. 5. [p. 88] Absorption curves with exposure of amino-acids of various concentrations and for different durations to ultra-violet rays.
A-1% aragine; I-control; II-10 hours; III- 20 hours; IV- 30 hours; B- 0.01% tyrosine; I-control; II-1 hour; III- 3 hours; IV- 6 hours; V- 24 hours; V-0.01% tryptophane; I-control: II-2 hours; III-3 hours; IV-6 hours; V-18 hours; VI-24 hours.

Tyrosine. Tyrosine solutions of 0.01 and 0.001% concentration showed already after a one hour exposure a light yellowish coloring which becomes slightly stronger after a longer exposure. In all the cases the tyrosine solutions being exposed remained clear and produced no sediment. As it is seen from fig. 5 B, when exposed for a prolonged period to ultra-violet rays the absorption curve characteristic for tyrosine changes drastically. However,

the formation of products causing this change is limited to a time interval between 12 and 24 hours, because after that the absorption per cent begins to decrease again which indicates the beginning of destruction of the generated products. That in the given case we really have a gradual destruction of the initial substance, follows from the experimental data on exposure of weaker tyrosine solutions. Already after a 3 hour exposure of such solutions the initial substance almost disappears; however, its complete destruction is observed only after a 24 hour exposure.

Tryptophane. In experiments with various concentrations of tryptophane it was possible to observe under the influence of exposure to ultra-violet rays, the different reactions in regard to coloring of solutions and formation of sediments. Solutions of 0.1% produced after exposure a yellowish coloring without formation of sediment, 0.01% solutions already after a one hour exposure produced a yellow coloring, apalescence and a loose brownish sediment, the amount of which increased with prolongation of exposure time. It was easy to centrofuge the sediment away and then the solution became absolutely clear. The 0.001% solutions remained clear and colorless even after a prolonged exposure. It is seen from fig. 5-V that already after a six-hour exposure such considerable changes take place in the tryptophane molecule that the = 280 m maximum characteristic for it disappears. With subsequent exposure the initial substance is gradually destroyed and the destruction proceeds so rapidly that after a 24 hour expsure its presence in the solution cannot be determined. In weaker tryptophane concentrations (0.001%) the characteristic = 280 m maximum disappears already after the first hour of exposure; a complete destruction of the initial substance takes place at the 9-12 hour of exposure. The given data demonstrate that photo-chemical changes in a tryptophane molecule proceed with a particular intensiveness.

Histidine. In experiments with histidine, its 1% solution, after a one hour exposure, acquired a saturated-yellow color but was absolutely clear; with lengthening of exposure time the solution became cloudy, the original yellow color changed gradually into light-brown, saturated-brown and dark-brown; a black sediment generated in large amounts. Measuring of absorption with histidine solutions which were exposed to rays could not be carried out because a complete absorption took place along the entire range of the ultra-violet spectrum. Same results were obtained from 0.1% solutions of histidine which, depending on duration of exposure, turned light-yellow to yellow-brown, were absolutely clear and produced no sediment.

## Conclusions

It is seem from the described results of experiments, that with the help of measuring of absorption in the ultra-violet part of the spectrum it is possible to establish the changes in molecules of amino-acids after exposure to ultra-violet rays in such negligible concentrations (for example in 0.001%) with which chemical analysis does not produce any results.

All the amine-acids which we studied after exposure to ultra-violet rays produced various changes determined by way of measuring the absorption spectra. On the basis of these data we can come to the conclusion that, first—in all the cases of examined amino-acids, after the exposure we observed considerable photo-chemical changes in their molecules and, second—the amino-acids are indeed very sensitive components of the living cell in regard to radiating energy.

Weak concentrations of amino-acid solutions showed a relatively rapid destruction of the initial substance under the influence of exposure to ultra-violet rays (tryptophane, tyrosine). Cysteine and cystine solutions which were exposed to ultra-violet rays were destroyed rapidly with isolation of hydrogen sulfide and formation of sediment. The sulfur-containing amino-acid--taurine--after exposure did not form hydrogen sulfide.

We find in literature an indication that with exposure to ultra-violet rays the amino-acids are destroyed with formation of aldehyde (4,5), with liberation of ammonia. The fact which we established about formation of hydrogen sulfide after exposure of cysteine and cystine to ultra-violet rays, indicates that the destruction of the amino-acid molecule can proceed in another way as well. Recently Bukhman and Manoilov (6) also published data on liberation of bubbles of hydrogen sulfide when cysteine powder was exposed to ultra-violet rays in a drop of vaseline oil.

We could point out varying reactions in regard to color change of amino-acid solutions, formation of cloudiness and sedimentation. To the first group of amino-acids, solutions of which remained colorless after exposure, we could refer the following: clycocol, alanine, taurine, d-leucine, arginine, lysine, asparagine. To the second group, which produced yellowing of solutions but remained absolutely clear, belong serine and tyrosine. To the third group which after exposure produced cloudiness of the solution and formation of a sediment belong cysteine, cystine, l-leucine, histidine, tryptophane. The character of sedimentation varied in all the cases. Some of these amino-acids formed cloudiness and a sediment only at a certain concentration (tryptophane at a 0.01%, histidine at a 1% concentration). Some amino-acid solutions after a many-hour exposure became again colorless (cysteine, cystine, l-leucine, serine), in other cases it was not possible to disclose this (histidine). Peculiar properties were noted in exposing l-leucine, solutions of which in various concentrations formed after exposure a long persisting foam and produced a characteristic rotten odor.

Of special interest to us seems to be the formation of intermediate products of decomposition, appearance of which depends on duration of exposure as well as, and in particular, on molecular structure of the given amino-acid. The cyclic amino-acids merit closest attention in this regard. Presence of intermediate products of decomposition of the amino-acid molecule, which in one way or another disturb the life process of a cell at the moment of their formation, can be of considerable importance for the life of the cell which is under the influence of radiating energy.

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# VARIABILITY OF FORMS OF EXISTENCE OF MICROBES AND VIRUSES OF INSECTS

More than 60 years ago the Russian scientist D. I. Ivanovskii (3) called attention to the filterable and infectious properites and the corpuscular nature of viruses, to their ability to be deposited in form of crystals in tissues of plant organisms. The discovery of filterable viruses enriched the world science. The science obtained new facts on the origin and development of life. This was a discovery in Nature of a new, not previously known, form of existence of albumin bodies which have no cell form, but possess all the properties of a living organism.

After D. I. Ivanovskii's discovery many diseases were disclosed in the plant and animal world which are caused by filterable viruses. At the same time it was noticed that there is some connection between viruses and known to us cellular forms of microbes. Thus, for example, Rickettsea—causal agents of typhus—form elementary corpuscles; they are larger than viruses, but considerably smaller than bacteria. Part of the microbes appeared to be able to change to a visual form, which possesses the property of producing a new visual cellular form of microbe.

But in spite of the accumulation of factual material which allows to record still new virus diseases of plants and animals, the nature of viruses, their origin remained a puzzle.

On the basis of the metaphysical dogma that "a cell is only from a cell", some scientists attempted to explain the origin of viruses by a simple breaking up of microbe cells. Others maintained that a virus is a dead substance which reproduces in cells of a living orgainism auto-catalytically, i.e. by way of a mechanical change-over of living albumin molecules

of an organism cell into a substance similar to it. The metaphysical character of these hypothesis is obvious. Nevertheless experimental proofs were required that viruses are not a result of a simple degradation of cellular forms of microbes or their breaking-up to albumin molecule, that viruses are not only a complex chemical substance which is outside the development process, but a living substance as well, from which during the development process more complex organisms can originate--microbe cells, and that the latter can generate a structurally simpler living substance--the virus.

A considerable step forward in solving the problem of origin of viruses were the works by O. B. Lepeshinskaia. In studying the non-cellular form of life, O. B. Lepeshinskaia (5) proved experimentally the possibility of origin of cells from a non-cullular living substance and thus refuted the idealistical and metaphysical notions of life which follow from Virchow's reactionary teaching.



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Considerable progress was achieved by V. A. Krestovnikova (9), L. I. Fal'kovich, V. V. Suknev, G. M. Bosh'ian (1) and other researchers who studied invisible forms of microbes of non-cellular structure and the possibilities of their changing (conversion) into visible cellular forms.

Our experimental work with pathogenic microbes of harmful insects which started in 1947 in the All-Union Scientific-Research Institute for Flax, demonstrated that micro-organisms are able to generate, in the process of their development, a non-cellular form of living substance which preserves the capacity of reversibility of the process.

The initial object of our work were caterpillars of the cutworm moths--gamma, which are dangerous pests of flax and of many other agricultural crops.

Observations of the cutworm moth-gamma development in 1935 and then in 1947 established, that a sharp change in temperature and frequent precipitation further epizootics which leads to a mass destruction of caterpillars, cocoons, butterflies and eggs of the cutworm moth-gamma.

The causal agent of the disease of this caterpillar remained

unknown until recent times, though there were indications (10) that the disease is caused by micro-organisms and that a mass dying of caterpillars is possible from polyhedral virus disease--yellow jandice.

In 1947 we isolated from organisms of diseased and dead caterpillars, cocoons, butterflies of cutworm moth--gamma a bactezium which differed in its cultural and biological properties from previously described pathogenic microbes causing disease of insects.

We called this microbe temporarily Bact. Gamma Sp. nov., which corresponds with the species name of the insect organism from which it is isolated.

In a 24 hour bacterial culture, the bacterial cells of Bact. Gamma sp. nov. have the aspect of rods up to 2.3 microns in length and 0.55 microns in width, which are distributed singly, in pairs and rarely in chains. The bacterium does not form spores, it is Gram-negative.

Changed (coccus-like and coccus-like with a transparent aureole) forms of cells start appearing on the third--fifth day after sowing of bacteria in nutrient media.

Bacterial colonies in meat-peptone agar slimy at first transparent, then cloudy-white. Bacterial cells developing in meat-peptone agar cause a surface dissolving of agar in connection with which a large amount of fluid is formed. Spreading on the surface of agar it furthers the formation of bacterial film which covers the surface.

Fig. 1 (p.90) Crystal-like corpuscles of yellow jaundice virus, which originated in the organism of a caterpillar inoculated with the microbe of Bacterium Gamma sp. nov.

Fig. 2 (p.91) Crystals generated in the bacterial film of Bact. Gamma sp. nov. in the liquid nutrient media

The bacterium which we isolated decomposes (with generating of acid) levulose, saccharose, galactose, mannite, arabinose.



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The Bacterium develops intensely in solutions of saccharose, levulose and maltose. It reduces nitrates, coagulates milk and dilutes gelatin, but it breaks down starch extremly slowly. Therefore sowing of bacteria

in potato agar produced a rather weak growth of colonies which stopped completely on the third-fifth day after the sowing.

Bacterial colonies grow only with a free access of air. Deep inside a solid nutrient media they did not form.

In a meat-peptone broth the growth of bacteria takes place also on the surface and then an uninterrupted thin dry film is formed which covers the surface. The cloudiness of the broth is moderate, constant, the residue is porous during the first 24 hours, after that--flaky. When the culture is kept long it (the broth) becomes thick and viscous.

Fluorescence of nutrient media was not observed. Optimum temperature for growth is 15 - 17°, maximum--30 - 35° and minimum--3 - 5°.

Testing of pathogenic properties of Bact. Gamma sp. nov. indicated that artificial inoculation of caterpillars of cutworm moth-gamma through food with pure bacterial culture causes mass disease and destruction of 84 - 100% (Table 1).

Effect of Artificial Inoculation of Caterpillars and Cocoons of of Cutworm Moth-gamma with Bact. Gamma sp. nov. (laboratory experiment 1948)

| Variant of experiment | Number of caterpillars in the experiment | Number of destroyed in | | Total of born butterflies | Total of destroyed caterpillars & cocoons | |
|---|---|---|---|---|---|---|
| | | caterpillar phase | cocoon phase | | Number | % |
| Inoculation of caterpillars with bacteria through food | 30 | 6 | 20 | 4 | 26 | 86.6 |
| Inoculation of caterpillars of cutworm moth-gamma by way of spraying the caterpillar with bacterial suspension. | 15 | 12 | 3 | 0 | 15 | 100.0 |
| Control (without inoculation). | 15 | 0 | 2 | 13 | 2 | 13.4 |

The most susceptible to inoculation appeared to be the caterpillars of the third and fourth generations. Inoculation of caterpillars of the fifth generation frequently caused their destruction during the pre-cocoon stage or during the phases of cocoon and butterfly.



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The butterflies which we bred from inoculated caterpillars, as a rule, were not able to deposit eggs and if they did it, then in very negligible amounts (not more than 20 - 30). Eggs deposited by diseased females produced no breed due to their inner decomposition by bacteria.

Tests demonstrated that the bacteria culture which we isolated is able to cause disease and destruction also of caterpillars of cabbage butterfly, cabbage cutworm moth, clover cutworm moth, nettle rash, potato cutworm moth.

Observing the pathological process in the organism of caterpillars we noticed, that with the decomposition of inner organs of caterpillars the microbe itself changes sharply. At the beginning of manifestation of disease symptoms of caterpillars we disclosed in their cavity-fluid microbe cells of rod-shape and then, when the decomposition of cells of hemolymph and fat-albumin body began, the bacterial cells became very small and in the cavity fluid appeared coccus-shaped cells with a transparent aureole. Further on the picture changed again and at the moment of complete decomposition of the inner organs of caterpillars their cavity-fluid was filled with a mass of clear rounded corpuscles which refracted light and which resembled elementary corpuscles of the virus. The development of such miniscule elementary corpuscles generated larger transparent light-refracting crystal-shaped corpuscles reaching 2 - 4 microns in size. These corpuscles were not colored by the method usually appled for bacteria, but reacted to coloring after being fixed with alcohol-formalin and to treatment with alkali-eosin. In this case they were colored red, i.e. they had similar properties with crystals of yellow jaundice virus of the silkworm and the oak silkworm, but differed from them in form as well as in size.

Thus the experiments showed that artificial inocculation of caterpillars with a pure bacterial culture causes formation of specific crystal-shaped corpuscles which possess properties similar to those of yellow jaundice crystals. And sowing of crystals in artificial nutrient media produced invariably manifestation of development of the initial

bacterial culture.

In connection with this a question confronted us: could not the observed changes in forms of bacterial cells in the caterpillar organism and the appearance of crystal-like corpuscles be caused by the change of form of microbe existence due to changes in the surrounding media?

This hypothesis followed not only from our observations of bacteria development in the caterpillar organism of cutworm moth-gamma, but from observations of other researchers as well who noted repeatedly the double infection of silkworm caterpillars with cellular as well as virus form of microbe. Thus according to Nikiforuk's data,



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in Bashkiriia was recorded with yellow jaundice infection of "unpaired" silkworm which was developed from silkworm eggs inoculated with Nosema; in 1944 V. P. Pospelov (6) discovered infection of caterpillars in first age with Nosema. During their further growth infection with Nosema and yellow jaundice was observed. V. P. Pospelov points out that flare-up of the Nosema disease during the second generation of the mulberry silkworm in the Ukraine in 1945, occurred also due to mixed infection with Nosema and polyhedral disease, under which conditions the adult butterflies contained Nosema and polyhedra but in their eggs were found only spores of Nosema.

Fig. 3 (p. 92) Change in crystal in solid nutrient media: A-substance of crystal obtains viscous structure of a living substance; B-crystal-like corpuscles.

Fig. 4 (p. 93) New formation of crystals in bacterial culture after sowing of crystal: A--sowed crystal; B--depression as a result of thinning of agar with the crystal; V--newly formed crystals.

Besides that, Pospelov and Noreiko noticed already in 1929, that it is possible to inoculate silkworms with yellow jaundice by feeding them with a culture of yeast Debaryomyces tyrocola con. isolated from caterpillars of the "Monashenka" silkworm which were sick with yellow jaundice. And at that time it was assumed that the yeast cells in entering the organism of the silkworm caterpillar change into the virus form of existance but this transition was not proved by the authors experimentally under artificial conditions.

Our observations of the development of Bact. Gamma sp. nov. in artificial nutrient media indicate that in the mentioned bacterial culture take place also changes in the forms of bacterial cells.

We fixed them directly in the organism of the insulated caterpillar of the cutworm moth - gamma. Simultaneously appeared in the bacterial culture transparent, light-refracting crystal-like corpuscles.

The consistency of these corpuscles which are formed in the caterpillar organism as well as in bacterial culture in artificial nutrient media, is at first viscous. In a fluid drop they have an oscillatory motion, being in a suspended state, and after a certain time they harden and take the form of crystals-tetrahedrons which are deposited.

The tetrahedrons which we detected in the organism of caterpillars which were inoculated with bacteria and in the bacterial culture itself in artificial nutrient media did not dissolve in alcohol, ether, acetone and weak solutions of acids, but readily broke down into elementary corpuscles in weak solutions of alkali.

Crystal-like corpuscles-tetrahedrons were formed in the organism of an insect and under artificial conditions at a 30 - 35° temperature



(begin p. 94)

as well as at 3 - 5%.

More intensive process of tetrahedron formation in caterpillars artificially inoculated with bacteria, was observed at 20 - 25°. At such temperature the breaking-down of a caterpillar organism through bacteria and formation of tetrahedrons was observed already 3 - 4 days after the inoculation and at 13 - 17° --on the seventh - ninth day and later.

At first appeared single specimens of tetrahedrons and with breaking-down of the organism their number increased. After a complete breaking-down of the caterpillar organism the amount of tetrahedron is so large that it cannot be calculated when a regular loop inoculum is taken.

Crystal-like corpuscles-tetrahedrons were formed in all the experiments with pure bacteria culture in organic media rich with albumin, as well as in inorganic ones with mineral sources of nitrogen. With the beginning of mass formation of these corpuseles in the caterpillar

organism as well as in artificial nutrient media, the bacterial culture frequently lost the intensiveness of growth when sub-cultured as if it lost its viability.

In a series of experiments the bacterial culture which lost the capacity of growing in artificial nutrient media was brought into an active state by passing through a living organism of a caterpillar. When caterpillars are inoculated artificially with tetrahedrons which were treated for various time periods (from 15 min. to 24 hours) with 96° alcohol, then already 24 hours after the inoculation in the hemo-lymph of the caterpillars begin to appear normally developed vegetative bacterial cells.

Soon after sowing of these cells in artificial nutrient media, a development of the initial bacterial culture became manifest. On the second day after the inoculation the amount of bacterial cells in the hemo-lymph of caterpillars increased considerably and a process of breaking-down of the organism began and on the fourth-fifth day the caterpillars were destroyed and a new - formation of crystal-like corpuscles-tetrahedrons was recorded.

Analysis of the intestines of caterpillars artificially inoculated with tetrahedrons showed that tetrahedrons pass with food up to the middle intestine where they undergo changes, break down and at the same time appear bacterial cells.

We checked under artificial conditions the possibility of obtaining bacterial cells from crystal-like corpuscles-tetrahedrons, and it was possible to record definite changes in tetrahedrons. 24 hours after the sowing some of them began to react to coloring with methylene blue: they became light-blue colored. After 3 days the number of tetrahedrons being colored increased considerably; in part of them the center was colored more intensely. On the fifth day we discovered tetrahedrons in which the intense coloring was in 8 - 12 spots on the periphery. At that time no uncolored tetrahedrons were discovered. On the seventh day bacterial cells were forming, chains of which looked like a coiled spiral while tetrahedrons disappeared. Resowing of these bacterial cells in nutrient media produced a manifestation of development of bacteria which were isolated previously from caterpillars of the cutworm moth-gamma.

On the basis of all the above mentioned we came to the conclusion that the crystal-like corpuscle-tetrahedrons which we discovered in the organism of caterpillars, butterflies and eggs of cutworm moth-gamma seem to present a specific form of bacteria existence.

In breeding isolated bacteria under artificial conditions in various nutrient media, together with the crystal-like corpuscle-tetrahedrons aggregate crystals were formed which, unlike the former did not dissolve in alkali but instead readily broke down



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in an albumin media with a weakly acid (p4-6.5) or neutral reaction into crystal-like corpuscle-tetrahedrons.

In various nutrient media the form of crystals was different: bipyramids, polyhedral rods, prisms, do-decahedrons, polyhedrons and druses. But in whatever media we bred the bacteria, the crystal-aggregates were generated and grew without fail in the bacterial colony itself or in the film. If the media was unfavorable for the development of bacterial colony, then the aggregate crystals were not formed.

When breeding in solid nutrient media, the crystals which were generated in bacterial colony grew into the depth of the nutrient media, frequently forming crystalline particles up to 1 cm. in size.

In liquid nutrient media the aggregate crystals were generated directly in the bacterial film and after it (the film) dropped to the bottom of the flask they appeared also inside the broth in the bacterial residue on the walls and the bottom of the container.

Studying biological properties of our isolated bacteria in various artificial nutrient media we established that decrease in temperature down to 9 - 13°--inhibits and rise to 17 - 22°--speeds up the process of crystal-formation.

At 3° the development of bacterial culture did not stop but proceeded extremely slowly. If at 13° and above the clouding of the meat-peptone broth was observed after 24 hours, formation of bacterial film after 3 days and formation of crystals 5 days after the inoculation, then at 3° it took place correspondingly on the 8th day, after 12 and 18 days.

In solid nutrient media the process of formation and crystallization takes longer than in liquid nutrient media. In the first case the crystallization process of the microbe's living substance lasted 55 days, in the second - 22 days.

The temperature factor which influences the intensiveness of development of the bacterial colony reflects also on the crystallization

process of the living substance--the virus. At a 23° temperature the beginning of crystal-formation in the bacterial colony was recorded on the third day after the sowing of bacteria, at 9°--on the eighth day and at 5°--on the twentieth day.

In studying the crystallization process in the bacterial film in liquid nutrient media, we found out that the amount of nutrient media in the container is of no particular importance but the size of the surface area of the broth in the container plays an essential role. The larger the area of the bacterial film, the more crystals are formed.

It was established also that crystal-formation in bacterial culture is in direct relation to the intensiveness of bacteria development, to pH and the qualitative composition of nutrient media. At the moment of intensive crystal-formation in the bacterial film takes place a change in the pH of the media towards neutralization (in a media with acid as well as with alkali reaction).

When the pH of the media changed sharply towards acidity or alkalinity, bacteria did not develop and crystallization was not observed. And when the media was changed gradually by the organism itself it appeared to be capable to change the form of its existence (up) to a virus and a crystal. With the formation of crystals, bacterial culture was able to stand a prolonged action of sun rays and high temperature.

Our experiments demonstrated that with the formation of crystals in the media, pasteurization at 70° and 2-hour sterilization at 120°



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stop the development of the culture, but after a certain time appears a cellular form of the microbe which generates crystals anew.

Table 2 (p. 96)

Effect of sterilization of meat-peptone broth inoculated with Bact. Gamma sp. nov. on subsequent manifestation of development of microbe's cellular form and new formation of crystals.

| Variant of experiment | Dates | | | Manifestation of development of microbe after sterilization | | | Manifestation of development when broth is resowed after sterilization | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sowing of microbe | Manifestation of crystalization | Sterilization | Clouding of broth | Formation of bacterial film | Formation of crystals in bacterial film | Sowing of broth | Formation of bacterial colonies | Beginning of crystal-formation in bacterial colony |
| Meat-peptone broth inoculated with cellular form of microbe. | 7/IX 1948 r. | 15/IX 1948 r. | 2/VI 1949 r. | 17/ VII | 28/ VII | 3/ VIII | --- | --- | --- |
| Sowing of inoculated broth in meat-peptone agar 2 days after sterilization in autoclave at 120° | --- | --- | --- | --- | --- | --- | 4/VI | None | None |
| Repeat sowing of broth in meat-peptone agar 4 days after sterilization. | --- | --- | --- | --- | --- | --- | 6/VI | " | " |
| Sowing of inoculated broth in meat peptone agar 6 days after sterilization | --- | --- | --- | --- | --- | --- | 8/VI | " | " |
| Sowing of inoculated broth in meat peptone agar 46 days after sterilization | --- | --- | --- | --- | --- | --- | 19/VII | 20/ VII | 25/ VII |
| Same | --- | --- | --- | --- | --- | --- | " | " | " |

In our experiments the artificial inoculation of caterpillars of cutworm moth-gamma with cellular and virus microbe forms as well as with crystals of living substance which were isolated from a bacterial culture caused their mass infection and death.

Dissection of diseased and dead caterpillars disclosed a characteristic picture of pathological process which takes place when caterpillars die under natural conditions. In cells of hemo-lymph and fat-albumin body a formation was observed of crystal-like corpuscles-tetrahedrons and in the cavity fluid of caterpillars was also disclosed a cellular form of microbe.

We checked the possibility of restoration of microbe life from crystals isolated from a bacterial culture in a series of tests in which we disinfected the crystals superficially (on the surface) with 96° alcohol and ignited them with the flame of burning alcohol. Experiments demonstrated that for restoration of life from crystals it is necessary to create such conditions in the media which would be favorable for a gradual change of the crystalline substance itself, its transition from the crystalline to the colloidal state. In the bacterial colony which developed from a crystal a rather intensive crystal-formation took place anew. Repeat tests always produced similar results.

Table 3 (p. 97)

Dynamics in dying off of caterpillars of cutworm moth-gamma in the experiment with artificial inoculation with the microbe of Bact. Gamma sp. nov. (1952; experiment conducted on September 11).

| Variant of inoculation of caterpillars | Total of caterpillars in experiment | Dead Caterpillars detected | | | | | | | | | | | Total dead caterpillars |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15/IX | 17/IX | 18/IX | 19/IX | 20/IX | 21/IX | 22/IX | 24/IX | 26/IX | 28/IX | 29/X | |
| Control (without inoculation). | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 12,5 |
| Through food with crystals from bacterial culture. | 40 | 6 | 3 | 14 | 14 | 3 | - | - | - | - | - | - | 100 |
| Through food with virus form of microbe. | 40 | 4 | 3 | 11 | 12 | 9 | 1 | - | - | - | - | - | 100 |
| Through food with cellular form of microbe. | 120 | 7 | 31 | 10 | 19 | 13 | 13 | 9 | 4 | 6 | | 2 | 97,5 |

Observing the development process of bacterial colony when aggregate crystal was sowed into the nutrient media, we established the following.

Already 4 hours after sowing of crystal-aggregate in nutrient media, the agar is liquefied in the spot where the crystal is located. In connection with this a crater-like depression is formed around the crystal which (depression) is filled with fluid, and the crystal still preserves its shape-it has no noticeable changes.

On the second day after the sowing, the crystal-aggregate loses its transparence, becomes dull-white and acquires from outside a non-crystalline viscous structure. At this moment the viscous mass being formed consists of minute crystal-like corpuscles-tetrahedrons which possess properties of liquid crystals very similar to crystals of the yellow jaundice virus of insects.

With the transition of the crystal into the indicated state its red coloring with alkali - eosin is possible, which is characteristic for crystal - like virus corpuscles - polyhedrons which are being found in insect organisms.

On the third day after the sowing of the crystal in the place of its location in the agar starts a formation process of bacterial cells which are at first roundly, coccus - like in shape and immobile and then stretch, acquire a rod-like shape and become mobile. The cells which acquired a rod-like shape reproduce intensely by way of paired division. With the appearance of the rod-like shape of cells which are mobile, bacteria spread over the surface of the solid nutrient media. On the fifth - seventh day after the sowing of the crystal, aggregate crystals are formed anew.

Bacteria culture from the crystal of aggregate which was generated in bacterial colony can be obtained also in liquid nutrient media. In this case the generating process of living bacterial cells proceeds considerable slower than when the crystals are sowed on the surface of a solid nutrient media.

When aggregate crystal was sowed in solid nutrient media, a bacterial colony developed intensively after 48 hours and new crystals were formed 5 days after the sowing of the crystal, and when similar crystals were sowed in liquid nutrient media, the intensive development of bacterial culture was recorded only after

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25 days, formation of bacterial film - after 35 days, new formation of crystals-after 38 days.

This phenomenon which we observed provid a basis for the assumption, that in order to obtain the cellular form of the microbe from crystals, not only a nutrient media is required but also a direct action of the air, which a parently furthers a speedier generation of such a process which makes a transition possible of the living substance from a crystalline state to a non-crystalline; together with this favorable conditions are created for formation of the cellular form of the microbe. Correctness of this is confirmed by the fact that the bacterial colony did not develop from the crystal directly into the depth of the solid nutrient media.

Table 4 (p. 98)

Effect of recrystallization of the living substance of the microbe of Bact. Gamma sp. nov. on development manifestation of the microbe's cellular form (1950)

| Variant of Solution of crystals and time of sowing | Date of sowing | Manifestation of development | | | | | |
|---|---|---|---|---|---|---|---|
| In 30% Sulfuric acid. Sowing of solution prior to neutralization NA OH | 30/VIII | - | - | - | - | - | - |
| In 30% Sulfuric acid + neutrilization of solution NA OH. Sowing prior to deposit of crystals. | 30/VIII | - | - | - | - | - | - |
| In 30% sulfuric acid + neutralization NaOh. Sowing of deposited crystals. | 30/VIII | 31/VII | 1/IX | 2/IX | 4/IX | 8/IX | 18/IX |
| In 10% hydrochloric acid + neutralization NaOH. Sowing of Deposited crystals. | 26/VII | 27/VII | 29/VII | 30/VII | 3/VII | 26/VII | - |

In order to test the possibilities of encystment (inclusion) in the crystal of the microbe of cellular form with preservation of its viability, we conducted experiments with crystallization of crystals. It was established that after dissolving the crystals in 10 and 30% solutions of sulfuric

and hydrochloric acid, reactivation of cellular forms of the microbe is impossible and after neutralization of the given solution with a 25% solution of alkali, i. e. after a return depositing of the crystalline substance, the latter is capable to produce cellular form of the microbe in artificial nutrient media. And the process proceeded in the same order as when the crystals, which underwent surface desinfection with alcohol, were sowed.

The experiment with recrystallization of crystals which were isolated from a bacterial culture was conducted with three and six repeats and in all the cases a similar picture of development manifestation was observed.

The experiment demonstrated that we are dealing here not with a simple entrapment(?) of microbe cells in a substance being crystallized, but with a

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formation in a bacterial colony of a substance which possesses properties of a living substance capable for reactisation and development into the cellular form of microbe.

Thus the living substance which passed into a crystalline state is like a transitory stage. Depending on conditions of the enviroment there can develop either a process of formation of albumin substances which have signs of life, or a process of decomposition as a result of which restoration of life is possible only under conditions of return depositing of living substance as crystals.

The results of observations of life restoration process from non-cellular living substance of the microbe which changed to the state of the crystal allow to maintain, that the development of a non-cellular living substance of the virus can be carried out only under certain conditions and when there is a uniformity with them. Outside of that the virus form can change to crystal form and through that become a lifeless body capable, however, to retain its structure and to renew the life activity when necessary conditions are restored.

The phenomenon of crystallization of the living substance gives a clear example of oneness of the living and non-living, when between the two there is no impassable abyss about which scientists are talking who occupy idealistical and metaphysical positions.

The above facts which demonstrate the possibility of transition of bacteria into virus and crystal forms in artificial solid and liquid

nutrient media, - confirm fully the correctness of opinions expressed by D. T. Tvanovskii, N. F. Gamabia, V. A. Krestovnikova, L. T. Fal'kovich and others on virus as on a result of variability of forms of existence of the microbe.

The development process of microbes under artificial conditions which we followed up from bacterial cell to virus form and aggregate crystal and vice versa from aggregate crystal to bacterial cell, is quite complicated and requires further more profound study.

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Nabliudeniia za lizisom aktinomitseta pod vliianiem aktinofaga pri pomoshchi elektronnogo mikroskopa

[Observation of lysis of Actinomycete under the influence of actinophage with the aid of an electronic microscope].

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(In Russian)

Observations with the help of an electronic microscope of lysis of Actinomycete under the influence of an actinophage.

The decomposition process of bacterial cell under the influence of bacteriophage was studied by many researchers.

But up to the present time there is no work in the literature with a description of the electronic-microscopic picture of the decomposition of Mycelium of Actinomycetes under the influence of an actinophage, in spite of the fact that phago-lysis of Actinomycetes is known since 1934, when for the first time a work appeared about it by the Soviet scientist Dmitriev (1). Krasil'nikov (2) described the microscopic picture of dissolving of colonies of some Actinomycetes and pro-Actinomycetes as well as the lysis process of their mycelium as a result of auto-lysis.

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In the works by Kriss, Rukina and Isaev (4) there are data on results of study with the help of an electronic microscope of the structure of a normal mycelium of Actinomycetes. In some works on study of actinophages are given only photographs of actinophages (7,8). At the same time the study of the decomposition process of mycelium under the influence of the phage is undoubtedly of interest for the understanding of some aspects of interrelation between the cell and the phage and of the generating mechanism of forms resistant to phages.

The purpose of the present work is the study through an electronic microscope of the decomposition process of the Actinomycete mycelium under the influence of actinophage. Act. globisporus culture suseptible to

actinophage and actinophage specific for it, was taken for this work.

In chosing a media for breeding Actinomycete and for phago-lysis we decided on glucose-asparagine media of the following composition: glucose— 1.0g., asparagine— 0.05g., $K_2 HPO_4$ - 0.5g, N'aCl - 0.5g., corn extract - 0.1 - 0.2g., distilled water - 100 ml. Sterilization of media was carried out by filtering through a Seitz's filter. The indicated media is favorable for the growth of Actinomycetes and for manifestation of lytic action of actinophage. The advantage of this media consists in the fact that in it the growth of Actinomycete and the process of its lysis under the influence of actinophage is slower than in media rich in albumin, which makes the observations of single stages of this process easier. The breeding was carried out under conditions of depth growth in flasks placed in a rocker (agitator) at a 26° temperature. Under these conditions, as observations showed, phago-lysis proceeded more completely than with surface breeding.

Experiments with phago-lysis were conducted in two variants. In some tests actino-phage was introduced into the media simultaneously with spores of Actinomycete; in others - actino-phage was added to the already grown 24-hour culture of Actinomycete. As a control served the culture of Actinomycete being grown in the same media but without actino-phage. Dialysis was carried out through collodion films by the method described by Kriss, Berezova and Zolkover (3) and lasted usually 15-16 hours.

Fixation of preparations for electronic - microscopic observations was done by dessication. For this purpose the material from flasks being studied was transferred with a loop directly on small collodion films which were stretched on special small metallic nets used for preparing of electronic-microscopic preparations.

(Begin p. 12) Preparations prepared by the indicated method were dried in the air at room temperature and then placed for dialysis.

## Results of experiments

Morphological peculiarities of actino-phage used in our experiments for the lysis of Act. globisporus culture are shown in fig. 1.

Caption for figures 1-10. 1) Actino-phage of the Act. globisporus culture. Dusting with Nichrome. 2) Spores of the Act. globisporus culutre. 3) Germination of Act. globisporus spores. 4) Same. 5) Normal 24-hour culture of Act. globisporus. 6) Act. globisporus spore germinated in presence of actino-phage which underwent a lysis after the formation of a short mycelium. 7) Mycelium grown from a spore in a media with actino-phage at the age of 24 hours. 8) Mycelium of Act. globisporus after one hour exposure to actino-phage.

In one section of the hypha the lysis has commenced. 9) Mycelium of Act. globisporus after one hour exposure to actino-phage. 10) Same.

It is clearly seen from the presented photograph that a particle of this actino-phage consists of an oval-shaped head and a tail. The head of the actino-phage is not homogeneous but consists of a lighter (in color) central part and of two more dense corpuscles at the poles.

The size of actino-phage's head when dusted with Nichrome is about 80-85 mm in diameter along its long axis; the length of the tail is about twice as long as that of the head.

