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BIOMEDICAL AND BEHAVIORAL SCIENCES

(FOUO 5/79)

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This serial publication contains articles, abstracts of articles and news items from USSR scientific and technical journals on the specific subjects reflected in the table of contents.

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AGROTECHNOLOGY

UDC 581.1.032.3

RAISING THE HEAT AND DROUGHT RESISTANCE OF SPRING WHEAT BY A COMPLEX METHOD

Moscow FIZIOLOGIYA RASTENIY in Russian No 3, 1979 pp 641-647

[Article by P. A. Genkel', K. A. Badanova, L. D. Prusakova, and K. S. Bokarev, USSR Academy of Sciences Institute of Plant Physiology imeni K. A. Timiryazev, Moscow]

[Text] A new method for raising the heat and drought resistance of cereal crops, a combination of presowing hardening of the seeds and semidry processing with retardants, is proposed.

Presowing drought-hardening of Albidum 43 wheat caryopses by Genkel's method coupled with their treatment by 5 percent CCC solution or 0.5 percent BES solution stimulated stem growth and maturation in the presence of both optimum soil moisture and dry soil. The combination of hardening and retardant processing caused the tillering node to develop deeper in the soil in comparison with control plants and with plants treated with retardants alone. This was accompanied by a rise in the resistance of leaf cells to dehydration and high temperature in the tillering and stem extension phases. Dry soil decreased the grain yield from unhardened plants grown in a phytotron by 85 percent, that from hardened plants grown from seeds treated with CCC by 20 percent, and seeds treated with BES by only 10 percent, which considerably reduced the yield loss in comparison with using each method for raising resistance to heat and drought separately.

Raising plant resistance to heat and dehydration continues to be one of the most important problems of plant physiology.

The resistance of plants to drought--that is, to heat and dehydration--is raised in many regions of the Soviet Union with the help of agrotechnical techniques, by breeding drought and heat resistant varieties, and by means

of other methods for raising plant drought resistance. Thus Genkel' (1) suggested a method for presowing drought hardening of seeds. This method, which relies on the significant adaptability of plants, concurrently increases heat and drought resistance.

The institute's laboratory of plant drought resistance has revealed a number of anatomical, morphological, and physiological characteristics of hardened plants which permit them to endure dry soil and atmosphere better. Owing to protective adaptive reactions, hardened plants maintain a high level of synthesis, which in turn insures high metabolism and causes changes in a number of properties of protoplasmic organelles, promoting high productivity of hardened plants exposed to drought (2-4). A mechanized seed hardening process proposed by one of the authors (5) permits broader application of the plant hardening method in the farming practices employed in arid regions of our country.

A new way for raising plant drought resistance appeared with discovery of retardants, chlorocholine chloride in particular (6). As we know, chlorocholine, chloride (CCC), which has an influence on many physiological processes in plants, inhibits stem growth and, at the same time, reduces the demand of stems for water and makes them more resistant to wilting (6,7). The resistance of plants treated with this preparation to drought increases because they experience activation of a number of protective adaptive reactions resulting in development of properties typical of drought resistant plants: a well developed, deeply penetrating root system, low height, reduced transpiration intensity, increased tissue water content, and reduced permeability of protoplasm to electrolytes (8-11).

A higher water content and better water metabolism were observed in plants sprayed with CCC. Good plant water metabolism promotes a rise in the water retention capacity of plants experiencing drought conditions, more economical water consumption, and greater activity of some key enzymes of respiration and of phosphorus and carbohydrate metabolism--ATP-ase, catalase, and cytochrome oxidase (12,13).

It can be hypothesized that the drought and heat resistance of plants could be raised even more by combining the physiological method for hardening caryopses against drought (wetting the seeds and subsequently drying them) with a chemical method (treating them with retardants before sowing). Because CCC has a favorable effect on water metabolism in plants experiencing drought, we could suggest the hypothesis that the retardant's effect on drought resistance would manifest itself even more in hardened plants.

Methods

Two retardants were used in the experiments-- crystalline trimethyl-β-chlorethylammonium chloride produced by the Kemerovo Nitrogen Fertilizer Plant, and a preparation synthesized by one of the authors of this article (14) named BES (dimethyl-β-bromethylsulfonium bromide). BES is a crystalline

substance that dissolves well in water and is similar in chemical structure to CCC. Its formula is

$$[(CH_3)\,SCH_2CH_2Br]^+\,Br^-.$$

The preparation demonstrated high retardant activity against peas and barley (14,15).

The research was conducted in 1975-1977. Seeds were subjected to semidry treatment by the method proposed by Zadontsev et al. (16).

The object of research was Albidum 43 spring wheat. Unhardened caryopses and caryopses subjected to presowing drought hardening according to Genkel's method were shaken together with the retardants for 6 hours in a revolving drum. Ten milliliters of 0.5 percent BES solution or 5 percent CCC solution was added to every 100 gm caryopses; caryopses sprayed with the same quantity of water served as the control. Following treatment, the caryopses were planted in pots of soil. The experiments were performed in a greenhouse following the design shown in Table 1.

Table 1. Experimental Design

(1) № варианта	(2) Предпосевная обработка семян			(5) Влажность почвы при выращивании растений
	закалка (3) по Генкелю	ССС	(4) БЭС	
1	−	−	−	70% все время (6)
2	+	−	−	То же (7)
3	−	−	−	40% с фазы трубкования (8)
4	+	−	−	То же (7)
5	−	+	−	70% все время (6)
6	+	+	−	То же (7)
7	−	+	−	40% с фазы трубкования (8)
8	+	+	−	То же (7)
9	−	−	+	70% все время (6)
10	+	−	+	То же (7)
11	−	−	+	40% с фазы трубкования (8)
12	+	−	+	То же (7)

Key:

1. Variant
2. Presowing seed treatment
3. Hardening, Genkel''s method
4. BES
5. Soil moisture content during plant growth
6. Throughout
7. As above
8. Beginning with the stem extension phase

The 1976 experiment differed from the others in that drought conditions were created in the soil at the end of the tillering phase and affected the beginning of the flowering phase. The plants were not watered for 7 days. Soil moisture in the pots was dropped to 10 percent. Then watering was resumed. The experimental plants were subsequently kept under observation, and their yield was determined at the end of the vegetative period.

The direct effect soil dryness had on experimental plants was studied in the homeostatic environment of a phytotron in January-March 1976, using the same experimental design. The plants were grown at 22° during the day and 16° at night. Plant illumination was 200,000 ergs·cm^{-2}·sec^{-1} for 16 hours (xenon lamps). In the ear formation stage the plants were subjected to soil drought (watering was interrupted for 11 days). This time soil moisture dropped to 35 percent of its total moisture capacity, which corresponded to a soil water concentration of 15 percent. The moisture content of the soil of control plants was kept at 70 percent during this time by watering.

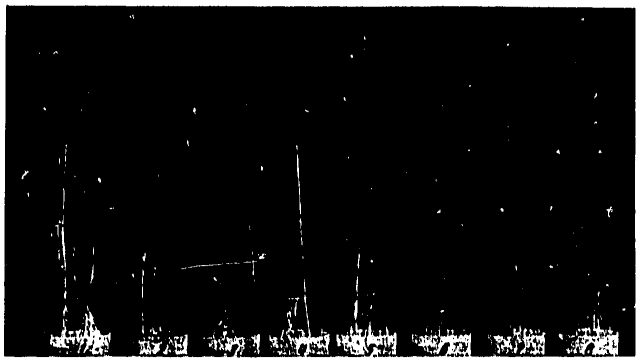
All experiments had a fivefold replication. Heat resistance was determined by Matskov's method (17) while resistance of cells to dehydration (18) was determined by the dessicator method, the criterion being the time of death of cells experiencing dehydration in the presence of diluted sulfuric acid in the tillering and stem extension phases. Determination of the length of the underground internode, which was an indication of the depth of the tillering node, involved fiftyfold replication.

Results

Hardened caryopses produced sprouts 2-3 days earlier than did unhardened plants. The retardants CCC and BES did not alter the sprouting time. Growth inhibition was observed in the first stages of growth of plants from caryopses treated with CCC and grown in the presence of 70 percent soil moisture; growth was stimulated in subsequent stages, and the height of the plants evened out (see Figure). In contrast to this, the retardant BES stimulated growth as early as in the first stages of plant growth, and it continued to have this effect until the end of vegetative growth.

Hardened plants in all experimental variants entered the tillering and flowering stages almost simultaneously, 3 days ahead of unhardened plants in similar variants. Deepening of the tillering nodes was observed in experimental plants even before the onset of drought (Table 2). The tillering nodes are the principal organs in which new shoots form, and when the above-ground organs and parts of the root system die, they can regenerate entire plants. This property obviously has special significance to mass death of plants in unfavorable conditions, particularly in droughts.

Considering that wheat plants with a deeper tillering node enjoy better conditions for root system development and for maintenance of viability in



Effect of caryopses hardening and presowing treatment on wheat growth in dry soil: Phytotron chamber, 1976. *1*--Unhardened (soil moisture content 70 percent), *2*--hardened (moisture content 70 percent), *3*--unhardened (moisture content 40 percent), *4*--hardened (moisture content 40 percent), *5*--unhardened, CCC (moisture content 70 percent), *6*--hardened, CCC (moisture content 70 percent), *7*-- unhardened, CCC (moisture content 40 percent), *8*--hardened, CCC (moisture content 40 percent)

Table 2. Depth of Tillering Node Following Drought Hardening of Seeds Treated with Retardants and Grown in Soil With a 70 Percent Moisture Content; Greenhouse, 1975

Variant	Treatment with	Tillering Node Depth, mm	Variant	Treatment with	Tillering Node Depth, mm
	Unhardened			Hardened	
1	Water	0	2	Water	15±0.08
5	CCC	15±0.05	6	CCC	30±0.10
9.	BES	10±0.06	10	BES	20±0.12

unfavorable environmental conditions, farmers usually try to plant the seeds deeply (7-10 cm); however, deep sowing weakens the sprouts as a rule. To the extent we know, causing the tillering node to develop deeper by presowing light and high temperature treatment of sprouting seeds (19) has not enjoyed practical application. We can see from Table 2 that treatment of seeds with

CCC deepened the tillering node of the plant by 15 mm, while BES treatment deepened the node by 10 mm. Combination of drough hardening of the plant and retardant treatment elicited even greater deepening of the tillering node--up to 30 mm with CCC and up to 20 mm with BES.