Looking through an electronic microscope at the spores of the Act. globisporus it is seen that they are predominatly cylindrical in shape (fig. 2), before germination the spores swell and become rounded (fig. 3) or retain their cylindrical shape (fig. 4).

In fig. 5 a normal mycelium of the culture is shown at the age of 24 hours. It is seen that at this age it has a homogeneous structure with single lighter sections.

In introducing into the glucose-asparagine media the spore material of Actinomycete simultaneously with the actino-phage the following picture is observed: at first the spores swell and germinate as in the control, i.e. in the media without actino-phage.

Part of the spores undergoes a lysis immediately after germination and formation of a short mycelium (fig. 6).

Another part of the spores continues its growth for some time after the germination and forms an entirely normal branching mycelium. In fig. 7 is shown such a culture at a 21 hour age.

Mycelium grown in media with an actino-phage can reach various sizes and be exposed at different stages of its development to the lytic effect of actino-phage.

The lysis of mycelium which grew from spores in the presence in the media of actino-phage, proceeds in the same way as the lysis of a mature mycelium to which the phage was added 24 hours after the sowing of spores. Therefore the description of mycelium lysis for the two cases is given together.

Changes in the grown mycelium of Actinomycete to which actino-phage is added during breeding in glucose-asparagine media and which are noticeable through the electronic microscope, can be usually detected an hour after introduction of actino-phage.

These changes begin in single sections of each hypha not covering at once the hypha as a whole. It can be noticed how in the spot of the hypha where the lysis starts being manifest, at first comes a certain thickening of it, the protoplasm becomes more dense and separates slightly from the wall. This is seen in fig. 8 where the state of the mycelium section is shown an hour after the exposure to actino-phage.

In fig. 9 and 10 are presented pictures of the state of mycelium which can be observed after one hour exposure to the phage and which indicate further development of the lysis process.

In single sections of the hyphae the protoplasm loses its homogeneity, spots appear with a noticeable granularity (fig. 9). By the same time there are single hyphae in which the lysis process has developed still further. In fig. 10 is shown the state of such a mycelium. Clearly seen is a sharply expressed nuclear granularity and presence of sections with a transparent content.

The character of further development of the lysis process can be judged (begin p. 13) from fig. 11, which demonstrates the state of some hyphae after a 2 hour exposure to actino-phage. It is clearly seen how the section of mycelium of a normal aspect changes sharply into a bysed section, in which the wall is already destroyed and the content broken down into a shapeless mass.

Captions for figures 11-18. 11) Hypha of the Act. globisporus mycelium after a 2 hour exposure to actino-phage. 12) The Act. globisporus mycelium after a 3 hour exposure to actino-phage. 13) Mycelium at the age of 27 hours in a media without actino-phage (control). 14) Mycelium of Act. globisporus after a 4 hour exposure to actino-phage. 15) Same. 16) Single hyphae of the Act. globisporus mycelium after a $4\frac{1}{2}$ hour exposure to actino-phage. 17) Same after a 5 hour exposure to actino-phage. 18) Same after an $8\frac{1}{2}$ hour exposure to actino-phage.

After a $3-3\frac{1}{2}$ hour exposure to actino-phage many hyphae are in a state of complete decomposition; in some of them are retained single small mycelium sections with a granular content which is in varying stages of decomposition (fig. 12).

The mycelium in the control flasks (without actino-phage) by this time retained fully its homogeneous structure (fig. 13).

After a 4 hour exposure to actino-phage the amount of fully lysed hyphae increases, but among them are found single hyphae which have fully retained the normal mycelium (fig. 14).

Figures 15 and 16 show the state of single hyphae of the Actinomycete culture after a $4\frac{1}{2}$ hour exposure to actino-phage.

In fig. 15 we see parts of two different hyphae; in one of them the lysis process terminated at one end with a tear in the wall and a break down into a shapeless mass and the other part still retained single sections of a mycelium of normal aspect.

In fig. 16 are illustrated three hyphae which are in various stages of lysis. On one of them (b) remains a small section with normal mycelium which changes into a lysed section; another hypha (a) is almost completely lysed, and the third hypha (d) contains extremely characteristic granular inclusions on the nature of which it is difficult to pass a judgement. After 5 hours of contact between the actino-phage and the mycelium, in preparations can be observed, together with lysed hyphae and hyphae completely broken down into a shapeless mass, also single sections of hyphae which retained entirely their normal aspect. Fig. 17 demonstrates the state of some hyphae after a 5 hour exposure to actino-phage. It is seen clearly in this figure how a normal section of one hypha terminates abrumptly and is sharply separated from the following entirely lysed part of the same hypha.

And after an $8\frac{1}{2}$ hour exposure of mycelium to actino-phage, when the number of the completely decomposed hyphae is already quite considerable, there are single completely preserved sections of hyphae (fig. 18).

Longer observations of the lysis process of Actinomycete indicate that together with a complete decomposition of some hyphae an appearance of a young mycelium at the expense of a secondary growth can be observed. The secondary growth orginates at the expense of germination of single mycelium sections which acquired resistance against the lytic action of the actino-phage. This is confirmed also through observations, which we conducted together with Peshkov, Sorokina, Cherednichenko and Sharkova, -of cytological changes of the Act. globisporus mycelium when it under went a lysis under the influence of actino-phage; observations were carried out with the help of the regular light microscope (6).

## Conclusions

Observations through the electronic microscope were conducted of the morphology of the actino-phage Act. globisporus and of the lysis process which it causes to the mycelium of the given Actinomycete. The following was exposed.

A particle of the actino-phage Act. globisporus consists of a small oval-shaped head and a tail. The head of the actino-phage which we studied is not homogeneous, but consists of a lighter (in color) central part and of more dense corpuscles at the poles.

When the Actinomycete spores are bred together with the homologous actino-phage, the lysis of a part of spores starts immediately after their germination and formation by them of a short mycelium.

(begin p. 14) Another part of spores can germinate first into a quite normally looking branching mycelium which later undergoes a lysis. Different hyphae of the same culture undergo a lysis at development stages

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# SOME PRINCIPLES OF BREEDING MICROORGANIMS FOR INDUSTRY

(Printed in connection with the discussion carried in the Mikrobiologiya (Microbiology) on problems of breeding microorganisms).

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The journal Mikrobiologiya has opened a timely discussion on the urgent problem of methods and principles of breeding microorganisms for industry (7).

In this article I wish to discuss certain general postulates which have been basic in the course of our many years of work on breeding industrial microorganisms.

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It seems to us that the most thorough understanding possible of their interrelationships with their environment, an understanding of the nature of their economically valuable properties, is basic and most important in the work of breeding microorganisms.

In the course of long evolution the properties of microorganisms have been formed under the natural conditions of existence. Industry is a new environment for microorganisms and they are changing continuously in the process of adapting to it. Depending upon the character of the influence of industrial conditions economically valuable properties of microbes can change in various directions. This is why knowledge of the technological processes of industry for whose needs the breeding work is being carried on and an understanding of its character of its influence upon the microorganisms is so important in the breeding of microorganisms.

We believe that it is this which should determine the course of research, which should take into consideration the specific features of industrial conditions in every specific instance.

Lengthy culture of microbes in some industries has improved their economically valuable properties. This has been observed primarily in cases where the direct adaptation of the microorganism to its industrial environment has been particularly useful to us, for example, the adaptation of more complex products of the hydrolysis of polysaccharides to deep fermentation. Thus, lengthy development of the products of amylolysis of starch has caused the ability to ferment some of the simplest dextrine in certain yeasts. It is a fact, as a number of research workers have pointed out, that we do not encounter in nature the yeast Saccharomyces ellipsoideus, which ferments must out of sugared starch so deeply, or the production strains which have been separated into the new species Sacch. cerevisiae primarily on the basis of this criterion (5). Our study of a large collection of yeasts gathered in various natural habitats in Central Asis fully confirms this position.

Therefore, lengthy breeding of cultivated strains of yeast in the liquor industry, which processes grain and potatoes, can improve their quality.

Similar results can be obtained in the work of adaptation of yeasts to media unfavorable for their components. Thus, Yakubovskiy (8) developed the "Ya" breed which gives splendid results in fermenting mashes made of molasses.

Repeatedly passing yeast through the production cycle of champagne making resulted in improving its quality (Ryabchenko (8), Kvasnikov and Khrolikova (4), and others).

We have also checked this method repeatedly in primary wine making. In this instance of working with musts seeded with ellipsoid yeasts there is never any assurance that the approved race is being improved, and not the development of a new strain with unknown properties (whose study would require lengthy and painstaking research). Turning to the form and structure of the yeast colonies is not justified since these characteristics change. Moreover, the final evaluation of the wine is made several months after it is prepared. Therefore, this method (in view also of the seasonal nature of the industry) can scarcely be widely recommended for the winemaking industry; it can be employed only in laboratory work.

Thus, improvement of races by means of repeated passages through production cycles provides positive results in those instances where

the latter improves certain properties in a directed manner at the same time that other, likewise economically valuable properties, correlate with this in a positive manner.

In industrial employment of this principle of breeding, direct return of the isolated cultures to the production cycle is possible only in industries which process sterile substrata; however, this is risky as the cultures developped require further study. In processing unsterile or provisionally sterile substrata, however, each of the isolated strains should be subjected to all-around study as in ordinary selection. It is impossible to avoid these checks as some strains of low quality may be among the isolated strains.

All this requires the use of more effective methods of breeding in these industries.

Industrial substrata do not influence the microorganism in a direction desirable for us and do not select forms whose quality is particularly interesting from the standpoint of the technological process in all industries. Under the artificial conditions of the technological process of some industries those forms which are less active in certain respects may turn out to be interesting. The qualities of physiological activity and great adaptability to the environment do not always coincide with the economic value of the forms. At times strains possessing weakened fermentative properties and which are physiologically inert are of particular interest to us (in the production of nonalcoholic beverages).

In some industries economically valuable properties of microbes are weakened during the technological process. This is observed in the employment of microorganisms for production of certain organic solvents, acids, etc. In this case frequent propagation of cultures carefully maintained under special laboratory conditions for industry is an essential requirement.

In some industries we are interested in properties of microbes which do not have a strongly adaptive character (By-Products of fermentation, especially the making of aromatics, etc). It is difficult to assume that industrial conditions have necessarily changed them in the desired direction.

Therefore, breeding of industrial strains only from industrial production may lead to unfavorable results in many instances.

Finally, in selecting microbes from industrial substrata, separating them from products of superior quality should not be regarded as the sole method. At times, on the contrary, spoiled and "sick" production may provide us with the needed material. In this instance

forms which have survived in competition with other species may be of interest. Most of all, forms with properties necessary for us can be formed in this case.

Thus, we have succeeded in developing strains of lactic acid bacteria from spoiled ensilage which possess great activity and show good results in industrial tests.

Thus, it is, of course, impossible to consider that races of microorganisms will necessarily change with improvement of the technological process in the direction desired by us.

At present microbiology has at its disposal many methods developed on the principles of breeding worked out by Michurin and Lysenko.

Nature is the inexhaustible source from which man, in the course of his history, has taken, is taking and will take living organisms for cultivation. The importance of studying the living wealth of nature in all branches of natural history has become all the greater now that Michurin biology has opened the greatest opportunities for the directed remodeling of nature. In this respect the world of microbes is particularly promising. Frequently, moreover, newly isolated strains which acquire a high degree of plasticity under new environmental conditions are favorable material for further improvement.

We note, for example, that the large amount of material that we have accumulated by comparative study of wine making strains of yeast isolated from their natural habitats in Central Asia (biocoenoses of vineyards and gardens, namely: from the soil, grapes, above-ground portions of plants, flower nectaries, etc), and strains isolated from the best wines have given curious results. It turned out that a number of industrially valuable characteristics, particularly the ability of forming insignificant quantities of volatile acids, are more widely prevalent among strains isolated directly from nature than from wines. A large number of the former gave splendid clarity of must and a dense precipitate very difficult to make turbid.

We (Kvasnikov and Khrolikova (3) isolated the Rkatsiteli-6 (Rts-6), which has given splendid results and has been extensively introduced into the industry in Central Asia, from grapes. It has also shown itself to be one of the best champagne breeds.

A high energy of chemical processes is inherent not only in yeasts, but also in a number of other groups of microorganism isolated from natural habitats in certain ecological regions. In this connection, strains developed in the sharply continental subtropical climate of Central Asia are of interest.

Thus, in breeding lactic acid bacteria for making ensilage fodder in Uzbekistan, in joint work with Sumnevich (2), we succeeded in isolating strains with an exceptional capacity for acid formation (up to 300-500 degrees absolute temperature).

Some economically valuable properties of microbes may be formed under wild natural conditions. In this instance searches for needed microbes in nature is not a haphazard but an entirely logical and justified method.

We note in passing that the enthusiasm for breeding local races of microorganisms from restricted regions for the needs of agricultural technology, which has been elevated to a godma, as it seems to us, is hardly justified. It is essential to approach each case individually, carefully taking into consideration specific local conditions. One must not ignore the possibility of introducting and acclimatizing breeds developed in other regions. Under new conditions they may at times display a number of valuable properties.

Thus, the 53rd strain of Azotobacter, which was isolated from Central Asian soils, has given good results in entirely different geographical regions and frequently surpasses local strains in respect to activity. Petrushenko (1) and I have isolated a number of strains of Azotobacter which markedly surpass the 53rd strain in respect to nitrogen-fixing ability from Central Asian soils. They have been turned over for testing in other ecologico-geographical regions.

Our Rkatsiteli-6 breed has given good industrial results in some localities in the western regions of the USSR. Its introduction into bottle champagnizing at the Moscow plant resulted in large economics. This breed possesses a clear-cut capacity for giving dense precipitates which do not become turbid and move smoothly along glass.

It is possible to cite many similar examples. Therefore, while devoting considerable attention to the breeding of local strains, it is essential to study strains whose economically valuable properties were formed in other regions.

Soviet microbiologists have achieved great success in the field of breeding microorganisms. Breeds isolated by them are working in plants belonging to different branches of industry.

At present we have at our disposal methods for intensifying the fermentative mechanism of microorganisms and for adapting them for fermentation of many substrata that are difficult to ferment; we have succeeded in obtaining marked shifts in the temperature boundaries of the life activities of microbes in accordance with industrial needs and are successfully acclimatizing microorganism to a whole series of antiseptics.

All this has been achieved primarily under laboratory experimental conditions combined with factors of industrial influence.

Living organisms possess greatest plasticity in periods of energetic acclimatization during stages when synthetic processes are predominant. At present a number of methods have been worked out in the laboratory (continuous cultures, etc.) in which these processes go on smoothly and rapidly, markedly surpassing the processes in industry (Savenko(9)).

By thoughtfully applying the methods of Michurin selection, selecting factors which loosen the heredity of the microbe cell and combining them with consequent directed conditioning it will be possible to obtain and to fix a number of industrially important properties of microbes under the effects of laboratory and industrial influences.

Work is scheduled on deep changes in the course of exchange of substances in the microbe cell, as correctly emphasized by Imshenetskiy at the All-Union Conference on Planning Microbiological Research.

Microorganism were among the first living substances to arise on the surface of the cooling planet. They have passed through a long path of evolution. Due to the peculiarities of their structure and exchange of substances microorganisms have been closely connected with the development of the organic world in the process of development. The properties of some microorganisms have been formed in company with other microorganisms. The peculiarities of the morphology and biochemistry of the microbe cell have made their mutual influence during joint development (in sludges, scum, colonies, etc.) particularly deep.

One may surmise that in the evolution of microbes heredity was enriched primarily by a primary (asexual) method, in particular, the penetration of some products of the metabolism of some cells into other cells.

Therefore, the most basic changes in the heredity of microbes, including the transformation of species, resulted from the action of other microbes. The ways in which this influence was exerted are already quite clearly outlined:

(1) Joint culturing of microbes in media;
(2) Culturing in the very same medium, but isolating them from each other by a semipermeable membrane;
(3) The influence of killed microorganisms on the life activities of other forms of microbes;
(4) Culturing some forms in media with the addition of products of the life activities of others, or in extracts taken from them, etc.;
(5) The influence of transforming substances (desoxyribo-nucleinic acid) obtained from microbe cells by chemical methods.

In industrial microbiology, due to the dogmatically accepted principle of use of microorganisms in pure cultures, this method of directed change in the hereditary properties of microbes has remained outside the sphere of research work up to the present time. In the meantime, however, it merits the most intent attention and promises inexhaustible possibilities in respect to deep changes in the exchange of substances in the microbe cell. In this connection the experience accumulated by medicinal microbiology should be taken into consideration in every way.

The course of "impressing" upon some industrial microorganisms properties of other microorganisms which possess valuable properties is very promising. Sexual hybridization is also of importance in the breeding of yeasts. (Kosikov (5))

A large amount of material on the use of mixed cultures of microorganisms has been accumulated in industrial microbiology. The hopes vested in these cultures have not been justified in some practical fields, in particular, in the selection of mixed cultures of yeasts for the wine making, liquar and beer brewing industries, and they have not found wide application in industry. It seems to us, however, that work in this direction should be intensified. Establishing more thorough ecologico-microbiological research projects could be of significant help. Greater attention should be devoted to study of certain biocoenological relationships between microorganisms in nature.

Our work on the study of the interrelationships of yeasts and lactic acid bacteria in natural, closely intimate fellowship have indicated to us the possibility of changing the biochemistry of the yeast cell through the effect of culturing them with certain species of lactic acid bacteria.

In particular, through lengthy culturing the Rkatsiteli-6 breed with lactic acid bacteria Lactobacterium buchneri, Strain 114, we succeeded in sharply reducing the capacity of yeast for accumulating volatile acids, which is especially important in wine making. The character of the precipitate of the yeast was also improved.

The RM breed developed by this method underwent industrial testing in two plants (of Sovkhoz No 1 imeni Khovrenko and Sovkhoz No 7 Uzbekvino). The valuable properties of this breed were displayed at this time. It is particularly important, however, that the directed acquisition of the capacity to form only insignificant amounts of volatile acids was also manifested under industrial conditions. At Sovkhoz No 1 the wine (Riesling) prepared with the RM breed had a maximum volatile acid content of 0.2 percent (with the initial Rkatsiteli-6 breed 0.5 percent); At Sovkhoz No. 7 the wine (Bayanshirey) prepared

with the RM breed contained 0.12 - 0.15 percent of volatile acids. The wine made with the new breed received a high mark.

At present we are studying the stability of the properties of this breed. Research is also being conducted on directed changes in the properties of industrial breeds under the influence of other strains of microorganisms and substances obtained from them.

We have discussed only certain principles which, it seems to us, play an important role in the complex and many-sided work of breeding microorganisms for industry. The deeper we look into the intimate side of the interrelationship of the microorganism with its environment and the more thoroughly we try to understand the nature of the formation of valuable properties in the process of formation and development, the more effective our methods of breeding will be.

In this instance practice will be the true criterion and the judge of the correctness of theoretical structures.

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From: Gigiyena i Sanitariya [Hygiene and Sanitation], No 1, January 1953, Moscow, Pages 58-60

# FAULTS OBSERVED IN THE RECOMMENDED METHODS OF DETERMINING HARMFUL SUBSTANCES IN AIR

A. A. Troitskiy

The extensive scale of operation of public health laboratories requires standard methods of examination. Uniformity in such methods should assure an exact picture of the state of the objects examined.

With this aim the Ministry of Health RSFSR advises industrial sanitiation laboratories to guide themselves, in their air-analysis work, by GOST's [State Standards] 5602-50 and 5612-50, and by the book written by M. V. Alekseyeva and associates Opredeleniye vrednykh veshchestv v vozdukhe proizvodstvennykh pomeshcheniy [Determination of Harmful Substances in the Air of Industrial Buildings].

Some of the methods in these handbooks, however, have misprints and ambiguities, and if they are used uncritically by persons inesperienced in gas analysis, they may give incorrect results, and thus have serious and undesirable consequences. For instance, in the above-mentioned GOST manual, on page 3, in describing the method for determining mercury in air, it is stated that the volume of the liquid in each absorption apparatus must be measured after taking the sample. The question suggests itself why this should be necessary, if according to the formula $X = \frac{(c_2 + c_1) \times 10}{V_0}$ (page 4) there must under all circumstances be 10 millimeters of liquid in each absorption apparatus. The text fails to point out that this formula can be used only if the sample solution is brought up to 10 milliliters by adding absorbing lipuid before the examination.

In this method of determining mercury the book leaves out the important note that 0.1 N iodine solution must be added to the sample to make its color the same as that of the scale. Unless this is done, the addition of the reagent solution to the sample will cause formation of a greenish precipitate owing to the sample's insufficiency of iodine, which is lost by volatilization when the air is blown through it. In this case it will be impossible to compare the sample with the scale.

The method for determining aniline gives the formula (page 8) $X = \frac{(g_1 + g_2)\ v_2}{v_1 \times v_0}$ in which $v_2$ is the total volume of the absorbing solution in millimeters. This is incorrect, since $v_2$ should denote the volume of absorbing solution in a single absorption apparatus. In the contrary case, the result will be double the actual aniline content of the air.

Page 15 fails to indicate the rate at which the air must be passed in taking samples for the determination of carbon bisulfide in air.

Page 20, line 17 from the bottom reads "0.06 ml of ammonia" instead of "0.06 mg of ammonia."

In the method for determining formaldehyde the following formula (page 26) is given: "$X = \frac{g \times v}{v_1 \times v_0}$, where v is the quantity of the test solution taken for analysis, in ml; $v_1$ the volume of all the liquid being tested in the first two absorbers, in ml." The formula would be correct if the value of $v_1$ were given for v, and that of v for $v_1$.

The following important remark is omitted in describing the method of determining nontoxic dust in air (pages 34-38):

"If the sample is taken from ventilating suction air-ducts, the velocity of the air drawn through the entrance orifice of the receiver equals the velocity of the air in the air duct."

In the method of determining dust in air (page 38) the following is given: "v is the volume of air passed through the apparatus in $m^3$." This should read: "$v_0$ is the volume of air passed through the apparatus in $m^3$, reduced to normal temperature and pressure."

In describing the method of determining carbon monoxide (page 60), the text reads: "2ml hydrochloric acid should be used for each 2 ml of barium hydroxide. Parallel determinations may be distinguished from each other by not more than 0.01 ml of hydrochloric acid."

We consider it unnecessary, for this test, to prepare an exactly normal barium-hydroxide solution, in view of the difficulty of doing so and of the instability of the concentration. What is important

is that no more than 2 ml hydrochloric acid shall be taken for each 2 ml portion of baryte taken; otherwise, somewhat less baryte must be measured off with the pipette.

In determining carbon monoxide, the temperature of the air being analyzed is taken at the time fo the test, since the volume of the air is measured in the laboratory. The GOST manual, however, (page 63), reads as follows : " t is the temperature of the room at the place the sample is taken."

In the same method (page 63) the following formula is given: "$v_0 = \frac{vt \times 273 \times (P - p)}{(273 + t) \times 760}$. It should read: "$v_0 = \frac{vt \times 273 \times (P + p)}{(273 + t) \times 760}$ since the pressure of the test air in the apparatus for determining carbon monoxide is greater than atmospheric pressure, not less than it.

In the recommended book by M. V. Alekseyeva and others, the chromate method of determining lead is described on page 94, with a reference to S. P. Sinyakova's papter "Micromethodology of Lead Determination" (Gigiyena bezopasnosti i patologiya truda / Hygiene of Industrial Safety and Occupational Pathology/, No 2, page 41, 1941), with the statement that a nephelometric determination is made by this method.

On page 96 there is a section "Performing the Determination," which consists of the following: to the solution containing lead, water is added, to make it up to 10 ml, 0.1 ml of 50 percent acetic acid added and mixed, 0.5 ml of 10 percent potassium bichromate added, ahaken, and the color after 5 minutes compared with the standard scale.

The following methods also refer to this same "Method of Performing the Determination": "Determination of Tetraethyl Lead" by B. Andronov (Otchet Moskovskogo nauchno-issledovatel'skogo instituta okhrany truda BTsSPS za 1940 g. / Report of the Moscow Research Institute for Labor Protection of the VTsSPS for 1940/ page 99) and Razdelnoye opredoleniye tetraetilsvintsa i svintsa pri sovmestnom ikh prisutstvii / Separate Determination of Tetraethyl Lead and Lead in the Presence of Both/ by B. Andronov, page 102.

Our own laboratory has reached the conclusion, after repeated tests of this method of determining lead, that scale solutions in test tubes, containing 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 mg, after addition of potassium bichromate and shaking, and after five minutes have passed, are very difficult to distinguish, not only from each other, but even from the control solution. Only in test tubes beginning with amounts of 0.05 mg lead and upwards does a faint turbulence appear, similar to the changing of an orange tinge to a yellow one. Consequently the claim that the method is sensitive to 0.005 mg in 10 ml is thus evidently shown to be incorrect, without a careful check of the technique.

To elucidate the cause for this inexactness, we decided to check it by direct reference to Sinyakova's paper, cited in the book, and found that it gave a somewhat different nephelometric method of determining lead (page 44): 2.5 ml potassuim bichromate (1:100), 5 drops of 50 percent acetic acid (or 2.5 drops of glacial acetic acid) and the lead solution are placed in a colorimetric tube, and mixed. After 20 minutes the solution is made up with water to 10 ml, agitated, and compared with the scale. Moreover it is mentioned on page 43 that to detect small quantities of lead the lead solution must be poured into the bichromate solution, and not the bichromate solution into the lead solution.

Thus in the Alekseyeva book the Sinyakova method has been modified, and not to its advantage, at that. Why the modification was made, and on what it was based, is unknown. In view of this our own laboratory determines lead by Sinyakova's method, and tetraethyl lead in the air by Gernet's chromate method described by A. M. Petrov in Pribory i instruktsii po opredeliniyu promyshlennykh yadov v vozdukhe / Instruments and Instructions for Determining Industrial Poisons in the Air/ (Gorkiy City, 1948, page 103), using Petri absorbers, mentioned in a note to that text, instead of indicator tubes.

In describing the method of total determination of hydrocarbons after A. S. Zhitkova (page 162 of the Alekseyeva book) it is stated that the air from the second vapor pipette is analyzed for carbon dioxide content of the following day, at room temperature. Consequently, with such a set-up, the anlysis will not take less than two days. We consider that if a gas analysis is made in a single working day, it should be more accurate, since in that case the meteorological conditions, the titer of the instrument (control experiment) and the time the gas is kept, should all be more comparable. Also having in mind the shortening of the analysis time, our laboratory first performs that section of the test, and then follows the order set forth in the book, and in this way succeeds in completing the analysis in one working day.

In the book's calculation of the quantity of hydrocyanic acid by the method of Iofinov-Gol'dfreyn and Gurvits (page 75), it takes into account the fact that on passing 50 liters of air in 2 hours through two absorbers with 15 and 10 ml of 0.1 NaOH, the volume of alkali in the absorption pipettes remains unchanged. We have, however, found in our practice that, if the volume of the alkali is measured after the air has been passed, and then the formula $\frac{gv + g_1v_1}{10 \times v_0}$ used in the calculation, where g = mg HCN in 10 ml of the first absorber, $g_1$ = mg HCN in 10 ml of the second absorber, v = ml alkali in the first absorber after passage of the air, $v_1$ = ml alkali in the second absorber after passage of the air, and $v_0$ = volume of air passed through the absorber, in liters, reduced to normal conditions,

then, by comparison with the method of calculation given in the book, the difference in some cases may reach 0.0001 mg/liter. Such a value for HCN cannot of course be neglected. We therefore consider the method of calculation given in the book to be inexact: it overestimates the actual HCN content in the air examined.

From: Mikrobiologiya (Microbiology), Vol XXI, No 1, January-February 1952, Moscow, Pages 77-82

# THE METHOD OF CONTINUOUS PRODUCTION OF MICROORGANISMS IN INDUSTRY

(Printed in connection with the discussion carried in the Journal Mikrobiologiya (Microbiology) on problems of breeding microorganisms).

I. M. Ryabchenko

The article by V. I. Kudryavtsev "The Continuous Breeding of Microorganisms in Industry" published in the journal Mikrobiologiya, No 2 of 1951 raises a question of great theoretical and practical interest. We had become familiar with the basic postulates of this article before this time--at the conference of microbiologists of the wine making industry held at Yalta in June 1950. At this conference Kudryavtsev aroused the interest of those present in the necessity of wider introduction of the method of continuous breeding of microorganisms into the practice of the fermentation industry.

We consider that this method is promising and that its proper use can give great industrial results. It is essential to exert all efforts to the end that this method be introduced in practice and the fermentation industry receive ever improving breeds of microorganisms, in particular yeast for wine making. It is essential to take adequate steps to popularize this method, subject it to wide discussion and improvement in details, also to coordinate it with other methods for improving microbes, especially yeast such as hybridization and directed breeding.

The method of continuous industrial breeding is an application of the basic postulate of Michurin science--the unity of the environment and the organism--to microbiological practice. It can become a method for combatting the inevitable deterioration (from the industrial viewpoint) of previously isolated pure cultures of microbes in the course of lengthy laboratory culturing under conditions differing sharply

culture from cans of baker's yeast from the Krasnodar bread baking industry (the development of the selected culture of baker's yeast takes place in these cans, under the protection of tars of hop decotion.) The pure cultures obtained are tested for fermentative activity--the rate at which dough raises in cylinders. Testing is conducted with the method we developed (3): a test tube with 10 milliliters of hopped wort is inoculated with the yeast to be tested and placed in a thermostat for fermentation; after 48 hours the fermented liquid is poured from the test tube into a flask containing 200 milliliters of sterile hopped wort of about 7 percent Balg. The flask is then placed in a thermostat set at 27-30 degrees. After 48 hours the yeast in the flask has multiplied to the extent that it can be used to make a batch of dough. In order to make a batch of dough 150 milliliters of the fermented liquid is poured into 200 grams of flour; the dough is mixed for 5 minutes, after which it is removed from the flask and placed in a 500-milliliter measuring cylinder. The times of placing in the cylinder and the beginning of the rise of the dough are noted and the cylinder is placed in a thermostat set at a temperature of 30 degrees. The fermentative activity of the yeast is judged by the amount of rise of the dough, which is noted at regular intervals of time. The best cultures are selected in accordance with the results of testing for fermentative activity and the selection is based not only on the rapidity of the rise, but on the aroma of the dough. The very best cultures of the selected strains are used in industry for the preparation of liquid yeasts. Then pure cultures (usually from 50 to 75), which are subjected to study and selection (the best of them) are again isolated periodically from industrial liquid yeast. Breed No 12, which possesses hereditarily fixed characteristics that are valuable in industrial respects, was obtained as a result of the work. This breed was lost during the War (1941-1945). After the War we again isolated a breed of yeast which possessed high industrial qualities by using the above method.

In the laboratory yeast for the bread baking industry is stored in hopped beer wort and is replanted every month. At the same time, a culture of yeast is also repagated periodically (every 3-4 months) in a sterile hopped brew of flour.

The Breeding of Yeast for Primary Wine Making and Champagne Production.

We have worked on the breeding of yeast for primary wine production since 1932, first in the training section of the Institute of Special Industrial Crops, then (since 1938) in the wine making enterprises of the Abrau-Dyurso Champagne Wine Combine.

Breeding was carried out by the following method: first, pure cultures of yeast were isolated from the yeast precipitates of the best wines obtained from fermentation of grape juice by a certain race of Saccharomyces ellipsoideus wine yeast.

Cultures were selected from the isolated pure cultures which displayed fermentative activity not less than the original breed in experimental fermentation under labortory conditions and when fermenting grape juice produced wine of good taste and some improvements in

respect to formation of precipitate, rate of clarification and increase of resistance to sulfur dioxide and to low temperatures. The selected pure cultures were used in the succeeding wine making season.

In laboratory storage pure culture yeast was developed in grape juice and also on a dense medium--grape juice plus agar. As a result of the work we did we obtained breeds with fixed industrially useful characteristics which did not change under laboratory conditions in the course of 2 to 3 years. On this basis it seems to us to be wholly permissible in certain cases to subject the breed used in primary wine making to industrial breeding not every year, but less often. This is done, however, only when the yeast is stored under conditions approaching the industrial.

Starting in 1938 we did similar work to obtain the most suitable champagne yeast. At first we used precipitates from unvented champagne wine obtained under artificial sterile conditions forperiodic isolation of pure cultures. Later we changed this method and began to isolate pure cultures from the yeast precipitates of industrial samples of champagne wine which showed good qualities in respect to activity of fermentation in champagnization, clarification, character of precipitate and rate of stirring (movement of yeast precipitate to the cork of a bottle of champagne wine).

The necessity for introduction of changes into the methodws brought about by the fact that in the champagnization of wine, in spite of a number of adopted measures (filtration of the wine utilized for preparing the drawn mixture; painstaking washing of communication tubes, barrels and bottles; maintenance of sterility in preparation of yeast samples), champagne wine is always contaminated to a certain extent by foreign microflora, including yeasts of different species which can exert an unfavorable influence on the development of a pure culture. Therefore, pure culture yeast should be adopted by industrial breeding to overcoming this influence too.

Every year the best breeds isolated are utilized after laboratory testing by means of champagnization, in production for mass drawing. The breeding we conducted under industrial conditions led to obtaining races with fixed properties that were favorable for champagne production. Thus, we obtained and improved No 7, which is being used successfully for champagnization in the champagne winery in Abrau-Dyurso (also in other wineries). In 1950 champagnization of wine in Abrau-Dyurso was conducted with Breed No 7, which insured even and active fermentation of the champagnized wine and good clarification, also the formation of readily stirred precipitate.

Thus, the results of our work indicate the expediency of utilizing the periodic or, according to "udryavtsev, continuous) industrial breeding method for maintaining and improving industrially valuable races of champagne yeast. For confirmation we are presenting figures on one of the stages of the work in which observations of the influence of industrial breeding on the improvement of several different breeds of yeasts were conducted in order to establish the general pattern. New cultures (derivatives) isolated fro yeast precipitates from wines

champagnized by corresponding breeds of yeasts (originals) are subjected to comparative testing in the process of champagnization. The test results (the averages of two repetitions) are presented in the following table.

| Breeds of Yeast | Pressure in Bottles (In Atmospheres) at 10 Degrees after a Period of 10 days | 15 Days | 25 Days | 8 Months | Character of Precipitate |
|---|---|---|---|---|---|
| Original No 7 | 2.5 | 3.5 | 4.0 | 4.8 | At first adherent, but upon ripening well separated from sides of bottle and well stirred. |
| Derivatives of No 7 | | | | | |
| No 7 | 2.8 | 3.8 | 4.3 | 5.0 | Same as above. |
| No 14 | 2.3 | 3.3 | 3.9 | 4.7 | At first very adherent, but upon ripening separated from sides of bottle with difficulty, poorly stirred. |
| No 16 | 2.5 | 3.2 | 3.9 | 4.8 | Same as above |
| Original Sh-2 | 0.2 | 0.8 | 1.5 | 4.5 | Precipitate dense, well separated from sides of bottle, well stirred. |
| (1) | (2) | (3) | (4) | (5) | (6) |
| Derivatives of Sh-2 | | | | | |
| No 9 | 0.5 | 1.2 | 1.9 | 4.6 | Same as above |
| Original Steinberg 1892 | 0.3 | 1.0 | 1.5 | 4.5 | Precipitate dense, grainy, well separated from sides of bottle, easily brought to the cork. |
| Derivatives of Steninberg 1892 | | | | | |
| No 8 | 0.4 | 1.2 | 1.[illegible] | 4.7 | Same as above |
| Original No 4 | 1.5 | 2.7 | 3.5 | 4.9 | Precipitate at first adherent, on ripening separated from sides of bottle withsome difficulty well stirred. |
| Derivatives of No 4 | | | | | |
| No 18 | 1.6 | 3.0 | 3.7 | 4.9 | Same as above |

The figures given in the table permit establishing the presence of a definite tendency toward the appearance of individual specimens that

possess higher industrial qualities within a breed of microorganisms.