The data presented here attest to the positive influence retardants have on the depth of development of the tillering node in wheat, which was noted in relation to CCC for the first time by Zadontsev et al. (16). A similar effect was observed for the first time when seeds were treated with BES retardant and the plants were drought hardened, and when drought hardening was combined with CCC and BES retardant treatment.

The tillering node of wheat in soil may develop deeper as a result of both low temperature and moisture content on one hand and agrotechnical techniques on the other, for example by changing the planting time or increasing the seed planting depth. The latter, however, may have an unfavorable effect on caryopsis germination.

Deepening of the tillering node is an important factor of plant drought resistance, especially in the early phase of plant development. When the tillering node is located deeper in the soil, plants receive an additional stimulus for growth; a larger tillering node forms, and the roots penetrate more deeply in the soil, which promotes fuller utilization of the moisture reserves available in the soil.

Table 3. Change in Heat and Drought Resistance of Leaves in Response to Seed Drought Hardening and Retardant Treatment (See Table 2 for the conditions; greenhouse experiment, 1975)

Variant	Treatment With	Temperature of Cell Death, °C		Time of Leaf Cell Death in Response to Dehydration, min	
		Tillering	Stem Extension	Tillering	Stem Extension
		Unhardened			
1	Water	58	74	95	100
5	CCC	60	77	105	110
9	BES	59	74	120	140
		Hardened			
2	Water	60	76	115	135
6	CCC	62	80	120	150
10	BES	59	77	140	165

Table 4. Effect of Seed Hardening and Retardant Treatment on the Structure of the Yield for Plants Grown in Dry Soil (Phytotron Chamber, 1976)

Вариант (1)	(2) Высота растений, см	(3) Длина колоса, см	(4) Число колосков	(5) Число зерен в колосе	(6) Вес колоса, г	(7) Вес 1000 зерен, г
(8) Незакаленные						
1. Вода — контроль (9)	95	6,1	13,4	12,0	1,3	55,0
3. » засуха (10)	73	4,2	7,0	1,5	0,2	40,0
5. ССС — контроль (11)	100	6,4	12,5	15,7	1,6	54,7
7. » засуха (10)	84	5,1	8,1	7,5	0,8	42,6
9. БЭС — контроль (12)	94	6,6	13,2	11,2	1,3	55,2
11. » засуха (10)	82	5,4	9,6	8,0	0,9	45,1
(13) Закаленные						
2. Вода — контроль (9)	104	6,7	13,8	13,5	1,3	58,2
4. » засуха (10)	89	4,8	7,8	10,0	0,8	42,8
6. ССС — контроль (11)	98	7,0	12,7	20,0	1,5	57,0
8. » засуха (10)	85	5,8	10,0	17,2	1,1	44,0
10. БЭС — контроль (12)	102	6,6	13,3	19,7	1,3	58,4
12. » засуха (10)	88	5,9	10,8	17,6	1,2	52,1

Key:

1. Variant
2. Plant height, cm
3. Ear length, cm
4. Number of ears
5. Number of grains per ear
6. Ear weight, gm
7. Weight of 1,000 grains, gm
8. Unhardened
9. Water--control
10. Drought
11. CCC--Control
12. BES--Control
13. Hardened

Table 5. Effect of Seed Hardening and Retardant Treatment on Relative Yield From Plants Grown in Dry Soil (Phytotron Chamber, 1976)

Variant	Grain Yield, % of Control	Variant	Grain Yield, % of Control
Unhardened		Hardened	
1. Water--control	100	2. Water--control	100
3. " --drought	15	4. " --drought	60
5. CCC--control	123	6. CCC--control	115
7. " --drought	60	8. " --drought	80
9. BES--control	100	10. BES--control	100
11. " --drought	70	12. " --drought	90

Table 6. Effect of Seed Hardening and Retardant Treatment on the Structure of the Yield From Plants Grown in Dry Soil (Greenhouse, 1976)

(1) Вариант	(2) Длина стебля, см	(3) Длина колоса, см	(4) Число колосков в колосе	(5) Число зерен в колосе	(6) Вес 1000 зерен, г
(7) Незакаленные					
(8) 1. Вода — контроль	64	6,4	11	21	38,3
(9) 3. » — засуха	48	5,4	11	11	28,2
(10) 5. ССС — контроль	63	6,4	13	24	40,0
(9) 7. » — засуха	45	6,0	12	13	36,1
(11) 9. БЭС — контроль	67	6,3	12	21	40,7
(9) 11. » — засуха	51	6,0	12	14	37,0
(12) Закаленные					
(8) 2. Вода — контроль	65	6,6	13	23	38,5
(9) 4. » — засуха	49	5,8	12	13	40,0
(10) 6. ССС — контроль	64	6,3	13	24	41,2
(9) 8. » — засуха	48	6,3	13	16	41,0
(11) 10. БЭС — контроль	71	7,0	13	24	38,4
(9) 12. » — засуха	52	6,9	13	18	40,5

Key:

1. Variant
2. Stem length, cm
3. Ear length, cm
4. Number of spikelets per ear
5. Number of grains per ear
6. Weight of 1,000 grains, gm
7. Hardened
8. Water--control
9. Drought
10. CCC--control
11. BES--control
12. Hardened

Determinations of plant drought and heat resistance showed (Table 3) that treatment of plants with CCC increases drought and heat resistance, while treatment with the retardant BES raises the dehydration resistance of leaf cells more. As we hypothesized, the heat and drought resistance increasing effect was more significant with plants that were drought hardened and retardant treated than with unhardened plants. Thus the results in Table 3 indicate that complex treatment of the plant elicits an increase in leaf cell resistance to high temperature and dehydration.

Soil drought initiated in the ear formation stage in the homeostatic conditions of the phytotron had a strong influence on ear formation (Table 4). Ear length and the number of spikelets and grains forming in the ear decreased, having an effect on ear weight. The weight of 1,000 grains also decreased. Empty ears and puny grains resulted in high yield losses. As a result the loss of grain from unhardened plants (Table 5) was 85 percent. The yield loss was lower when caryopses were subjected to retardant treatment before planting. The loss due to soil dryness was only 40 percent for unhardened plants treated with CCC, and even lower--30 percent--for plants treated with BES. Drought hardened plants experienced a 40 percent yield loss due to drought. The yield loss was decreased even more by combining plant hardening with retardant treatment. The yield loss of hardened plants treated with CCC retardant was 20 percent, while that of plants treated with BES was only 10 percent (see Table 5).

Our observations showed that soil drought led, among plants that were not drought hardened, to a greater decrease in stem growth rate, a decrease in ear length and weight, and a drop in the number of spikelets per ear and the number of grains per spikelet, in comparison with hardened plants (see Table 6 and Figure). Hardened control and retardant treated plants were taller and the ear grain content was higher, as a result of which the plants suffered less from drought and produced better grain.

As we can see from these results, complex treatment of wheat before planting raises the drought and heat resistance of the plants. Apparently the integrated effect strengthens protective mechanisms against the injurious action of tissue dehydration.

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ENZYMOLOGY

UDC 577.15.036

INFLUENCE OF EFFECTORS ON THE FOLDING OF AN IMMOBILIZED PROTEIN (TRYPSIN)

Moscow MOLEKULARNAYA BIOLOGIYA in Russian Vol 13, No 3, 1979 pp 673-680
manuscript received 28 Jul 78

/Article by V. V. Mozhayev, K. Martinek and I. V. Berezin, Department of Chemical Enzymology, Moscow State University imeni M. V. Lomonosov/

[Text] A study has been made of the folding of immobilized trypsin in the presence of various effectors of its enzymatic activity. The trypsin, covalently bound to Sephadex G-200 or Sepharose 4B, was folded in a concentrated solution of urea with simultaneous splitting of its S-S bonds. with the help of dithiotratol. The preparation was then separated from the splitting agents, one of the effectors of enzymatic activity of the trypsin was added (boric acid, benzamidin, pancreatic and soy inhibitors of trypsin, ethyl ether of N-benzoyl-L-arginine, methyl ether of N-tozyl-L-arginine) and the reaction of the immobilized enzyme in the absence of catalysts of thiol-disulfide exchange was studied. It was shown that the reactivation of the trypsin in the presence of specific substrates and protein inhibitors takes place with the same yield (2-5%) as in their absence; in the presence of benzamidin and boric acid, the reactivation yield of the immobilized trypsin is increased by factors of 5 and 12, respectively, and amounts to 15 and 40%. In a comparison of this result with the statistical probability of formation of 6 native S-S bonds from 12 SH-groups, it is evident that even in the absence of a catalyst of the thiol-disulfide exchange (i.e., under conditions of irreversible formation of S-S bridges) it is possible to increase the reactivation yield of the enzyme several thousand times if the enzyme is immobilized on a carrier and is treated with a soluble effector. A model is proposed according to which the action of various effectors of trypsin on its folding is explained by their attachment to intermediately folded forms of the protein and by a subsequent shift of equilibrium

toward the "correct" conformers. According to this assumption, even at early stages of folding, when S-S bonds have not yet been formed, a drawing together takes place of catalytically-active groups of serine-histidine pairs and a sorption sector is organized; finally, the formation of an active center of the trypsin (a drawing together of the catalytic and sorption sectors) takes place at the last stage of folding.

The problem topic, biosynthesis of proteins, is a fundamental task of contemporary molecular biology /1/. The question has as yet not been resolved on how folding of a synthesized polypeptide chain into a functionally-active protein takes place. To resolve this it seemed fruitful to study folding of proteins in vitro /2-4/. It is no longer doubted that the native conformation of a protein has been programmed in its initial structure /3/. However, the mechanisms which should function in the living cell and promote a more effective (in particular, a faster) folding of proteins--as compared with model experiments in vitro--are not yet clear. It was shown earlier /5/ that a positive role in the process in vitro can be played by natural immobilization of the polypeptide chain on ribosomes. In the present article, a study has been made--on a model of trypsin, covalently connected to Sephadex or Sepharose--of the influence of various effectors on the process of folding of an unfolded protein (reactivation of the enzyme).

A multitude of metabolites are dissolved in the living cell. It is well known that some of them are capable of affecting the structure and function of proteins /6/, hence it is completely probable that they can participate in folding. Actually, there have been isolated, from cells, compounds of a low-molecular (enzymes) /8,9/ nature, capable of effectively catalyzing the thiol-disulfide exchange in proteins and substantially accelerating their reactivation. Nevertheless, folding, in vitro, of some enzymes into the native conformation does not occur even under optimal concentrations of catalysts of the thiol-disulfide exchange. Reactivation can be realized, with a large yield, only in the presence of substrates, cofactors or such metallic ions (Ca^{2+}, Mg^{2+}, Zn^{2+}) without which enzyme functioning is not possible. Thus, reactivation of takaamylase in the presence of Ca^{2+} /10, 11/, lactate-and malate-dehydrogenases /12/, glyceraldehyde-3-phosphate-dehydrogenase /13/ in the presence of NAD, enolase, fumarase, aldolase and several dehydrogenases /14-16/ in the presence of their specific substrates proceeds with yields exceeding 50%. In the folding of heme-containing proteins /17,18/, a necessary stage, according to Fischer, et al. /19/, is coordination of the porphyrine ring of heme and (to a lesser degree) coordination of the iron atom.

These results /10-19/ indicate that, for some proteins, reactivation with high yield is possible only when the information about the native conformation, contained in its initial structure, is supplemented by the action

of effectors. The mechanism of this influence upon the folding of proteins can be represented, according to Teipel and Koshland /15/, in the following way: in the folding of a polypeptide, there is formed both "correct" and, also, "incorrect" intermediate conformers, and these are in a state of dynamic equilibrium. Addition of an effector which reacts predominantly with the "correct" intermediate conformer should (if the activation barrier of the transition between the conformers is not too great) lead to the desirable shift of equilibrium and, in the last analysis, to an increase in yield of the reactivated protein.

The present article has several new aspects. First, study has been made of the influence of soluble effectors on folding of immobilized protein; earlier /10-19/, for this purpose, only free enzymes were used. The essential distinction in our work is that, earlier, in the enzyme which was unfolded in solution by a denaturing agent, either the S-S bonds were not split at all /12-19/, or, when they were split, reactivation was carried out by addition of glutatione /10,11/. Our model (an enzyme immobilized on a carrier) makes it possible to trace the reoxidation of the split S-S bonds even in the absence of catalysts of the thiol-disulfide exchange, where the S-S bridges are formed irreversibly and, consequently, the intermediately-forming "incorrect" (catalytically inactive) conformations of the enzyme molecule are also irreversibly fixed. As the result of immobilization, the yield of reactivated protein under these conditions*, which are not favorable for folding, reaches several percent /5/; this is quite enough to stay within the limits of the usual methods of measuring catalytic activity. Second, study has been made of the dependence of yield of reactivation on the mechanism (the method) of combining the effector with the enzyme. Earlier /10-19/ it was shown that an increase in yield of the native form of proteins during folding in vitro is caused by the addition of effectors which act, basically, in the finishing stages of the process. Thus, for example, in study of the intermediately-folded forms of takaamylase, it was found that the first 3 of the 4 native S-S bonds are formed during the folding, independent of the presence in the system of Ca^{2+} ions. However, formation of the fourth disulfide bridge, and the structural reconstruction in the macromolecule, which lead to the catalytically-active conformation of the takaamylase, were seen only after addition of the Ca^{2+} ions /11/. On the other hand, it is known that, during renaturation of carboxypeptidase, the centers of combining of the various effectors of catalytic activity (in particular of inhibitors) are formed at different stages of the process of folding of the polypeptide chain /20/. Hence, it is of interest to study the influence of such effectors, too, which might react with the protein precisely in the early stage of its folding.

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* In the presence of catalysts of the thiol-disulfide exchange (when the "incorrect" S-S bonds are split, so as to form, in the end, the "correct" bridges corresponding to the thermodynamically stable, native conformation of the protein) the yield of reactivated enzyme approaches 100% /5/.

Influence of effectors of catalytic activity of trypsin on reactivation of the enzyme immobilized on Sephadex G-200.

20°, pH 8.0, 5 x 10^{-7} M enzyme, in the absence of catalyzers of the thiol-disulfide exchange

1 Параметры реактивации	2 Эффекторы						
	3 без эффектора	4 панкреатический ингибитор трипсина	5 соевый ингибитор трипсина	6 этиловый эфир N-бензоил-L-аргинина	7 метиловый эфир N-тозил-L-аргинина	8 бензамидин	9 борная кислота
Константа диссоциации комплекса эффектора с нативным ферментом, *M*	—	$8 \cdot 10^{-12}$ [29]	$3 \cdot 10^{-9}$ [30]	10^{-5} [31]*	$8 \cdot 10^{-6}$ [32]*	10^{-2} [33]	10^{-1} [22]
Концентрация эффектора, *M*	—	10^{-5}	10^{-5}	10^{-1}**	$5 \cdot 10^{-1}$**	10^{-2}	10^{-2}
Выход реактивации, %	3	2—4	3—5	2—4	2—5	15	40

* Значение константы Михаэлиса, характеризующее образование ацилфермента.
** Начальное значение концентрации; через 30 *час* расходование субстрата не превышало 20—30%.

Key 1. Reactivation parameters.
Constant of the dissociation of the complex of the effector with the native enzyme, M.
Concentration of effector, M.
Reactivation yield, %.

2. Effectors.
3. without effectors; 4. pancreatic inhibitors of trypsin; 5. soy inhibitors of trypsin; 6. ethyl ether of N-benzoyl-L-arginine; 7. methyl ether of N-tozyl-L-arginine; 8. benzamidin; 9. boric acid.

* Value of Michaelis constant, characterizing the formation of the acylenzyme.

** Initial value of the concentration; after 30 hours, consumption of the substrate did not exceed 20-30%.

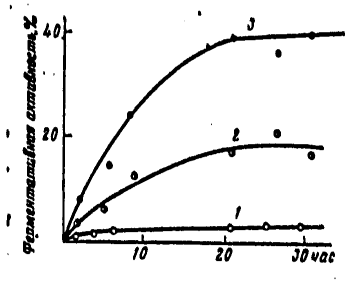

Fig. 1

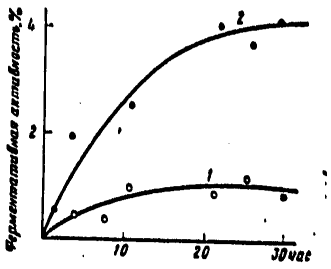

Fig. 2

Fig. 1. Growth of specific activity of trypsin immobilized on Sephadex G-200 during its folding in the absence of catalyzers of thiol-disulfide exchange. 1.--without effector; 2--10^{-2} M benzamidin; 3--10^{-1} M boric acid; pH 8.0, 20°, 5 x 10^{-7} M enzyme.

Fig. 2. Growth of specific catalytic activity of trypsin, immobilized on Sepharose 4B, during its folding in the absence of catalyzers of thiol-disulfide exchange. 1.--without effector; 2--10^{-1} M H_3BO_3; conditions as in Fig. 1.
ordinate: enzyme activity, %; abscissa: hours.

In order to find such effectors, we tested a number of compounds which clearly differ in character of reaction with the active center of trypsin. It was found that boric acid, which is an inhibitor of alpha-chymotrypsin /21/ and of other serine proteinases, including trypsin /22/, forms a complex with two catalytically-active groups contained in the nucleophil-active center,--the OH-group of the serine and the imidazol radical of histidine /23/. Benzamidin, a competitive inhibitor of trypsin, reacts electrostatically with the asparagine radical contained in the hydrophobic sorption "pocket" of the active center /24-26/. Specific substrates (ethyl ether of N-benzoyl-L-arginine and methyl ether of N-tozyl-L-arginine) are attached to this same sorption "pocket" (electrostatically, with an additional hydrophobic interaction), and also form, with the active center, a hydrogen bond (with participation of the serine radical) /24-27/; further, in the acylenzyme there additionally appears a covalent bond of the substrate radical with the OH-group of the component nucleophil /24-26/. Protein inhibitors (pancreatic and soy), which form complexes with trypsin, "close" not only the active center but, also, the more important part of the surface of the enzyme globule /24-26,28/.

PROCEDURE

We have described earlier /5/ the substances and the methods of covalent attachment of trypsin to Sephadex G-200 and Sepharose 4B used in this work.

A typical experiment in study of the folding of a protein was the following: the S-S bonds in an immobilized enzyme were split, as described earlier /5/. Then the preparation was separated from the splitting agents and one of the effectors of enzymatic activity of trypsin was added: boric acid (Soyuzreaktiv, USSR), benzamidin ("Sigma", USA), specific substrates or protein inhibitors of trypsin ("Reanal", Hungarian Peoples REpublic), and reoxidation of the S-S bonds was carried out by the method described earlier by us /5/. After a certain interval of time we took samples of the immobilized trypsin and determined the enzymatic activity (using the specific substrate ethyl ether of N-benzoyl-L-arginine), following the familiar procedure /5/.

RESULTS AND DISCUSSION OF THEM

The influence of effectors on reactivation of immobilized trypsin in the absence of catalyzers of the thiol-disulfide exchange: the effectors used were added in excess with respect to the enzyme in concentrations significantly exceeding (in the case of protein inhibitors of trypsin and specific inhibitors) or commensurate (for low-molecular-weight inhibitors) with the dissociation constants of their complexes with an active center of native enzyme (Table). Like the protein inhibitors, the highly-specific substrates have practically no influence on folding of the immobilized enzyme: the kinetics of reactivation in all cases, is described by a curve of Type 1, in Fig. 1; for comparison, the Table presents the corresponding magnitudes of reactivation yield.