In this connection the questions arise as to how to isolate these specimens, and what quantity of new pure cultures must be isolated in order to obtain the improved variant of a given breed. In order to be more assured of isolating specimens showing changes in the better and desired direction we have made use of the following method: a drop of the yeast precipitate from wine which has shown good production results in champagnization is transferred to a medium somewhat "heavier" than usual "on the average) in industry, namely, wine (with an alcohol content of 12 percent by volume) plus sugar (5 percent), which is then held at low temperatures (12-14 degrees). Under these conditions, of the enormous number of cells introduced into the wine medium with the drop of yeast precipitate the specimens which adapt themselves best to the given medium begin to grow the most rapidly. About 50-75 pure cultures are isolated from the fermented liquid, using the upper layer where the most active specimens are found, then the best cultures are chosen.

The problem of the method of storage for selected breeds of yeasts is also of great importance. We usually store champagne yeast in a wine medium with replanting every month. Prior to use in industry the yeasts are "activized" by passing through experimental champagnization at low temperatures (12-14) under properly sterile conditions. The bottles in which champagnization of wine has proceeded in a normal manner are used to provide the original pure culture for industrial use.

It is essential to note at this time that we have observed an increase in the fermentative activity of champagne yeasts as a result of laboratory storage in hermetically sealed vessels in a wine medium at increased carbon dioxide pressure and at low temperatures (10-12 degrees).

The method of continuous breeding of microorganisms in industry can be of importance not only in the fermentation industries, but also in other fields where microorganisms are utilized, for example, in the use of Azotobacter to obtain Azotobacterin. A previously isolated breed (for example, 54) which has been stored for years under conditions different from those in which it is to develop (soil, plants) is propagated for the practical preparation of Azotobacterin, which is used for many crops and under various soil and climatic conditions. It is possible that the uncertainty of favorable results from use of Azotobakterin is explained by this to a certain extent. We hold to the opinion that in this case it would be expedient periodically to regenerate the breed of Azotobacter by means of isolating active specimens from production (field) conditions and storing the cultures of Azotobacter in a medium that approaches as closely as possible that in which the given breed should develop.

Taking into consideration the specific influence of the root system of individual species of agricultural plants upon the microflora of that region, it is essential to cultivate breeds of nitrogen-fixing bacteria with specific objectives--for a certain crop

conditions prevailing in the champagne industry likewise have established that Steinberg 1892 yeasts have changed their favorable industrial characteristics for the worse by this time. Pure culture yeasts isolated from "wild" yeast material change especially rapidly in respect to culturing and biochemical characteristics in laboratory storage. Thus, breeds of yeast isolated by Tyuning (4) during a study of the distribution of yeasts in the Kuban Area (Breed No 89) rapidly lost their industrially valuable characteristics under conditions of laboratory storage. The good Tu-pseikh I race of wine yeast isolated by Drobogiav from "wild" yeasts of Anapskiy Rayon has suffered the same fate. It is apparent in these instances that the yeasts were not hereditarily fixed, as a result of which their storage under laboratory conditions, different from those under which they were created, led to rapid changes and loss of certain characteristics useful in industry.

Taking into consideration this lability of microorganisms, their ability quite rapidly to adjust to environmental factors acting for a prolonged period, we should hail the extensive introduction of the method of continuous industrial breeding into practice--a method directed toward the retention and improvement of industrially useful biological characteristics in microorganisms. Only under industrial conditions are all the essential prerequisites created for acquisition and fixing of characteristics valuable for the corresponding industry.

Applying the method of continuous breeding of microorganisms in industry (for example, in the wine making industry) will make it possible to improve breeds of yeast, since breeding by this method yeast of the retained and improved breed will be developed under complex industrial conditions in which changes in the chemical composition of the medium take place not only as a result of fermentation of grape sugar, but also as a consequence of the formation of products from the exchange of substances by other microorganism that have entered the grape juice by various means. Under such conditions stronger specimens of yeast of the improved breed may be developed which will successfully cope with the harmful influence of the increasing alcohol concentration and with the products of the life activities of competing species of microorganisms in the grape juice. The influence of competing species is known to be very important.

Under inter-species conflict, depending upon the peculiarities of the environment, individual specimens acquire properties which make them more adaptable in the ecological sense and more useful for industry.

In connection with the question raised by Kudryavtsev concerning the necessity for utilizing the method of continuous breeding of microorganisms in industry, we consider it fitting to present some material from the results of work we carried on for a period of many years on the isolation and improvement of yeasts for both the bread-baking industry and the wine making industry.

The Breeding of Yeasts for the Bread Baking Industry

From 1929 through 1946 we periodically isolated yeast of pure

from industrial conditions.

When stored under laboratory conditions microbes are subjected to environmental influences entirely different from those in which a definite type of nourishment was formed and the biochemical specificity of the breed was developed. Due to the length of the course of such influences marked changes in the biological peculiarities of the microorganism appear as a result of adaptation. These changes become hereditarily fixed and determine the character of further development. Microorganisms, like macroorganisms, as T. D. Lysenko stated, "are closely linked with environmental conditions and are not only linked, but also definitely adjested to the medium in which they live." Thus, changes in environmental conditions logically lead to adequate changes in the biological peculiarities of microorganisms--at a slower or more rapid rate depending upon the lesser or greater conservatism of the nature of the given organism. The unity of microbe organism and their environment is well defined by Meysel' (1): "Peculiarities of the mutual interrelationship of one-celled organisms with their environment has led, in the process of evolution, to the rise of cells which are specially adapted to changed conditions of existence and which possess very stable protoplasm and a well-expressed capacity for adaptation to changing environmental conditions. The stability of these organisms is successfully combined with physiological activity and with a wide amplitude of individual variability."

Such variability on the part of microorganism in accordance with culturing conditions is of great importance from an industrial point of view. Very frequently the direction of the changes in the biological peculiarities of microorganisms maintained in collections is such that their industrially valuable properties are lost. Wine making practice has been witness of many examples which confirm this position. Thus, deterioration of industrial characteristics in good breeds of Steinberg 1892 wine yeast as a result of laboratory storage have been noted repeatedly. Research by Podoprigora (2) provided interesting material on this problem. The author made a comparative study of Steinberg 1892 yeast acquired by him fro different laboratories: the Leningrad Institute of Microbiology, the Odessa Experimental Station for Viticulture, the Magarach All-Union Scientific-Research Institute for Viticulture and Wine Making, the Anapskiy Rayon Experimental Station for Viticulture, and the Rostov Champagne Winery.

It was established as a result of these studies that yeast of the very same breed differed in respect to fermentative activity, culturing characteristics, sporulation, etc. The author came to the following conclusion: "In connection with this, one may state with conviction that yeast of this breed has changed its properties in different directions under the influence of the different conditions of existence to which it has been subjected. Thus, we may assume that the yeasts known in various regions as the pure Steinberg 1892 culture are not identical, but have actually acquired new properties; some have deviated to a greater, others to a lesser extent from the original breed isolated by Mueller-Turgau."

Our observations of the behavior of this breed of yeast under

or for certain soil and climatic conditions, using local breeds as the original material.

The small amount of material presented here confirms the importance of the method of continuous (or periodic) breeding of microorganisms in industry, a method which stems logically from the basic postulates of Michurin science.

By applying this method and combining industrial and laboratory influences through its use, it will be possible to obtain breeds of microbes more adaptable to the specific conditions of a given industry.

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(In Russian)

# BACTERIOPHAGE PHENOMENA IN BACTERIAL DISEASES OF PLANTS

## Experiments in Bacteriophage Prophylaxis and Therapy in Plant Bacteriosis



The use of bacteriophage as a prophylactic and therapeutic control measure against bacterial plant diseases is of great importance. In testing bacteriophage for the purpose of disinfecting seed stock and for therapeutic and prophylactic action of plant organisms, the following methods are employed: the seed is soaked in bacteriophage at various temperatures and for different periods of exposure, plants are sprayed and irrigated at different stages of their development, the roots of seedlings are soaked in bacteriophage, compresses of bacteriophage, and injections of bacteriophage into the internal tissues of the plant are administered. In many cases experiments in the application of bacteriophage have produced positive results.

Thus, experiments conducted by other investigators in disinfection of cotton seed (Lebedeva) against Xanth. malvacearum, and in disinfection of corn seed by bacteriophage against B. stewarti, corroborate that bacteriophage can be utilized successfully for disinfection of seed grain.

Experiments in the application of bacteriophage as a prophylactic and therapeutic factor in the control of bacterial canker [rak] of plants, conducted by a number of authors (Izrail'skii, Vinogradov and others), as well as against potato diseases (Bel'tiukova and others) produced positive results. Spraying makhorka [Nicotiana rustica] plantations with bacteriophage against Ps. tabacum (Novikova) effected considerable reduction in the wildfire disease in comparison with control [plants].

The positive effect of the works indicated was obtained in a vast majority of cases as a result of direct bacteriophage action on pathogenic bacteria. Methods of immunizing plants with bacteriophage did not, in the majority of cases, produce positive results.

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The diapason of the action of many bacteriophage specimens on the causal agents of bacteriosis in plants is extremely narrow. There are frequently encountered bacteriophages producing lysis of only one strain of bacteria isolated from the same section of the sick plant from which the bacteriophage was obtained. In working out the methods for bacteriophage prophylaxis and therapy, particular attention must be paid to obtaining a rich preparation of bacteriophage. It must possess a high titer and must be active with respect to the largest possible number of strains of the causal agents of disease obtained from different localities.

Thus, for instance, it is expedient to use a bacteriophage preparation obtained from a mixture of the largest possible number of bacteriophage specimens complementing each other on diapason of action. In the control of Bacterium tabacum of makhorka, bacteriophage specimens were selected which caused lysis of 98% of the 241 strains of B. tabacum tested with them. (Novikova). Regardless of the big prospects, the methods for mass application of bacteriophage to control of bacterial plant diseases have not yet been worked out. In this sphere of knowledge the extensive cooperation of scientific research institutions and industrial organizations is extremely necessary.

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Izmenenie nukleinovykh kislot v khode oplodotvoreniia u gorokha

/Change of nucleic acids during the process of fertilization of peas/

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(In Russian)

## CHANGE OF NUCLEIC ACIDS DURING THE PROCESS OF FERTILIZATION OF PEAS.

At the present time it can be considered definitely established that the ribo-nucleic (RNA) and desoxy-ribo-nucleic (DNA) acids participate in the synthesis of protein in animal and plant organisms (13, 9, 8, 3, 20, 19). The problem of the synthesis of nucleic acids in the process of embryonic development interested many authors, but the obtained results are still contradictory and do not give a clear idea of the course of the synthesis.

In recent years it was demonstrated(14, 16, 7) on a series of animal objects that in the process of fertilization and of embryonic development of the organism the nucleic acids were consumed during the synthesis of protein and can in turn, be synthesized by living substances. The present work is dedicated to the problem of the course of synthesis of nucleic acids in plants during the fertilization process. At the same time some aspects of the fertilization process are clarified.

### Material and Methods

The research was carried out on peas of the Moskovskii 116 variety. In order to investigate changes of the unfertilized embryo sac in connection with its aging, the flowers were castrated while in the state of green buds. In this case the ovaries were fixed at once and 2, 5, 8 days after the castration. In order to study the processes which take place in the fertilized embryo sac the ovaries of flowers which did not undergo castration were fixed 8, 12, 24, 48, 72, 96, 120 and 144 hours after the beginning of blossoming. As fixative agents were used Carnoy's fluid, a mixture of formalin, acetic acid and alcohol, as well as 96% alcohol. The pouring was done into parafin sections 10-12 microns thick were treated according to Feulgen with additional staining with light green for detecting of DNA and with methyl green with pyronine for finding of RNA. Part of the preparations were treated according to Brashe (Roskin's modification). After such treatment only staining of the nuclear material with methyl green was observed.

## Results of the research

### A. Content of nucleic acids in the embryo sac before fertilization.

Before fertilization the embryo sac is stained red with pyronine. In the sexual apparatus and the polar nuclei there is more RNA than in the cells of the integument of the ovule. With the aging of the egg-cell distinct changes in the DNA content are observed. Thus the nucleus of the young egg-cell does not produce a Feulgen reaction (fig. 1), i.e. it is oxychromatic [adject. ?] according to O. B. Lepeshinskaia's terminology. As it is known, this is characteristic for nuclei of young cells. On material fixed 2 days after castration, small lumps of DNA are seen distinctly in the nucleus of the egg-cell and their number increases with the aging of the latter (fig. 2). At the same time the polar nuclei remains always achromatic and the nuclei — synergid-chromatic.

Some authors as, for example, V. E. Kozlov, interpret the picture of DNA distribution in the nucleus of the egg-cell, which exists prior to fertilization, as [begin p. 164] a picture of assimilation of the sperm. According to Kozlov, after entering the nucleus the sperm forms a bead-like thread. In reality the structures described under that name correspond to the little lumps of DNA which is contained in the nucleus of the egg-cell even before fertilization. The increase in the quantity of DNA in the nucleus of the egg-cell can be connected according to its aging with the genetic data (18, 6, 2, 5), according to which the growth stage of the egg-cell during crossing reflects on the results of fertilization. Of course the process of fertilization is conditioned by many factors, but probably the absence or presence of DNA in the nucleus of the egg-cell is of great significance in the process of mutual assimilation of gametes.

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Fig. 1 [p. 164]
Embryo sac. Young egg-cell. Nucleus of egg-cell and polar archromatic nuclei. Synergid chromatic nuclei. Stained with methyl-green and pyronine. Ocular x7, ob. im. 90

Fig. 2 [p. 164]
Embryo sac. Old ebb-cell. Nuclei of egg-cell and Sinegrid chromatic nuclei. Stained according to Feulgen. Ocular x15, ob. im. 90

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### B. Changes of nucleic acids during the process of fertilization.

In the pollen grains were found basophilia not only in the plasma but in the sperms as well. When the pollen tubes grow into the style of the postil and the cavity of the ovary, an uneven distribution of RNA is observed but its content does not change. After the pollen tube enters the embryo sac its plasma stains more intensely. This fact indicates

that the metabolism of the pollen tube in the embryo sac increases. No changes in the content of DNA during the growth process of pollen tubes were noted.

After fertilization takes place, the amount of RNA begins to increase in the plasma of the egg-cell and in the area of polar nuclei. The content of RNA reaches a maximum during the metaphase of the first division of the zygote and the endosperm. This fact as well as the observations by Levinson and Kavarskaia (12) do not confirm Brashe's data on the decrease of RNA during the metaphase. During the fertilization process the DNA in the sperms gradually decreases and, finally, disappears, simultaneously the RNA content increases mainly in the nucleolus which is being formed. When the egg-cell and the secondary nucleus are fertilized by one sperm, one additional nucleolus is formed in the nucleus, but where two sperms participate in this process — two additional basophilic nucleoli originate (fig. 3).

The RNA increase in the nucleus of the egg-cell and in the secondary nucleus where with double fertilization, probably causes speedier metabolism of the zygote and endosperm and, therefore their vitality. Directly after the termination of the fertilization the first nucleus of the endosperm appears to be oxychromatic, the nucleus of the zygote, depending on the growth stage is a chromatic or weakly chromatic.

During the prophase of the first division of the zygote and endosperm, the amount of DNA increases, apparently at the expense of a synthesis of nucleic acids which takes place at that time. It should be pointed out, that such increase in DNA takes place parallel to the increase in the volume of the nucleus. The highest content in DNA is observed during the metaphase.

Here should be mentioned in passing, that in the already fertilized embryo sac there was observed the entry of one, two and more additional pollen tubes. On the basis of Morganistic positions, Maheshwari (20) maintains that the pollen tubes enter the embryo sac in which the embryo develops only in a case when the egg-cell develops parthenogetically . Our data demonstrates that additional pollen tubes can enter the embryo sac also at the moment of the uniting of male gametes with elements of the embryo sac (fig. 3). This phenomenon entry of additional pollen tubes into the embryo sac— can be considered regular. It takes place up to the time when unevenly maturing pollen reaches the stigma. In our preparations additional pollen tubes were detected in an embryo sac 6 days after the fertilization when the embryo and the endosperm are multinuclear, so that natural self-pollination in peas can be considered multiple self-pollination (fig. 4).

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Fig. 3 -/Embryo sac. When two sperm are assimilated in the nucleus of the egg-cell there appears two additional nucleoli. Additional pollen tube in the embryo sac. Stained with methyl-green pyronin. Ocular x15, ob. im. 90

Fig. 4 /p. 165/ -
Embryo sac. A large embryo and an endosperm develop 6 days after pollination. At the micropylar end is an additional pollen tube. Stained with methyl-green pyronin.
Ocular x7, ob. 20

These pictures can be connected with genetic data /begin p. 166/ on hereditary characteristics of two and several male parent forms (17, 1, 11) and, on the other hand, — with an increase in vitality in the process of fertilization. T. D. Lysenko considers that "by way of uniting nuclei of reproducing cells into one nucleus a quality difference /"paznokachestvennost'"/ of a living body is created"(14). We think that the vitality can increase also as a result of assimilation, by the egg-cell as well as by the zygote and embryo, of the content of additional pollen tubes as a living substance. The biological role of large amounts of pollen which are separated by the organism, consists not only in producing progeny, but also in preserving and increasing the vitality of the organism and this is probably of particularly great importance for self-pollinators.

C. Change in nucleic acids in the development process of embryo and endosperm.

When nuclei are being formed in the embryo and endosperm, the nucleic acids disappear almost entirely: nuclei are not stained with pyronine and DNA is found in a negligeable amount in an atomized state. In the interkinesis in peas achromatic nuclei are not observed fully. In subsequent prophases takes place a simultaneous accumulation of RNA (basophilic nucleoli) as well as of DNA.

In peas at the two-cell stage, their difference of quality appears. Thus the upper cell which is located closer to the micropyle is older and can be, for example, in late prophase or metaphase, while the lower cell, a younger one — in the state of early prophase. The quality difference of the two-cell stage is observed also in later stages of the division. These data on a plant object confirm observations by O. B. Lepeshinskaia(13). P. V. Makarov(15). and others on animal objects. They give a key for an explanation of the course of origination of physiological quality difference of the organism. At later stages of the division other peculiarities in distribution of nucleic acids are also observed. In the suspensors of the embryo the RNA is found in a smaller amount than in lower cells of the embryos. The lower embryo cells are younger, their growth is more intensive than that of the cells in the suspensors.

Consequently during the process of fertilization and embryonic development of peas RNA and DNA are consumed during the synthesis of protein i.e. with the formation of new nuclei and cells, and then they are synthesized now in newly forming cells from their living substance. This cycle of operations is found also in later development of the embryo and endosperm. Thus the formation cycle of the

cell nucleus and cells in the embryonic development is connected with the cycle of nucleic acids. Besides that the accumulation of RNA and DNA in the process of embryonic development in peas takes place simultaneously, which speaks of independent courses of their synthesis.

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Mikrokhimicheckoe izuchenie iadernogo apparata tsianofitsei i nekotorykh bakterii s nomoshch'iu fermentov gruppy nucleaz

[Microchemical study of nuclear systems in blue-green algae and some bacteria with the aid of nuclease-type enzymes].

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Micro-chemical study of the nuclear apparatus of Cyanophyceae [ blue-green algae ] and some other bacteria with the help of enzymes of the nuclease group.

In research of recent years' on cytology and caryology [ nuclear studies ] of bacteria (Peshkov, 8, 9, 10, 11, 15; Robinow, 18; Caspersson, 13) an hypothesis was brought forward to the effect that bacteria possess an apparatus of a specific type represented by one chromosome cautiously called "Pekar's nucleoid" [?]. While heteromorphous forms (Gamaleia, 14) are formed, a delay takes place in the division of the protoplast of the bacterial cell while it is growing uninterruptedly and the "nucleoids" reproduce. This process can lead to an appearance of multinuclear forms similar to swarming Proteus or specimens of Caryophanon (15). The non-separation of reproducing "nucleoids" leads to the formation of poly-energid, (7) ribbon-shaped or netted nuclei which were considered erroneously by Pietschmann (16) and Inshenetskii (5, 6) to be the result of separating out of diffuse chromatin in dying forms. On the basis of a series of physico-chemical properties of these structures I suggested (7) that the structure of these ribbon-shaped and sometimes spiral formations can be best compared with those of chromosomes of higher forms. Therefore it seemed to me very interesting to test on these formations the effect of enzymes of the nuclease type, thus repeating Frolova's (12) work on the study of chromosome structure of Diptera and some other organisms with the help of the same enzymes.

As objects were taken trichomes of blue-green algae from the Oscillatoria group (fig. 1) the nuclear apparatus of which, according to data by Polianskii, Petrushevskii (17) and Spearing (19) is represented by a small chromatic net similar to thread and reticular nuclei of heteromorphous bacteria forms and, in particular, heteromorphous spherical "cold" forms Achromobacter Epsteinii (Peshkov, 7) with well manifest reticular nuclei (fig. 2, 3). Then were taken also

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normal forms of Caryophanon (fig. 4) "nucleoids" which are most similar in their behavior and organization to chromosomes of ordinary nuclei (Peshkov, 9, 15).

In contrast to the Mazia and Iaeger (14) methods I applied the method developed by Frolova (12) in which aceto-carmine preparations of objects being investigated are subjected to the effect of the nuclease. The obtained aceto-carmine preparations of Oscillatoria, Achromobacter Epsteinii and Caryophanon were photographed (fig. 1, 3, 4): The position of the given section of the preparation was recorded by vernier and the preparations, after letting the cover glass stand in 96° alcohol, were placed for 24 hours in the juice of the spleen of cattle.

The juice was prepared by grinding fresh spleen for an hour with crushed glass until a uniform paste was obtained which then was diluted with five volumes of water. After a 4-hour steeping in cold temperature, the mixture was centrifuged at 5 thousand RPM for half an hour, the obtained juice was adjusted with 0.1 N. NaOH to pH=7 and was tested for activity with the help of a test-object of aceto-carmine preparation of saliva glands of Drosophila Melanogaster. /begin p. 342/ After a 15-hour action of juice in presence of chloroform at 37°, the test-objects were treated according to Feulgen simultaneously with the untreated aceto-carmine preparation of saliva glands. Feulgen negative reaction in the preparation treated with the juice was an indicator of the presence of a sufficient amount of nuclease; such juice was used for the work. Its activity did not diminish during 2-3 weeks.

Experiments with nuclei of blue-green algae and bacteria were conducted according to the following scheme: the photographed aceto-carmine preparations were placed in active juice. Simultaneously a test was conducted (aceto-carmine preparation of chromosomes of saliva glands). After an 18-hour exposure all the preparations were treated according to Feulgen. Simultaneously a second aceto-carmine preparation of glands which served as a control for the correctness of the course of Feulgen's reaction was subjected to treatment.

Usually after the effect of the juice the chromosomes of the saliva glands as well as the nuclei of Oscillatoria, Achromobacter Epsteinii and Caryophanon no longer gave a Feulgen's reaction. In analoguous preparations which did not undergo the effect of nuclease, Feulgen's reaction was without fail positive and produced the usual sharp pictures of specific coloring of the nuclear substance (Polianskii and Petrushevskii, 17; Peshkov, 7, 9).

Preparations which were exposed to the effect of nuclease and produced afterwards a negative Feulgen reaction, were additionally colored with acid fuchsin by the Frolova Methods (13). According to the vernier were located the previously photographed spots and they were photographed again at the same magnification. Prints of photographs of aceto-carmine preparations were compared with those treated with nucleases and then according to Feulgen (fig. 1-4), which made it possible to come to the following conclusions:

Fig. 1. In the upper frame a is seen a short trichome and hormogones of Oscillatoria sp.

In each of the 14 cells of the trichome the nuclear apparatus stands out clearly. In some cells the division of the nucleus is seen. In the lower frame b it is seen that after the nuclease treatment the Feulgen reaction was negative but after additional coloring with acid fuchsin, all the nuclear formations seen in the aceto-carmine preparation were preserved in spite of a certain shrinkage of the material which always occurs in the process of hydrolysis with N HCI after the action of the nuclease.

Fig. 2. In frame a are pictured living gigantic globular specimens of Achromobacter Epsteinii which were obtained as a result of incubating the culture at 10°. The picture was obtained by the method of combined negatives (Peshkov, 8). Clearly seen are the white netted more refractive nuclei filling almost the entire cavity of the specimen and enclosed by a narrow dark protoplasm. Among the spherical specimens are distributed a few bacillary forms.

In fig. 3 in frame a is pictured a hetero-morphous divisible form of Achromobacter Epsteinii with a netted nucleus reminiscent of the prophase stage. Well seen is the protoplasm. After the effect of nuclease and ith a negative Feuglen reaction the nuclear structures are fully preserved (frame b) and finally, the structure of multi-nuclear specimens of Caryophanon depicted in frame a (fig. 4) is manifest much more sharply after the treatment with nuclease and additional staining with acid fuchsin. Chromosome-like nucleoids having also Caryophanon shape of ring or horse shoe (Peshkov, 9, 15), are seen with more details than in an aceto-carmine preparation.

Comparing the data which I obtained in regard to nuclei of Oscillatoria, Achromobacter Epsteinii and Caryophanon, with those by Nazia and Jaeger (14) and by Frolova (12) who treated with nucleases the chromosomes of animal and plant objects, it is possible to come to /begin p. 343/ a basic and most essential conclusion: specific netted or horseshoe-like structures found in cells of blue-green algae and of such bacteria as Achromobacter Epsteinii, Caryophanon, produce a distinct true Feulgen reaction (Peshkov, 9). Their division precedes the division of protoplast and the type of division, in the case of the horse-shoe-like nucleoids of Caryophanon reminds one mostly of the lengthwise splitting of chromosomes of mitotic nuclei. These structures behave also in regard to nuclease action as true formations.

## Conclusions

The present work had only one aim: to find out the chemical similarity between the nuclear structures of some lower plant organisms of the Schizophyta group and those of true chromosomes of plants and animals. Without going into a discussion of connections severed by nucleases in the prosthetic group of the nuclear nucleoproteid (?), it is possible to maintain that this problem is solved in the positive sense and the chemical similarity of materials from which was

built the main mass of metaphatic chromosomes of plants and animals and of "nuclei" of Schizophyta so distinctly demonstrated by Belozerskii's work (1,2,3) can be considered proved according to one more criterion-Feulgen negative reaction after the action of the specific group of enzymes of the nuclease type.

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Fig. 1 [p. 342a] Effect of nuclease on the trichome Oscillatoria. Explanation in the text. X 2000.

Fig. 2 Living globular "cold" forms of Achromobacter Epsteinii. Netted nuclei are seen (light sections). Contrasting according to Burinskii-Peshkov. Apochromate 2mm, ocular K 18. Light accord. to Koler, X 2400.

Fig. 3 Effect of nucleases on heteromorphous forms of Achromobacter Epsteinii similar to those depicted in fig. 2. a.- fixation and coloring with aceto-carmine, b-preserving of structures (of the chromatin net) after the effect of nuclease (Feulgen's negative reaction) and additional coloring with acid fuchsin. X 2400

Fig. 4 Analogous case. As an object is taken a culture of Caryophanon latum. X 1300.

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Trans. 501
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By:
A. Antik

Klechkovskii, V. M., and Evdokimova, T. P.

O radioavtograficheskom onpredelenii lokalizatsii radiozotopov v rasteniiakh

/On radioautographic determination of radio-isotope localization in plants/

Akad. Nauk SSSR. Dok. 79:629-632, Aug. 1, 1951.
511 P444A

(In Russian)

## ON RADIO-AUTOGRAPHIC DETERMINATION OF RADIO-ISOTOPE LOCALIZATION IN PLANTS.

With the appearance of the radio-isotope method, a rather wide use began to be made in biological research of contact radio-autographic picture images of isotope localization in tissues. In experiments with plants, the leaves, roots as well as slices of fruits, stem, etc. serve as objects of obtaining of radio-autographs.

In cases when the radio-autography is used for a demonstration of a general presence of radio-active isotope in certain tissue sections, the interpretation of the picture seems very simple; the spots of blackening of the sensitive film indicate the content of radio-active radiator in corresponding places, and in reprints from such "negatives" the spots of radio-isotope accumulation look like white or altogether lighter spots on a black background. The intensiveness of blackening of the "negative" and the sharpness of light spots in the reprint, can serve in some cases as an indication of greater or lesser concentration of the radiator in corresponding places of the object being investigated. Thus the radio-autographic pictures of lengthwise cuts of a corn stem in fig. 1 (see glued-in piece), distinctly demonstrate an accumulation of radio-phosphorus in nodes especially at the base of the stem, as compared with the adjoining sections of the internode. In fig. (2) the localization of radio-phosphorus in the seeds and its more or less diffuse distribution in the flesh of the apple /fruit/ is seen very distinctly. The radio-autographs in fig. (2) were obtained in experiments for the study of distribution of tagged phosphorus in the crown of the apple tree when it entered through isolated sections of the root system; these experiments were conducted in 1950 in the orchard of the Experimental Fruit Station of the Moscow Agricultural Academy imeni K. A. Timiriasev.

Starting from the notion of the direct connection between the intensiveness of blackening of sensitive film and the content of radio-isotope in corresponding sections of the tissue, the possibility is examined of quantitative determinations with the help of photometric radio-autographs (1). In the literature there are indications of a development of methods of determining in this way the contents of radio-active elements "in

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individual organs and points of the plant" (2). At the same time, even without a quantitative analysis, by way of simple comparison of radio-autographic pictures obtained in experiments by introduction into the plants of radio-isotopes of various elements, conclusions are sometimes made on their one-type or uneven distribution in tissues. Thus on the basis of such a comparison A. A. Drobkov (2) came to a somewhat unexpected conclusion on similarity of distribution in the plants on such sharply different, in their chemical nature, substances as natural radio-active /begin p. 630/ elements on one hand and radio-phosphorus - on the other. By way of an analogous comparison, conclusions were drawn on uneven distribution in plant leaves of radio-isotopes of molybdenum and potassium (3). Different distribution type in the leaves was noted also (4) on the basis of comparison of radio-autographs for isotopes of phosphorus $P^{32}$ and sulfur $S^{35}$.

As examples of characteristic differences of this kind there can be considered the radio-autographs of corn leaves presented in fig (4); the leaf containing $P^{32}$ gives a picture which shows on the outside a completely different character of distribution of radio-isotope than the picture of the leaf containing $S^{35}$. However, in evaluating similar kinds of radio-autographic pictures it is necessary first of all to keep in mind that the intensiveness of the radiation which falls on a sensitive layer depends on the concentration volume of radio-isotope in the tissue as well as on its amount per area unit, and this amount is in relation to the distribution of the bulk of the tissue in the surface of the picture. Besides that the intensiveness of radiation which falls on a sensitive layer is influenced by the absorption of radiation into the tissues , the volume of which /absorption/ in turn is determined from one side by the mass of the tissue per unit of area and on the other hand, by the type of disintegration of the radio-isotope and the energy of particles radiated by it. And then, it is particularly important to keep in mind that, for example, depending on the energy of the Beta-particles and therefore on the uneven absorption of radiation into tissues, the unevenness of distribution of the mass of the tissue on the surface of the picture has a different influence on the character of the radio-autograph which is obtained. Without consideration of these factors the judgement of peculiarities in localization of radio-isotope, for example, in the leaf tissue, may prove erroneous.

In several experiments we carried out determinations of the radio-phosphorus content in individual parts of fruits and leaves which served for the obtaining of radio-autographs. The data in table (1) demonstrate that the results of determination of phosphorus (tagged as well as total) content in seeds and flesh of apple tree fruits are in complete accordance with the radio-autographic pictures of cuts of the same fruits presented in fig (2). Slightly different results were obtained from analogous determinations of tagged phosphorus content in the central vein and then the separated from it, leaf blades of the apple tree. Radio-autographs of which are shown in fig. (3). It is seen from the data in table (2) that the amount of tagged (and total) phosphorus in ratio to a unit of the tissue's dry bulk in the central veins of the leaf was even somewhat lower than in the blade.

Table (1) - page 630.

Content of total and tagged phosphorus in seeds and flesh of apple tree fruits (per 1 g. dry subst.)

| Fruits taken for analysis | Total phosphorus in mg $P_2O_5$ | | Tagged phosphorus in thous. imp./min. (?) | |
|---|---|---|---|---|
| | seeds | flesh | seeds | flesh |
| 1 | 14.0 | 2.0 | 13.0 | 3.0 |
| 2 | 12.0 | 1.5 | 27.5 | 5.3 |

Table (2) - page 630.

Content of total and tagged phosphorus in leaves of apple tree (per 1 g. dry substance).

| Leaves | Total phosphorus in mg $P_2O_5$ | | Tagged phosphorus in thous. imp./min | |
|---|---|---|---|---|
| | Central vein | Blade | Central vein | Blade |
| 1 | 2.6 | 3.8 | 15.0 | 18.2 |
| 2 | 3.2 | 3.8 | 23.0 | 27.2 |

The seeming discrepency between the data in table (2) and fig. (3) can be explained by the unevenness of distribution of the tissue mass of the leaf in the picture surface, because there is a several times larger mass per unit of exposed area in the central vein than in the blade. /begin p. 631/

For various isotopes with unequal energy of Beta-particles such an effect, as mentioned before, must manifest itself to various degrees. Since radio-phosphorus radiated Beta-particles of high energy, the absorption of radiation in the thickness of the object being examined (leaf, slice) reflects comparatively little on the intensiveness of radiation reaching the sensitive layer. Other conditions are created in experiments with the radio-isotope of sulfur $S^{35}$, the maximum energy of the Beta-particles of which is approximately 10 times lower and therefore their absorption into the tissues is much stronger.

The following test demonstrates that these differences are sufficiently clear with such objects as leaves. From a corn leaf containing $S^{35}$ there were cut out 4 blades with a 1 $cm^2$ area each, which were then placed singly or 2, 3, 4 blades together (one above the other) in front of the window of the meter for the measuring of activity. Identical measurements were taken of blades cut out of a leaf containing $P^{32}$. The results of this

experiment showed distinctly that in the leaf blade only an insignificant part of radiation $P^{32}$ is absorbed, while the absorption of radiation $S^{35}$ if very great.

| Number of blades | Impulses per minute | |
|---|---|---|
| | Test with $P^{32}$ | Test with $S^{35}$ |
| 1 | 210 | 195 |
| 2 | 413 | 259 |
| 3 | 543 | 259 |
| 4 | 710 | 235 |

In fig. (4) are shown radio-autographs of corn leaves from vegetative experiments with $S^{35}$ and $P^{32}$. If the radio-phosphorous produces in radio-autography a characteristic picture of an accumulation in the vascular bundle (most distinctly manifest in the central (axial) part of the leaf), then in a case with radio-sulfur the opposite takes place; in the axial, the central part of the leaf on the print can be observed an even darker (corresponding to a lesser blackening of the "negative"). Analysis of individual parts of such leaves indicated, however, that in reality there is no sharp difference in the distribution of $S^{35}$ and $P^{32}$ between the central vein and the side parts of the leaf blade. In the ratio per 1 g of dry substance in both cases the radio-isotope content was about three times higher in the blade than in the central vein (see table 3).

Contents of radio-phosphorous and radio-sulfur in corn leaves

| Parts of the leaf | Thous. imp./min (?) per 1 g of dry substance | | Thous. imp./min per 1cm$^2$ of leaf area | |
|---|---|---|---|---|
| | Test with $P^{32}$ | Test with $S^{35}$ | Test with $P^{32}$ | Test with $S^{35}$ |
| Central vein | 57.6 | 16.1 | 0.78 | 0.25 |
| Leaf blade | 141.6 | 45.6 | 0.35 | 0.13 |

Comparison of table (3) and fig. (4) demonstrates clearly the fact that in the given case the character of the radio-autographic picture of the leaf containing $P^{32}$, is conditioned not by concentration of radio-phosphorus in the central vein but by a greater bulk of tissue and therefore by a larger amount

amount of $P^{32}$ per unit of picture area in this part of the leaf. And in the case of $S^{35}$, on the contrary the larger amount of radio-isotope per area unit in the central part of the leaf does not cause increase in intensiveness in this part of the picture due to relatively more complete absorption of $S^{35}$ radiation in the thickness of the tissue. /begin p. 632/

Thus unequal energy of Beta-particles appears to be a factor determining to a high degree the character of radio-autographic picture localization of radio-isotopes of various elements in leaves of the plants. Therefore the comparison of pictures obtained in experiments with different isotopes, is possible only with definite relation between the thickness of the object being examined and the energy of Beta-particles radiated by isotopes. It is understood that in studying localization in plant tissues of isotopes differing in the type of radio-active disintegration, the marked factors acquire an even greater significance.

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Fig. 1
Localization of radio-phosphorus in corn stem.
1, 2, 3 - order of nodes from the bottom.

Fig. 2
Loca lization of radio-phosphorus in apple-tree fruits.

Fig. 3
Radio-autographs in apple-tree leaves containing $P^{32}$.

Fig. 4
Radio-autographs of corn leaves containing:
(A)- $P^{32}$, - $S^{35}$ (from test with isolated feeding /? nutrition?/

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By:
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Primenenie metoda mechenykh Atomov k izucheniiu ustoichivosti klopa-cherepashki k dvum fosfororganicheskim insektitsidam i opyt izucheniia proniknoveniia ikh v rasteniia

/Utilization of tagged atoms in studying the resistance of Eurygaster integriceps in two organic phosphage insecticides and method of studying their penetration into the plant/

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511 P444A
(In Russian)

## UTILIZATION OF TAGGED ATOMS IN STUDYING THE RESISTANCE OF EURYGASTER INTERGRICEPS TO TWO ORGANIC PHOSPHAGE INSECTICIDES AND METHOD OF STUDYING THEIR PENETRATION INTO THE PLANT

Increasing importance in the control of pests of agricultural crops is acquired by the organic phosphorus insecticides, in particular, by diethyl-4-nitrophenylthio phosphage or compound -(NIUIF) 100 (thiophos) which is a fluid with a 115° boiling temperature at 0.03 mm "rt. st." (?) $d^2)_4$ 1.2704, $n^{20}D$ 1.5374, "t. pl." (temperature of melting) + 6°.

In manufacturing "thiophos", ethyl-4,4'-dinitro-diphenyl thio-phosphate can also be obtained, which is a crystalline, little-volatile substance, with 125° melting temperature.

In our laboratory a study was undertaken of these two compounds tagged with the help of the radio-active isotope of phosphorus, for the purpose of clarifying the causes of varying destruction of insects and, in particular, of the harmful Eurygaster integriceps Put. due to these insecticides, as well as for explanation of penetration of the "thiophos" compound into the plants.* The adult bugs were dusted with weighed portions of dust containing 1% of the active element of organic phosphorus insecticide and there observations of their dying were conducted as well as analyses, in regard to intensiveness of radiation, of amounts of insecticides penetrated into the eurygaster's body. Prior to that the body of the eurygaster was

NOTE- Toxicological examinations were carried out by K. A. Gar and V. T. Chernetsova, the synthesis of preparations — by Ia. A. Mandel'baum and K. D. Shvetsova — Shilovskaia under M. N. Mel'nikov's direction.

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washed thoroughly. The results of calculations are shown in table (1), in which data are given of two series of experiments conducted at different times with bugs gathered in different areas.

Table (1) - page 1189)

Amount of phosphorus (on conversion to diethyl-4-nitrophenyl thio-phosphate and ethyl-4.4'-dinitrodiphenyl thio-phosphate) which penetrated into the eurygaster body at varying degrees of poisoning

| Preparation | Sex | State of the eurygaster | | | | | |
|---|---|---|---|---|---|---|---|
| | | Living | | Paralyzed | | Dead | |
| | | Exper.1 | Exper.2 | Exper.1 | Exper.2 | Exper.1 | Expr.2 |
| | | Amount of active element penetrated into the eurygaster's body in (micrograms per gram) | | | | | |
| Diethyl-4-nitro-phenyl-phosphate (1% dust) | ♂ ♂ | 1.1 | -- | 2.1 | -- | 6.4 | 10.2 |
| | ♀ ♀ | 2.2 | -- | 7.4 | 6.5 | 9.5 | 10.9 |
| Ethyl-4.4' dinitro-diphenyl thio-phos-phate (1% dust) | ♂ ♂ | -- | 14.7 | 5.8 | 28.7 | -- | 54.6 |
| | ♀ ♀ | 3.8 | 30.0 | 7.3 | 48.8 | 15.3 | 56.9 |

In spite of the different resistance to the effects of poisons which detected in bugs during the two series of experiments, the results from the given data are as follows:

1) There is a direct relation between the amount of phosphorus detected in the eurygaster's body and the degree of its poisoning (within each of the experimental series).

2) There is a considerable difference in the resistance of bugs of different sexes to the effect of the insecticides. We observed that when wheat plantings were dusted with a 1% dust of "thiophos" in one of the experiments, the destruction of eurygaster females constituted 60% and of males — 77.6%. However at that time it was not possible to prove that a lesser destruction of females is conditioned by their greater biological resistance; it was possible to assume that ecological peculiarities of the eurygaster's mode of life had also a bearing. Experiments with tagged insecticides indicated clearly that the eurygaster females are biologically

more resistant to the effects of insecticides than the males, which is directly confirmed by D. M. Fedotov's observations of greater resistance of the eurygaster females to unfavorable conditions.

3) From the comparison of amounts of diethyl-4-nitrophenyl thio-phosphate and ethyl-4.4'-dinitrodiphenyl thio-phosphage penetrating the eurygaster's body and causing destruction, it follows, that death took place with smaller doses of diethyl-4-nitrophenyl thio-phosphate which coincides well with the data on the lesser toxicity of ethyl-4.4'-di-nitrodiphenyl thio-phosphate (2).

However different quantities of dead eurygasters can be due both to different toxicity of applied compounds as well as to their different penetration into the body of the insects. The results of our experiments indicate that the difference in the destruction of the eurygaster from these two compounds is due just to the fact that the latter have different toxicity for the eurygaster. Apparently the different penetration capacity of insecticides into the eurygaster body is not a passive diffusion process but is closely connected with the metabolism and life activity of the insect. This is confirmed by the fact that in treating dead specimens the penetration of insecticides decreased sharply and the difference in the penetration of insecticides into the bodies of bugs of different sexes disappeared.

Penetration of diethyl-4-nitrophenyl thio-phosphate (thiophos) into plants.

Lately much attention is paid in literature to the problem of penetration of organic phosphorus insecticides into plants, special insecticides are being developed which readily penetrate the plant tissues. There are many attempts to use thiophos as a "systemic" insecticide (5). However the problem of dynamics of penetration and isolation of thiophos from plants has not been studied sufficiently up to the present time or is based only on the method of biological tests. At the same time this problem is of great practical and theoretical importance because the possibility of imparting insecticidal properties to plants without the danger of poisoning humans and warm-blooded animals by feeding them intoxicised plants or fruit from these plants presents a very attractive perspective in the field of chemical protection of plants.

For solving the problem of determination of insecticides in plants in an undestroyed form we applied the methods of extraction of $P^{32}$ from plants treated with tagged insecticides. As a basis for the methods served the fact that the organic phosphorus insecticides being studied are insoluble in water but are adequately soluble in organic solvents (3). In hydrolysis the organic phosphorus insecticides break down to phosphoric acid which is soluble in water (4). For the determination of the total amount of isotope $P^{32}$ which penetrated the plant, the methods of calculating phosphorus in cuts from leaves was applied.

In fig. (1) are shown the results of determination of thiophos penetration [begin p. 1191] into chrysanthemum leaves through roots when the plants were watered through the soil with an aqueous emulsion of 50% thiophos concentrate in the amount of 50 ml. per flower pot. The curves

1 and 4 correspond to, with a 0.05% concentration of the emulsion, the curves 2 and 5 with a 0.1 concentration, and 3 and 6 — 0.2% concentration. The curves 1, 2, and 3 correspond to the amount of thiophos found in plants, which is calculated according to the total contents of phosphorus; the curves 4, 5, and 6 indicate the amount of thiophos found in plants, taking into consideration its hydrolysis (fraction dissoluble in dichlorethane).

Fig. 1
Diethyl-4-nitro-phenylthiophosphate — Duration of Exposure — 16 days

Shown in the graph is also the destruction of aphids (Aulacorthum pelargonii Kalt.) on treated plants:
(a) absence of destruction.
(b) slight destruction.
(v) destruction of 20-30% of specimens.
(g) destruction of more than 50% of specimens.

As it is seen from the given data, the use for watering of the plants of the emulsion even in a 0.2% concentration did not secure a complete destruction of aphids. At the same time the amount of penetrated insecticide reached in this case — taking into consideration the decomposition — 20-30 mg/kg, or 0.003% of the green mass of plants. In another test, when the plants remained in the shade at lower temperatures the penetration of insecticides into the plants reached 60 mg/kg. of which 50 mg/kg. were soluble in dichlorethane. In this case the destruction of aphids after 2 days reached a considerable volume. Analysis of aphids fallen off the poisoned plants disclosed phosphorous (on conversion to thiophos) in amounts of 22 mg/kg. of the live weight of the aphids.

Apparently the penetration of the insecticide into various plant species does not proceed uniformly. However, when plants of beets and cineraria were watered, the amount of insecticide which penetrated them corresponded approximately to that which penetrated chrysanthemum leaves (at different concentrations of the emulsion). In a subsequent experiment cabbage plants infested with grey aphids (Brevocaryne brassicae L.) were watered with thiophos emulsion. The amount of penetrated insecticide is indicated in table (2).

In spite of considerable amounts of penetrated insecticide, the destruction of aphids took place. This observation speaks of a considerable resistance of this aphid species. It is necessary to point out that depression and drying out of plant leaves was noted when they were watered with the emulsion in a 0.05% and higher concentration. In the cabbage test as well as in the test with chrysanthemums a considerable hydrolysis of thiophos was noted in plants. In practice /begin p. 1192/ 30 days after the watering, only products of hydrolysis in the plants remain and they are

soluble in water. Analogeous observations were made in regard to chrysanthemums and hydrangeo when they underwent analysis during a month, and the hydrolysis proceeded somewhat faster in plants which were exposed to direct sun rays.

Table (2)

Penetration of phosphorus from the thiophos compound when cabbage plants were watered

| Days after watering | Amount of penetrated phosphorus when plants were watered with emulsion on conversion to thiophos (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.025 | | | 0.05 | | | 0.1 | | |
| | Total amount mg/kg | Fraction soluble in dichlorethane, % | | Total amount mg/kg | Fraction soluble in dichlorethane, % | | Total amount mg/kg | Fraction soluble in dichlorethane, % | |
| 1 | 5 | — | | 32 | — | | 21 | — | |
| 2 | 5 | 91 | | 102 | 89 | | 107 | 75 | |
| 3 | 55 | — | | 85 | — | | 188 | — | |
| 5 | 53 | — | | 90 | — | | 237 | — | |
| 8 | 107 | — | | 142 | — | | 287 | — | |
| 18 | 56 | 31 | | 80 | — | | 230 | — | |
| 21 | 64 | 15 | | 107 | 11 | | 114 | 28 | |
| 29 | 69 | 0 | | 55 | — | | 158 | 13 | |
| 49 | 26 | 0 | | 100 | — | | 98 | — | |

An experiment was conducted for determination of thiophos penetration into leaves of plants when they were dusted with 1% dust marked $P^{32}$. Bean plants which were in a green-house (in November 1950) and were seldom exposed to sun, were dusted with a 1% dust in the ratio of 4 g. of compound per 1 $m^2$. After some time one leaf at a time was cut from the plants; the leaves were weighed and washed thoroughly until the remains of the compound were completely removed from the surface, thereafter extracted with water and dichlorethane. In analyzing the leaves, after 7 days there were found in them 8.6 mg/kg of phosphorus on conversion to diethyl-4-nitrophenyl-thio-phosphate, after 16 days — 13.5 mg/kg and after 23 days — 3.6 mg/kg; and at that, on the 7th day 72% of phosphorus was converted into dichlorethane and on the 16th day — 58%. In repeating the experiments under bright sun light (in May 1951) the entire phosphorus which penetrated the bean leaves was converted already on the 4th day, into an aqueous fraction. In wheat leaves the compound was detected only on the 2nd day after the treatment was absent.

The given data on the whole coincides with data in literature on results of chemical analyses (5) and they indicate that thiophos is not a "systemic" insecticide adaptable for the destruction of insects through plants, both by watering the soil as well as by treating the leaves, which is due to insufficient penetration and retention of it in plants. On the other hand this circumstance makes it possible to

permit treatment of fruits and vegetables before harvesting, without the danger of poisoning humans and animals who feed on fruits from treated plants.

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Scientific Institute of Fertilizers
on insectifungicides.

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Sravnitel'no-tsitologicheskoe issledovanie pyl'tsevykh trubok lilii martagon na zhivom i fiksirovannom materiale

[Comparative cytological investigation of pollen tubes of Lilium martagon on living and fixed material].

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Comparative - cytological research on pollen tubes of Lilium martagon on living and fixed material.

In the history of our country's plant embryology we find a series of names of which we can be rightly proud. Due to classical works of Russian scientists already in the last century our knowledge was formed of morphology of such an important process as fertilization. Thoroughness of research, independence of thought allowed our country's scientists to disclose the fallibility of ideas of a number of great foreign scientists and to present convincing proofs of correctness of their observations. Thus I. N. Gorozhankin's (1880, 1888) research on the development of the pollen tube and fertilization among the gymnospermae impelled Strasburger (1884) to renounce the theory of fertilization of the nucleus with a dissolved substance which he accepted earlier and to agree with I. N. Gorozhankin's proved statements on penetration of female sex elements by formed male gametes.

However no research of the last century had such an influence on further development of the embryological science as S. G. Navashin's (1898) discovery of double fertilization among angiospermae.[1]

1. V. V. Finn (1948) reports interesting data on circumstances connected with the discovery of double fertilization.

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Brilliant in execution technique, daring in wide generalization, it had a tremendous influence on the scientific biological thought. Even now, 50 years after this discovery, it retains its significance and in the light of the Michurin theory it is a starting point for new works (Prezent, 1948a, 1948b).

[begins p. 127

Unsurpassed master in the execution of preparations and drawings, S. G. Navashin strived to greater perfection. Thus he pointed out the desirability of investigation of living objects. Not accidentally did the students of S. G. Navashin suggest new methods which improve the cytological technique, not by accident did from Navashin's laboratory come the numerous investigations of the living material not altered by fixative fluids (Chernoiarov, 1929; Kostriukova and Chernoiarov, 1938; Kostriukova, 1939 - 1949; Kostriukova and Benetskaia, 1939).

Examination of spermatogenesis in living pollen particles and pollen tubes of a series of plants carried out by this author dealt also with such classical objects of previous cytological - embryological works as the lily. These examinations permitted the solving of a number of problems, among them the problem of structure of the male gametes of angiospermae which has been debated for longer than a half--century. Of decisive significane in these works was the application of methods of observation of living non - colored material with the use of large magnifacations of the microscope (x 1000, x 1500).

The successes' of these investigations brought forward a new task: to improve the technique of developing preparations in such a way as to obtain on fixed material a development picture of the generative cell and sperms similar to observations in living state. It was particularly important to obtain such a picture of Lilium Martagon, investigated by S. G. Navashin himself.

In preparing fixed preparations we used artificial cultures of pollen tubes. For living state observations the pollen tubes were grown on cover glasses spread with the fluid which appears on the stigma when it matures (Kostriukova, 1949a). It could be expected that when the cover glasses with pollen tube cultures are submerged into the fixative fluid, the results would be good, because the fixative fluid

will have a direct action on the object. But an apprehension arose, that large pollen grains of the lily only slightly glued by the secretion of the stigma, will be washed off when treated with an aqueous solution and will carry along the pollen tubes. Therefore the treatment time of the preparation from the moment of fixation and up to the inclusion into Canadian balsam was reduced to a minimum: thus the objects were keep in the fixative fluid for half an hour, in alum - 20 minutes, in hematoxylin 10 minutes. It was necessary to give up all staining. The entire treatment was carried out with cover glasses with the cultures in a horizontal position.

Of greater importance was the choice of the fixative fluid. We decided upon a chondriosome fixative with osmic acid already tested earlier (Kostriukova, 1935) which appeared to be excellent in regard to preservation of various thin cyto-plasma formations. The choice of the fixative is conditioned by the purpose of the research: to preserve in preparations the thin easily destroyed formations which we discovered during observations on material in living state - the cyto-plasm of sperm--cells, polarly located curved canals of the nucleoids or inclusion bodies [vakuom], etc.

The results of the adapted technique of treatment justified the expectation allowing to bring considerably closer to the pictures in a living state those observed in fixed preparations.

In the given research with an abundance of material we posess the best criterion - comparison with observations made [on material] in living state.

In our preparations the pollen grains were found to be inadequately fixed, probably the solid exine was an unsurmountable obstacle for penetration of such a fixative as the chondriosome fixative [begin. p. 14] which we used. But the fixed pollen tubes presented an abundant and excellent material in regard to finesse of obtained pictures we succeeded to observe all the division stages of the generative cell which allowed us to compare our data with data obtained earlier by other authors as well as with observations "in vivo".

As it is known the lily species which we took for our research represents a classical object for embryological research. The spermatogenesis of this lily was described by such renown cytologists-embryologists as Guignard (1891), Sargant (1897), Koernicke (1906), Strasburger (1908) and later - Welsford (1914) and O'Mara (1933). It was generally recognized that all the preceding as well as subsequent works are surpassed by the classical research by S. G. Navashin (1911) and neither in the completeness of the research nor in the perfection of the included drawings can there be any comparison between them, especially in regard to the study of the division stages of the nucleus. This circumstance was to a considerable degree the reason why the problem of structure and development of male gametes of the lily was considered solved by S. G. Navashin.[1]

In studying the preparations which we obtained it was possible to establish that the generative cell of the lily crosses over into the pollen tube usually at the stage of early prophase. This division stage is characterized by a marked curvature of the chromatin thread. The prophase is a prolonged stage during which the generative cell becomes considerably longer. Also the prophatic spindle lengthens, and becomes less compact. Due to staining it is readily possible to distinguish the abrupt curves of the thin chromatin thread, which when placed in one optical plane appear as short curved sections (fig. 1,a).

The cytoplasm of the generative cell is distinct from that of the vegetative one: it is characterized by a very fine, even granular structure and by grayish or yellowish staining. Due to large sizes of the nucleus and a considerable elongation of the cell, the cytoplasm is well seen mainly at the elongated ends of the cell; in the remaining part it covers the nucleus with a very thin layer. The thin surface layer of cytoplasm is not stained and does not have a granular structure. This is the so - called skin or film layer of the cytoplasm (fig. 1,a,b). S. G. Navashin mentioned in his work that he did not succeed in observing in his preparations a non - granular even very-thin layer which sets boundaries to the cell body from the outside. In investigating pollen tubes while they are in living state, particularly in certain positions of the generative cell, this layer can be well noticed.

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1. It is stated erroneously in Maheshwari's r=sume' (1949 that Cooper (1936) investigated the Lilum Martagon. Cooper's objects were Lilium regale, Auratum [?], "filippinenze" [?]

At some time we succeeded in obtaining microphotographs of a living generative cell of this lily in which the outer skin layer of the cyto-plasm is seen distinctly (Kostriukova, 1949, table IV, Micro-photo 3).[1]

Thus the methods adapted in the present research allowed us to bring closer together the pictures observed in fixed preparations and in living state.

[begin p. 15]

In preparations there remain - true, very seldom - such readily destructable formations as a peculiar nucleoid or inclusion body [vakuom] of the generative cell and sperms which we described in living cultures of pollen tubes under the name of colored bodies of the lily (Kostriukova, 1939a, 1940b). Many authors described specific inclusions in the cytoplasm of the generative cell under various names. Thus Guignard, Sargant and Welsford described centrosomes in the body of the lily's generative cell, Mottier (1898) - extra - nuclear nucleoli, Koernicke - rod - shaped corpuseles. S. G. Navashin examined in detail the formations described before him and pointed out that he too had the opportunity to observe up to five nucleolate corpuscles. Sometimes these formations entered inside symmetrically located nuclear or foamy [frothy] bodies which had a "remote resemblance to the "blefaroplast"[?] of the cycad family". Later Anderson (1939) described mito-chondrin in the cytoplasm of the generative cell.

In our investigations of living pollen tubes we did not discover any other formations besides colored bodies - peculiar symmetrically located inclusion bodies of the cell. In fixed preparations these bodies were found as formations blackened by osmic acid and only extremely seldom was it possible to discern in them some structures. On fig 1,a are shown these polarly located bodies. In one of them the reticular nuclear structure

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1. We shall point out, that we never observed in living pollen tubes a cavity surrounding the generative cell and sperms of the lily, of which Johnston (1941) speaks. He dealt probably with dead or dying cells. As to the fixed material there a lagging behind of the generative cytoplasma in comparison with the vegetative was actually observed due to the shrikking of the fixed material.

is seen clearly, the second one has become dark colored, but on its surface revolutions of the spiral can be detected in some spots. But in the majority of cases only remains of the destroyed inclusion bodies in the form of dark colored granules (fig. 1, 2) or bands (fig. 1,b) were found. We shall point out that of all the researchers S. G. Navashin came closest to the interpreting of the actual structure of colored bodies noting their polar distribution and frothy structure "similar to complex vacuoles". The diversity of inclusions in the body of the generative cell described by other authors is apparently connected with various stages of their destruction.[1]

However in spite of a somewhat more perfect preservation of these formations in our preparations, the vagueness of the inner structure would not permit one to interpret them without observations in living state. Only in living pollen tubes at high magnifications (x1500) was it possible to distinguish in the greenish - yellowish polarly located formations, the very fine winding small canals filled with a colored content, drawn tightly together and forming bodies of quite definite contours.

Thus in regard to preservation of these peculiar structures, the cytological technique for executing of fixed preparations is still far from perfect.

Further transformations of the generative cell lead to a thickening and shortening of the chromatin thread, to its breaking up into sections—chromosomes. Very complicated pictures can be observed in these transformations. S. G. Navashin called attention to the fact that the lengthiness of the little knot, its breaking up into sections in the presence of already formed cuttings, may remind one of the anaphase of division. In fig. 1,b is depicted one of such peculiar prophases. The little knot was broken up into two unequal parts. The chromatin thread is complexly curved. In the smaller part of the little knot a section of the chromatin thread is seen, one end of which winds around the other end. The characteristic figure formed by this section is similar to the violin key. [begins p. 18]. A peculiar picture is presented in fig. 1,b.

1. Apparently a similar explanation has to be given to the regeneration of the centrosome described by Ellengorn and Svetozarova (1949), in the male gametes of an object unidentified by them.

The chromatin thread has already divided into 12 chromosomes which are easy to count. The chromosomes have the shape of curved elongated corpuscles. Interesting are the shapes of the extreme left chromosome curved like a corkscrew and of the twisted one on the top right. The chromosome's shape itself indicates that these are not unchangeable, congealed, solid bodies. Such forms of chromosomes led S. G. Navashin to the notion of their independent mobility. We shall note here, that even though S. G. Navashin held the position of the chromosome theory of heredity, thorough observations of division pictures impelled him to renounce the general notion of chromosomes as hard "cleavable" bodies which only mechanically change their places in the cell, and to see in their transformations an evidence of a life process.

In the metaphase the straightened out chromosomes come close together in the central part of the cell and they are distributed symmetrically in two groups of six chromosomes each in relation to the middle plane (fig. 1, 2,b). Very often in the elongated cells this plane is obliquely oriented which was first noted by S. G. Navashin. [1] In fig. 1, 2, where a very late prophase is presented, the oblique position of the equatorial plane is already outlined. In living material the straightening out of chromosomes, characteristic for the metaphase, and their orientation in relation to the middle part of the oblique plane is well observed, but due to the mobility and transparence of the chromosomes their lengthwise division is not disclosed. In our fixed preparations it is noticeable due to the cleftlike space between the chromosomes and the forking of their ends.

During observations in living state it was sometimes possible, especially in recently dead cells, to see in the chromosomes of this stage lengthwise rows of granules described by S. G. Navashin. He indicated that he could detect these granules only with strong differentiation. We succeeded while solving other problems, to obtain in well preserved cells usually dark colored chromosomes, but thereafter it was possible to disclose the fine structure of the cytoplasm. As to the living objects, the juicy, flexible, thick chromosomes of our objects did not show any inner structure.

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1. Maheshwari (1949) remarks erroneously that S. G. Navashin did not observe a correct equatorial plane and also erroneously attributes the description of the oblique position of the equatorial plane to later foreign authors.

At this stage the cytoplasm preserves a fine-granular structure and an even staining. Only infrequently do granules occur which are stained black with osmic acid the remains of the destroyed inclusion bodies. No successive vacuolation leading according to Navashin, to the destruction of the body of the generative cell, was noticeable in our preparations. As the observations in living state indicate the generative cell in the metaphase of division somewhat shortens in comparison with the preceding stage. Its elongated ends are stretched symmetrically, at the poles are located colored bodies (Kostriukova, 1949 b, fig. 1. table IV).

Very interesting is the anaphase of the dividing nucleus. It is possible to follow up in fixed preparations the severance of daughter-chromosomes. Fig. 1,e, depicts a very early anaphase. All the chromosomes lie as yet in the cental part of the cell. Among them can be distinguished quite symmetrical pairs of stretched sister-chromosomes. Two pairs are caught at the moment of separation. Particularly interesting are two chromosomes located on the top right. These are converging sister-chromosomes; one of them has twisted in sliding off the other, each of them is directed to the opposite pole. Under this pair lies another /begin p. 19/ separating pair. Like S. G. Navashin, we did not succeed in seeing even once either in this or in the preceding as well as in the following stage the threads of the spindle in spite of applying a thin cytoplasm fixative. The threads of the spindle are not seen in living material either. It can be assumed that the spindle threads described by Koernicke in the body of the generative cell of the lily represent an artifact as it was pointed out by S. G. Navashin.

In the telephase of division the chromosomes which gathered at the poles lose their independence and in the forming daughter-nuclei a tender chromatin net is formed. The small daughter-knots pictured in fig. 2,a, are dark colored and the inner structure cannot be distinguished. But according to the outlines of the knots it can be seen that the shaping of the nuclei is not yet terminated. According to S. G. Navashin's data the cytoplasm of the generative cell at this stage becomes evenly frothy and gradually disorganizes. In our preparations no symptoms of disorganization of the cytoplasm are noticeable and the body contours of the generative cell are quite distinct. The forming daughter-nuclei have at first a roundish-oval shape. Later on they stretch out considerably and at the same time they become thinner. Fig. 2 (b-e) depicts successive development stages of sperms. A tender chromatin net is formed in the nuclei. It can be seen clearly that it consists of granules. If the

preparation is differentiated less strongly, this granulated condition becomes unnoticeable. In the nuclei of the sperms shown in fig. 2, b, the granular structure of the chromatin thread was particularly well noticeable. These sperms were located outside the pollen tube, apparently they overflowed from it together with the cytoplasm of the pollen tube at the moment of fixation.

In living cultures we had the opportunity to observe more than once how out of a burst pollen tube its content pours out. And at that time the sperms usually became deformed and instead of being elongated became round in shape. It is seen in fig. 2, b, that the elongated shape of the sperms is well preserved, because the tube burst at the moment when the preparation was immersed into the fixative fluid. This was what conditioned the preservation of the sperm shape.

In living pollen tubes the inner morphology of the sperm nucleus is seen not as distinctly though the division stages of the generative cell can be well observed. It is only that the nuclei of the sperms are less transparent, than the reposing nuclei of somatic cells.

Unlike S. G. Navashin's observations in our preparations the sperms represent well formed cells. The cytoplasm of the sperms is similar in its structure with the cytoplasm of the generative cell: it is uniformly fine-grained and grayish colored. At the poles there sometimes occur dark - colored granules—the remains of a destroyed inclusion body. (fig.2 b) The body of the male cells as well as their nucleus, elongates, the ends of the cells become pointed, as is well seen in fig. 2. g. The nuclei of late development stages of sperms are very elongate (fig. 2,e), close in length to those depicted by S. G. Navashin in the embryo sac (Navashin, 1898) with the difference that the sperms in our preparations represent cells. Thus the best obtained fixative fluid secured the best preservation for sperm structure. The possibility of preservation in fixed preparations of cytoplasm of sperms is a big step forward and is a great achievement of our suggested method.

As we mentioned in our previous works (Kostriukova, 1939 v, 1940 b.) in living cultures, in growing pollen tubes, the sperm - cells were always clearly differentiated, particularly due to the polarly situated inclusion bodies. / begin p. 20/ The difficulty of observation was conditioned by the unusual length of the pair of sperms and the rapidity of their shifting in the moving cytoplasm of the pollen tube. The sperms proved to be connected with each other, which was especially well seen during their movement. On the border - line between them, always very distinctly seen, is a thin lustrous membrane partition which separates the body of one sperm from the

other. It is morphologically similar to the skin - like layer of the generative cell and is apparently similar to the very fine membrane placed at the border between two male gametes (Kostriukova, 1941). In fixed preparations of the membrane we did not succeed in detecting the separating sperm-cells which are noticeable in observations in the living state. However it should be mentioned that Johnston independently, according to his work, describes membrane which separates the sperms in a tulip, camelia, forsythia and one of the amaryllidaceae. Johnston considers that he succeeded in discovering this formation due to the use of staining with gametoxylene according to Delafield.

Unlike the observations on a living state, in our fixed preparations are found completely isolated sperm - cells (fig. 2, в - д). On living material we happened to observe disunion of sperms only in one object - amaryllis Hymenocallis americana.

A few words on vegetative nucleus and vegetative cytoplasma. The vegetative nucleus is preserved until the late development stages. It is very large, elongated, its chromatin is dispersed in the nucleus in form of lumps of irregular shape (fig. 1, г, 2, а). It is pliable and can take on very peculiar shapes. Its morphology and behaviour indicate that it is a formation of life activity. We did not have a chance to observe symptoms of its destruction either on living or on fixed material. The weaker coloring of the vegetative nucleus does not at all indicate its degeneration or destruction as Maheshwari (1949) assumes, just as weaker coloring of the so - called resting nucleus as compared with the nucleus passing over to division does not indicate degeneration. In both cases the weaker coloring indicates only a different character of life activity. The change of form of the vegetative nucleus depending on conditions of place changing in the pollen tube shows that it is pliable, that it is able to react corresponding to the conditions of the surroundings, i. e., it also indicates that it is a life activity formation. The degeneration pictures of the vegetative nucleus described in some works, as well as the descriptions of destruction of cytoplasm of the sperms are due probably to the inperfect technique in preparing the material.

The vegetative cytoplasm in fixed preparations has a loose netted character as it is described by S. G. Navashin. In places the structure of the cytoplasm is so fine and uni - typed that it does not leave any doubt as to the good preservation of the living state morphology However it is not so. In living material the cytoplasm of the lily's pollen tube is overfilled with drops of different sizes and has the aspect of an emulsion. Besides that it is in uninterrupted motion. The fine, loose, congealed

net in fixed preparations does not at all reflect its real structure. These observations bear witness again of the exceptional significance of living material investigation for the understanding of the morphological pictures.

Summarizing the conducted research we shall point out that the methods which we applied allowed us to make a step forward in the matter of bringing closer to structures in living state of those observed in fixed preparations. At the same time these investigations showed the danger which / begin p. 21 / is hidden in a non-critical study of fixed material and the enormous importance of observations in living state as a contol for investigation of preparations.

We indicated that in cultures of living pollen tubes together with the growing and developing pollen tubes can always be observed also dead and dying ones. Towards the end of the development, at the time of sperm formation, there are considerably more dead tubes than living. Their content is at different stages of destruction and the order of this destruction is always quite definite. Longest preserved are the chromosomes which become particularly noticeable on the coarse - granular substrate of the pollen tube content. In fig. 2, a is depicted a part of the generative nucleus in a late prophase of division. Since the generative cytoplasm spread out and is not distinguishable from the vegetative it can be maintained, that the destruction went quite far, but the chromosomes as the most resistant formations, are well seen. In the three polarly distributed chromosomes the contours of their bodies are seen clearly, they are dark stained and their inner structure is not distinguishable. Of great interest are the other three elongated chromosomes. Their body is destroyed but thereafter their inner structure becomes remarkably clear. It is seen clearly that the chromosomes contain a spiral thread.

How far the non - critically perceived pictures of disintegration can lead is demonstrated in works by the cytologist Strasburger (1908). S. G. Navashin examined attentively the causes of Strasburger's errors. We shall mention only one. Strasburger described the disintegration of the body of the generative cell at the metaphase stage and the subsequent formation of naked sperm - nuclei. But he did not confine himself to this: he maintained that further then takes place disintegration of the nucleus and liberation of the chromosomes which he described as lying freely in the pollen tube. Unfortunately the imperfect methods of treatment lead some of the Soviet scientists also to such conclusions: described are not only the naked sperm nuclei but also the naked chromosomes and even naked spiral threads of sperms (Gerasimova - Navashina, 1947).

In terminating the present report we allow ourselves to point out again the particular importance which was attributed by the classics of our country's plant embryology to thoroughness of research and perfection of technique in executing preparations. Armed with Michurin's understanding of plant organism, our science, making use of lessons of history, will proceed on a new fruitful road to which it is obligated by the exceptional possibilities which are open to scientific research in the great country of socialism.

Kiev Botanical Garden imeni academician A. V. Fomin

Fig. 1 /p. 16/ Division of the generative cell of the pollen tube of Lilium Martagon.

a. prophase of division of the generative cell. The cell is greatly elongated. The elongated prophatic knot is depicted in an optical profile. In the drawing are seen only short sections of the chromatin thread due to the fact that its curves are very abrupt. On the poles of the nucleus are seen two elongated bodies dark colored with osmic acid, which represent peculiar inclusion bodies of the lily's generative cell; b. - late prophase of generative cell division. The chromosomes are formed and they straightened out somewhat. 12 chromosome can be counted; v.-prophase of generative cell division. The knot is divided into two sections. The chromatin thread is winding. In the upper part of the drawing a figure is seen which is similar to a violin key, and a dark strip - the remains of a colored body; 8. - a very late prophase of a generative cell division. The chromosomes straightened out and are distributed in the central part of the cell symmetrically to a certain oblique plane. At the frontend of the cell are seen two dark-colored nucleolus-like corpuscles-remains of the destroyed inclusion bodies. d. metaphase of the generative cell division. The pollen tube is considerably wider than the others. The shape of the generative cell indicates that it has been fixed soon after death. The chromosomes form a regular equatorial plate. The lengthwise division already took place, which is seen from the forked ends of some chromosomes. In the front (left part) is seen a part of the vegetative nucleus; e.-early anaphase of generative cell division. Beginning of separation of chromosomes. In the drawing are shown three pairs of chromosomes which are symmetrically located (lower part of the drawing). On the top at the right there are two pairs of humological chromosomes which are directed towards opposite poles. The ends of the cell are considerably elongated.