At the same time, low-molecular weight inhibitors significantly increase the yield of reactivation of the immobilized enzyme--by a factor of 5 or 10

with addition of benzamidin (Fig. 1, curve 2) and of boric acid (curve 3), respectively. A positive influence of these inhibitors on reactivation of trypsin was found by us with immobilization of the enzyme not only on Sephadex G-200 (Fig. 1) but, also, on Sepharose 4B (Fig. 2, data for H_3BO_3).

From data on the dependence of the yield of reactivation of trypsin, immobilized on Sephadex G-200, on concentration of the most active effector--boric acid (Fig. 3)--it appears that lowering the concentration down to 10^{-6} M practically does not decrease the yield of reactivation of the trypsin (30-40%). It can be assumed that the constant of combining the boric acid with the folding protein should be of the same order ($\lesssim 10^{-6}$M). With native enzymes--alpha-chymotrypsin /21/, subtilysin, alkaline peptidase, pancreatic lipase, pencillinamidase /23/ and trypsin /22/, the boric acid attaches substantially worse (constant of dissociation of the complex with the active center is 10^{-2} - 1 M). Explanation of the reasons of so great differences in the combining of the ligand with native and folding proteins (more than four decimal orders with respect to the constant of dissociation of the corresponding complexes) requires further study.

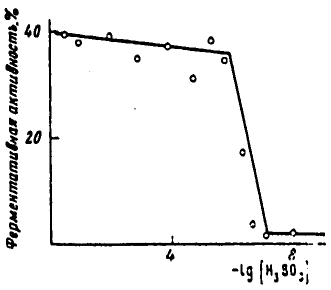

Fig. 3

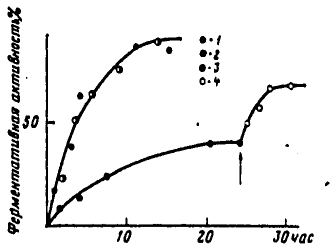

Fig. 4

Fig. 3. Dependence of the end yield of reactivation of trypsin, immobilized on Sephadex G-200, on concentration of boric acid added during folding of the protein in the absence of catalyzers of thiol-disulfide exchange. Conditions as in Fig. 1.

Fig. 4. Growth of specific catalytic activity of trypsin, immobilized on Sephadex G-200, during its folding in the presence of catalyzers of thiol-disulfide exchange (4 x 10^{-4} M of oxidized and 4 x 10^{-3} M of reduced glutathione) (1,2,4) and in their absence (3). 1,3,4--10^{-1} M H_3BO_3; 2--without effector. Conditions as in Fig. 1.

ordinates and abscissas as in Figs. 1 and 2.

Reactivation of immobilized trypsin in the presence of catalyzers of thiol-disulfidine exchange: If folding of immobilized trypsin is carried out in the presence of glutathiones which catalyze thiol-disulfide exchange, the yield of reactivation reaches almost 100% even in the absence of effectors (due to one effect of the immobilization) (Fig. 4,1). As for the kinetics of the process, reactivation in the presence of glutathiones proceeds more rapidly than in their absence (compare curves 1 and 3 in Fig. 4). This agrees with the idea that the reaction rate is limited by the chemical stages of the thiol-disulfide exchange /7/.

Addition of glutathiones at the end of the folding, carried out in the presence of H_3BO_3 (Fig. 4,3), leads to further increase in catalytic activity (curve 4). This result indicates that attachment of the boric acid to the folding protein does not control the fixing of all of the native S-S bonds; some of them are formed incorrectly. Since, in this experiment, catalyzers of the thiol-disulfide exchange were added without addition of urea (without supplementary unfolding of the protein) it can be thought that the following redistribution of the S-S bonds, accompanied by an increase of catalytic activity (Fig. 4,4) does not require substantial conformational changes in the protein.

Mechanism of the influence of effectors on the reactivation yield in the absence of catalyzers of the thiol-disulfide exchange: The results obtained can be explained in the following way. In the early stages of the folding, both "correct" and "incorrect" intermediary conformers are already being formed. They are in dynamic equilibrium, the mobility of which is determined by the magnitude of the activation barrier. With addition to the folding protein of an effector able to form a complex only with "correct" intermediary conformer, shift of equilibrium should take place to the side of increase in concentration of the "correct" conformer due to the free energy of combining:

"incorrect" conformer $\rightleftarrows$ "correct" conformer $\rightleftarrows$ complex of the "correct" conformer with the effector

However, such a mobile equilibrium can be seen only at early stages of folding, when the polypeptide chain is still sufficiently mobile (in particular, is not fixed by "incorrect" S-S bonds). In this case, i.e., in the complex with an effector, the "correct" conformer, continuing to fold, is fixed by the correct (native) disulfide bridges, as a result of which active trypsin is formed with a higher yield than in a complex without effector. Such a situation is realized, apparently, only in the presence of boric acid or benzamidin. It can be assumed that these inhibitors positively influence reactivation of trypsin, precisely for this reason, that they are capable of combining with the folding protein even at the early stage of the process. This means that even in early stages of folding of trypsin there are formed separate fragments of the active center which react with the inhibitors of catalytic activity, i.e., a drawing together occurs of the catalytically-active groups of a serine-histidine pair with which the boric acid reacts and a sorption sector is organized on which the attachment of benzamidin takes place.

Another situation is seen in addition of specific substrates or protein inhibitors. These compounds are able to react with sufficient efficacy only with a completely-organized active center. Moreover, formation of a complex of the enzyme with a protein inhibitor assumes a definite organization of a rather large section of the surface of the globule near the active center. The fact that these effectors do not influence the reactivation of trypsin (Table) indicates, evidently, that they are capable of combining with intermediary conformers of the protein. This means that formation of the active center finishes only at the last stage of the folding. In this case, those molecules which have already formed the "incorrect" intermediate conformations (have been fixed by "incorrect" S-S bonds) cannot be reconstructed into correct conformers--this would require (in the absence of catalyzers of thiol-disulfide exchange) insurmountably-large energy expenditures on splitting of covalent S-S bonds. Hence, effectors, highly-specific to the active center of trypsin, are not able even to shift the conformation equilibrium to the side of enzyme renaturation.

Study of reactivation of immobilized enzymes in the presence of effectors is important at the very least from three points of view. First, studies of the system model the folding of proteins in vivo. The model suggested by us has merit because it takes into account that polypeptides, in biosynthesis, are "immobilized" on ribosomes /1/. Trypsin, in this model, is seen as "simply" a protein, its specificity is considered only in selection of effectors. It is known that trypsin, in vivo, is not directly folded; it is formed by limited proteolysis of the inactive predecessor trypsinogen /34/. Nevertheless, even for such an "unfortunate" (from the point of view of folding) protein, as trypsin, it has been possible in this work to show that the immobilization and an effector, in their common action, substantially increase the effectiveness of reactivation. In comparison with the statistical probability of formation of six native S-S bonds from 12 SH-groups (0.01%) the reactivation yield increased by a factor of several thousand, reaching 30-40%. It must be emphasized that folding of protein was studied by us precisely in the absence of catalyzers of the thiol-disulfide exchange. Hence, it is possible that in living nature the redistribution of S-S bonds in folding of protein does not play so substantial a role as could be thought on the basis of experiments carried out in vitro with ribonuclease /35/, lysozyme /36/ and the pancreatic inhibitor of trypsin /37/. These proteins could be reactivated in the free (not immobilized) form by a fundamentally different mechanism: through intermediate products with a non-native bank of disulfide bonds with their subsequent breakup in reactions of thiol-disulfide exchange and formation as the result of conformation restructure of native S-S bridges. As shown in the present article, it is possible by immobilization, with mutual action of an effector, to completely avoid the intermediate formation of incorrect S-S bonds.

Second, it is known that some proteins, after unfolding, with splitting of intramolecular S-S bridges, are not able to renature /3/. It can be hoped that, as the result of immobilization,it is still possible to bring about renaturation of such proteins if the corresponding effector can be selected.

In solving this problem, it is possible to try to reactivate irreversibly denatured enzymes based on the known scheme /38/. This would permit multiple use of enzyme preparations under technological conditions /39/.

Third, study of renaturation of enzymes should reveal mechanisms of their denaturation which have as yet not been discovered. This has complicated creation of scientific bases of stabilizing enzymes and, consequently, inhibits, to a definite degree the introduction of biocatalysis into practise /39/.

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MARINE MAMMALS

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ON THE PROBLEM OF CHEMORECEPTION IN DOLPHINS

Kiev VESTNIK ZOOLOGII in Russian No 3, 1979 received by editors 27 Dec 78 pp 3-11

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/Text/ The ability to differentiate chemical stimuli by means of taste and smell is characteristic of all mammals to a greater or lesser degree. Representatives of the Cetacea order were considered an exception, because the peripheral sections and conduction paths of the olfactory analyzer, as well as the gustatory papillae on the surface of the tongue, are reduced in them (Kukenthal u. a., 1889; Rawitz, 1903; Sonntag, 1922). However, observations of the behavior of dolphins under natural conditions and oceanariums (Yablokov, 1961; Lilly, 1965) made it necessary to revise the formed opinion. In connection with the absence in Odontoceti of some anatomical structures of the olfactory analyzer specialized exteroreceptives perceiving chemical stimuli can be only in their oral cavity and, most probably, in the mucous membrane of the tongue. A. V. Yablokov (1957, 1961), describing the recesses on the root of the tongue of the white whale, characterized them as organs of chemical sensation. Experiments conducted according to special methods (Sokolov et al., 1971; Kuznetsov, 1974; 1978a; 1978б) have shown that dolphins can differentiate a number of substances dissolved in water (indole, camphor, trimethylamine, valerianic and caproic acids, secretion of anal and prostate glands and so forth). It should be noted that many of the substances very well perceived by dolphins are inadequate stimuli for the gustatory receptors of the tongue of other mammals. Along with this, substances possessing a purely gustatory effect, with the exception of bitter substances, were poorly distinguished by experimental animals. In connection with this the opinion (Kuznetsov, 1978б) that stimuli in low concentrations are perceived by special gustatory receptors belonging to the system of the trigeminal nerve was expressed.

*The report data were presented at the Seventh All-Union Conference on Marine Mammals on 20 to 23 September 1978 in Simferopol'.