Fig. 2 /p. 17/ Division of the generative cell of the pollen tube of Lilium martagon.

a. - part of the nucleus in the late division prophase of the dead, disintegrated generative cell. Three chromosomes in the left part of the drawing preserve the usual outlines, from the other three chromosomes

remained a very fine spiral thread. The lower right side chromosome preserved unchanged only the right bent end; (b)- late telophase of the generative cell division. The daughter nuclei are not yet formed. The body of the cell is distinctly outlined; (v)- sperm cells from the discharged contents of the pollen tube. Between the cells is the section of discharged cytoplasm of the pollen tube. The stretched ends of the cells are bent, the nuclei with the fine-granular chromatin net are oval; (g)- later stage of sperm formation. The cells are fully separated from each other. They are considerably elongated; their ends are pointed, the nuclei with a fine chromatin net are also elongated. Attention is called to the difference in sizes of gamete nuclei; (d)- sperms-cells with intensely stretched, leaning on each other inner ends. Nucleolus-like corpuscles are seen — the remains of a destroyed inclusion body. In the front is a large vegetative nucleus, with lumps of chromatin; (e)- sperms-cells with very stretched dark colored nuclei. All the drawings are executed from fixed and colored pollen tubes of Lilium martagon with the help of Abbe's drawing apparatus with Zeiss's objective 90 and a compensating ocular 4, with the exception of figures 12 and 2a, executed with Zeiss's objective 90 and ocular 10.

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Prezent, I. I.

Dvoinoe oplodotvorenie i
zhiznennost'

/Double fertilization and vitality/

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## DOUBLE FERTILIZATION AND VITALITY

The greatest event in the history of plants is the acquiring by highest representatives of the plant world of the ability for double fertilization, i.e. ability to create through fertilization not only an embryo but also supply food necessary for its development.

In the preceding article dedicated to the problem of biological essence of double fertilization (Prezent, 1948) we mentioned that the endosperm, formed by fertilization and being preserved in seeds of many plant forms of higher flowering plants, is food of special kind which is an additional resource for the increase in biological plasticity and at the same time a morphological typicalness in specimens of species and varieties.

Formation of food through fertilization is the finest "method" historically developed in plants which provides heterogenity, inconsistency in the food itself and therefore serves as an additional lever for higher vitality with all the features characteristic for this rise.

Double fertilization like any fertilization increases the plasticity of living beings, their adaptability to new conditions.

But a distinction should be made between plasticity and "pliability". With limited pollination among self-pollinators and forced self-pollination among cross-pollinations, their response to the minutest fluctuations in the environment also increases. But this responsiveness of inbred plants and plants from limited pollination is only a manifestation of their instability, lowered resistance to factors of the environment which they are unable to adapt as their conditions of existence. In the minutest fluctuations in the environment the inbred plants respond with a change in their form due to which the followers of Morganism promised the selectionists /breeders/ to use inbreeding for creating of new varieties of field crops. But the Morganists reasoning abiologically, ignored the law, established already by Darwin, of biological harm caused by forced self-pollination of cross-pollinators and by prolonged self-pollination of self-pollinator plants. Ignoring the problem of vitality the Morganists saw only a certain abstract form without considering the degree of its vitality.

In reality the subjection of an organism to changes can be of two kinds and at that opposite in their biological essence: it is possible to increase the variability of an organism by strengthening its vitality and in an

opposite way — by weakening its vitality. The first way is — increasing the adaptability of living beings, of their ability to reconstruct their nature through acceptance of new environmental factors, through transformation of these new factors into conditions of their development and existence. The second road is — a lower adaptability of living beings, lowering of the ability to transform new environmental factors into conditions of their existence, lowering of the resistance to exterior influences and therefore a greater subjection to the latter.

Depriving the seed of the endosperm, formed by fertilization, leads to results of the same order as the inbreeding among cross-pollinators and the limited pollination among self-pollinators. (Begin p. 60)

In an article in 1948 we reported that growing of self-pollinator plants from an embryo without an endosperm, which was removed from slightly swollen seeds (further-on such seeds are called for shortness "seeds without endosperms") led to a variety of plant forms caused by their instability to fluctuations of the environment. Even when grown in the same bed a series of changes was observed among plants being tested. A particularly sharp deviation from the norm was in one wheat plant of the Al'bidum 604 variety which was bred from a seed without an endosperm. In it features formed, characteristic for the Veliutinum species, i.e. the spike was white awnless, pubescent and the grain was red. It is true, the pubescence of the covering scales in this plant was not continuous as in Veliutinum, but was only along the edge of the covering scales (fig. 1).

Fig. 1. (p. 60) Right - pubescent spiklet scales of an Al'bidum 604 variety plant grown in 1947 from an embryo isolated from the endosperm; left - non-pubescent spiklet scales of the control plant of the same variety.

Table 1 (p. 60)
Progeny of the embryo of spring wheat Al'bidum 604, isolated in 1947 from the endosperm.

| Year of sowing | Amount of obtained | |
|---|---|---|
| | Plants | Varieties |
| 1947 . . . . | 1 | 1 |
| 1948 . . . . | 8 | 4 |
| 1949 . . . . | 546 | 22 |
| 1950 . . . . | 32052 | 31 |

It could be assumed that the appearance of a plant with such sharp deviations is the result of a mechanical or biological contamination of the original material, i.e. that the original seed producing this plan was a hybrid or a mechanical admixture. True, such supposition was little probable due to the fact that each grain was examined when the endosperm was removed and among the original treated grains of Al'bidum 604 wheat no red grains were disclosed.

In order to find out whether the sharp deviation occurred actually because the embryo of the Al'bidum 604 seed was deprived of the endosperm, we

analyzed one after another (in 1948, 1949 and 1950) the progeny of this original seed.(1) Each year the sowing was carried out with whole (with endosperms) seeds, in families, i.e. seeds of one plant were sown in separate rows. The ancestor of each individual family being analyzed is an individual grain of the original deviated plant. Thus, from the plants sharply deviating from the original were gathered after threshing in 1947 nine grains of which one perished after sowing, in 1948 eight families were formed each one of which was (begin p. 61) analyzed in its varietal make-up during the subsequent generations. Data on numbers of plants grown in families and of originated varietal forms are given in tables 1 and 2.

From the original deviated plant types of variety Veliutinum, in 1948 were obtained 4 varietal forms, in 1949 - 22 and in 1950 - 31 (table 2) (2)

The results of the analysis of these varietal forms carried out every year, impel us to renounce the hypothesis that the original seed which gave in 1947 a plant with sharp deviations, was a mechanical admixture or a hybrid. If, for example, one of the families in 1948 (family 7) was a type of Veliutinum and the white coloring of the spikes remained the same among all the plants in the progeny of 1949, then in 1950 in the progeny of this family, along with plants having white spikes, there appeared also plants with red spikes i.e. from a recessive character "chipped out" (?) a dominant one.

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(1) Participating in all the experimental works described in this article were E. S. Strutsovskaia, Z. H. Khokhlova and particularly closely participating was A. G. Lazareva.

(2) The varietal make-up of forms obtained in the progeny was checked by E. M. Iakubtsiner - a specialist on wheat taxonomy - for which I extend to him my thanks.

Table 2 (p. 61)

Varieties of the progeny of the Al'bidium 604 wheat embryo which was separated from the endosperm in 1947.

| 1947 | 1949 | |
|---|---|---|
| 1. Veliutinum | 1. Veliutinum | 11. Eritrospermum |
| | 2. Liutescens | 12. Ferrugineum |
| 1948 | 3. Pyrotrix | 13. Meridionale |
| | 4. Barbarossa | 14. Gostianum |
| 1. Veliutinum | 5. Al'bidum | 15. Turtsikum |
| 2. Liutescens | 6. Al'borubrum | 16. Subgrekum |
| 3. Pyrotrix | 7. Mil'turum | 17. Suberitrospermum |
| 4. Barbarossa | 8. Leukospermum | 18. Subferrugineum |
| | 9. Del'phi | 19. Submeridionale |
| | 10. Grekum | 20. Subgostianum |
| | | 21. Subturtoikum |
| | | 22. Subbarbarossa |
| 1950 | 10. Grekum | 21. Subturtsikum |
| | 11. Eritrospermum | 22. Subbarbarossa |
| 1. Veliutinum | 12. Ferrugineum | 23. Al'binfliatum |
| 2. Liutescens | 13. Meridionale | 24. Tsinereum |
| 3. Pyrotrix | 14. Gostianum | 25. Geratikum |
| 4. Barbarossa | 15. Turtsikum | 26. Tsianotrix |
| 5. Al'bidum | 16. Subgrekum | 27. Eritroleukon |
| 6. Al'borubrum | 17. Suberitrospermum | 28. Tsezium |
| 7. Mil'turum | 18. Subferrugineum | 29. Rubromarinum |
| 8. Leukospermum | 19. Submeridionale | 30. Suberitroleukon |
| 9. Del'phi | 20. Subgostianum | 31. Subtsezium |

The same was observed also in a series of other families in which white-spike plants (recessive character) in a number of cases generated red-spike (dominant character) progeny.

The plant of family 1 in 1948 was awned (recessive character). In 1949 in the progeny of this family along with awned forms there were formed also awnless ones, i.e. dominant forms. In turn the plants of this family which were awned in 1949, produced in 1950 a progeny in which there were not only awned but awnless forms as well.

The same was noted in other families as well. The plant of family 5 was non-pubescent in 1948 and in 1949 in its progeny together with non-pubescent i.e. recessive forms were also pubescent, dominant forms. In turn, in 1950 in the progeny of non-pubescent plants of the same family there appeared non-pubescent as well as pubescent plants.

(begin p. 62)

The same has been noted in regard to the coloring of the grain. In 1949 in the progeny of the plant which in 1948 had a red grain(dominant), there were formed together with plants having red grains also plants with white grains (recessive) and the latter produced in 1950 not only plants with white grains but also plants with red grains, i.e. dominant according to the characteristics of color (tables 3-6).

Table 3 (p. 62)
Appearance of awnless plants from awned ones in the progeny of the embryo of spring wheat Al'bidum 604 which was separated in 1947 from the endosperm*.

| No. of family | 1948 Second generation | | | | 1949 Third generation | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of plants | of them awned | semi-awned | awnless | Number of plants | of them awned | semi-awned | awnless | |
| 1 | 1 | 1 | - | - | 138 | 136 | - | 2 | (continued below) |
| 2 | 1 | - | - | 1 | 33 | 7 | 13 | 13 | |
| 3 | 1 | - | - | 1 | 50 | 13 | 12 | 25 | |
| 4 | 1 | - | - | 1 | 125 | 23 | 70 | 32 | |
| 6 | 1 | - | - | 1 | 106 | 12 | 52 | 42 | |
| | 5 | 1 | - | 4 | 452 | 191 | 147 | 114 | |

| No. of family | 1950 Fourth generation (progeny of awned forms obtained in 1949) | | | |
|---|---|---|---|---|
| | Number of plants | of them awned | semi-awned | awnless |
| 1 | 10043** | 9996 | 28 | 18 |
| 2 | 396*** | 362 | 18 | 5 |
| 3 | 654 | 647 | - | 7 |
| 4 | 1136 | 1131 | 1 | 4 |
| 6 | 410**** | 397 | - | 12 |
| | 12639 | 12533 | 47 | 46 |

* In this and in tables 4, 5, 6 is presented an analysis of features of the spike in the progeny from one changed plant of first generation (1947) which had an awnless pubescent white spike and red grains(variety veliutinum).

** Among them one plant which had awned and semi-awned spikes

*** Among them eleven plants with shortened awns

**** Among them one plant which had awned and awnless spikes.

Thus in the progeny of one original plant changed (altered) during the growth from a seed without an endosperm, there was formed an enormous number of varietal forms and all the basic varietal features were changed: color of the spike, awns, pubescence, color of the grain. All the varietal features which according to their character were recessive, in the progeny of a number of families became dominant, which compels one to reject the hypothesis of hybrid character of the original grain.

The diversity of appeared varietal forms increased each year. In 1950 among them were such little distributed forms as the Tsianotrix, but the spikes had not the characteristic for Tsianotrix, smoky gray coloring on a red background, but a dark brown one. Besides the 31 varietal forms, in the 1950 yield were also 43 plants of Rigidum type with a very coarse, brittle spike, hard scales and grains difficult to thresh, 55 plants of Subrigidum type, i.e. semi-coarse type, and inflated forms with inflated scales, and very short awns bent like a sickle.

In the progeny of the changed plant of Al'bidum 604, 14 plants in 1950 had branching stems (from the first true stem node, there branched out additional stems). Particularly important is the circumstance that 9 plants in 1950 formed within the limits of one plant both awnless as well as awned spikes. Among these plants was one which formed an inflated spike together with two awned spikes of the Gostianum variety (fig. 2, 3). One of these nine plants various (within the limits of one bush) spikes (begin p. 63) formed an awned spike on an additional stem, which branched out of the first true above-ground node, while its other stems had awnless spikes. This fact compels one to reject the hypothesis that plants which had within the limits of one bush spikes of different varietal forms intertwined or grew together their root systems. Besides such a hypothesis was in itself little probable, because each grain was sown into the soil separately (under its recording number).

Table 4 (p. 63)

Appearance of pubescent plants from non-pubescent ones in the progeny of the embryo of spring wheat Al'bidum 604 which was separated from the endosperm in 1947.

| Number of family | 1948 | | | 1949 | | | Fourth generation (progeny of non-pubescent forms obtained in 1949) 1950 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Second generation | | | Third generation | | | | | |
| | Number of plants | of which pubescent | of which non-pubescent | Number of plants | of which pubescent | of which non-pubescent | Number of plants | of which pubescent | of which non pubescent |
| 1 | 1 | 1 | - | 138 | 124 | 14 | 801 | 15 | 786 |
| 2 | 1 | 1 | - | 33 | 24 | 9 | 555 | 4 | 551 |
| 3 | 1 | 1 | - | 50 | 38 | 12 | 684 | 124 | 560 |
| 4 | 1 | 1 | - | 125 | 87 | 38 | 2214 | 584 | 1630 |
| 5 | 1 | - | 1 | 20 | 1 | 19 | 1414 | 17 | 1397 |
| 6 | 1 | 1 | - | 106 | 79 | 27 | 1162 | 29 | 1133 |
| 7 | 1 | 1 | - | 6 | 4 | 2 | 94 | 1 | 93 |
| 8 | 1 | 1 | - | 68 | 49 | 19 | 953 | 42 | 911 |
| | 8 | 7 | 1 | 546 | 406 | 140 | 7877 | 816 | 7061 |

Table 5 (p. 63)

Appearance of plants with colored spikes from plants with white spikes in the progeny of the embryo of wheat Al'bidum 604 which was separated from the endosperm in 1947.

Table 5

| Number of family | 1948 Second generation | | | 1949 Third generation | | | 1950 Fourth generation (progeny of forms with white spikes obtained in 1949) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of plants | of which with spikes | | Number of plants | of which with spikes | | Number of plants | of which with spikes | |
| | | colored | white | | colored | white | | colored | white |
| 1 | 1 | 1 | - | 138 | 68 | 70 | 5671 | 113 | 5558 |
| 3 | 1 | - | 1 | 50 | - | 50 | 2574 | 27 | 2547 |
| 4 | 1 | 1 | - | 125 | 100 | 25 | 1323 | 30 | 1293 |
| 5 | 1 | - | 1 | 20 | 2 | 18 | 1333 | 41 | 1292 |
| 6 | 1 | - | 1 | 106 | - | 106 | 3762 | 112 | 3650 |
| 7 | 1 | - | 1 | 6 | - | 6 | 274 | 16 | 258 |
| | 6 | 2 | 4 | 445 | 170 | 275 | 14937 | 339 | 14598 |

The results of the described experiment demonstrate how great an importance the endosperm has for the plant's vitality and that the depriving the seed of the endosperm (begin p. 64) has the same effect as the limited pollination which usually deprives the plant of stability to fluctuating conditions of the environment and therefore creates variety of forms just because of the weakening of their vitality.

What effect will be produced by back crossing of all this enormous diversity of varietal forms obtained from the original seed Al'bidum 604, will it be favorable (in the sense of higher vitality).---further research will show.

We also conducted work on clarification of biological significance of the endosperm for rye. For the experiment was taken the winter rye of Viatka, Tulunskaia green-grained and spring rye Onokhoiskaia.

Table 6 (p. 64)

Appearance of plants with red grains from plants with white grains in the progeny of the embryo of spring wheat Al'bidum 604 which was separated from the endosperm in 1947.

 



Table 6

| Number of family | 1948 Second generation | | | 1949 Third generation | | | 1950 Fourth generation (progeny of forms with white grains obtained in 1949) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of plants | of which with grains | | Number of plants | of which with grains | | Number of plants | of which with grains | |
| | | red | white | | red | white | | red | white |
| 4 | 1 | 1 | - | 125 | 117 | 8 | 397 | 2 | 395 |
| 6 | 1 | 1 | - | 106 | 90 | 16 | 724 | 20 | 704 |
| | 2 | 2 | - | 231 | 207 | 24 | 1121 | 22 | 1099 |

Researchers point out that with inbreeding, in rye usually there appear plants with most diverse deviations from the norm. Thus, for example, according to data by V.F. and V.I. Antropov (1929) among the inbred rye plants which they grew were forms without a wax film, forms not able to tiller, albinos, plants with curling leaves, plants in shape of spike similar to couch grass, plants of winter crop type, plants with a changed color of grain etc.

Deviations in the form of plants analoguous to the results of inbreeding were obtained by us when growing rye from seeds without the endosperm. In sowings of 1949 and 1950 were found albinos, multicolored plants, spikes without seeds, grains without embryos, plants without a wax film, dwarfs and semi-dwarfs, plants in which the first spike is shorter than the additional ones[3], plants of "winter crop" type, which tillered during six months remaining at the tillering stage until their harvest in October. In sowing with whole seeds from plants of seeds without endosperm, a certain diversity of forms was also noted. But in sowing of such progeny again with seeds without endosperms the diversity of plants particularly in the length of the growing period increased considerably.

The examination of experimental plants of the spring wheat Onokhoiskaia indicated a very great variety of spikes on various bushes. Among them were clavate (club-shaped) spikes, narrow loose and narrow solid ones, as well as spikes with very short (begin p. 65) awns. In the control such diversity was not disclosed.

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[3] Among the 204 recorded plants grown from seeds without endosperms there were 57.8% in which the first spike was shorter than the following ones. In the control only 10% of the recorded 100 plants had a shorter first spike.

Fig. 2 (p. 65) Spikes of plant no. 1 (from plot no. 205), of the fourth generation from the embryo of seed Al'bidum 604, separated in 1947. X

The central spike is from a plant of inflated form, variety Geraticum; the two side spikes are from the same plant of the Gostianum variety (Yield 1950).

Fig. 3 (p. 65) Spike of plant no. 12 (from plot no. 440) of the fourth generation from the embryo of seed Al'bidum 604, separated in 1947.

Spike of form Al'binflatum; left-front side, right-side view; center-schematic drawing of spiklet scales of the given spike. (Yield 1950).

It is very interesting, that in sowing with whole seeds the progenies of plants grown the preceding year from seeds without endosperms, deviations from the norm were also discovered. For example, six plants were found without a wax film on stems, leaves and spikes, and 25 plants were dwarfed and semi-dwarfed. One of the dwarf-plants had a light-green stem with narrow leaves and a dark-green stem with wide leaves. A case took place when flowers blossoming at different times appeared on one plant: on one side of the spike they finished blossoming by July 19, 1950 while on the other side of the spike the flowering had not even started. One side of this spike had short awns and awns of medium length, and the other--long ones.

* * *

In order to clarify the significance for the vitality of plants of the endosperm as a body formed by fertilization (double fortilization), it was necessary to verify experimentally the same order of nature of the effect caused by removing the endosperm and the effect caused by inbreeding, as well as limited pollination, not only according to morphological characteristics but according to indicators of viability and productivity as well. It was necessary also to find out the importance of such a method as intravarietal crossing for increase in vitality not only for the nature of the embryo but for the endosperm as well. For this purpose a series of experiments were conducted.

Experiment 1. Comparative survival rate of rye plants of the spring variety Onokhoiskaia which were grown from whole seeds and from seeds without endosperms was examined.

In the control from whole seeds (control plants as well as the experimental ones were grown first in paper cups with soil and then in the

X The plants in second, third and fourth generation demonstrated in this figure, as well as in figure 3 were reproduced from whole seeds.

field, in the ground) at the moment of planting into the ground 8% of plants were eliminated and at the moment of heading--12%. And in the experiment (sowing of seeds without the endosperm) at the moment of planting from the paper cups into the ground there was eliminated 28.9% of plants, and at the beginning of heading--56.6%. Growing of the next generation (begin p. 66) also from seeds without endosperms reduced the viability of plants even more: at the moment of heading 64.8% of this series were eliminated.

Experiment 2. It was investigated how the depression from limited pollination reflects on productivity of rye plants grown from whole seeds and from seeds without endosperms. The work was conducted with winter rye Viatka. For a common isolotor were taken the spikes of ten bushes.

With limited pollination the plants which were grown from seeds without endosperms proved to be less productive than plants from whole seeds. Due to special care, the plants from seeds without endosperms were more tillered (bushy), they had on the average, 15.5 spikes per bush, while in the control the average per bush was 13.1 spikes. Therefore the possibility of cross-pollination among plants from seeds without endosperms was greater. In spite of that the productivity of plants from seeds without endosperms proved very much lower than in the control. While in the control the average per one spike was 10.6 and per one plant--138.8 grains, in the experiment the average per spike was 6.2 and per plant--96.1 grains.

Experiment 3. The plants of winter rye Tulunskaia green-grained, were grown:

1) from whole seeds from free pollination;
2) from seeds without endosperms from free pollination;
3) from whole seeds obtained from inbreeding within the limits of one spike;
4) from seeds without endosperms obtained from inbreeding within the limits of one spike.

Plants of all the variants were compared according to indicators of winter-resistance and productivity (table 7).

Table 7 (p. 66)

Comparative winter-resistance and productivity of plants of rye Tulunskaia green-grained, grown from seeds without endosperms and from whole seeds of cross-pollination and inbreeding; 1950-1951.

Table 7

| Origin of seed | Sowing material | Sown seeds or embryos in 1950 | Germinated | % of germination | Hibernated plants (record 19.IV 1951) | % of hibernated plants in ratio to number of sown plants or embryos | Harvested plants | Average height of plants in c m | Average number of spikes per 1 plant | Average weight of one plant in g. | Weight of 1000 grains in g. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| From free repollination | Whole grain | 64 | 61 | 95,3 | 54 | 84,4 | 48 | 142,9 | 7,7 | 4,68 | 21,81 |
| Same | Germ | 71 | 66 | 93,0 | 20 | 28,2 | 16 | 120,3 | 5,1 | 3,01 | 16,82 |
| From inbreeding (within the limits of one spike) | Whole grain | 62 | 49 | 79,0 | 28 | 45,1 | 25 | 121,3 | 6,8 | 3,35 | 16,38 |
| Same | Germ | 66 | 40 | 60,6 | 4 | 6,0 | 3 | 75,0 | 2,7 | X in isolators | |

X The spikes of the plants in the given variant were taken under the isolator for further tests and therefore their productivity was not calculated.

The obtained results demonstrated one type character of the negative effect of inbreeding and removing of endosperms. Inbreeding as well as growing of plants from seeds without endosperms leads to lower vitality according to indicators of winter-resistance and productivity. In the variant in which (begin p. 67) the negative effect of inbreeding is aggravated by the negative effect of growing plants from seeds without endosperms, the vitality drops catastrophically.

Experiment 4. The productivity of the progeny of plants from seeds of the spring rye Onokhoiskaia, inbred and without endosperms was studied. During the test year (1951) the plants were grown from whole seeds in all the variants. The following variants were in the experiments:

1) second generation of inbred seeds of the 1949 yield;
2) second generation of plants grown in 1949 from seeds without endosperms;
3) second generation of plants grown in 1949 from seeds without endosperms and subjected the same year to inbreeding;
4) first generation of plants grown in 1950 from seeds without endosperms;
5) first generation of plants grown in 1950 from seeds without endosperms, derived (?) in 1949 from inbreeding;
6) first generation of plants which were grown during the two preceding years (1949 and 1950) from seeds without endosperms;
7) first generation of plants grown in 1950 from inbred seeds without endosperms (in 1949 the parent plants were grown also from seeds without endosperms);
8) control plants reproduced in the same years and in the same plots as the experimental ones.

Table 8 (p. 67)

Productivity of plants of spring rye Onokhoiskaia depending on the reproduction method of preceding generations. Yield of 1951, sowing with whole seeds.

Table 8

| Variant of the experiment | Recorded plants | Yield of grains in g. total in the variant | Yield of grains in g. average per one plant | Weight of 1000 grains per g. |
|---|---|---|---|---|
| 1. Second generation of plants inbred in 1949 | 55 | 275.5 | 5.1 | 25.90 |
| 2. Second generation of plants grown in 1949 from seeds without endosperms | 252 | 569.3 | 2.26 | 24.33 |
| 3. Second generation of plants grown in 1949 from seeds without endosperms and subjected to inbreeding in the same year | 23 | 62.4 | 2.71 | 16.82 |
| 4. First generation of plants grown in 1950 from seeds without endosperms | 1067 | 3538.35 | 3.32 | 26.66 |
| 5. First generation of plants grown in 1950 from seeds without endosperms; in 1949 the parent plants were inbred | 107 | 517.6 | 4.84 | 26.84 |
| 6. First generation of plants grown in 1950 from seeds without endosperms; in 1949 the parent plants were grown also from seeds without endosperms | 1121 | 2767.65 | 2.47 | 25.40 |
| 7. First generation of plants grown in 1950 from seeds without endosperms; in 1949 parent plants were grown also from seeds without endosperms and were inbred | 40 | 142.6 | 3.57 | 25.55 |
| Control..... | 138 | 392.5 | 2.8 | 23.83 |

/begin p. 68/As it is seen from table 8 the greatest ( in comparison with the rest of the variants and the control) productivity was in the first variant, i. e. in the second generation from inbred seeds. It is known that the first normal crossing after inbreeding gives higher productivity which was confirmed also in our experiments. Very close to indicators of the first variant are the indicators of the fifth variant, i. e. the first normally grown progeny from parents which were grown from seed without endosperms obtained from inbreeding. This was the result of the fact that after the depression caused by inbreeding and removing of endosperms, the plants were grown from normal seeds with endosperms derived /?/ from normal pollination. Increased productivity as compared with the control is the result of a return to normal growing of plants after the depression of parential forms.

Of particular interest is the fourth variant because it gives a possibility to clarify the existence of parallelism in the behavior of the first progeny normally formed from inbred parents and from parents grown from seeds without endosperms. As the experiments demonstrated the first progeny formed normally (from whole seeds) from plants grown from seeds without endosperms, is more productive than the control. This speaks of the fact that the return to normal conditions of reproduction causes in the progeny of parent-plants grown from seeds without endosperms, a higher productivity analoguous to that which is observed in progeny grown from normally derived seeds of inbred parents.

During a repeated normal reproduction (second variant) the plants came in productivity close to the control. This return to normal is of the same order as the return to normal during a repeated normal reproduction by cross pollination of the progeny of inbred plants.

It should be pointed out that in the sixth variant, i.e. in the progeny of plants which were grown for two generations from seeds without endosperms, albinism of nodes (in 26 plants) was observed.

The removal of endosperms leads, like inbreeding, to depression and the return to normal reproduction (i. e. to sowing with whole seeds), like the return to cross-pollination among inbred plants--to an increase of productivity which disappears in subsequent generations as it disappears in subsequent generations, of a normally pollinated progeny of inbred plants.

Experiment 5. The problem was studied whether not only the embryo but the endosperm of the seed as well is a bearer of depression inherent to inbred seeds. For that purpose to the inbred or regular embryo of spring rye Onokhoiskaia was added an inbred or regular endosperm. In the experiment were compared:

1) regular seeds - control (conventional sign K);
2) seeds with a regular embryo and added to it regular endosperm of other seeds of the same variety (conventional sign K/K);
3) seeds with a regular embryo and added it inbred endosperms (conventional sign K/I);
4) whole inbred seeds (conventional sign I);
5) seeds with an inbred embryo and an added to it inbred endosperm of another seed (conventional sign I/I);
6) seeds with an inbred embryo and an added to it regular endosperm (conventional sign I/K);

The results of the experiment are presented in table 9.

As it is seen from table 9 the germination of seeds is higher in the first variant, i. e. among the regular control seeds K; then it gradually decreases in variants K/K, K/I, I/K, I/I.

(Begin p. 69) Thus adding of the inbred endosperm K/I to the control embryo decreased the germination as compared with seeds in which to the control embryo was added a control endosperm K/K. Addition of the control endosperm I/K to the inbred embryo increased the germination as compared with seeds in which to the inbred embryo was added the inbred endsoperm I/I. Due to the additional transplanting of an inbred endosperm to a regular embryo K/I the productivity of plants (average yield, average number of seeds per one plant) decreased.

As to the adding of the control endosperm I/K to the inbred embryo, the absolute weight of grain somewhat increased due to that, but the average yield of grain and the average number of seeds per one plant was even less than in the variant I/I. This fact which is not in accordance with others we explain as follows: in the variant with the addition of an inbred embryo to an inbred endosperm I/I only five plants remained for harvesting, which, of course, were the most vital ones in the given variant, and as a result the average yield per one preserved plant was relatively high.

Table 9 (p. 69) Germination and productivity of plants of spring rye Onokhoiskaia as an indicator of the role of endosperm of inbred seeds in the development of plants (inbreeding within the limits of one spike)

..Yield of 1951..

| Sowing material | Variant (conventional sign) | Average weight of sowing seeds in g. | Sown seeds | Sprouted plants | % of germination |
|---|---|---|---|---|---|
| Whole seeds | K | 29.4 | 18 | 18 | 100 |
| Embryo with added to it endosperm of another seed of the same variety | K/K | 30.4 | 20 | 18 | 90 |
| Embryo with added to it inbred endosperm of another seed of the same variety | K/I | 32.4 | 27 | 23 | 85.2 |
| Whole inbred seeds | I | 29.7 | 19 | 15 | 78.9 |
| Inbred embryo with added to it inbred endosperm of another seed of the same variety | I/I | 32.1 | 19 | 9 | 47.4 |
| Inbred embryo with added to it an endosperm from control seeds of the same variety | I/K | 34.6 | 27 | 22 | 81.5 |

| Sowing material | Preserved and recorded plants in harvesting | Average yield of grain per 1 plant in g. | Average number of seeds per 1 plant in g. | Weight of 1000 grains in g. | Average yield for sown grain in g*. |
|---|---|---|---|---|---|
| Whole seeds | 16 | 10.90 | 397.0 | 27.44 | 9.68 |
| Embryo with added to it endosperm of another seed of the same variety | 16 | 7.39 | 283.9 | 26.02 | 5.91 |
| Embryo with added to it inbred endosperm of another seed of the same variety | 21 | 6.02 | 254.1 | 23.66 | 5.52 |
| Whole inbred seeds | 13 | 4.90 | 198.8 | 24.66 | 3.54 |
| Inbred embryo with added to it inbred endosperm of another seed of the same variety | 5 | 4.16 | 180.2 | 23.09 | 1.22 |
| Inbred embryo with added to it an endosperm from control seeds of the same variety | 18 | 3.82 | 154.6 | 24.79 | 2.86 |

* In calculating the yield in regard to the sowed grain, not calculated were the grains, the plants from which were destroyed by the Swedish fly or due to mechanical damages.

With a calculation of the screenings the average yield per one sown grain in variant I/K is higher than in variant I/I.
(begin p. 70)

Final conclusions on experiment 5 can be drawn only after repeating it on a wider scale and after studying the progeny of plants of all the variants.

Experiment 6. The purpose of the experiment is to clarify the significance of an intra-varietal crossing as a method increasing the vitality of self-pollination plants not only for the embryo of seeds but for their endosperms as well.

The endosperms in seeds of spring wheat variety Tulun ZA/32 obtained from intra-varietal crossing were removed and in their stead were added endosperms from other seeds of the same variety obtained also from an intra-varietal crossing (conventional sign V/V). Simultaneously to the embryos of regular (control) seeds instead of their own were added endosperms of seeds of the same variety but derived from intra-varietal crossing (conventional sign K/V.) The regular seeds of the control underwent a similar treatment: to their embryo was added a foreign endosperm from control (regular) seeds of the same variety (conventional sign K/K).

The results of the experiment are presented in table 10.

The results of transplanting embryos of spring wheat Tulun ZA/32 on endosperms of seeds of other plants of the same variety. The transplanting took place in the spring 1951.

Yield 1951

| Indicator | Embryo from intra-varietal crossings transplanted on endosperms of seeds | | Embryo of control seeds transplanted on endosperm of seeds | |
|---|---|---|---|---|
| | of the control | from intra-varietal crossings | from intra-varietal crossings | of the control |
| | V/K | V/V | K/V | K/K |
| Number of plants | 15 | 10 | 15 | 15 |
| Average height of plants in cm | 105.8 | 106.4 | 102.4 | 103.13 |
| Average total weight of plants in g | 13.8 | 12.5 | 10.4 | 9.9 |
| Average number of stems per bush | 8.4 | 8.2 | 7.5 | 7.0 |
| Average number of spikes per bush | 8.2 | 8.0 | 7.4 | 6.4 |
| Of this number-productive | 7.1 | 6.9 | 5.8 | 5.0 |
| Average length of main spike in cm | 9.5 | 10.1 | 9.6 | 8.8 |
| Average number of grains in main spikes | 36.4 | 40.4 | 35.3 | 33.2 |
| Average weight of grains in main spike in g. | 1.33 | 1.25 | 1.09 | 1.06 |
| Average number of grains per bush | 141.6 | 145.3 | 127.8 | 113.5 |
| Average weight of grains per bush | 3.46 | 3.67 | 3.1 | 2.82 |

As it is seen from table 10 the average length of the main spike, the average number of grains in it, the average number of grains and the average weight of the grain per one plant, i. e. the basic indicators of seed productivity, are best in the variant V/V where the embryo and the added endosperm are obtained from intra-varietal crossing. Adding of the embryo or the endsoperm from an intra-varietal crossing to regular endosperm or embryo also increases the productivity in comparison with the control.

The data of the experiment indicate that not only the embryo, but also the endosperm of seeds from intra-varietal crossing increase the vitality of plants. The selectivity of fertilization reflects not only on the embryo but also on the product of the double fertilization--the endosperm, by the results of which is the vitality of the plant produced increased.

It is very significant that at the beginning after the fertilization the endosperm outstrips in its development the development of the embryo. This is indicated (begin p. 71) by the results of a series of anatomical-embryological investigations (V. G. Aleksandrov, 1937; V. A. Poddubnaia - Arnol'di, 1947). And only after the endosperm begins

to turn into a reserve nutrient substance does the embryo begin to develop vigorously.

What is the biological significance of the fact that the endosperm and the embryo change places in the speed of development: that the vigorously developing endosperm is later on left behind in development tempo by the embryo which begins to develop vigorously?

The biological meaning of this regularity is in the fact that the endosperm "serves" the development of the embryo and therefore it develops vigorously up to the moment when it becomes ready to be used by the embryo. Only from this moment can the embryo start its development using the endosperm as nutrition.

The embryo draws out of this nutrition formed by fertilization a specific kind of supplementary variance in connection with which its vitality slightly increases. And inasmuch as the embryo - as it is indicated by the anatomist - embryologist prof. Aleksandrov--uses for its development not only stored substances of the endosperm, but also its tissue, cover, nucleus and protoplasma, it is comprehensible what a large additional stimulus of variance and therefore of vitality is drawn from the endosperm by the embryo and the little plant growing from the seed.

And at that, if the endosperm belongs to another seed even of the same variety, its role as an additional lever of variance of development of the embryo and the young plant is still greater.

The embryo draws from the endosperm, which is prepared to be assimilated, an additional stimulus of vitality already from the first moments of its embryonic development and not only from the beginning of plant development from the embryo. From here follows a very important theoretical and methodical conclusion: the sooner the embryo will be provided with nutrition saturated with great variances--the endosperm, the greater will be the vitality of the embryo and the little plant developing out of it.

For an experimental confirmation of this statement an immature embryo which had not finished its development was transplanted on an endosperm of another grain of the same variety during the stage of early wax ripeness. The thus obtained heterogenous seeds were kept through fall and winter until sowing[4] time. In the spring in other seeds of

4) The experiment was conducted with grains of spring wheat Lutescens 62. When transplanted during the early wax ripeness stage the embryo is so strongly attached to the endosperm that the grain can be very well preserved until spring of next year, even not under sterile conditions.

the same variety of the same reproduction the endosperms were also replaced by endosperms of other grains of the same variety. Seeds of both transplanting dates (fall and spring) were sown simultaneously.

Even the fall, i. e. comparatively late (according to stage of seed formation), intra-varietal transplanting of the foreign endosperm has an advantage as compared with the spring transplanting, i. e. transplanting of a mature embryo on a mature endosperm. In plants from grains of fall transplanting, more productive stems are formed and they are all more vigorous and productive than the control plants and the plants of the spring transplanting. This is the result of the use by the embryo of the foreign endsoperm which was taken for transplanting from a not yet absolutely mature seed.

Seeds of plants from "heterogenous" seeds of fall and spring transplanting were sown the following spring separately in fractions without (begin p. 72) any surgical interference (sowing with whole seeds). In 1951 the first generation from whole seeds was obtained --the generation of original "heterogenous" seeds of fall transplanting of 1949 and spring-- of 1950. (table 11).*

Yield of 1951

| Indicator | First generation from transplanting: fall (1949) | spring (1950) | Control |
|---|---|---|---|
| Number of plants | 120 | 100 | 100 |
| Average height of plants in cm | 122.69 | 115.87 | 120.65 |
| Average weight of plants in g | 12.13 | 10.30 | 8.52 |
| Average number of stems per one plant | 5.53 | 4.21 | 3.91 |
| Average number of spikes per one plant | 5.20 | 4.06 | 3.72 |
| Average number of productive spikes per bush | 4.23 | 3.33 | 3.54 |
| Average length of main spike in cm | 9.00 | 9.06 | 8.63 |
| Average number of grains in the main spike | 38.26 | 41.9 | 33.85 |
| Average weight of grains of the main spike in g | 1.78 | 1.75 | 1.40 |
| Average number of grains per one plant | 133.45 | 121.14 | 100 |
| Average weight of grain per one plant in g | 4.89 | 4.53 | 3.43 |
| Mass heading | 6-10/7 | 8-11/7 | 8-11/7 |

As it is seen from table 11, the basic indicators of productivity (average weight of plants, average number of stems, average number of grains and average weight of grain per one plant) in the progeny of "heterogenous" seeds of fall transplanting is higher not only than in the control but higher than in the progeny of "heterogenous" seeds of spring transplanting as well.

*Results of transplanting embryos of spring wheat Liutscens 62 on endosperms of seeds of other plants of the same variety. Sowing with whole seeds. /Table 11, p. 72/

Thus, it is established that in "heterogenous" seeds the added endosperm effects the process of embryo development itself, raising the vitality of the seeds which later reflects on the progeny from whole seeds.

In order to increase the effect of "heterogeneity" of seeds it is expedient to perform the transplanting of embryos not in mature seeds, but in those which are still in the process of maturing.

The total experimental data which we obtained confirms the statement that the endosperm which is formed by fertilization is extremely important for the plant's vitality; that the depriving the seed of the endosperm is, on the same order, in its effect, as inbreeding and limited pollination and that in the increase of vitality or, on the contrary, in depressiveness of plants participates (depending on the reproduction method) not only the embryo of the seed, but also the endosperm.

* * *

The endosperm is nutrition, but nutrition of a particular kind: it is created by the most complex life function--fertilization, and, carries in itself the features of a living body and its peculiarities, it plays a big role in the life of a plant individual and species.

(begin p. 73) The problem of biological significance of double fertilization must attract the intent attention of biologists. This problem is of serious importance for further development of the entire problem of vitality.

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/Importance of isotopes and other newer
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(In Russian)

# IMPORTANCE OF ISOTOPES AND OTHER NEWER RESEARCH METHODS IN BIOLOGY FOR THE SOLVING OF AGRICULTURAL PROBLEMS[x]

One of the leading tasks of the socialistic agriculture is the further increase in yield capacity of all the species of food and technical crops. Extensive government measures in mechanization and electrification of agriculture, in construction of irrigation systems, in draining of swamps in supplying the agriculture with all kinds of fertilizers — create possibilities for obtaining of high and steady yields in all the climatic zones of our country.

However, in order to use skillfully these possibilities it is first of all necessary to know the requirements of plants, to learn to satisfy these requirements with the greatest benefit for the yield.

Great representatives among soil-specialists, agro-chemists, physiologists, selectionists, geneticists and other branches of biology are conducting research in directions important for agriculture. Great progress has already been achieved on the basis of which further rationalization of soil cultivation is being put into practice, more perfect methods for the care of agricultural crops are being created, changes in their hereditary nature are being achieved and new highly productive plant varieties are being introduced into agricultural production.

In connection with the great tasks set up by the September Plenum of the TsK KPSS /Central Committee Communist Party of the Soviet Union/, the Soviet biologists have to penetrate even more courageously and deeply into the process of the plants' life activity and to show on this basis to the agricultural workers the even more efficient methods of nutrition and plant growing, to equip them with new knowledge and methods for increasing the yield capacity of agricultural crops.

[x] Report at the General meeting of the AN SSSR on 22 October 1953

The Soviet biologists approach this responsible work after a fundamental review of many general problems of biology. Notions proposed by Soviet scientists on non-cellular forms of life, on inheritance of acquired characteristics and properties, stages of development, inter-relation of organisms with their life conditions and some others have a profound influence not only on the development of all the divisions of biological science, but also on the solving of practical problems of agriculture, medicine etc.

Together with this, the progress of isotope, chromatographical, optical, electron-microscopical, electrophoretical and other research methods widely used at the present time by the Soviet science, opened to biologists new technical possibilities, leaning on which they can approach the solving of problems which earlier seemed to be inaccessible for direct study.

Thus at the present time the Soviet biologists are well equipped by progressive theoretical ideas and powerful research methods in order to s solve successfully and rapidly the new problems confronting them.

//*Begin p. 9/ Extremely wide possibilities for such kind of works are presented by tagged atoms, which in combination with partition chromatography on paper and some other methods allow to observe directly and accurately the processes which take place in the soil, the use by plants of nutrient elements, the movement of nutrient juices in their tissues an and finally, the finest reactions of metabolism taking place in their cells.

Only very little time passed since the radio-active and non-radio active isotopes entered for the first time the laboratories of biological and agricultural research institutions. However, even during this short interval the Soviet biologists who study the processes of life activity of plants, had the possibility of visualizing considerably fuller and in many cases with an entirely new approach the life of the plant and its relation with the environment.

Further research with the use of tagged atoms promises more new and unexpected discoveries. But even now on the basis of analyzing and summarizing of the results of works by a series of research groups of the Academy of Sciences of the USSR and of other scientific institutions of the country, it is possible to outline a picture of nutrition of plants as it appears to us now due to the use of isotopes and of methods of

partition chromatography connected with them.

The most easily directed aspect of physiological plant activity is the root feeding and agriculture has at its disposal many effective methods of influencing it. This is the reason why most of the researchers working with isotopes concentrate their attention on problems of distribution and conversion of nutrient substances in the soil and on their assimilation by plants.

Experiments with radio-active phosphorus, calcium, sulfur, heavy nitrogen and organic substances tagged radio-active carbon, in a short time considerably widened and made more precise our knowledge of plant nutrition through roots. In particular, the use of tagged phosphorus allowed us to change the previously existing notion of low (10-12%) assimilation of phosphorous fertilizers by plants. Such a notion was formed as a result of comparison of the total content of phosphorus in yields of plants grown in fertilized and non-fertilized soil, without dividing it into phosphorus of the soil itself and phosphorus introduced with the fertilizer. At the same time experiments demonstrated that in introducing into the soil of phosphate fertilizers with tagged P32, for example- double superphosphate, the wheat and other plants first of all use 48-68% of the phophorus of the fertilizers at the same time reducing the absorption of phosphorus from the soil itself.

This result gives evidence of a considerably higher assimilability of phosphorous fertilizers by the plants than it was supposed and compels us to search for methods which increase the use by plants of phosphate supplies from the soil itself.

Easily and accurately can now be solved also such an important agricultural problem as a rational distribution of fertilizers in the soil, which would provide the most rapid and complete assimilation of stores of fertilizers by roots of young plants. This problem is particularly essential for granulated phosporous fertilizers which recently are being widely distributed. Placing in different sections of the soil granules of fertilizers tagged by radio-active phosphorus, the Soviet scientists demonstrated that even 15-20 minutes after the contact of the rootlet with the source of P 32, the isotope is detected in leaves. Therefore by watching for the appearance of the first signs of radio-activity in leaf blades, it is possible to establish the moment the roots reach the store of fertilizer in the soil and by observing the subsequent increase in radio-activity it is possible to evaluate also the speeds of assimilation of the given supply.

/Begin p. 10/ The importance of correct distribution of fertilizers for the feeding of plants is indicated, for example, by experiments with oats which demonstrated that during the introduction of phosphorus tagged by an isotope into the soil at a depth of 3-4cm under the seeds, the "con-

tact" of rootlets with the supply of fertilizer starts already 2.3 days after the germination of seeds and when the granules are placed 5-6cm distant from the seeds, this "contact" is retarded by 3-4 weeks and therefore the beginning of the phosphorous feeding which is so necessary for young plants is also retarded. With the help of this method it was even possible to clarify the optimum distribution of some kinds of fertilizers and there is no doubt that in the future the isotopes will play an even greater role in the evaluation of various methods of mechanized introduction of fertilizers.

The given method can be used also in observations of the determination of the distribution of the root system in the soil which was formerly adhieved by labor-consuming and far from perfect methods of digging out of roots and freeing them from the soil. By distributing concentrations of fertilizers tagged by a radio-active element into different layers of the soil, the researchers can easily observe the penetration of roots into any horizon without disturbing the completeness of the plant by watching for the appearance of radio-activity in its leaves. In this way can be studied the development of roots in plants depending on soil cultivation, on methods and dates of irrigation, on temperature and other factors, which is necessary for a correct organization of plant feeding applicable to concrete conditions.

Use of tagged phosphorus (P32) for the study of the absorbing function of the roots disclosed in this seemingly long known process new interesting peculiarities which allows us to substantiate theoretically the use of granulated fertilizers. It was demonstrated on a sample of spring wheat that during the contact with a granule of phosphorus of any single small rootlet which, let us assume, constitutes 4-5% of the total root system, the absorbing function of such a rootlet increases immediately 20-30 times as compared to an ordinary one and therefore this rootlet alone provides to a considerable degree for the needs of the entire plant in this element. It appeared that the root system of plants as an organ of absorption possesses a large reserve of potential power which however is realized only locally, when the rootlets come in contact with concentrations of nutrient substances. The fact that the nutrient substances absorbed bu any single fibrous root are redistributed in cereals in the so-called tillering nodes and are directed from it to all parts of the plant according to their requirement in the given source of nutrition is very essential. (fig.1).

Fig. 1 [p. 10] Scheme of the use by the plant of a granule of phosphorous fertilizer.

Thus the root system appears to us now not as an evenly functioning mechanism which sends water and nutrient subsrances into above ground parts. but to a high degree a labile and operative organ, the activity of which in various parts changes rapidly depending on the presence of nutrient substances and the requirements of the plant. These peculiarities of roots allow them

to use readily the local concentrations of nutrient substances, in particular of granuated fertilizers.

Fig. 2 Radio-autograph of a beet plant (a) and of tomato (b) after additional fertilizing wit radio-active phorphorus through the leaves.

Fig.3 Radio-autograph of a 12 day old bean plant enriched with radio active carbon as a result of absorbing $C^{14}O_2$ throygh the roots.

Fig. 4 Radio-autography of 6 days old shoots of wheat grown from soil with rotting radio-active remains of plants.
left-during the period of intense emission of $C^{14}O_2$ by radio-active remains;
right- atthe end of the disintegration of organic remains.

[begin p. 11] During certain periods of development which usually begin after the setting of the fruits the plants loose to a considerable degree the ability to absorb phosphorus and some other nutrient elements through the roots. Meanwhile the processes of movement of plastic substances and their deposit for reserves, which are dominant in the plants at the given period, are still in need of an intake of nutrient salts and can be strongthened by free access of them [salts] to the plant.

Agronomical science already found the solution of this problem having applies the so called non-root additional fertilizing, i. e. spraying or dusting of the plant's above-ground parts with the necessary nutrient substances. In particular, it is possible with the help of leaf feeding with phosphorus, to increase the yield of sugar beet, potato, cotton, and with leaf feeding of ammonium salts to increase the yield of cabbage and other vegetables, especially in the North, where the low temperatures of the soil interfere with a normal intake of nitrogen through the roots.

The use of tagged atoms assisted in the given case as well in regulating and substantiating of this useful measure. Thus the applying of salts of radio active phosphorus as leaf feeding allowed us to observe the penetration of the fertilizer through the leaves into the plant and its distribution in the tissues.

Particularly graphic is the picture of such distribution in radio autographs where the plants seemingly "photograph" themselves by means of the radio active element which penetrated into its tissues. In fig. 2 is shown the distirbution in the tissues of P 32 introduced in the form of a salt solution through the leaves of beets and tomatoes: phosphorus, after penetrating inside the plant, accumulates, for example, in a young beet

plant in the root, and in tomatoes, in the fruits, depending on the requirement of individual parts for the given element. It is natural that guided by such documentary pictures agricultureal workers can determine considerably more accurately the dates and norms of leaf feeding.

Having applied radio active carbon ($C^{14}$) in combination with chromatography on paper, the Soviet biologists discovered a new function of the root system which consists in the absorption by the roots of carbon dioxide from the soil and in transmitting it into leaves and other green parts of plants. It appears that here, under exposure to light, the soil $CO_2$ is used together with that absorbed from the air for a synthesis of sugars and other products of assimilation(Fig.3). Thus was disclosed an additional source of carbon nutrition of plants the existnece of which was not known before. This fact has not only a theoretical but a practical importance as well because it points out the important role of humus and of microbiological processes in the soil for the feeding of plants with $CO_2$ and it warns against a one-sided understanding of problems and possibilities in the use of mineral fertilizers.

By introducing legumes into the soil which are tagged in their organic part by radio-active carbon, it is possible to observe the decompesition of organic remains according to the speed of liberation of the $C^{14}O_2$. Such observations are of great practical value because they allow one to judge the intensiveness of the humus-forming processes taking place in the soil depending on its cultivation, humidity, temperature and other factors. At the same time by growing plants in a soil with activ mulch it is possible to make direct observations of absorption and use by them of the carbon dioxide of rotting remains. In fig.4 is given a radio autograph of a wheat shoot grown in soil fertilized with remains of radio active plants, during which the conditions of the experiment excluded the possibility of another way of entering the plant by $C^{14}O_2$ except through the roots.

In the general carbon feeding of plants the specific significance of carbon dioxide entering through the roots apparently can vary. /begin p. 12/ Clarification of this problem is of great practical interest and deserves serious attention. In the table below are given some preliminary data obtained in the summer of this year under field conditions during enrichment of the soil with carbonates.

| Crop | Yield c/ha | | Increase in yield due to $CO_2$ | |
|---|---|---|---|---|
| | N P K | NPK+$CO_2$ | in c | in % |
| Potato | 291,1 | 311,1 | 20,0 | 6,9 |
| Barley | 21,2 | 25,0 | 3,8 | 18,0 |
| Beans | 40,0 | 46,96 | 6,96 | 17,4 |

These results are for orientation, however they indicate that soluble and non-soluble carbonates introduced into the soil together with basic elements of nutrition can in a number of cases noticeably increase the yield of beans, barley, and potatoes. In some cases the absolute increase in yield can even exceed the amount of carbon dioxide introduced into the soil (for example, in experiments with potatoes) which indicates a more complex relation.

Indeed, detailed study of the biochemical aspect of assimilation of soil carbon dioxide reveals its close connection not only with the carbon nutrition but also with many other important aspects of the physiological activity of the plant.

At the present time due to the use of radio-active carbon ($C^{14}$) and partition chromatography, it was possible to understand rather in detail, the inner mechanism of this previously unknown phenomenon and its significance in the life of the plant.

In fig. 5 there is schematically presented a plant and next to it are chemical formulas which demonstrate the course of transformations in which the carbon dioxide of the soil participates.[1] Sugars formed in the leaves by assimilation of $CO_2$ from the atmosphere, move down along the bark (phloem) and having reached the roots they penetrate into their finest and most active ramifications. The speed of this downward movement determined with the help of marked carbon is, for example, in sugar beets from 0.7 to 1.5 m per hour.

In plant roots the sugars undergo a step-by-step glycolytic disintegration as a result of which pyroracemic acid is formed. This is the substance which picks up by means of a specific enzyme the soil carbon dioxide which unites in the form of carboxyl to the pyroracemic acid, converting it into oxalacetic acid. The latter, which is easily reduced, is converted into malic acid which is the first comparatively stable substance that carries carbon dioxide of the soil. This first stage of fixation of soil carbon dioxide can be illustrated by fig. 6. Radio-autography presented in this picture indicates that with additional fertilization (for example of bean roots) with tagged $CO_2$, the radio-activity concentrates first of all in the malic acid and only later on, due to inter-conversion of acids is it partly manifest in citric and keto-glutaric acids.

It should be pointed out that penetration of $CO_2$ as a carboxyl group into organic acids does not in practice raise the free energy of the substance /begin p. 13/ and therefore cannot yet be considered as nutrition of plants with carbon dioxide. However the organic acids which are formed in the roots

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1. In formulas (in figures and in the text) the carbonic acid of the soil is marked with an asterisk (C*).

and which carry the soil carbon dioxide ascend upwards through the plant and penetrate into the green fruits, the growing point and especially into leaf blades (see fig. 5). The speed of this ascending movement even in grass plants is 3 to 7 cm per minute or 2 - 4 m per hour, due to which the $CO_2$ of the soil very rapidly reaches the assimilating tissues where it is freed again as a result of the action of the decarboxylizing enzyme, and being reduced in the process of photsynthesis, it forms carbohydrates, protein and other products rich in energy.

Part of the sugars thus created are in turn directed towards the roots for the purpose, after being converted there during the respiratory process into pryoracemic acid, of accepting new portions of $CO_2$ from the soil and delivering them to the leaves. Such is the basic cycle of this process: however on it are superimposed a number of supplementary phenomena which connect the root feeding of carbon dioxide with other aspects of the physiological plant activity.

Fig (5) [p. 13]
Cycle outline of sugars and organic acids in a plant with the participation of soil carbon dioxide. (The continuous line indicates the downward movement of sugars, the dotted — the upward movement of organic acids).

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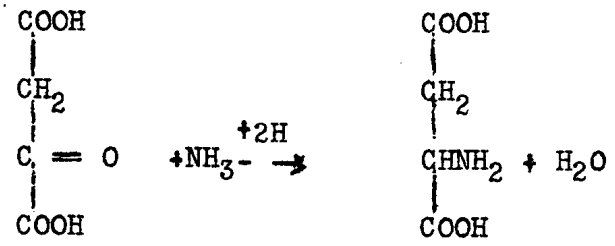
The method of tagged atoms allowed us to establish, that part of the carbon dioxide, which moves in compounds of organic acids from the soil, is used for photosynthesis even by the green cells which form a layer around the conducting tissues of the stems and the leaf petiole. As a result, in the solid tissues which are usually not easily accessible to outside air, there forms a considerable amount of oxygen which is necessary for the support of very intense respiration inherent to conducting tissues. Thus there was shown the puzzling role of the chlorophyll-bearing tissue around [begin p. 14] the vascular-fibrous bundle which usually caused perplexity and contradictory statements by botanists. Now it became clear that the development and functioning of this tissue has an important significance for oxygen nutrition of the conducting system, and consequently, for the movement of substances in the plant and for accumulation of stored products. It, in particular, is known that in plants of spring wheat varieties which give high yields, the chlorophyll layer is developed considerably stronger than in plants of lower yielding varieties.

A certain amount of carbon dioxide absorbed from the soil enters also green fruits which frequently have solid covers excluding the possibility of the penetration into them of atmospheric oxygen. Therefore here, also the physiological significance of chlorophyll consists first of all in

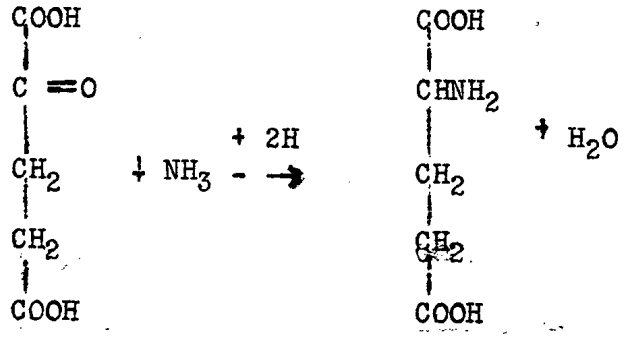
providing normal oxygen nutrition of fleshy tissues during the process of $CO_2$ assimilation. Thus, we come to a conclusion seemingly paradoxical at first, that the oxygen nutrition of thick organs and tissues of plants with little access to the outer air is achieved with the help of carbon dioxide which penetrates through the roots.

Absorption of carbon dioxide from the soil is in direct relation also to such important functions of the root system as nutrition with nitrogen and phosphorus. Works by Soviet scientists demonstrated that the primary assimilation of ammonium nitrogen is accomplished by way of direct amination of keto acids — such as pyroracemic, oxalacetic, and keto-glutaric, which are converted at that time into alanine, asparic, and glutamic acids and with subsequent reexamination into other amino-acids as well. Having adopted partition chromatography on paper, Soviet scientists discovered that sap, i.e. the fluid sent up by roots into above-ground organs, contains a large amount of various amino-acids — the basic components for the building of protein. Such a result compels us to recognize that the root system has an important function in the protein metabolism of the plant.

The role of the soil carbon dioxide consists [in this process] in carboxylization of products of incomplete decomposition of sugars, which are formed by roots in the so-called cycle of two and three carbon acids which leads to a formation of keto-acids — oxalacetic acid and alpha-keto-glutaric acid which are the main acceptors of the nitrogen of ammonium fertilizers:



Oxalacetic acid Asparic acid



Alpha-Keto-glutaric acid Glutamic acid

However this entire system can function in roots only during a sufficient supply of phosphoric acid, because phosphorus which enters the roots participates directly in the formation of the necessary organic acids from [begin p. 15] sugars. Therefore if, for example, the plant suffers a deficiency in phosphorus, then in its roots the formation of keto-acids ceases and therefore the absorption of $CO_2$ from the soil also ceases. Such plants make limited use of nitrogen

fertilizers even in cases when the soil is sufficiently rich in them. As an illustration in fig. (7) and (8) are shown chromatograms of the sap of a pumpkin which was grown in nutrient solutions with normal and insufficient content of phosphorus. Normally the roots of a pumpkin produce, and send into the above-ground organs, 9 to 12 amino-acids which can be detected in the sap of these plants by the method of paper partition chromatography (see fig. 7). At the same time in plants suffering from deficiency in phosphorus the sap contains almost no amino-acids. Instead of those a large amount of sugars is found in it (see fig. 8), which are usually not characteristic of sap of normal plants. A conclusion can therefore be drawn that sugars which move from the leaves to the roots in absence of phosphorus do not undergo necessary conversions and return to the leaves in unused state. This is the more probable since when such plants are fertilized additionally with phosphorus, the sugar disappears rapidly from their sap (see fig. 8), and in its stead from the roots and into the growing tissues and leaves, moves a mixture of amino-and organic acids which carry in their carboxyls carbon from the soil carbon dioxide (fig. 9).

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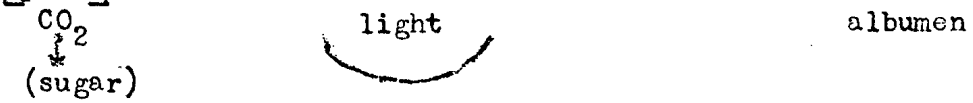
Fig. (6) -
Radio-autography of organic acids formed in roots of beans during absorption of soil acids.
(1)- malic, (2)- keto-glutaric, (3)- citric acid.

Fig. (7) -
Partition chromatogram of amino-acids contained in the sap of pumpkin:
(left)- in complete nutrient mixture, (right)- lacking phosphorus. Each stripe corresponds to a definite amino-acid: (1)- cystine, (2)- lysine, (3)- asparagine and asparic acid, (4)- serine, (5)- glycoco, (6)- glutamic acid, (7)- alanine, (8)- threonine, (9)- gamma-amino-butyric acid.

Fig. (8) -
Paper chromatogram of sugar (glucose) contained in the sap of pumpkin:
(1)- in absence of phosphorus, (2)- in complete nutrient media, (3)- 24-hours after renewed phosphorus feeding of the plant, (4)- one hour after administering phosphorus.

Fig. (9) /p. 15/




Cycle outline of sugars and organic acids in a plant with the participation of the soil carbon dioxide in relation to phosphorus and nitrogen nutrition. (continuous line indicates downward movement of sugars. The dotted — upward movement of organic and amino acids).

/Begin p. 16/

Such appears to be the present time picture of one of the central links in plant nutrition and this example demonstrates particularly convincingly the power of the isotope and chromatographic methods, and it is their combination which allowed the Soviet scientists to penetrate in a short time the nature of the most intimate aspects in life activity of plants.

Differently appear now also the speeds of the processes taking place in the plant. Use of tagged atoms allowed us to determine accurately the course of some of them and compelled us to renounce the notion of sluggishness of phenomena taking place in a plant. Thus by way of heavy nitrogen ($N^{15}$) introduced into the soil in form of ammonium sulfate it was demonstrated that the proteins in all the plant organs are continuously renewed and at such speed that the life duration of a protein particle in growing rye, for example, is considered (according to preliminary data) only as lasting a few hours. Even the nitrogen of the chlorophyll which enters the very center of the molecule of this pigment, in winter rye, for example, is renewed up to 50% during 24 hours and in leaves of sorrel it is completely changed in 3 days. The progression of substances in plants takes place also considerably faster than it was assumed before. In particular, the so-called downward flow of plastic substances from leaves to the root or the fruits takes place at a speed of 0.7 to 1.5 m per hour, due to which the products of assimilation in the majority of agricultural plants usually reach, after 30-60 minutes, the points of growth and the organs of supply deposits. Even faster move the substances up the plant — usually 2-4 m per hour.

Finally the water, particularly in some woody plants, as experiments with radio-active phosphorus and iodine demonstrated, covers along the wood 14 m and more per hour.

All this indicates a great tension of the plants' physiological activity and the agricultural workers in taking care of plants have to take into consideration the tempo of their life activity.

Radio-active carbon ($C^{14}$) is now being used successfully also for observations of the direction in the movement of organic substances in a plant — of their distribution and redistribution in tissues and organs.

These phenomena are of great importance for the formation of the body of the plant and for accumulation of reserve substances which in the end determines the volume and quality of the yield. Up to recent times this important problem remained almost undeveloped due to a lack of methods for direct observation of the movement of substances in plants.

Using the method of tagged atoms in experiments or irrigated lands in the Trans-Volga area, Soviet biologists demonstrated, for example, that the assimilates which are formed in wheat leaves from tagged carbon dioxide are distributed considerably more rapidly and fully into seeds of watered plants than of plants suffering a deficiency of water (fig. 10). This kind of uncomplicated observation method can be successfully used in agricultural practice for clarification of plant productivity in relation to their varietal characteristics and applied agrotechnical measures. With the help of tagged carbon dioxide it was also discovered that under conditions of the Extreme North the products of $CO_2$ assimilation are directed from the leaves mainly into stems and points of growth, which causes vigorous growth of

their leaves /"botva"=leaves of root-bearing plants; haulm, stem (of grass)/. and the flow-off of nutrient substances into tubers is sluggish and very late, due to a long day and low soil temperature, which determines the comparatively low starchiness of local potatoes. At the present time the workers of northern agriculture are searching for ways of overcoming this negative phenomenon by using the method of tagged atoms for an evaluation of the effectiveness of the applied measures.

/Begin p. 17/

Among the different means affecting the growth and the shape-forming processes in plants, steadily wider distributed in the practice of agriculture are special chemical preparations, for example, the 4-iodo-phenoxy-acetic acid which, being applied in small doses on the raceme and ovary of tomatoes, sharply increases the yield of fruits (fig. 11). The biological aspect of this peculiar phenomenon began to be clarified only recently due to the use of tagged atoms. In particular, by introducing into a tomato plant the solution of this acid tagged with radio-active iodine ($I^{131}$), it is possible after a certain time to determine accurately its localization in plant tissues. Particularly significant is the accumulation of the iodine preparation in flowers and young fruits which creates a peculiar polarization due to which the nutrient substances are directed from leaves, roots and other parts of the plant into these organs, securing their good growth and accumulation of reserve products.

However the method of tagged atoms allows one to observe not only the movement of substances in plants but also the course of their further conversion. Thus applying a saccharose solution tagged with radio-active carbon ($C^{14}$) on leaves of kok-saghyz. Soviet researchers discovered that in the latex of such plants there soon appears radio-active rubber (fig. 12). By the same token was shown the origin of rubber from carbohydrates which was disputed earlier. This fact is interesting also because it makes it possible to explain the post-harvest accumulation of rubber in kok-saghyz, which attracted the attention of practical workers for a long time.

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Fig. (10) - /p. 17/
Outline of movement of products of assimilation from leaves into seeds of wheat growing in non-irrigated (left), and irrigated (right) soil. (At the bottom — an apparatus for field tests with tagged carbon dioxide).

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Simultaneous use of tagged atoms and of partition chromatography allows us to approach also the solving of many other problems connected with the synthesis in plants of economically valuable products. The tagged atoms open up a particularly wide field of activity to researchers who study the nature of photo-synthesis — this /begin p. 18/ remarkable physiological process which leads to the accumulation of solar radiation in organic compounds formed by plants.

Fig. (11) - /p. 18/
Bunch of tomatoes. (left)- not treated, (right)- treated with preparation (4-iodo-phenoxy-acetic acid) during the period of flowering).

Fig. (12) - /p. 18/
Outline of an experiment which proves formation of rubber from saccharose — (asterisk indicates the radio-activity)

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In using isotopes the Soviet scientists achieved here as well, a number of successes, of which the most essential one for the understanding of the mechanism of photo-reduction of carbon dioxide is the discovery of photo-lysis of water carried out with the help of heavy oxygen ($O^{18}$). These works demonstrated that oxygen liberated by plants in the process of photosynthesis is freed from water and the hydrogen of the water is used for reduction of carbon dioxide.

Now the Soviet scientists come close to the solving of the problem of primary organic substances formed in the cells during photosynthesis. The use of tagged carbon dioxide in combination with paper chromatography adds exceptional accuracy to such experiments and allows one to detect tagged products of photosynthesis already 0.5 sec. after the beginning of exposure. Two low molecular substances were detected , the nature of which is now being investigated. So far it is only known that the first does not contain phosphorus and therefore is not phospho-glyceric acid as the foreign scientists assumed. These and many other works prepare a decisive step towards revealing the secret of photosynthesis and for mastering of this process which might occur even in the near future.

We should halt at one more problem which deals with the direct products of photosynthesis.

Having used radio-active carbon ($C^{14}$) in the form of carbon dioxide, and heavy nitrogen ($N^{15}$) in the form of ammonium salt, Soviet biologists demonstrated that not only carbohydrates, as it was assumed earlier, can be direct products of photosynthesis in plant leaves, but proteins as well, among which catalytically /begin p. 19/ active combinations, i.e. enzymes, are possible. Depending on the species of the plant, its age and condition of existence, the composition of products of photosynthesis are altered considerably. The strongest influence on this process is exerted by the spectral make-up of light and its intensiveness which, in combination with (mineral nutrition can change substantially the composition of primary products formed in leaves. In particular, in the red-yellow part of the spectrum mainly carbohydrates are synthesized, while the blue light furthers the formation of protein. The establishing of this fact has a principal significance for biologists and at the same time it opens practical possibilities for influencing the development and characteristics of plants by way of changing the composition of the primary products of photosynthesis which are being formed. Particularly realistic and promising is the application of this discovery in hot-house economy where without great

expense light sources varying in their spectral make-up can be created, with the help of which the agronomist will have the possibility of directing not only the quantitative but also the qualitative aspect of photosynthesis.

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Of course, the present survey does not exhaust all the new data obtained by Soviet biologists during recent years with the help of the isotope method and the chromatography but describes only the works connected with plant nutrition which acquire now a particular importance in the light of decisions of the September Plenum of the TsK KPSS.

This survey demonstrates that Soviet biology can solve now considerably more complicated problems than before, and that the old limits of our scientific notions have been seriously widened during recent years and partially and radically changed by Soviet scientists who use atomic energy for purposes of peaceful construction.

New discoveries and achievements of Soviet biology are already partially applied in the solving of practical problems of agriculture. However, this is only a beginning. Soviet biology has the possibility and must penetrate considerably deeper into the laws of the life activity of plants and to light more courageously and brightly the road for agricultural workers in order to obtain higher and steadier yields.

The decision of the September Plenum of the TsK KPSS outlined a drastic rise of the entire socialistic agriculture. Such a rise is possible only under the conditions of a wide use in agriculture of modern techniques and a courageous introduction of the newest scientific achievements into agricultural practice.

Trans. 506
(In full)
By:
A. Antik

506

Poddubnaia-Arnoldi, V. A.

Issledovanie zarodyshei u pokrytosemennykh rastenii u polrytosemennykh rastenii v zhivom sostoianii

/Investigation of embryos in living angiospermous plants/

Moscow Glav. Bot. Sad. B., v. 14, p:3-12, 1952
451 M854

(In Russian)

# Ivestigation of embryos in living angiospermous plants.

In connection with the development of the Michurin theory which shows the problem of reproduction in a new light, interest in embryology of plants has increased in recent years. A need arises for a development of new research methods which would allow studing more precisely and rapidly the vast and diversified material in order to approach differently previously described phenomena. Pictures obtained from preparations of fixed and stained material prepared by slow as well as by accelerated microtechnical methods, are by far not always satisfactory. Quite frequently in order to throw light upon the finest details of development and structure of reproducing organs, observations "in vivo" (on living material) are required, because fixation and staining frequently distort greatly the natural structure.