Investigating the histology of the mucous membrane of the tongue of dolphins, V. Ye. Sokolov and O. V. Volkova (1971) established that the epithelial lining of the deep cavities at the boundary between the root and body of the tongue has a distinctive structure, which makes it possible to assume that these formations participate in the perception of chemical stimuli. It was possible to detect structures similar to gustatory papillae and bulbs on the bottom and in the lateral walls of the above-mentioned cavities (Suchowskaja, 1972; Suchowskaja et al., 1973; Bel'kovich et al., 1976; Gilevich, 1978; Khomenko et al., 1978). The abundance and diversity of the intraorgan innervation of the tongue of toothed whales is an indirect proof of its chemosensory function (Gilevich, 1975; Valiulina et al., 1976). Thus, it was proven that chemoreception occupies an important place in the vital activity of toothed whales.

Nevertheless, some aspects of the problem of chemoreception in cetaceans require a further study. The available morphological data are insufficient for an explanation of the characteristics of dolphins' perception of various chemical stimuli established by experimental investigations. Furthermore, there are contradictory opinions concerning the classification of chemical sensation in toothed whales. Some authors identify it with smell, while others consider it a well developed sense of taste.

On the basis of the above-stated we set for ourselves the task of studying the micromorphology of tissue structures of the mucous membrane of the tongue of the bottle-nosed dolphin.

The investigation was conducted on the tongues of 12 adults, 2 newborns and 3 9- to 11-month old fetuses. The material was taken 1 or 2 hours after the animals' death and was fixed in 12% neutral formalin. Staining with hematoxylin-eosin, carmine and alcian blue and impregnation with silver nitrate according to Bielschowski-Gros and Kampas were used.

The mucous membrane covering the tongue of the bottle-nosed dolphin is smooth, does not contain papillae so characteristic of other mammals and is closely joined with the underlying layers. A weakly pronounced submucous layer is found only on the root at the transition to the oropharynx. The thickness of the mucous membrane is not the same in different sections, increasing from the top to the root, that is, on the top, 1.8 to 2 mm, in the middle of the body, 2 to 2.3 mm and on the root, 2.5 to 3 mm. Basically, the mucous membrane thickens at the expense of t. propria. The epithelium covering the dorsal surface of the tongue is flat and stratified. The external keratinized layer is represented by three or four rows of flattened cells with dense nuclei and the boundaries between them are poorly discernible. The epithelial lining of the cavities on the root is formed by a small number of rows of cells with clearly differentiated, round nuclei. The keratinized layer is absent here.

Papillae in the tongue of the bottle-nosed dolphin are located at the bottom and in the lateral walls of root cavities. These are protuberances of the mucous membrane, which do not rise above the surface of the tongue, because

their height (100 to 200 μm) is smaller than the depth of the cavities. In the tongue of the bottle-nosed dolphin we detected two types of papillae--fungiform and conical. The fungiform papillae (fig. 1) are the largest and have a narrowed base and an expanded top. They are of two types--with a concave and convex top. The stroma of these papillae is rich in cellular elements. Small secondary papillae penetrate into the epithelium. Some fungiform papillae are surrounded with a torus. The conical papillae are smaller. There are two varieties of conical papillae--with a pointed and rounded top (fig. 2).

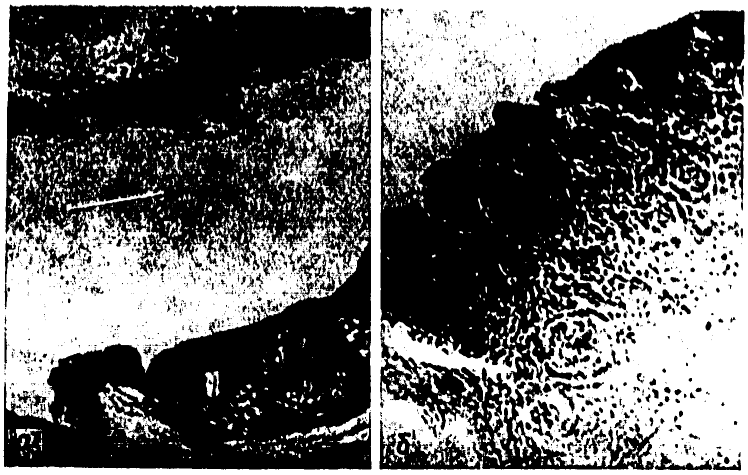

Fig. 1. A Fungiform Papilla in the Tongue of the Bottle-Nosed Dolphin

а--hematoxylin-eosin (ob. 6.3, oc. 7); б--impregnation according to Bielschowski-Gros (ob. 20, oc. 10).

In addition to the described two types of papillae in the tongue of the bottle-nosed dolphin there are elongated ribbon-shaped papillae, as well as papillae of an incorrect form. Structures resembling taste bulbs are very rare. We found them only in some fungiform papillae (fig. 1б). They are bright formations of a round or oval form. The gustatory canal is not quite clearly pronounced in them and sometimes it is not seen at all.

Investigating the tongues of fetuses and newborns of bottle-nosed dolphins at our disposal, we detected that their papillae are situated not only in cavities, but also on the surface of the mucous membrane. They are localized (fig. 3) in the area of the root and along the lateral edges of the body, their number ranging from 20-25 to 25-35. Some papillae can be grouped with fungiform papillae, while others, those surrounded with a groove and torus, can be classified as typical grooved papillae. Filiform papillae are found occasionally (fig. 4).

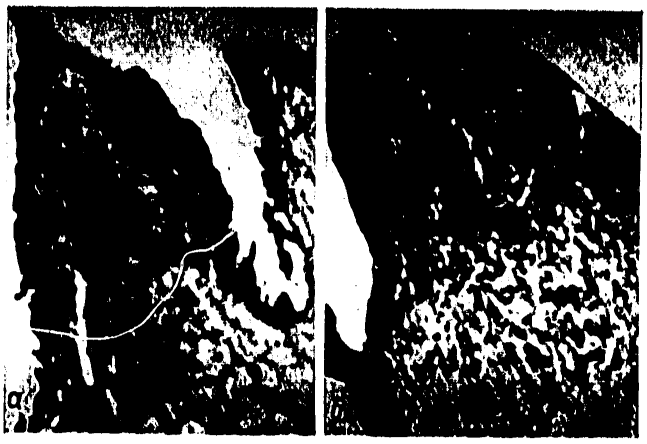

Fig. 2. Conical Papillae in the Root of the Tongue of the Bottle-Nosed Dolphin (Hematoxylin-Eosin, ob. 10, oc. 7):

a--with a pointed top; б--with a rounded top.

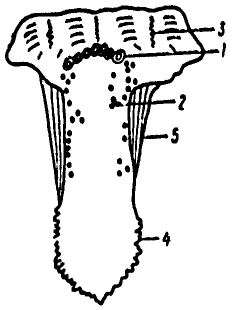

Fig. 3. Diagram of Distribution of Papillae Over the Surface of the Tongue of Fetuses of the Bottle-Nosed Dolphin: 1--large cavities at the boundary between the body and root of the tongue; 2--papillae on the surface of the tongue; 3--root of the tongue; 4--fringe of the anterolateral edge of the tongue; 5--mucous membrane of the lower surface of the tongue.

In the tongue of the bottle-nosed dolphin there is a large number of glands. They are absent on the top and along the lateral edges. The bulk of the glands is situated in the area of root cavities and behind them. Here they form continuous fields divided by thin connective-tissue interlayers (fig. 5). The glands of the tongue of the bottle-nosed dolphin are simple tubular-alveolar. The end sections of large glands consist of a large number of lobules with narrow interlobular interlayers. They lie in the deep layers of the mucous membrane. The main excretory ducts reach a considerable length and their lumen is wide. Small glands consisting of two or three lobules lie more on the surface. The excretory ducts of glands open into large and

small cavities on the surface of the tongue. The glandular formations in the tongue can be divided into two groups not only according to morphological characters, but also according to the nature of secretion. The bulk of the glands, when treated with alcian blue, produces a sharply positive reaction to mucin. The small glands located on the surface are not stained with this dye, which makes it possible to assume the protein nature of secretion.

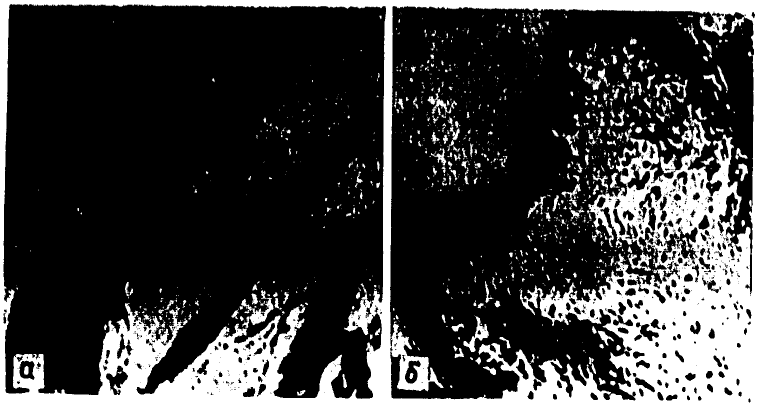

Fig. 4. Papillae in the Tongue of a Newborn Bottle-Nosed Dolphin (Hematoxylin-Eosin, ob 6.3, oc. 7):

а--filiform; б--grooved.

The study of the intraorgan innervation of the tongue of the bottle-nosed dolphin has shown that in the deep layers of the internal layer of the mucous membrane there is a thick nerve plexus consisting of bundles of a large and medium size. A thin plexus is situated on the surface. Afferent nerve fibers form endings of various types. In the connective tissue layer there are free (coils and clusters) and nonfree (Krause's bulbs and encapsulated and unencapsulated glomeruli) receptors. Nerve endings in the form of long, gradually thinned filaments, coils and loops are especially characteristic of the epithelium. The zone of root cavities is characterized by the most abundant nerve supply. The fact that it has a large number of thin unmyelinated sensory fibers forming free receptors of a considerable length is noteworthy (fig. 6).