How important is the application of methods of studying the development processes on living material is seen from the fact mentioned by K. Iu. Kostriukova (1940), that the use of only fixed and stained material, artificially concentrated the attention only on nuclear structures, which are better preserved and made clear by this method than plasma structures which are frequently destroyed by fixation and escape from observation. And this, in turn, furthered the corroboration of notions of the nucleus as the only important cell organ and supported the theory of the monopoly of the nucleus in phenomena of heredity. Study of embryonic processes on living material allows one to surmount the limits of study of only the nucleus or of only chromosomes, which up to recent times was widely accepted and led to a

one-sided, metaphysical approach to complex and many-sided generative processes.

At the present-day development stage of embryology the need arises for a deeper knowledge of physiology and bio-chemistry of embryonic processes, it becomes particularly necessary to develop and improve methods of observations during the life time of pollen, pollen tubes, embryonic sacs, embryo and endosperm as well as of pollination and fertilization processes. However it would be wrong to propagandize for these purposes only the study of living material because these methods have also their shortcomings. Only a parallel complex application of different research methods, of slow as well as of accelerated ones, on living material as well as on fixed, provides a possibility to understand most completely one or another embryonic process (begin p. 4),

some embryological characteristics or others in their development, change and interaction with the environment. It is obvious, that the more perfect and diverse the research methods are, the more completely, precisely and manifoldly they will reflect the processes being observed, the more profoundly it will be possible to penetrate their nature and the faster—to master them in order to direct knowingly the heredity which is the goal of active Michurin biology.

Many researchers have long since shown interest in observations on living material. The authors whose work took place during the period when the use of microtome was not yet introduced into botanical practice, used extensively observations during the object's lifetime. At the present time these methods are undeservedly forgotten, and in the contemporary literature we find only single reports on embryological works carried out on living material. Among these works first of all should be mentioned the research by the Soviet scientists M. V. Chernoiarov, K. Iu. Kostriukova, G. K. Benetskaia who studied pollen and pollen tubes in a series of living angiospermous plants, which allowed us to deepen and widen considerably our notions on the structure of generative cells and sperm cells.

Extending of such research to other processes of embryonic development can contribute very much to the knowledge of their nature. But in studying on living material the development and structure of the embryonic sac, of processes of pollination and fertilization, the researcher is confronted with great difficulties. This is conditioned by the fact that the pistil and the ovary--due to their massiveness, and the embryonic sac--due to its location in the depth of the ovule and the difficulty of its isolation from the surrounding tissues,--are considerably less favorable for study in

a living state than the pollen particles which are readily isolated from the mother plant, which, besides, have frequently a transparent wall (capsule) and can be easily cultivated in artificial media.

On the other hand withaa gradual change of the ovule into a seed and of the ovary into a fruit, the study of the embryo-genesis with the help of the microtome technique becomes more difficult because the ovaries and ovules, in connection with the gradual formation in them of hardening covers (capsules), are becoming more difficult for fixation and for cutting on the microtome. And the hardened mature ovaries and seeds, as a rule, are not at a all saturated with a fixative and making of preparations from them does not produce good results. Therefore the embryologists usually terminate their research on embryos with the initial phase of cotyledon formation. The later development stages of the embryo are so far not sufficiently studied. The result of this gap is that the peculiarities of the embryo's structure in representatives of various families are not fully enough clarified ; thus a wrong impression is created of great uniformity in their structure, while in reality there are substantial differences in this respect. Being similar in the general outline of development and structure, the embryos of various species disclose greater or smaller deviations in details. Study of embryos in living state allows one to find out very rapidly not only the different stages of their development, but also their form, the veining of cotyledons, coloring etc. which is almost impossible on fixed and thinly sliced material.

N. V. Tsinger (1947, 1951) in her histo-chemical research undertaken for the purpose of studying bio-chemistry and physiology of fruits and seeds, as well as other authors, used the methods of preparing crude slices with subsequent treatment of them with various reagents depending on (begin p. 5)

the presence of certain substances and the duration of certain processes being studied at the time. The embryo and the endosperm at this time were cut. The possibility of isolating the embryo and the endosperm from the ovule allows us , so it seems, to resort to other methods in which the embryo and the endosperm could be treated by various reagents while they are whole, not cut. In many cases, particularly during early development stages, the embryo and the endosperm are so minute, tender and transparent which are readily saturated with various reagents. Besides that, the possibility of isolating the embryo and endosperm from the ovule allows one to study their physiology and bio-chemistry separately from the mother tissues. Microscopically small amounts of material enclosed in the embryo and the endosperm, particularly

during early development stages, apparently can be studied with the help of micro-methods. This will open prospectives for further study of bio-chemistry and physiology specifically of the embryo and endosperm because it will allow us to study the accumulation and disappearance of many substances, as well as the character of the course of various bio-chemical and physiological processes on embryonic stages of plant development. The development and application of the above mentioned methods is a task for the near future.

Observations indicate, that the study of embryo-genesis on living material is methodically considerably simpler, than the study, by the same methods, of embryo sacs, of pollination and fertilization processes, because the embryos can be readily separated from surrounding tissues and besides that, they can be cultivated in artificial media. Due to the fact that the study of development and structure of embryo sacs as well as of pollination and fertilization processes on living material presents great difficulties, we undertook, as a first stage of the work, to study the development of the embryo among some of the angiospermae, to which this article is dedicated. In research conducted during a number of years under our direction by E. V. Ivanovskaia on culture in artificial media of embryos of some cereals and lebuminous plants, as well as in our subsequent work, it was found that the isolating of embryos in a series of plants and their examination, at different development stages, as a whole, not cut into thin slices, is quite possible and in many cases very convenient. This allows one to follow up rapidly the various stages of their development, to establish their form, size, veining of cotylendons, tempo of development, etc. Application of accelerated research methods with the help of fixation and staining with a mixture of aceto-carmine and glycerin, allowed us at a certain time to trace rapidly and in succession various development stages of the embryo in kok-saghyz (Taraxacum kok-saghyz) decorative tobacco (Nicotiana alata) and buckwheat (Fagopyrum esculentum).

As objects for the present research served = flax (Linum usitatissimum), peas (Pisum sativum, vetch (Vicia sativa), lupine (Lupinus luteus), sunflower (Helianthus annuus) kok-saghyz (Taraxacum kok-saghyz), krym-saghyz T. hybernum), buckwheat (Fagopyrum esculentum), tobacco (Nicotiana tabacum and N. alata), hemp (Cannabis sativa), wheat (Triticum vulgare and T. durum), couch grass (Agropyron intermedium and A. elongatum), some decorative orchids (Cattleya sp., Cypripedium insigne, Dendrobium nobile, Calanthe veitchii and Phalaenopsis schilleriana), pyrola (Pirola minor) and "votlianitsa" (Monotropa hypopitys).

The method which we adapted of studying embryo-genesis on living material is very simple and rapid. It consists of carefully isolating with a fine tweezer or needle of the ovules from the ovary, slightly cutting them on top or bottom without hurting the embryos which /begin p. 6/ were then squeezed with a light pressure out of the ovule. Thus, then we placed on the objective the isolated embryos and examined it in a weak solution of sugar or in vaseline, paraffin or castor oil. And we examined the object under a cover glass or without it, at different magnifications under

a microscope or with binoculars. In a number of cases it was possible by this method to squeeze out undamaged, not only the embryo, but the entire embryo sac with the endosperm and the embryo inclosed in it, which allowed us to examine in a living state not only the embryo, but also the endosperm.

Applying the above mentioned methods it was possible to trace rapidly and easily the embryo-genesis in a number of plants, beginning with a multicellular embryo — and in a series of orchids even with a zygote — and finishing with a completely formed embryo. On the average 2-3 days were spent on the study of each object.

At the same time we do not consider it unnecessary to mention here that the picture of development and structure of the embryo which was observed on living material completely corresponds with that which is always described in many angiospermous plants on fixed and microtome cut material. The difference is that when the first methods are applied the embryos are studied as a whole, as volumetric massive bodies and when the second methods are used — the study is conducted on thin slices. The first method facilitates the study of some sides of the embryo's life activity (for example, generating of chlorophyll), of the process of its formation as a whole, the second method gives an idea of the anatomical-cytological structure of the embryo of the character of its various tissues and cells. Both methods mutually supplement each other.

Going over the description of the obtained results we consider it necessary to point out the fact that in a number of examined representatives of angiosperamous plants belonging to remote families (Linaceae, Leguminosae, Cruciferae, and Orchidaceae) embryos at various development stages are green colored (fig. 1). The color of embryos at early development stages is yellowish-green or light-green and at later stages the embryos, particularly the completely formed ones are more or less dark green. However with maturation and formation of the seed skin the embryos of a number of species which we studied, apparently due to reduction of chlorophyll, gradually lose their green color and at the moment of maturing of the seed they become light green. Later on chlorophyll originates again in the cotyledons only after the germination of the seeds and formation of shoots. The seed capsule which becomes browner and coarser apparently hinders the penetration of light and oxygen to the embryo, which furthers the gradual disappearance of chlorophyll. According to the opinion of a number of researchers the pigment of the seed coat protect the seed from light, functioning as light-filters and the light deficiency is one of the factors of chlorophyll reduction. Apparently by the same measure, the accumulation of stored substances in the embryo terminates due to the maturing of the seed and it enters the anabiotic state, its photo-synthetic activity ceases. With the appearance of chlorophyll in the organs of the embryo during the seed germination, the photo-synthetic activity resumes.

The presence of chlorophyll in embryo tissues is a very interesting fact. It apparently indicates that already from the earliest development stages the embryo can feed and accumulate stored substances not only with the help of substances of mother tissues but partly also by way of photo-synthesis. Such activity at first gradually increases and then gradually — and to the moment of complete maturation of the seeds even fully /begin p. 7/ stops, in order to resume when the seed germinates and forms a shoot.

Indications of the presence of chlorophyll in tissues of the embryo are found in literature as well. According to Lubbock's data (1892) a green embryo has been found among representatives of the following families. Cruciferae, Malvaceae, Tiliaceae, Linaceae, Zygophyllaceae, Geraniaceae, Celastraceae, Sapindaceae, Anacardiaceae, Leguminoceae, Plumbaginaceae, Polemoniaceae, Convolvulaceae and Orchidaceae. Netolitzky (1926) points out the getting green of embryos not only among representatives of the above mentioned families, but of others as well, namely among representatives of the families: Aponogetonaceae, Scheuchzeriaceae, Araceae, Loranthaceae, Basellaceae, Caryophyllaceae, Nymphaeaceae, Ceratophyllaceae, Capparidaceae, Oxalidaceae, Burseraceae, Euphorbiaceae, Aceraceae, Rhamnaceae, Violaceae, Combretaceae, Myrtaceae, Cornaceae, Ebenaceae, Hydrophyllaceae, Lentibulariaceae, Valerianaceae and Dipsacaceae. In one of his works on plant embryology Soue'ges (1934b) mentions in passing: "It is known that some embryos have chlorophyll in their tissues". An extensive research work on embryos in living state is being conducted by M. V. Chernoiarov, according to his oral report, he found the presence of green embryos among representatives of many families of angiospermous plants.

We have not discovered in literature a detailed and consecutive description of development and structure of embryos in which special attention was called to chlorophyll. An opinion was even stated that in all the embryonic tissues the plastids are, as a rule, in a state of deep degradation and do not contain chlorophyll. Unfortunately at present, chiefly due to methods, research on the problem of chlorophyll of the embryo cannot be exposed with sufficient completeness. Since it is of great importance for the understanding of biochemistry and physiology of the embryo, specific research should be undertaken. It seems to us essential to throw light in the future on the following problems: what is the functional significance of chloroplasts for the embryo; does the chlorophyll-bearing apparatus of the embryo undergo reduction, as it was observed in fruits by N. V. Tsinger (1947); what is the specific import of independent photo-synthetic activity of the embryo in its general nutrition balance; what is the phyllo-genetic significance of chlorophyll presence in embryo cells, is this characteristic primitive or progressive, etc.

Our studies of embryo-genesis among some decorative orchids, for example, Calanthe veitchii and Dendrobium nobile, on living material indicate that green plastids form very early. Chloroplasts were discovered among these plants in the zygote. It is possible that they are there already in the non-fertilized egg-cell, or even at early development stages of the embryo sac. We expect to answer this later when we shall examine the development of the embryo sac in a living state.

With the growing number of cells in the embryo of Calanthe veitchii and Dendrobium nobile, the number of chloroplasts in them increases and at the same time the intensiveness of the green color increases, which reaches its maximum at the moment of complete formation of the embryo. However, when the seeds of these orchids fully mature, the green color of the embryos have a yellowish or yellowish-green color.

Discovering of green embryos among representatives of remote families apparently indicates that formation of [begin p. 8] chlorophyll in tissues of an embryo represents a quite widely spread phenomenon, which it is not

possible to explain when embryo-genesis is studied on fixed material, because under the influence of the fixatives used at the present time, the chlorophyll is fully destroyed and the green color disappears. Further studies on living material of the development and structure of the embryo among a possibily large number of representatives of various families of angiospermous plants are necessary. However, already from the meager data at our disposal at the present time it is apparent that the development of green plastids in cells of the embryo is not characteristic for all plants. In a number of representatives of angiospermous plants the embryos do not remain green colored during early as well as late stages of their development, but are milky-white or ivory colored during the entire course of their development. Such embryos were discovered in wheat, couch grass, kok-saghyz, krym-saghyz, sunflower, tobacco, hemp, buckwheat, poppy, apple, tomato, potato, pumpkin, cucumber, water-melon, melon and many others. (fig. 2). In these species, with the exception of cereals, the cotylendons of embryos begin getting green only after the germination of seeds. Together with green and milky-white embryos, yellow, brown, pink and even red ones are found. The latter were discovered among representatives of the families Sterculiaceae and Myrtaceae.

It is natural to assume that the physiological and bio-chemical processes taking place in green as well as not green embryos of various representatives of angiospermous plants are to a known degree different, but up to now there were almost no attempts of studying embryo-genesis from the point of view of physiology and bio-chemistry. This has to be the task of further research.

One of the first steps in the direction of study of physiology and bio-chemistry of fruits and seeds during their development and with consideration of their anatomical structure was made by N. V. Tsinger (1947, 1951) who applied histo-chemical methods. In her study of fruits and seeds she threw light upon such important problems as carbohydrate metabolism, activity of various enzymes, dynamics and some physiologically active substances (ascorbic acid, sulfhydryl groups, etc.) dynamics of some mineral substances, etc. A natural development of the very important and interesting works of N. V. Tsinger would be an expanded study of embryo-genesis in an analogous direction. This would allow us to deepen and detail the knowledge of bio-chemistry and physiology specifically of the embryo during its development and interaction with the endosperm. It is true that in studying histo-chemistry of seeds, N. V. Tsinger and other authors at the same time payed attention to the study of the embryo and endosperm in this direction, but never-the-less very little has been done so far in this respect. Being a new just originating field, physiological histo-chemistry could not yet produce all that it is promising for the future.

Fig. 1 /p. 6a/
Various development stages of a flax embryo. (a)-(b)-section of the endosperm with globular, multi-cellular embryo; (v)- section of the endosperm with an embryo in which cotyledons are outlined; (g, d, e)- various development stages of the embryo; (zh, z, i)- later development stages of the embryo; (k)- fully formed embryo.

possible to explain when embryo-genesis is studied on fixed material, because under the influence of the fixatives used at the present time, the chlorophyll is fully destroyed and the green color disappears. Further studies on living material of the development and structure of the embryo among a possibily large number of representatives of various families of angiospermous plants are necessary. However, already from the meager data at our disposal at the present time it is apparent that the development of green plastids in cells of the embryo is not characteristic for all plants. In a number of representatives of angiospermous plants the embryos do not remain green colored during early as well as late stages of their development, but are milky-white or ivory colored during the entire course of their development. Such embryos were discovered in wheat, couch grass, kok-saghyz, krym-saghyz, sunflower, tobacco, hemp, buckwheat, poppy, apple, tomato, potato, pumpkin, cucumber, water-melon, melon and many others. (fig. 2). In these species, with the exception of cereals, the cotylendons of embryos begin getting green only after the germination of seeds. Together with green and milky-white embryos, yellow, brown, pink and even red ones are found. The latter were discovered among representatives of the families Sterculiaceae and Myrtaceae.

It is natural to assume that the physiological and bio-chemical processes taking place in green as well as not green embryos of various representatives of angiospermous plants are to a known degree different, but up to now there were almost no attempts of studying embryo-genesis from the point of view of physiology and bio-chemistry. This has to be the task of further research.

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Fig. 2 /p. 8a/
Various development stages of a sunflower embryo. (a)- globular, multu-cellular embryo; (b)- beginning of formation of cotyledons in the embryo; (v, g)- various development stages of the embryo; (d, e, zh)- later development stages of the embryo; (z, i)- fully formed embryo.

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In plants with green and non-green embryos which we examined, we did not observe that the endosperm and the suspensors of the embryos were green colored. It was noted lately that in a series of other plants the endosperm is colored green which indicates the presence in it of chlorophyll. Thus according to Ioffe's data (1952) the presence of chlorophyll was established in a number of representatives of the mustard family (cruciferae). At the same time there are indications in the literature that chlorophyll is present in the cells of the suspensors of some anigospermous plants. In examining the endosperm "in vivo" we discovered that at earlier development stages it is transparent and gelatinous and at later stages — milky-white and solid. In all the plants which we examined, with the exception of cereals, the endosperm in developing /begin p. 9/ is assimilated early by the embryo and at the moment of the maturing of the seed it disappears almost completely, only a very thin milky-white film remains of it and the embryo fills the entire inside of the seed.

In flax and sunflower the suspensors of the embryo is very clearly seen during all the development stages up to the complete development and the suspension looks like a transparent narrow and short column.

In cases when the embryos are green-colored and when the embryo sacs are studied in a living state, particularly at early development stages, they /the embryos/ are clearly seen through the endosperm. In cases when the embryos are not green-colored they are also seen, but considerably less clearly. Therefore, on living material the embryos can be examined separately from the endosperm as well as with it. And at that, it is necessary to remember, that it is not sufficient to examine even in a living state only the isolated embryo and endosperm. It is necessary to study them also in connection with the surrounding tissues of the ovule, ovary and fruit, because the embryo and endosperm are in a close physiological interaction with them. Therefore particular attention should be directed towards the study of the ovule, ovary and fruit by various methods, including that in a living state. It seems to us that such approach opens wide perspectives for further physiological, bio-chemical and morphological study of various development stages of the embryo, endosperm, ovule, ovary and fruit in their inter-relation with each other and the environment.

As it follows from our work and from data of a large number of works by other authors, the types of development and structure of embryo among

different plants are extremely diverse.

The embryos differ in shape, color, size, development degree of individual plants, development speed, position in the seed, structure of suspensors, veining of cotyledons and their behavior during seed germination. These characteristics can be used successfully for purposes of taxonomy and in a certain part they already are being used in establishing connections in relations between various systematic plant groups.

However, the above mentioned features of development and structure of embryos do not by far exhaust all the varieties possible in this respect, because the embryo-genesis among different representatives of angiospermous plants is far from having been sufficiently investigated. And then of tremendous interest for toxonomy is not only the morphology of the embryo in its nature completed form, but also the dynamics of its forming and the changes through which the embryo passes during the development of the seed. Only at the present time, applying investigation of seed development on living material and with the help of other accelerated methods, which allow us to observe the embryos as a whole, we obtain the possibility to start on an extensive study of embryo-genesis of a possibly larger number of representatives of the angiospermous plants. This will help to reveal the existing variety of structure and development of the embryo and, on the other hand, to observe the general regularities of development. At the present time the classification of embryo types is premature, because due to the incompleteness of our information it cannot reflect in a due measure their existing multiformity. Classifications suggested by Schnarf (1929) who mentions the existence of five types namely: Cruciferae-, Asteraceae-, Chenopodiaceae-, Caryophyllaceae-, and Solanaceae- types) and by Johansen (1945) who recognizes the existence of six types (namely: Piperad-, Onagrad-, Asterad-, Caryophyllad-, Solanad- and Chenopodiad- types) cannot satisfy us because they do not encompass the diversity in development and /begin p. 10/ structure which are known to us even now. Besides that at the basis of these classifications lies the very vast, but not sufficiently deep and many-sided data by Soue'ges. This scientist artifically limited the study of embryo's development and structure by examining only the initial stages of its development, not taking into consideration the entire embryo-genesis as a whole, and at that he ignored the position of the embryo in the endosperm and the seed. Even though Soue'ges studied the development and structure of embryos among representatives of many families of angiospermous plants and thus gave an impetus to their further investigation, he considers mechanistically the laws of the embryo's development and structure, reducing them to laws of geometry and does not study the embryo-genesis in all its completeness and multiformity.

It is absolutely clear to us, that various biological processes, among them also the embryo-genesis, cannot be reduced to mathematical laws and formulas because these processes have their own specificity, which cannot be expressed by mathematical laws. Types of embryos cannot be judged only on the basis of initial stages of their development, while not taking into consideration the entire embryo-genesis as a whole, in its interaction with the surrounding external conditions. Even though our idea is that it is still too early to give a classification of types of embryos, it is already clear, as it was particularly distinctly shown by

the Soviet scientist M. S. Iakovlev (1950), that a characteristic of development and structure of the embryo, together with other embryological characteristics is of great significance for taxonomy. Study of embryogenesis on living material will, no doubt, be very useful because it will facilitate the establishing of /close/ relationship between various plant groups and the clarification of paths of their historical development. Besides that these methods can be applied not only in the interest of taxonomy, but also for work on some problems of selection and genetics due to the fact, that the character of metabolism during the embryo-genesis reflects on the fruitfulness and hereditary nature of the organism. A more thorough notion on the character of metabolism has to help in establishing the requirements which the embryo presents to the environment at different moments of its development and determining conditions which are the most favorable for the overcoming of the conservatism of heredity and for the obtaining of directed changes.

In works on clarification of the effect obtained as a result of adapting various methods of pollination, during works on remote hybridization and on clarification of causes of incompatability and sterility, and of methods for overcoming them, on establishing dates of maturing and quality of seeds of cultivated and useful wild-growing plants — it is not infrequently necessary to conduct an investigation of embryo-genesis, which is considerably easier and faster by the application of methods for studying it on living material.

In the light of further development of the Michurin—Lysenko teaching, the study of various embryonic processes among them also of embryo development, is of great interest. Knowledge of these processes is necessary in creating new practical and valuable varieties of cultivated plants, because it must help a conscious directing of heredity. Hereditary characteristics of a living organism originate and change under the influence of life conditions; therefore, change in nutrition also influences hereditary properties. It is self-explanatory that this is applicable not only to a grown plant but to its embryo as well. In changing the life conditions of the embryo, feeding it differently; it is possible to change its hereditary nature. Taking into consideration I. V. Michurin's statement on great plasticity of a young organism as compared with an old one, there is hope that influences on a plant when it is /begin p. 11/ in an embryonic state (i.e. the youngest) with the purpose of a directed change in its hereditary nature, will be the most successful /ones/. At the present time we still know too little about what conditions it is necessary to create and at what moment, in order to direct changes in the hereditary basis of one or another plant, what is the character of metabolism among different species and in different parts of the embryo, beginning with the earliest and finishing with the latest development stages. The problem of direct rearing of the embryo of a new organism, beginning at the moment of fertilization of the egg-cell and up to its emergence from the seed — is very real, but nevertheless not at all developed.

It is clear from here, how important the further study is of just the embryonic stages of plant development, with the use of possibily more perfect and varying research methods among which the study /of plants/ in a living state is of great interest and has great advantages in comparison with

other methods.

In conclusion it should be emphasized, that in applying the grafting method, the possibility of isolating from mother tissues undamaged embryos at various development stages, allows one to make grafts at very young development stages, when the plant is the most pliable, the most capable for changes. Therefore, it should be attempted to make grafts of more or less developed embryos, which will allow us to influence a younger organism which therefore is more flexible to changes.

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USSR Work on Application of Tracer Atoms in Microbiology

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# USSR WORK ON APPLICATION OF TRACER ATOMS IN MICROBIOLOGY

The Institute of Microbiology, Academy of Sciences USSR, conducted at the end of November 1953 a conference on the application of isotopes in microbiology. More than 300 scientific workers participated in this conference. Among them were microbiologists, plant physiologists, agricultural chemists, and bio-physicists, who came from many cities of the USSR.

A. A. Imshenetskiy, Director of the Institute of Microbiology USSR and Corresponding Member of the Academy of Sciences USSR, in this introductory address indicated that the method of tracer atoms, which has already contributed much to the understanding of metabolism, the physiology of the nutrition of microorganisms, and the chemistry of the formation by them of economically valuable products, opens still wider prospects for the development of scientific and practical microbiology in the future. He added that it is timely, for that reason, to summarize the results of the investigations carried out with the aid of isotopes and to evaluate the advantages and shortcomings of this promising method, and also to determine ways for its future development, improvement, and inculcation.

A number of reports presented at the conference were devoted to the critical evaluation of methods of applying isotopes as well as results of scientific investigations carried out with the aid of this method in the fields of microbiology, virology, and immunology.

Doctor of Biological Sciences M. N. Meysel' (Institute of Microbiology, Academy of Sciences USSR) in his report expressed the opinion that the application of tracer atoms is justified in the following cases: (1) when the problem under investigation cannot be solved by any other method, (2) when tracer atoms increase the precision of the determination, and (3) when the application of tracer atoms shortens the time necessary for the investigation as compared with other methods. It is most expedient to apply isotope methods in combination with other recently developed chemical and physical methods, as for instance fractionation, chromatographic separation, ion-exchange methods, and the newest optical methods (particularly microscopic methods). On the example of investigations carried out at the Institute of Microbiology, Academy of Sciences USSR, Meysel' illustrated the possibilities of the isotopic method applied to the investigation of the laws of the biosynthesis of proteins and vitamins by microorganisms, to the development of rapid methods for the

quantitative determination of biologically active substances, and to the elucidation of interrelationships between animal organisms or plant organisms and microorganisms.

In an extensive report replete with new factual data, V. L. Ryzhkov, Corresponding Member of the Academy of Sciences USSR, illustrated the progress in the investigation of phytopathogenic viruses which has been achieved to a considerable extent by the application of isotopic methods. In this report particular attention was paid to bacteriophages and their metabolism. Research carried out with the aid of tracer atoms made it possible to establish that bacteriophages, regarded by Ryzhkov as viruses affecting bacteria, are formed from low-molecular substances such as amino acids, purine bases, and pyrimidine bases. The nucleic acid of bacteria is included in the composition of a bacteriophage only after the preliminary decomposition and rearrangement of this acid. In Ryzhkov's opinion, the work in question demonstrates the fallacy of the assumption that multiplication of bacteriophage proceeds autocatalytically.

Professor V. I. Tovarnitskiy (Institute of Virology imeni V. I. Ivanovskiy) pointed out that the isotope method, notwithstanding its great possibilities, has not yet been applied adequately in the investigation of the biochemistry of animal viruses. Tovarnitskiy summarized the results of laboratory research carried out in the USSR and abroad which pertain to changes in the phosphorus metabolism of animal bodies under the influences of a virus developing in them. Particularly interesting in this respect are the investigations of the adenosine triphosphoric acid metabolism during the propagation of the influenza virus.

A report by S. Z. Roginskiy, corresponding Member of the Academy of Sciences USSR, attracted great attention. This report dealt with the characteristics of the mechanism and kinetics of the isotopic exchange in solutions. Roginskiy discussed in detail the possibilities and significance of the isotope exchange that is observed and also the various types of the mechanism of this exchange. He emphasized that the magnitude of isotope exchange is very small for the majority of the compounds with which biologists have to deal. Roginskiy added that the possibilities of isotope exchange in catalytic reactions which play a considerable role in the metabolism of the body deserve special study.

V. L. Troitskiy, Corresponding Member of the Academy of Medical Sciences USSR, presented a review of the use of bacteria containing tracer atoms and of the active substances formed by these bacteria and producing immunobiological reactions for the study of the pathogenesis of infectious diseases and of immunological phenomena. Troitskiy clearly illustrated his general assumptions on the example of his investigations on the immunology and pathogenesis of dysentery that were carried out in collaboration with M. A. Tumanyan.

A considerable number of reports dealt with the experimental investigation of metabolism in microorganisms that are important from the standpoint of their practical use in industry and agriculture.

Of particular interest in this series of reports was one presented by Professor I. Ya. Veselov (Scientific Research Institute of the Beer-Brewing Industry). Veselov's report dealt with the phosphorus and carbon metabolism of yeast during the fermentation of malt wort. Veselov established that during the period of active fermentation, when multiplication of the yeast takes place, radioactive phosphorus is not eliminated from the yeast cells. It was further shown in the investigation described by Veselov that the products of the fission of carbohydrates, particularly acetaldehyde, are utilized for the synthesis of substances that enter into the composition of yeast cells. These facts, which are so important for science and industry, could be established only with the use of compounds containing tracer atoms.

Candidate of Chemical Sciences R. D. Gal'tsova (Institute of Microbiology Academy of Sciences USSR) reported on the results of the application of radio-active sulfur in the investigation of the velocity and extent of the formation of protein by yeast organisms. In this investigation, the inclusion of sulfur into proteins and individual amino acids of yeast cells could be followed with great precision. It could also be shown that the synthesis of proteins can be considerably accelerated by vitamin $B_6$ (pyridoxine).

Professor G. M. Frenkel' (Institute of Microbiology, Academy of Sciences, Ukrainian SSR) stated that the application of radioactive iron made it possible to establish the existence of significant differences in the resorption of iron by aerobic as compared with anaerobic microorganisms. This investigation was conducted principally on acetone-butyl alcohol bacteria.

N. A. Pomoshchnikova, Scientific Associate of the Institute of Microbiology, Academy of Sciences USSR, presented extensive data on the phosphorus metabolism of yeast under aerobic and anaerobic conditions. By using phosphorus compounds containing radioactive phosphorus and chemical fractionation methods, she established that a number of delicate changes take place in the phosphorus metabolism of microorganisms under the effect of certain physical influences exerted on these microorganisms.

A number of reports touched upon very important problems of photosynthesis and chemical synthesis. The method of tracer atoms has played a considerable role in the clarification of the fine mechanisms involved in the processes studied in connection with these problems.

Professor S. I. Kuznetsov (Institute of Microbiology, Academy of Sciences USSR) made the well-founded assertion in his report that application of radioactive carbon dioxide is the only suitable method for the clarification of the significance of chemosynthesizing bacteria in the formation of organic substances in water basins. By using very apt experiments, Kuznetsov proved that the principal source of the primary formation of organic substances in water basins is the photosynthesis carried out by phytoplankton. The chemical synthesis carried by bacteria results in the formation of not more than 1-2 percent of the organic matter present in water basins.

Candidate of Biological Sciences U. I. Sorokin (Institute of Microbiology, Academy of Sciences USSR) told about the application of radioactive phosphate in the investigation of the autotropic assimilation of carbon dioxide by sulfate-reducing bacteria. He established that anaerobic oxidation of hydrogen is accompanied by oxidative phosphorylation. He further established that the process takes place in two stages; in the first stage there is phosphorylation, during which the energy of the oxidation of hydrogen is absorbed by the organic phosphates; in the second stage the assimilation of carbon dioxide takes place at the expense of the energy absorbed by the organic phosphates.

Candidate of Biological Sciences I. S. Skalon (Natural Science institute imeni P. F. Lesgaft) told about some observations on the resorption of radioactive carbon dioxide by lactic acid bacteria and on the inclusion of carbon tracer atoms into the composition of organic acids.

At the present USSR investigators are paying particular attention to the role of extraradical soil microflora in the nutrition of higher plants. The possibility of exerting an active effect on the development and yield of agricultural crops through the medium of the microbe population of the soil depends to a considerable extent on the solution of the problems involved here. These problems were disucssed in several reports presented at the conference.

Professor A. I. Akhromeyko (All-Union Scientific Institute of Timber and Forest Economy) outlined in detail the results of the investigation of the role of extraradical microorganisms on the process of the assimilation of phosphorus by oak and ash seedlings. According to Akhromeyko's data the microorganisms first reduce the assimilation of phosphorus by the higher plants, because they resorb it and retain it in their own bodies. Later, as the microorganisms die and liberate the phosphorus bound by them, there is an increase in the resorption of this element by the higher plants.

Candidate of Technical Sciences N. M. Shemakhanova (Institute of Microbiology, Academy of Sciences USSR) reported on the state of research on the role of mycorrhiza in the nutrition of ligenous plants. The use of tracer atoms made it possible to investigate in greater detail the mutual exchange of nutritive substances between higher plants and mycorrhiza. Preliminary data on the participation of mycorrhiza in the assimilation of phosphorus by the higher plants were given in this report. The investigation was carried out on oak seedlings both in the presence of mycorrhiza and in the absence of mycorrhiza.

New data pertaining to the resorption of phosphorus and sulfur by microorganisms and to the transfer of these elements to higher plants by the microorganisms were contained in the reports presented by the Candidate of Biological Sciences A. Ye. Fomin (Institute of Agriculture of the Southeast of the USSR) and Candidate of Biological Sciences, V. V. Kotelev (Moldavian Affiliate, Academy of Sciences USSR).

A substantial report which was presented by the representative of the L'vov State University, G. M. Shavlovskiy, and which dealt with the participation of microorganisms in the amino acids and vitamin nutrition of plants was listened to with great interest.

Using amino acids and vitamins containing tracer atoms as well as bacteria and the products of the metabolism and dissociation of bacteria containing tracer atoms, Shavlovskiy investigated the regularities of the distribution of methionine and vitamin $B_1$ in higher plants as well as the transfer of these substances from bacteria to higher plants.

N. P. Plotnikov, Scientific Associate at the Ural Affiliate of the Academy of Sciences USSR, presented a report on the investigation of the brucellosis infection with the use of bacteria containing tracer atoms.

The reports presented at the conference were discussed in detail. Particularly lively discussions developed on methodological questions. The resolutions passed by the conference indicated the fundamental scientific problems for the solution of which the application of isotope methods is advisable. Furthermore, measures for the improvement and perfection of methods and techniques used in work with tracer atoms were indicated.

At the conclusion of the conference, Candidate of Biological Sciences I. N. Verkhovskaya demonstrated for the participants contemporary methods of work with tracer atoms.

Smorodintsev, A. A. (Prof.) and
Kriviskiy, A. S.

USSR Work on Modification of Viruses

Priroda, Vol. 42, no. 12, pp: 34-44



# USSR WORK ON MODIFICATION OF VIRUSES

In the resolution passed by the Conference on the Problem of Noncellular Forms of Life that was called by the Department of Biological Sciences, Academy of Sciences USSR, in conjunction with the Presidium of the Academy of Medical Sciences USSR and held in May 1953, it was noted that there is neither complete understanding of the biological significance of filterable forms of microbes nor sufficient knowledge of the problem of the connection between filterable forms of bacteria and viruses and bacteriophages. It was also indicated in the resolution that among the most timely problems to be solved in connection with noncellular forms of life are those of the modifiability of viruses and that of the biological significance of noncellular forms of bacteria.

In regard to filterable forms of bacteria and their connection with viruses and the stage (or phase) forms of development, Soviet scientists V. V. Silmev amd V. D. Timakov have definitely proved the wide occurrence in nature of diverse noncellular forms of life, the so-called filterable forms, among cellular microbes, and the possibility of the transition of noncellular into cellular forms. On the other hand, viruses are not changed into cellular forms or bacteria. To insist that there must be transition of bacteria into cellular forms is to apply Virchowian ideas to the understanding of the evolution of that extensive and independent class of life, noncellular organisms.