We observed two types of innervation of tongue papillae. In the first the papilla base includes one or two-three nerve trunks of an average size, which then separate into thinner trunks and together with vessels form a thick

plexus in the stroma. In the second type innervation is ensured by a large number of thin unmyelinated fibers, which in almost parallel threads enter the papilla, where they anastomose and form the thinnest plexus (fig. 7). In some fungiform papillae, along with thin fibers, there are thick, sharply argentophilic myelinated fibers (fig. 8).

Fig. 5. Mucous Glands of the Tongue of the Bottle-Nosed Dolphin (ob. 10, oc. 10)

Fig. 6. Preterminal Sensory Fiber in the Subepithelial Region of the Zone of Root Cavities of the Tongue of the Bottle-Nosed Dolphin (Impregnation According to Bielschowski-Gros, ob. 40, oc. 10).

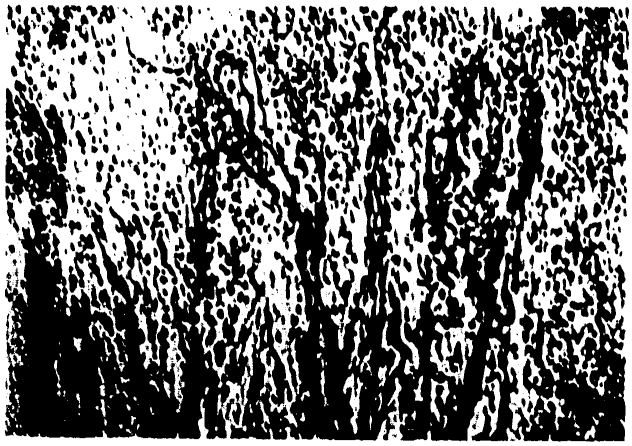

Fig. 7. Innervation of the Papilla of the Tongue of the Bottle-Nosed Dolphin (Impregnation According to Kampas, ob. 40, oc. 10)

Fig. 8. A Sharply Argentophilic Fiber in the Stroma of the Fungiform Papilla of the Tongue of the Bottle-Nosed Dolphin (Impregnation According to Bielschowski-Gros, ob. 20, oc. 10)

In the mucous membrane of the bottle-nosed dolphin we detected a thick ganglionic apparatus represented by a large number of nodules of a diverse form--round, bean-shaped and oval. The number of cells in these ganglions varies from 3-5 to 40-50. Most ganglions have a clearly pronounced capsule.

It is absent in some cases. The cells forming part of ganglions have an oval or pear-shaped form and a large nucleus (fig. 9). Some of them are unipolar with a thick process, but in the bulk of the cells processes have not been detected.

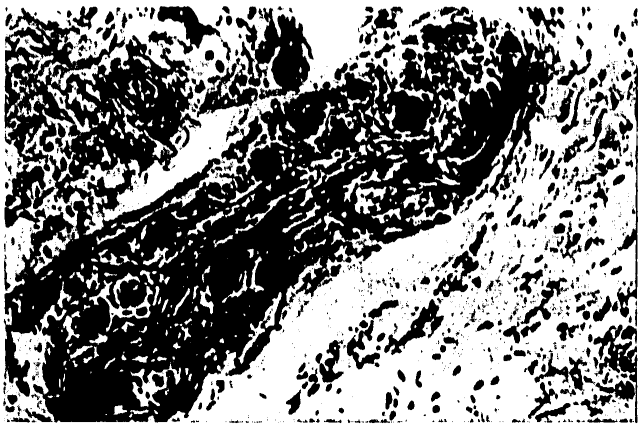

Fig. 9. Microganglions in the Root of the Tongue of the Bottle-Nosed Dolphin (Impregnation According to Bielschowski-Gros, ob. 20, oc. 10)

Analyzing the distribution of nerve, vascular and glandular elements, we single out in this organ a region of increased functional activity--a reflexogenic zone. This is the zone of root cavities. The bulk of the receptors, neurons and glands present in the tongue of the bottle-nosed dolphin is concentrated at this section. The data of our investigation make it possible to state that the tongue of the bottle-nosed dolphin is a functionally important organ, one of the functions of which is the participation in the perception of chemism of the environment.

Taking into consideration the data on the structure of the tongue of the bottle-nosed dolphin obtained by us and the information on other toothed whales (porpoise, white-sided dolphin, white whale and sperm whale) available in the literature, it can be assumed that chemoreception in toothed whales is of a special nature and cannot be ensured only by gustatory papillae, which in the tongue of dolphins, as compared with other mammals, are underdeveloped and the number of bulbs contained in them is very insignificant. At the same time, the range of stimuli perceived by these animals is quite wide and the sensitivity of the tongue to many of them is quite high. A number of these substances have not so much a gustatory as an olfactory effect. In our opinion, in addition to gustatory papillae and bulbs, some other structural components of the tongue participate in the perception of chemical stimuli in dolphins.

In the opinion of some authors (Semenov, 1965; Polacek, 1965), free nerve endings can perform the function of chemoreceptors in thin multilayer epitheliums. The same authors consider gustatory bulbs only a distinctive "breach" in thick multilayer epitheliums ensuring an access of liquid stimuli to free nerve endings.

The epithelium lining the root cavities of the tongue of dolphins is thin and without a horny layer. Therefore, water soluble stimuli can fully penetrate into free afferent endings, which can function as chemoreceptors in this region. We believe that the investigated animals perceive most of the chemical stimuli available to them in this way. It can be assumed that neurons of intraorgan ganglions of the tongue, some of which, on the basis of their morphological characters, we are inclined to consider sensitive, as well as glands, play a certain role in this process. The secretion of glands discharged into root cavities, possibly, participates in the adsorption of the specified substances. It is well known (Bronshteyn, 1950) that the olfactory section of the nasal cavity is abundantly supplied with mucous glands.

In the literature there is information that the system of the trigeminal nerve participates in the perception of aromatic substances (Bronshteyn, 1950; Devitsina et al., 1978). The branches of the trigeminal nerve innervating the tongue are well developed in cetaceans. In connection with this we believe that it is possible to assume that trigeminal chemoreception, the perceiving structures of which are represented by free nerve endings of the epithelium and of the subepithelial region of root cavities, is the prevalent form of chemical sensation in dolphins.

Evidently, gustatory papillae and bulbs ensure a particularly gustatory sensitivity, which in dolphins, basically, is manifested with respect to bitter substances (Kuznetsov, 1978б). First, the well known fact that a bitter substance is best perceived by the papillae of the tongue root and, second, the presence of thick, sharply argentophilic fibers in the stroma of the fungiform papillae of the tongue of the bottle-nosed dolphin attest in favor of this assumption. As some researchers (Filatova, 1940; Shubin, 1946; Lavrent'yeva, 1960) believe, such fibers are connected with the perception of a bitter taste.

The certain reduction in the gustatory sensitivity in dolphins can be explained by the distinctive method of feeding these animals (quick swallowing of a whole fish). The sufficiently good perception of the bitter taste alone, apparently, is connected with defense reactions, because most substances unfavorable for the body are bitter.

The diversity of tongue papillae in fetuses and newborns, as well as their location on the tongue surface, can attest to the fact that the relatively simplified structure of the tongue in adult dolphins and the partial reduction of gustatory sensitivity in them are secondary, are of an adaptive nature and are connected with the uniform nature of food and with the feeding method.

As a result of the analysis of our own data and the data in the literature we can conclude:

Dolphins have a distinctive organ of chemical sensation, which is represented by recesses at the boundary between the body and root of the tongue;

apparently, the free receptors of the epithelium and subepithelial region of root cavities have the dominating role in the perception of stimuli of chemical nature;

gustatory papillae, owing to their historically formed narrow specialization, perceive only substances possessing a particularly gustatory effect;

the tongue of dolphins, along with the participation in the process of feeding, to a great extent also performs the functions of an olfactory organ.

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PLANT PHYSIOLOGY

UDC 581.14; 582.542.1.037

DETERMINATION OF THE THRESHOLD OF WHEAT SPROUT AND ROOT SENSITIVITY TO MAGNETIC FIELD INTENSITY

Moscow FIZIOLOGIYA RASTENIY in Russian No 3, 1979 pp 620-624

[Article by N. I. Bogatina, B. I. Verkin, V. M. Kulabukhov, V. M. Litvin, and V. F. Nikulina, Ukrainian SSR Academy of Sciences Physico-Technical Institute of Low Temperatures, Khar'kov]

[Text] The effect of magnetic fields in a broad range of intensities ($2 \cdot 10^{-4} - 10^{4}$ Oe) on growth of the roots and stems of wheat caryopses was studied. The threshold of magnetic field sensitivity was discovered to be about $2 \cdot 10^{-2}$ Oe. A dependence was found between the distribution of the extension effect in a magnetic field on time of year: Maximum extension is observed in roots in spring; in all other times of the year it is approximately the same for roots and stems.

There is considerable interest in the effect constant magnetic fields have on plant growth, but despite intensive research conducted by different authors over long periods (more than 10 years), unambiguous results have not been obtained. This pertains especially to weak magnetic fields, on the order of the Earth's magnetic field and lower. We can assume it proven that plants are not indifferent to magnetic fields in a rather large range of intensities (10-10,000 Oe). Changes in the rate and dynamics of the vital activities of plants are observed in response to them (1-8). However, the papers cited here pertain to magnetic fields having intensities that significantly exceed that of the Earth ($H_{horiz} = 0.17$ Oe; $H_{vert} = 0.48$ Oe in the vicinity of Khar'kov). Little work has been done, meanwhile, on magnetic fields having intensities on the order of the terrestrial magnetic field. They are devoted mainly to orientation of rootlets and sprouts in the Earth's magnetic field, and the results are contradictory (9-14). As far as works systematically analyzing the effect of magnetic fields with intensities below that of the Earth's field are concerned, we were unable to find any.

It should be noted that all investigations of magnetic fields of different intensities discussed above made comparisons with control experiments

conducted in all cases in the Earth's magnetic field. This was not a sufficiently pure experimental design from the physical point of view. The fact is that in addition to a constant component depending on the geographical latitude at which the experiment was conducted, orientation in relation to it, and the state of the Earth's surface (presence or absence of magnetic anomalies), the Earth's magnetic field has a significant variable component typified by a large assortment of frequencies and amplitudes depending on time, Sun state, the Earth's magnetosphere, proximity to industrial facilities, and so on. Thus the nonreproducibility of the results can be explained by the inadequacy of control experiments.

The main goal of the method used in our work was to create adequate, satisfactorily reproducible experimental and control conditions. Another objective of our work was to clarify the following questions: 1) What is the minimum constant magnetic field intensity causing changes in growth? 2) What is the nature of a magnetic field's effect on plants: Has it do with information or with energy? 3) Are seasonal effects on plant growth associated with change in the external magnetic field, or are these internal effects inherent to the plants themselves? By answering these questions we can come closer to an understanding of the mechanism of action of magnetic fields upon biological objects.

Methods

We studied the influence magnetic fields of different intensities (in a broad range, $10^{-4} - 10^{4}$ Oe) have on wheat caryopsis growth rate. The experimental method differed from that commonly employed: All control experiments were performed within Permalloy cells (15), in the cell's residual magnetic field which, as constant monitoring with a iron-probe magnetometer showed, remained constant during the experiment.

Wheat caryopses (Mironovskaya 808 variety, 1976 harvest, 99 percent germination in the early germination stage of up to 3 days) sprouting at a temperature of 22° served as the object of research. The temperature was stabilized during the experiment with a precision of up to ±0.5°. The caryposes were grown in the same way as in previous experiments (16)--in Petri dishes on a thin layer of cotton with a strictly determined quantity of double-distilled water, and in an artificially created magnetic field.

All experiments were performed in a horizontal magnetic field with the flux lines oriented in the plane of the Petri dish--that is, in the plane of the roots, perpendicular to the gravitation field. The magnetic field was artifically generated within the Permalloy cells; its intensity was varied from 0.5 mOe to 7.5 Oe. Shielding against the Earth's magnetic field was provided by double-walled Permalloy cells with flat bottoms. The cell's residual magnetic field was measured with an iron-probe magnetometer. The horizontal component was $H_{horiz} = 0.2$ mOe and the vertical component was $H_{vert} = 0.5$ mOe. Variation of the residual magnetic field in the cell did not exceed 0.07 mOe during the experiment. A 0.5 mOe-7.5 Oe magnetic field was artifically generated within the Permalloy cells by solenoids inside

the cells; in this case the variability of the volume occupied by the Petri dishes did not exceed 1 percent, field instability did not exceed 0.1 mOe, and monotonous changes in the artificial magnetic fields did not exceed 0.1 percent of their rated value during the experiment.

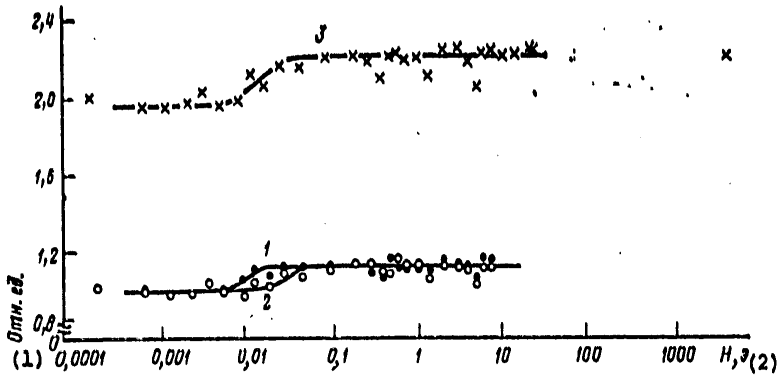
It should be noted that different magnetic field intensities were associated with different experiments, the field intensity being constant within any single experiment. Three experiments could be conducted simultaneously in magnetic fields of three different intensities, and a fourth--in the residual magnetic field--served as the control. We will refer to all experiments performed with magnetic fields of different intensities but at the same time as one series of experiments; we will refer to a set of experiments performed with the same magnetic field intensity but at different times as a one magnetic field intensity series of experiments, and we will refer to identical series of experiments involving the same assortment of magnetic field intensities (within one series) but conducted at different times as identical series.

Measurements in fields having intensities greater than 7.5 Oe were made near the permanent magnet. It should be noted that the vertical component of the magnetic field equalled zero only in the center of the gap between the poles of the magnet, such that the experiment was not entirely valid when Petri dishes were placed near the magnet: As the dishes are brought closer to the magnet, the vertical component becomes significant and comparable to the horizontal component of the magnetic field. In relation to the dimensions of the Petri dishes employed, fields with intensities of 7.5-50 Oe were rather uniform close to the magnet (nonuniformity did not exceed 10 percent).

Thus the method we employed has a large number of advantages over the commonly accepted one: The conditions of the control experiment are fully reproducible and they do not depend on changes in the Earth's magnetic field. It should be noted that the Permalloy cells were themselves placed within grounded copper cells, which provided shielding against the variable components of the Earth's electromagnetic field and shielding against the Earth's constant electric field with a shielding coefficient of not less than 10^6. All of this significantly reduces the scatter of the experimental results.

Results and Discussion

We measured the length of wheat roots and sprouts. The results are shown in the figure below. The magnetic field intensity is plotted on the abscissa in logarithmic scale, and dependencies $l_4(H)/l_4(0)$, $l_{1+2+3}(H)/l_{1+2+3}(0)$, and their sum are plotted on the ordinate. Here l_4 is coleoptyle wheat length, l_{1+2+3} is the cumulative length of three wheat roots, H is the intensity of the magnetic field, and 0 in this case means a residual magnetic field close to zero. We can see from the figure that all of the dependencies we studied (curves *1-3*) are threshold dependencies. The threshold is associated with very weak magnetic fields, where H is about 0.01-0.03 Oe. Each point on the curves represents not less than six



Dependence of relative coleoptyle length, $l_4(H)/l_4(0)$ (*1*), relative cumulative root length, $l_{1+2+3}(H)/l_{1+2+3}(0)$ (*2*), and total effect on wheat roots and coleoptyles, (*3*), on the size of the magnetic field's horizontal component, H: l_4-- wheat coleoptyle length, l_{1+2+3}-- cumulative length of three wheat roots. Symbols l_4 (0) and l_{1+2+3} (0) correspond to a residual magnetic field with $H \approx 0$, having a vertical component of 0.5 mOe and horizontal component of 0.2 mOe

Key:

1. Relative units
2. H, Oe

series of experiments with one magnetic field intensity. The results of identical series of experiments may differ somewhat from each other, but the general trend of the curve remained constant for all series. It may be noted that results obtained in spring at certain magnetic field intensities differed significantly from results of experiments with the same magnetic field intensities performed at all other times of the year. Thus, in spring the magnetic field affected mainly root extension (the effect attained 25 percent), while having no influence on stems. At all other times of the year the effect was uniformily distributed between roots and stems. The cumulative effect does not depend on time of year: It is much more stable. Curve *3* in the figure does not depend on time of year; curves *1* and *2* are averaged in relation to all seasons of the year except spring.

The significance with which curves *1* and *2* deviated from 1.00 and curve *3* deviated from 2.00 at particular points is shown in the table below. We can see from the figure that in the range of field intensities $H > 0.03$ Oe curves *1-3* do not depend monotonously on magnetic filed intensity: Some of the points deviate from the trend. However, this deviation is significant (98 percent) only at a magnetic field intensity of $H = 5$ Oe.

(1)	(2)	(3)	(4)
H, Э	Достоверность отклонения кривой *1* от 1,00	Достоверность отклонения кривой *2* от 1,00	Достоверность отклонения кривой 3 от 2,00
0,0007	73,30	78,87	81,65
0,0015	57,55	24,34	25,58
0,0025	66,27	12,71	76,99
0,004	34,73	34,73	68,28
0,006	44,43	20,51	42,45
0,01	95,45	90,27	45,07
0,015	100	63,19	100
0,02	99,73	0,00	99,73
0,03	100	98,02	100
0,05	98,91	98,76	99,95
0,1	98,07	99,97	100
0,2	98,76	99,40	99,95
0,3	98,02	100	100
0,4	99,73	83,85	93,12
0,5	100	99,95	100
0,6	100	99,95	100
0,75	97,55	100	100
1,0	86,64	100	97,63
1,5	80,61	88,27	93,27
2	99,07	99,40	99,94
3	98,36	100	99,98
4	99,24	99,24	99,97
5	68,27	25,86	52,23
6	100	100	100
7,5	100	97,75	100

Key:
1. *H*, Oe
2. Significance of deviation of curve *1* from 1.00
3. Significance of deviation of curve *2* from 1.00
4. Significance of deviation of curve 3 from 2.00

Concurrent research on the effect of magnetic field orientation (the seeds were oriented with the embryo pointing north, south, east, and west in each Petri dish) on wheat caryopses showed that a positive effect does occur in all seasons except spring (both stems and roots grow more vigorously when the embryo is oriented southward), being 8-10 percent on the average. In spring the magnetic field orientation effect changes sign to equal (-3) - (-4) percent. It should be noted, however, that there are great difficulties in studying the dependence of the magnetic field orientation effect on magnetic field intensity, since the measurements must be highly precise; it should also be noted that the figures presented above are not precise.

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SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

SECOND ALL-UNION CONFERENCE ON PROBLEMS IN BIOMECHANICS

Riga MEKHANIKA KOMPOZITNYKH MATERIALOV in Russian No 3, 1979, pp 560-562

[Article by I. V. Knets]

[Text] On the initiative of the Scientific Council of the USSR AN [Academy of Sciences] on problems in biomechanics, the Latvian SSR Academy of Sciences and the Latvian SSR Ministry of Health, the Second All-Union Conference on Problems in Biomechanics was held in Riga from 18 to 20 April 1979.

Three hundred sixty seven works of Soviet and 19 works of foreign scientists were selected by the organizational committee for presentation at the conference. All the works were grouped by 54 separate themes for each of which a survey report was presented. In making the selection the organizational committee gave preference to works in which fundamental or practically important problems of applied biomechanics were examined.