Experiments which have been conducted proved that there is no spontaneous modification of viruses into bacterial forms. The most precise results were achieved by the method of cultivating viruses in tissues explantates or in developing chicken embryos. Under these conditions many viruses are capable of intensive development on newly generated or surviving embryo cells. Here the most favorable conditions for the development of the most exacting microbes (spirochetes, hemoglobinophilic bacteria) exist. If transformation of viruses into microbes actually takes place, cellular microbes would definitely be detected under these favorable conditions. However, the extensive practical experience in cultivating the most diverse viruses and rickettsiae in tissue explantates convincingly proves that the microorganisms preserve their qualitative species characteristics in the process of repeated reseedings carried out over a great number of years. If the tissue cultures are skillfully handled, no accumulations of bacteria are obtained.

In experimenting with allantois cultures of the viruses of influenza, mumps, and tick encephalitis, we found that the virus perishes within two or three days and that no bacterial cells originate. Experiments with dying tissues also showed that the viruses perish and that no bacterial cells originate. The results of these experiments refute the assumption that in dead tissues viruses are regularly transofrmed into bacterial modifications.

The modification of viruses can be best studied on the example of phages, which are a kind of bacterial virus. However, investigations (A. S. Kriviskiy, 1950-1951) have shown that, notwithstanding the cellular structure of bacteriophage, the manifestations of its heredity and modifiability are subject to the laws of heredity common to all kinds of living matter. The modification of bacteriophages is a phenomenon that occurs extensively in nature. It can be easily observed under laboratory conditions. The phage particles, after being adsorbed on hereditarily changed bacteria and penetrating them, change under the conditions encountered in the bacterial cells, adapt themselves to reproduction in the modified cells of bacterial variants, and transmit hereditarily their newly acquired properties.

By investigating the interaction of phages with secondary phage-resistant cultures which have been obtained after the dissolution of the initial phage-sensitive cells, one may trace the whole process of the adaptation of the phage to a resistant culture. One observes that some phage particles succeed in adapting themselves to reproduction in cells which are resistant to the remaining mass of the phage. Initially the degree of this adaptation is small: the modified phage particles do not adsorb effectively on cells, multiply weakly in them, and form turbid, barely visible sterile spots. But during subsequent multiplication, individual particles arise in the modified phage population which progressively increase their degree of adaption to development in bacterial cells. Then individual sterile spots which become increasingly clearer appear in the cultures. Reseeding from these sterile spots gives rise to new variations of the phage. Finally, the most perfectly adapted and virulent variations of the phage develop. Then, one often observes a reduction of the virulence toward the initial phage-sensitive bacterial strain. The virulence toward this strain often disappears completely.

Under the circumstances, the phage acquires entirely new properties, one of which is the capacity to dissolve a culture which was resistant to the initial strain of the phage. The capacity to dissolve the initial bacterial culture is often lost. Artificial selection, in other words, isolation of the most virulent variation of the phage from individual sterile spots, considerably accelerates the process of adaptation.

Like cellular organisms, some variations of phages have a heredity which persists under various conditions of existence (i.e., existence on bacteria belonging to various strains), while others are less stable in regard to their heredity. These less stable return to their initial state when they have been transferred to the original conditions of

existence. A reversion of this type also takes place as a result of the development of individual phage particles which are identical with the initial type of the phage rather than by a modification of the whole phage population. Therefore, if constant selection is applied, in other words if isolation of the most characteristic sterile spots is consistently carried out, one may preserve during tens of generations any strain of the phage and in this manner establish definitely that newly arising modifications are inherited. The reversion to the initial type is most easily realized in the beginning of the process of adaptation. Among variants isolated during this period are strains which are distinguished by an exceptional capacity for rapid reversion. These strains seem to have an unstable, weakened heredity. The variants which are most remote from the initial type do not yield by reversion a type completely identical with the initial type. If the phage variant has completely lost its capacity to multiply on the cells of the initial bacterial culture, reversion becomes impossible.

The modification of the hereditary basis of viruses is of great practical significance. One of the most important practical problems of medical virology is the creation of living vaccines which are harmless to human beings and are capable of inducing the formation of an intensive and prolonged immunity against various mass infections. To bring about a change in the nature of the virus, which is necessary for the creation of a live vaccine and which is aimed toward the removal of pathogenic properties on the one hand, and preservation and reinforcement of immunogenic characteristics on the other hand, it is necessary to weaken the hereditary basis of the virus by changing radically the conditions of its individual development that have been established in the course of phylogenesis. Then the modification of the virus must be guided in such a manner that there is a weakening of the virulence to human beings, while the antigenic properties which are typical for the virus as the causative factor of the disease are preserved. Finally, one must also consolidate and perpetuate the newly acquired characteristics at the desired level. Experimental methods of exerting effective artificial action upon the organism of susceptible animals that would result in sufficiently thoroughgoing changes in the metabolism of viruses have not been developed as yet. For that reason the necessary new conditions of the existence of viruses are best obtained by the adaptation of the viruses to developing chicken embryos, to the embryonic tissues of other animals, to some sensitive organs of resistant animals, to tissues of experimental malignant tumors, or to the organism of a new species of susceptible animals which do not participate in the natural cycle of transmission of the causative factor.

These are viruses the hereditary characteristics of which are not stable. Any changes in the conditions of their existence, for instance, a short sojourn in the body of a different host, is accompanied by a thoroughgoing and irreversible modification of the pathogenicity and of other initial characteristics. A classical example of this group of highly plastic viruses is the virus of influenza. Even a short sojourn of a strain freshly isolated from human beings in white mice, ferrets,

or developing chicken embryos results in a thoroughgoing modification of the properties of the virus in relation to its principal host, i.e., man.

In view of the fact that any effective living vaccine must induce the multiplication of the weakened virus in the organism of human beings who have been inoculated, excessive suppression of the capacity to multiply makes the vaccine ineffective. For that reason, viruses which have the most strongly modified heredity require the briefest and most carefully controlled change of natural conditions of existence to new conditions. For example, after only a few passages through the organisms of developing chicken embryos, the strains of the influenza virus are usually weakened sufficiently to make them suitable for the production of living vaccines.

To obtain a living vaccine active against influenza, the procedure of carrying out brief passages of epidemic strains through developing chicken embryos proved to be the best according to our data.

If the virus has been excessively weakened and is no longer capable of multiplying in the human body, it is necessary to increase its activity. This is best achieved by cultivating the virus on human tissues according to the method proposed by A. A. Smorodintsev.

Another group of viruses has more stable hereditary characteristics. They lose their initial pathogenic properties with greater difficulty when the conditions of existence have been changed. However, the modifications produced experimentally in them are more firmly consolidated. To this group belong the viruses of rabies, smallpox, and yellow fever. The most effective living vaccines for these diseases have been obtained.

The third group of viruses possesses an exceptionally stable basis of heredity. To change their hereditary characteristics, prolonged efforts are necessary. Examples of such viruses are some causative factors of neuro-virus infections of the tick encephalitis and Japanese encephalitis type. Viruses of this type preserve their pathogenic and antigenic properties under the most varied conditions of natural circulation as well as under experimental conditions.

For instance, after prolonged adaptation to the organisms of white mice, which has continued for many years, the strains of viruses of tick encephalitis preserve a very high infectiousness for man and monkeys or apes. These viruses do not modify their characteristics as a result of natural or artificial changes in the hosts and carriers. They exhibit stable retention of their pathogenic characteristics in cultivation under conditions unusual to them, as for instance, in developing chicken embryos or in the testicular tissue of resistant animals (for instance, white rats or guinea pigs). For that reason it is very difficult to obtain from such stable viruses strains with a weakened heredity, in other words, strains which would be suitable for further modification of their pathogenic and immunogenic properties. A large amount of inventiveness in the selection of new conditions of existence for such viruses is necessary. However, when viruses of this type have been successfully subjected to a

thoroughgoing modification, they yield the most persistent production strains that retain their modified hereditarily transmissible characteristics for a great number of years.

At present, our scientific and medical institutions are concentrating on the creation of live vaccines effective against a number of mass infections produced by viruses. This includes influenza, measles, mumps, pappataci fever, tick encephalitis, Japanese encephalitis, and other diseases. In this work, our scientists assume that it is possible to modify the nature of viruses sufficiently and to consolidate the changes which have been obtained on the necessary level. This task would be entirely unrealistic if the process of modification that has been invented by pleomorphists really operated in nature. In that case, no efforts could prevent the newly created immunogenic strains, which have been suitably weakened, from undergoing transformations that would be irreversible and that would destroy them.

The success of further work in the direction indicated depends on the most rapid accumulation of facts pertaining to the existence of limits to natural and directed modification of microbes and viruses, development of principles and concrete methods necessary for controlling the phenomena of modification, and study of the interrelationships between cellular forms of microbes, viruses, and bacteriophages which exist and operate in nature.

# STUDIES ON THE ORAL TOXICITY OF CLOSTRIDIUM BOTULINUM TOXIN, TYPE A[1]

By Ivan W. Coleman[2]

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## Abstract

Three aspects of the reported oral toxicity of Clostridium botulinum toxin, Type A, were investigated. No demonstrable migration of the crystalline toxin from the lumen of the intestine into the blood stream of the dog could be found. Evidence indicating that the crystalline toxin was inactivated by pepsin and chymotrypsin was obtained, but the toxin was found to be resistant to the action of trypsin. Comparison of the oral toxicity and the intraperitoneal toxicity of the crystalline toxin revealed that the product was not orally toxic. A spraydried crude preparation of the toxin demonstrated a low oral toxicity.


## Introduction

The toxin of Clostridium botulinum has been described by Pappenheimer in his review of the proteins of pathogenic bacteria (11) as the only bacterial toxin which remains active when administered orally. Diphtheria and tetanus toxins, the only toxins of comparable toxicity, are both inactive orally, being rapidly destroyed by the proteolytic enzymes of the gut. Botulinum toxin is described as remaining toxic after in vitro treatment with pepsin and trypsin.

The toxin of C. botulinum has been crystallized by Abrams et al. (1) and Lamanna et al. (8). The type A toxin has been shown by Putnam et al. (15) and Kegeles (7) to be a protein of molecular weight 900,000 as determined by sedimentation and diffusion experiments. It is homogeneous in the ultracentrifuge at most pH values below pH 3 (19) and shows but one zone of migration in the electrophoresis apparatus over a pH range from 3 to 7 (14). The isoelectric point was shown (9) to be at pH 5.6

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Elemental and amino acid composition of the toxin by Putnam et al. (15) revealed a protein of nitrogen value 16.29%. On the basis of a molecular weight of 900,000, this group advanced the amino acid residues as follows, according to the convention suggested by Brand and Kassell (2); Gly.166, Ala.394, Val.406, Leu.708, Isoleu.820, Prol.203, Phen.64, CySH.20, $(CyS_2)_{40}$, Met.64, Trp.82, Arg.239, His.60, Lys.477, Asp.$NH_2$ 370, Glut.953, Ser.374, Thr.642, Tyr.672. This composition accounts for 100.2% of the total nitrogen.

The toxicity of the material is phenomenally high; per milligram of nitrogen it contains 1,200,000 toxic doses (minimum lethal dose per kilogram of animal) for a guinea pig, and 620,000 toxic doses for a mouse, on intraperitoneal injection (17). It is stable only at acid pH values, and is rapidly inactivated in neutral or alkaline solution.

It is difficult to reconcile the known physical properties of the toxin with the reported oral toxicity. The toxin is one which inhibits conduction at the myoneural junction (5,18), death usually resulting from paralysis of the respiratory muscles. This requires that the toxin be absorbed from the intestinal lumen into the blood stream or lymphatic system, a process which would necessitate a protein of molecular weight 900,000 migrating across the gut membranes. It is difficult to imagine how a protein with such a molecular weight could penetrate the membranes of the gastrointestinal tract which are impermeable to proteins of less molecular weight. Dent (6) has reported evidence that dog serum proteins can be so absorbed, but these were restricted to serum proteins of the same dog. Part I of this report consists of an attempt to demonstrate the ability of the toxin to migrate across the gut membranes.

The amino acid constituents of the toxin do not supply evidence which would indicate a lack of the amino acids requisite to the formation of peptide linkages specifically opened by the action of the proteolytic enzymes, pepsin, trypsin, and chymotrypsin. A study of the action of the enzymes on the crystalline toxin comprises Part II.

In Part III a comparative study of the intraperitoneal and oral toxicities of the crystalline toxin and a crude preparation on mice and rats is reported.

## Part I

## Migration of C. botulinum Toxin Across the Gut Membrane

### Materials

A sample of crystalline C. botulinum toxin, Type A, which was electrophoretically homogeneous, was available. The preparation in gelatine-phosphate diluent (1, 8) was found to have a minimum lethal dose by intraperitoneal route of 0.0001 µgm. per 20 ± 2 gm. mouse.

### Method

Dogs 8 to 15 kgm. in weight were anesthetized with nembutal (40 mgm./kgm.). A loop of ileum was isolated through a small abdominal incision,

and approximately 15 cm. tied off with two loops of surgical silk, leaving the circulation intact. One hundred micrograms of crystalline toxin in citrate buffer at pH 6.0 was introduced into the lumen of the intestine in one milliliter with a No. 22 needle, using a No. 18 needle as a trocar. The incision was then closed, and venous blood samples of 5 to 10 ml. in volume withdrawn under heparin at half-hour intervals for two and one-half hours. At the end of this period, 1 ml. of toxin containing 100µgm. was injected intravenously. The animal was allowed to rest overnight without food or water. A terminal blood sample was taken 24 hr. after the first injection, before the animal was sacrificed. Plasma was separated from the blood cells and the toxicity tested by injecting 0.5 ml. into each of four mice. Deaths among these mice, 96 hr. after the injection, are recorded in Table 1.



TABLE I

Absorption of C. botulinum Toxin from Isolated Segment of Gut

| Time of sample, hr. | Deaths among mice injected with 0.5 ml. plasma from: | | | |
|---|---|---|---|---|
| | Dog No.1 14.5 Kgm., male | Dog No.2, 14.1 kgm., female | Dog No.3, 15.4 kgm., female | Dog No.4, 8.7kgm. female |
| 0* | 0/4 | 0/4 | 0/4 | 0/4 |
| ½ | 0/4 | 0/4 | 0/4 | 0/4 |
| 1 | 0/4 | 0/4 | 0/4 | 0/4 |
| 1½ | 0/4 | 0/4 | 1/4 | 1/4 |
| 2½ | 0/4 | 0/4 | 0/4 | 0/4*** |
| 20** | 4/4 | 4/4 | 4/4† | 3/4 |

*Control sample before introduction of toxin into lumen.

**Time of intravenous injection of 100 µgm of toxin in citrate pH 6.0.

***Gut lumen was washed out with 2.0 ml. of gelatin phosphate solution which was tested for toxicity with four mice. Two mice died.

†0.5 ml. of filtered urine was injected into each of four mice, with no deaths resulting in 96 hr.

The results indicate that, in the blood samples examined, only two deaths occurred in a period of two and one-half hours after the introduction of 100 µgm. of crystalline toxin into the gut lumen. However, after the intravenous injection of the same quantity, deaths occurred even when the blood samples were taken 20 hr. after the injection.

Discussion

In the dogs examined with the previously described technique, no evidence could be found indicating the migration of the toxin in an active form into the blood stream. Injection of the toxin intravenously indicated in every case the presence of the toxin in the plasma as long as 20 hr. after the time of injection. The parenteral dose applied was of the order of 200,000 mouse lethal doses, which, if 8% of the dog's body weight is blood (1.2 to 1.5 liters), would mean that an absorption of as little as 0.3 µgm. of the toxin is detectable. This represents an amount absorbed from the isolated gut lumen of less than 0.5% of the toxin introduced. Since conditions were such that a high concentration was maintained in the isolated lumen, it seems reasonable to expect that absorption would be of a higher order than 0.5% in two and one-half hours.

This argument takes no cognizance of the in vivo behavior of the toxin. Because no information exists on (a) the rate of tissue removal of the toxin from the blood stream, (b) the rate or extent to which the toxin can be absorbed on cell surfaces, (c) the reactions of detoxification possibly occurring in liver, kidney, or other body sites, no allowance can be made for that fraction of absorbed toxin which is by any mechanism removed from the plasma and hence not available for analysis by this method. It seems likely, however, that the necessary correction for such reactions is small, since no difficulty was found in detecting the toxin in plasma 20 hr. after intravenous injection, but the rate of such reactions may still be such that something more than 0.5% of the toxin could have been absorbed from the gut lumen without detection.

## Part II

## The Digestion of C. botulinum Toxin by Pepsin, Trypsin, and Chymotrypsin

Methods

Buffered substrates of crystalline C. butoulinum toxin, Type A, were prepared at 1 mgm./ml. concentration using 0.01 M potassium chloride-hydrochloric acid buffer at a pH of 1.4 for pepsin digestion, and 0.01M phosphate buffer at pH 6.5 for trypsin and chymotrypsin. To 15 ml. of each substrate, 5 ml. solution containing 1mgm./ml. of Armour crystalline pepsin, trypsin, and chrmotrypsin was added. One 15 ml. portion of toxin substrate at pH 1.4 was retained as a control for the pepsin digestion, while a similar volume at pH 6.5 was retained as the undigested control for the trypsin and chymotrypsin digests. Five milliliters of water was added to each of the controls to maintain equivalent toxin concentration. Digestion was performed at 37°C. and allowed to proceed for 72 hr. One milliliter samples were withdrawn at regular intervals,

and the toxicity determined by injections of 0.5 ml. samples intraperitoneally into mice. By serially diluting the digests sample (1:10 dilution at each step), the number of mouse lethal doses per milliliter of digest was determined. Groups of four mice were used at each dilution. They were arranged in weight groups: 20 ± 2, 25 ± 2, and 30 ± 2gm. A minimum of 24 mice was used for each estimation of the toxicity of a digest sample. This required, with the controls, that 120 mice be used at each sampling time. Since groups of mice of the same weight in this number were not available, the three weight groups were chosen. The number of mouse lethal doses per milliliter of digest is therefore reported per kilogram of mouse.

Results

The toxicity of the crystalline pepsin, trypsin, and chymotrypsin preparations used was determined by injection of 0.5 ml. intraperitoneally into mice in graded concentrations to a maximum of 1.0 mgm./ml. No deaths resulted in any of the mice tested, even at the highest concentration used. This step was taken to ensure that deaths resulting from the assay of the toxin digests could be ascribed to the toxicity of the toxin alone.

The results of the analysis of the digests and contents are shown in Fig. 1, in which the number of lethal mouse doses per kgm./ml. of digest is plotted against the time of digestion.

The results indicate that the toxicity of the controls decrease to approximately one-tenth of the starting value in 72 hr. This is in agreement with the findings of Reed and Muench (16).

Fig. 1. Toxicity of crystalline botulinum toxin, Type A, after digestion with proteolytic enzymes: Curve A, control pH 1.5; Curve B, control pH 6.5; Curve C, trypsin digest; Curve D, chymotrypsin digest; Curve E, pepsin digest.

During the same period, the toxicity of the pepsin and chymotrypsin digests decreased rapidly after a short lag, to a value at 72 hr. at which the digests were nontoxic (at 1.0 mgm. toxin per ml.). The fugitive increase in toxicity of the chymotrypsin digest from zero to six hours may be significant. A similar process has been described by Bridgeman (3), Peterman (12), and Peterman and Pappenheimer (13), on enzymatic digestion of antibody molecules. These authors report that digestion of antibody molecules may proceed to the point of yielding residues of one-fourth the molecular weight of the original antibody without loss of the specific antibody activity. The transitory increase in the toxicity of the chymotrypsin digest may reflect such a process.

The action of trypsin was not as striking. At the end of the digest period the toxicity of the trypsin digest was about one-fourth the toxicity of the control at the same time. This may indeed represent the inertness of the toxin to trypsin digestion. However, since the terminal value of the toxicity is below the control, trypsin certainly exerts some action on the toxin. The lack of complete inactivation by trypsin may well be due to the inability to operate the experiment at or near the optimal pH trypsin digestion. The value of pH 6.5 used was necessitated by the rapid inactivation of the toxin at pH values more alkaline than 7.0. The digestion of the toxin at pH 8.0 to 8.4, the optimum pH of trypsin, would proceed more rapidly than shown here.

The uniform decrease in toxicity of the digests in comparison with the controls at zero time is probably due to the time interval between the withdrawal of the sample and the performance of the assay. The interval was of the order of one hour.

Summary

Pepsin and chymotrypsin digests of crystalline botulinum toxin, Type A, at pH values of 1.4 and 6.5 respectively, resulted in the inactivation of the toxin, such that the injection into mice of a volume of digest equivalent to that containing 1.0 mgm. of toxin (approximately $10^4$ lethal mouse doses) had no effect.

Trypsin digestion did not result in complete inactivation when digested at pH 6.5, but in 72 hr. the digest showed a toxicity of approximately one-quarter of the similarly treated, but undigested, control.

## Part III
## The Comparison of Oral and Intraperitoneal Toxicities of Crystalline Botulinum Toxin Type A

Methods

A fresh solution of Type A crystalline botulinum toxin was prepared in citrate buffer pH 6.0. Several dilutions of the stock were made in gelatine phosphate diluent to yield six solutions ranging in concentration

from 10 to 0.001 µgm. of toxin per ml. of solution. One milliliter of each dose was injected intraperitoneally into four mice in each group. For oral administration, 0.2 ml. was administered by stomach tube. The tube consisted of a flexible, 2-in. No. 26 needle, ball-tipped with silver solder. Ten animals were used in each oral dose group. The mice were kept in separate cages without food for the period of 48 hr., and the survivors were fed on regular rations until the conclusion of the assay period of 96 hr. Mice used were all females of 20 ± 2 gm. in weight.

The results of this comparison are shown in Table II, Section I.

The method previously described was repeated, using rats of 110 to 130 gm. in weight. The maximum oral dosage was increased to 1000 µgm. The results are also shown in Table II, Section II.

Spray-dried preparations of botulinum toxin, which had been prepared by precipitating the cell-free cultures by adjusting to pH 5.6, were available. The samples had been stored for three to four years, and still showed an $LD_{50}$ of 0.002 µgm. per 20-gm. mouse by intraperitoneal route. Studies of the oral and intraperitoneal toxicity of this product were repeated on the rat in the manner previously described. The maximum oral dose of this crude preparation was increased to somewhat less than 7000 µgm. The results are shown in Table II, Section III.

Results

With crystalline toxin, application by the oral route of a dose which exceeded $2 \times 10^4$ times the minimum lethal intraperitoneal dose failed to cause any deaths in the mice tested. With the rat, increase of the oral dose to a value exceeding $10^4$ times the minimum lethal dose intraperitoneally failed to show any effect on the rats tested. There is reason to believe that, with the crystalline product, the oral dose could be raised still further without being toxic. With the crude preparation there was a demonstrable oral toxicity at a level which represented $10^4$ times the minimal effective intraperitoneal dose.

Discussion

The lack of demonstrable oral toxicity of crystalline botulinum toxin, Type A, is in agreement with the results obtained in the permeability study and with the inactivation of the toxin by pepsin, chymotrypsin, and probably trypsin. It is, however, in sharp contrast with the results reported in the literature (10,11) and others which described the toxin as being effective orally. The previous observations of the oral toxicity have been made by many authors and are well documented in the clinical cases of fatal botulism in which the oral route could be the only method of admission of the toxin. However, it must be remembered that all the observations have been made (to the best of the author's knowledge), on either intact cultures of C. botulinum or on cell-free

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TABLE III

Results of the Oral and Intraperitoneal Administration of Botulinum Toxin Type A

| Section 1 | | | | | | Section 3 | | | | | | Section 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crystalline product Mouse 20, 2 gm. | | | | | | Crystalline product Rat 120, 10 gm. | | | | | | Crude product Rat 120, 10 gm. | | | | | |
| Oral | | | Intraperitoneal | | | Oral | | | Intraperitoneal | | | Oral | | | Intraperitoneal | | |
| Deaths per group | Animals per group | Dose, µgm | Dose, µgm. | Animals per group | Deaths per group | Deaths per group | Animals per group | Dose, µgm. | Dose, µgm. | Animals per group | Deaths per group | Deaths per group | Animals per group | Dose, µgm. | Dose, µgm. | Animals per group | Deaths per group |
| 0 | 10 | 0* | 0 | 4 | 0 | 0 | 10 | 0* | 0* | 10 | 0* | 0 | 10 | 0* | 0* | 8 | 0 |
| 0 | 10 | 2.10 | 1.0 | 4 | 4 | 0 | 10 | 1000 | 1.0 | 8 | 8 | 10 | 10 | 6870 | 68.7 | 8 | 8 |
| 1 | 10 | 0.2 | 0.1 | 4 | 4 | 0 | 10 | 100 | 0.1 | 8 | 8 | 3 | 10 | 687 | 6.8 | 8 | 8 |
| 0 | 10 | 0.02 | 0.01 | 4 | 4 | 0 | 10 | 1.0 | 0.01 | 8 | 8 | 0 | 10 | 69 | 0.68 | 8 | 8 |
| 0 | 10 | 0.002 | 0.001 | 4 | 4 | 0 | 10 | 0.1 | 0.001 | 8 | 3 | 0 | 10 | 6.9 | 0.07 | 8 | 8 |
| 0 | 10 | 0.0002 | 0.0001 | 4 | 3 | 0 | 10 | 0.01 | 0.0001 | 8 | 0 | 0 | 10 | 0.069 | 0.007 | 8 | 8 |
| | | | | | | | | | | | | 0 | 10 | 0.07 | 0.0007 | 8 | 1 |

* Controls of gelatino-phosphate diluent.

filtrates. The toxicity of the crude products has then been ascribed wholly to the toxin. It is only this conclusion that is erroneous. If the hypothesis is advanced that in the process of isolation and purification of the crystalline toxin a second oral potentiating factor of unknown properties is lost, the results of this work and the previous reports can be reconciled. Evidence in favor of such an hypothesis has already been advanced by Bronfenbrenner and Schlesinger (4). These workers found that the addition of ammonium sulphate to cell-free cultures of C. botulinum reduced the oral toxicity of the culture to one hundredth of its previous value, although the intraperitoneal toxicity remained the same. On addition of the material precipitated by ammonium sulphate, the oral toxicity of the reconstituted media returned to its original value. The results obtained with crude spray-dried botulinum toxin tend to support this concept. The oral toxicity of this product can be explained by assuming that it still contains some of the postulated factor.

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Osbennosti Obrazovaniia Kletochnykh iader v tkaniakh vegetativnykh gibridov

[Characteristics of the Formation of Cell Nuclei in the Tissues of Vegetative Hybrids].

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(In Russian)

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The study of the characteristics of cell formation and of the development of nuclei in plant organisms with culturally induced changes in sexual and vegetative hybrids, possessing, as is known, increased diversity in the characteristics of cells and tissues and wide morphological variation (1-4, 8, 9), holds out great theoretical interest.

During the first phase of the work we decided to investigate the process of the formation and development of cells and of their nuclei in vegetative hybrids with the objective of turning, later, to sexual hybrids and to organisms altered by breeding conditions.

The study of this process was conducted on shoots of the first seed generation of vegetative hybrids obtained from crossing winter barley with winter rye. The winter barley variety "Kruglik 21" (Hordeum vulgare L. var. pallidum) served in the capacity of the scion, and the two winter rye varieties of "Kazanskaia 546" and "Datnuvo-Aukshtein" (Secale cereale L. var. vulgare) in that of the stock. The grafting was accomplished by transplanting the embryo of the barley onto the double endosperm of the winter rye according to the method described earlier (8, 9).

The pure-variety seed of the original forms — barley Kruglik 21, rye Kazanskaia 546 (control), and of the first seed generation of the vegetative hybrids $\frac{\text{Kruglik 21}}{\text{2 Kazanskaia 546}}$ and $\frac{\text{Kruglik}}{\text{2 Datnuvo—Aukshtein}}$ — were grown on a moist substrate until shoots appeared. The root tips (rootlet meristem) which had formed were fixed with Bruin's fluid and with mixtures of a 3% solution of chromic acid and neutralized formalin. After the usual cytological treatment, sections (5-8 microns thick) were stained with hematoxylin and acetocarmine.

A detailed study of all the preparations showed that the formation of new cells in the meristem of shoots of the control series was accomplished with an obvious predominance of mitosis (62 - 68%) (See table 1).

An entirely different correlation in the occurrence of mitosis, amitosis and cases of new nuclei formation in the nuclei of maternal cells is observed in the meristem of shoots of seed generation of investigated vegetative hubrids (Table 1). It was found that here the mitotic index equalled 8.5 — 10%, while amitosis, instances of nuclear budding, and the appearance of some new nuclei within one nucleus of a maternal cell reach 69-73%.

In vegetative hybrids amitosis is encountered more often in the form of a direct division of the nuclear substance into two parts. In the literature this type of division is considered as a typical, complex amitosis. Fig. 1 shows that the new nuclei thus formed are connected with each other by a thick bridge. The initial phases of complex amitosis are represented in Fig. 2 v,d. Thus it is obvious that the appearance of new nuclei is preceded by the formation of two nucleoli, the separation of which indicates the beginning of the partition of the remaining nuclear substance. The division of the nuclear substance into two approximately equal parts manifests the morphological characteristics of such amitosis.

The other process in the formation os new nuclei is the budding of the nucleus. In this instance, the nucleolus at first splits into two parts. One of the nucleoli leaves the nucleus, and a new but small nucleus forms around it (Fig. 2, a,g,z). The cell becomes binucleate, and then the daughter nucleus, separated by budding migrates from the nucleus of the mother cell, and the formation of the membrane of a new cell takes place. Sometimes the budding of the daughter nucleus precedes the formation of nucleoli (Fig. 2, g,k). In such a case there occurs an unequal division of the nucleus: the smaller part of the nuclear substance goes to the daughter nucleus, and the larger part remains in the mother nucleus. More rarely there occurs a process of new nuclei formation that could be designated as "multiple budding" or as the decomposition of nucleus into several parts.

Table 1.

| Series | Mitosis (all phases) in % | Amitosis and new formation of nuclei in % | Mononuclear cells and indistinct forms of development in % |
|---|---|---|---|
| I. Winter rye, Kazanskaia 546 . . . . . | 68 | 12.6 | 19.4 |
| II. Winter barley, Kruglik 21 | 62 | 16.5 | 21.5 |
| III. Kruglik 21 / Datnuvo—Aukshtein | 8.5 | 69.5 | 22 |
| IV. Kruglik 21 / Kazanskaia 546 | 10.6 | 73 | 16.4 |

According to our observations such a method of nuclear formation occurs, as a rule, in cells of large dimensions possessing large-size nuclei. While nuclei are being reproduced by this method in a large-size nucleus, there appear several nucleoli followed by fission of the nuclear substance into three daughter nuclei (Fig. 2, b,c) in conformity with the number of the newly developed nucleoli, or the nuclear substance separates into a larger number of parts than the number of the nucleoli defined (Fig. 2, zh). There were observed instances of separation of the original nucleus into four new nuclei (Fig. 2, k).

Fig. 1,
Straight division of nuclear substance in a meristem cell of the shoot of a vegetative hybrid (Series III). The newly formed nuclei are joined by a bridge.

The forms of cell reproduction described above and the pictures of new nuclei production within the old nucleus denote nothing specially new at this time. Similar pictures of such development of new nuclei have already been described in the literature (10, 12).

In our opinion, attention should be paid to the reasons for the predominance of amitosis and the formation of cells through the formation

of new nuclei in the tissues of investigated vegetative hybrids as compared with the tissues of the original varieties, utilized as controls, in which the predominant form of cell formation was mitosis.

On the basis of our own observations, and taking into account a large accumulation of material covering this subject in the literature, we are ready to advance the hypothesis that the reason for such predominance probably is a metabolic disturbance caused by the union of remote intergeneric components.

It is generally known that vegetative hybridization is one of the powerful means for upsetting heredity, of eliminating its conservation, i.e. the established, stabilized type of metabolism. From a purely chemical angle, metabolism represents the sum total of comparatively simple reactions of oxidation, reduction, decomposition, synthesis, transfer of separate atomic groupings. However, the specific thing for the organism is that these reactions are accomplished in it in co-ordination, in strictly determined succession.

The specific characteristics of metabolism determine permancy, the qualitative constancy of species, genus, variety, forms of living organisms under appropriate conditions of environment. In so far as the result of vegetative hybridization is a complete modification of the character and the type of metabolism in the seed generation causing changes in its properties, characteristics and phenomena (11), it must be assumed that this modification determines also a change in the process of cell production.

Fig. 2
Various forms of nuclei propagation in the tissues of vegetative hybrids (series IV).
(a) - formation of a new nucleus by means of budding;
(b) - dividing nuclear substance in 3 daughter nuclei;
(v,d) - initial phases of dividing nucleus into 2 daughter nuclei of equal size;
(e, zh) - decomposition of nucleus into 3 daughter nuclei of varying size;
(z) - new budding begins in nucleus after the formation of one daughter nucleus;
(i) - in the nucleus appear embryos of new nuclei (buds), but no nucleoli have formed in them as yet;
(k) - multiple budding of one nucleus; formation of 4 daughter nuclei of varying sizes.

Proceeding from such an hypothesis, it can be assumed that in the case of an undisturbed, stabilized type of metabolism the predominant form in the formation of new cells would have to be mitosis, as it is the more complex form of cell reproduction, capable of equal distribution

of living substance between the mother and daughter cells. In other words, mitosis reflects coordination, regular sequence of metabolic reactions, and amitosis is an indicator of a disturbance in the constant type of metabolism — of shattered heredity. Namely, it is, precisely, thanks to the unequal distribution of living substance during amitosis or nuclear budding and the formation of new nuclei that diverse tissue properties are formed, thus causing the morphological diversity in the progeny of vegetative hybrids (1,3,8,9).

The direct relation between metabolic distrubance and the processes of cell reproduction was discovered by P. S. Revutskaia (10) who established the fact that in mesotholium which settles out in ascitic fluid, in cases of cancer of internal organs, the amitotic index equaled 91.3%, and in mitotic 0.6%.

In studying the seed sprouts of rye, wheat, Crepis tectorum, after a 5-6 year storage period, and following the action of high temperature (54-55°C) on dormant dry seed for a period of 20-44 days, M. S. Navashin and P. K. SHkvarnikov (7) established an almost complete absence of typical mitosis. Together with this they discovered in the cells of both series the presence of additional nuclei, fragmentation of nuclei and, most interesting of all, a predominance of amitosis. It is true that the authors treated these facts as an example of "chromosome mutations", "chromosome translocation", yet in studying the microphotograph it becomes clear that there actually occurred amitotic division of nucleii, or that nuclear budding took place. It is clear that the reason for this was a deep disturbance of metabolic type caused by prolonged storage of the seed or by sublethal temperature.

A series of important observations of the process of metabolism in vegetative hybridization was carried out by D. Kostov (4). He found that in cases of transplantation there occurs an accumulation of starch and other nutritive substances, and that enrichment of cells by cytoplasm, cytolysis (nucleolysis, proteolysis, hydration, etc) and necrosis which are the result of increased enzyme activity, could be seen. It is significant to note that these changes in the physiological processes caused a series of morphological changes in cell structures, particularly there occurred an abnormal increase in the size of cells, then hypertrophy of nuclei and an increase of the number or size, or size as well as number, of the nucleoli.

If our hypothesis will be substantiated also in regard to seed progeny of sexual hybrids and in regard to the progeny of organisms whose nature had been changed by cultural conditions, then it could be assumed that the predominance of the coefficient of amitosis and the new formation of nuclei in the nucleus over the mitotic coefficient is one of the authentic characteristics (indicators) that the nature of an organism is unstable, unsettled. In the reverse case — in an absolute preponderence of mitosis as a form of cell reproduction, one will be able to assert that instability has definitely been overcome, that a stable type of metabolism has been established, which means, asserting that there is a certain stability of form, class, species, and variety of the organisms under investigation.

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