At the first plenary session four survey reports were heard. V. K. Kalnberz' report examined current problems in medical biomechanics associated with the application of compression-distraction apparatus in treating bone deformations, in osteosynthesis and in restoration of joint function. In V. I. Shumakov's report the current status of development of the artificial heart was analyzed in detail. Different designs for its power supply and regulation were examined, and the biomechanical properties of the blood circulation system were evaluated in order to improve the construction of artificial hearts. G. A. Lyubimov's report was devoted to a new and rapidly developing area of biomechanics--respiratory mechanics. The main problems associated with development of an effective mathematical model of respiration were investigated, and the influence of the mechanical properties of the respiratory organs on the biomechanics of the respiratory process was evaluated. In his report, A. P. Gromov gave an account of the basic aspects of biomechanics in head traumas and evaluated the influence of not only the mechanical but also the morphogeometric properties of the skull on the character of injuries to it.

Six survey reports were given on fundamental problems of biomechanics of compact bone tissue. In his report I. V. Knets examined deformation and destruction of compact bone tissue from the viewpoint of the mechanics of a hard, deforming body. The degree of its anisotropy and physical nonlinearity were evaluated, and questions of the viscoelastic behavior of bone tissue were presented; the role of the piezoelectric effect in bone was analyzed. E. P. Podrushnyak's report was devoted to general questions of the aging of bone tissue and the morphological and biomechanical changes in bone associated with this process. In his report, G. O. Pfafrod evaluated the effect of a different type of influence--which includes reduced blood supply, weightlessness and radiation--on the mechanical properties of human and animal bone tissue. In S. S. Tkachenko's report current problems in the formation of regenerated bone in fusion of fractures was examined, the influence of electric polarization and local mechanical vibration on this process was evaluated, and the degree of change in the acoustic and mechanical properties of bone during regeneration was analyzed. A. K. Muyzhulis' report was devoted to complex questions of biomechanics in treating fractures by internal osteosynthesis, evaluation of allo- and xenogenous bone supports and steel pins. In I. K. Vilka's report biomechanical problems arising during the period of rehabilitation were examined. in detail. They are related to teaching of proper walking, compensation for the motor defect and optimization of control of movements.

Six survey reports were presented on questions in the biomechanics of endoprosthetics. In Kh. A. Yanson's report general biomechanical aspects associated with endoprosthetics were examined and the feasibility of applying crystal insulators as structural biomaterials for endoprostheses was analyzed. In Ya. M. Shersher's report the durability and strength of endoprostheses of different joints were evaluated. Ya. B. Kutsenko's report was devoted to evaluation of the positioning, form and orientation of different bone elements in the joint by means of roentgenography. In his report V. S. Shargorodskiy analyzed the basic biomechanical principles for treatment of articular pathology of the lower limbs. Such current problems as determination of the biomechanical criteria for restoration of the functions of the knee joint depending on the method of plastics of the ligamentous apparatus and the coxofemoral joint following arthroplasty were examined. T. A. Revenko's report was devoted to examination of different statodynamic indices in orthopedic diseases and traumas of the lower limb. B. S. Rozenshteyn gave an account of the general biomechanical criteria for standing and walking following endoprosthetics of the knee or coxofemoral joint and gave an evaluation of the electric activity of the thigh following endoprosthetics of the knee joint using Sivash's method.

Three survey reports were read on the biomechanics of the vertebral column. V. Ye. Raykhinshteyn presented a review of data on deformation and destruction of the elements of the spinal column--the vertebrae, the intervertebral discs and the dura mater spinalis. Analysis of the statics and dynamics of the intervertebral discs under the influence of different loads was presented and the weight-bearing capacity of the human spinal column and vertebrae was evaluated. In Ya. L. Tsiv'yan's report the results of biomechanical

evaluation of the mechanogenesis of the vertebral column were presented and the problems of treating its post-traumatic and degenerative deformations were discussed. Biomechanical criteria were proposed for selecting the optimal variant of bone plastics of the vertebral column. V. E. Belen'kiy's report was devoted to problems of the biomechanics of mechanogenesis and problems in treating scoliosis, including the biomechanical basis for operations on the intervertebral discs and the vertebrae in scoliosis.

New models of biological systems and methods of investigation in biomechanics were examined in two survey reports. A. S. Vitenzon reported on the principles of the physical modeling of the elements of pathological gait, presented a model of the electrical resistance of bone tissue, evaluated injuries to the vertebral column from the perspective of biomechanics and examined the functions of the foot. In R. A. Gurevich's report the questions of the investigation of the biomechanical properties of the lower limbs of man by the methods of vibration experiments and ultrasound, measurement of the pressure between bone fragments, increasing the information content of statographic investigations and application of qualitative electromicrography were examined.

One survey report touched on general questions of biomechanics and zoology. The speaker, S. F. Manziy, analyzed in detail the properties of deformation and destruction of bone tissue, ligaments and muscles of animals, took notice of the characteristics of the statolocomotion of the locomotive apparatus of terrestrial vertebrates and evaluated the patterns of change in the structure of bone tissue of animals during ontogenesis.

Ten reports were presented on the mechanics of soft biological tissues and their substitutes. In his interesting survey of the mechanics of skeletal muscle L. V. Nikitin examined different mathematical models of muscle, analyzed the deformation of muscle as a function of its activation and evaluated its viscoelasticity. The subject of artificial muscles was discussed separately. V. I. Vorob'ëv's report explored the biomechanical problems associated with the structure and mechanical properties of one of the basic components of biotissues--collagen; the influence of its structural organization on the mechanical behavior of collagenous fiber was evaluated, among other things. Yu. Zh. Saulgozis' report was devoted to the characteristics of deformation of skin, the sclera and other types of soft tissues and to analysis of the influence of different mechanical conditions on the regeneration of soft biotissues. R. P. Kikut examined the biomechanics of the arteries and tissue of the brain, paying particular attention to thrombogenesis in the cerebral arteries. In his report V. V. Dzenis not only analyzed in detail the characteristics of the transmission of ultrasound to the sound biotissues but also presented interesting data on the application of ultrasound for characterization of coxarthrosis and fractures of the bones of the knee and thigh. V. A. Kas'yanov gave a concise account of the characteristics of the mechanical behavior of the major blood vessels. He reported on the most general mathematical models of these vessels which describe deformation of them under different types of mechanical influences and presented extensive experimental material. In V. Ya. Isanov's report

an analysis of the general problems of the mechanics of the myocardium was given; among other things mathematical modeling of contraction of the myocardium, the viscoelastic behavior of cardiac muscle, and the mechanical properties of the ventricles of the heart were described. N. B. Dobrovaya's interesting report was devoted to problems of practical importance in investigation of the properties of the mechanical behavior of the valves of the heart and the creation of prostheses of them; an integral evaluation of the hydrodynamic indices of the valves was given, and the dynamics of their operation were analyzed. B. A. Purinya's report was devoted to questions of selection of substitutes for the blood vessels; she examined the feasibility of using xenotransplants and synthetic prostheses for replacement of injured vessels. In A. M. Movshovich and N. S. Gavryushenko's report the role of the influence of aggressive bioenvironments on the mechanical properties of different polymeric implants was evaluated. The authors presented a mathematical model of the destruction of implants in the bioenvironment and gave an evaluation of the strength and antifriction properties of bioinert thermoplasts for endoprosthetics of joints.

Five reports were presented on the mechanical properties of biological fluids and the problems of biomass exchange. V. A. Levtov analyzed in detail the characteristics of the aggregation and orientation of erythrocytes, compared the viscosity of blood in healthy and sick people, evaluated the influence of additives on the indices of the dynamics of circulation and examined the sinovial flow through the capillaries at different rates of displacement. V. M. Zaiko's report was devoted to questions of the hydrodynamics of circulation. A mathematical model of the movement of blood in cavities with irregular geometry and in a tube with distorted walls was examined in detail, and experimental data on blood flow in the venous bed was presented. Problems in the biomechanics of microcirculation were interpreted in S. A. Regirer's report; he gave a detailed review of the current status of the problem and analyzed a number of problems, such as mathematical modeling of myogenetically active blood vessels, investigation of the isometric contraction of smooth muscle, modeling of vascular tension. M. A. Khanin's report was devoted to questions of modeling the vascular bed and evaluation of the peripheral resistance of the vessels. V. N. Akimov analyzed problems associated with extracorporal metabolism of biological substances, chiefly with artificial blood circulation.

Twelve reports were presented on questions in the biomechanics of movement of humans and animals. In his report V. V. Beletskiy gave an account of the principles of modeling bipedal walking and examined control of the inertial leg during the phase of transfer and parametric optimization of bipedal walking. Ye. A. Devyanin's report was devoted to problems in modeling of hexapodous walking. In it the timing of the forces in the legs of the walking apparatus was also evaluated, use of a range-finder in order to control walking robots during movement on complex sites was analyzed and a model of an integral walking apparatus was presented. In M. V. Kudryavtsev's report the results of experimental investigation on the locomotion of humans and animals were reported, an evaluation of the dynamics of movements using special counter-computer systems was given,

and the distribution of the key reactions in a system of the exoskeleton type was examined. A. Ye. Kobrinskiy's report was devoted to the biomechanics of workers' movements--the questions of the maximum endurable loads on the body of a person using a protective assembler's belt, the mobility of human hands and improving precision movements in youths with training. V. M. Zatsiorskiy examined the problems of biomechanical analysis of human locomotion and gave an evaluation of the radioisotope method for determining biomechanical characteristics of segments of the human body. In his report V. K. Bal'tsevich gave an evaluation of the external and internal determination of the parameters of the development of locomotive systems during human ontogenesis and a description of the integral criteria for evaluating the level of achievement of the objective in sport movements. A. N. Laputin's report was devoted to problems of developing technical means for shaping movements using automated control in teaching athletes motor training. I. M. Kozlov examined the characteristics of muscle activity in different athletic movements. I. P. Ratov gave a concise statement of the principles of controlling the movements of athletes, discussed spectral analysis of movement and modeling of the processes of control of movement by the biomechanical components of man. V. T. Hazarov's report was devoted to questions of the synthesis of athletic movements and vibrostimulation of muscles in training athletes. F. K. Agashin gave an account of problems of wave biomechanics and gave an evaluation of training on biomechanical machines. In the report of the Polish scientist E. Marynyak general problems of mathematical modeling in biomechanics of movements were examined; among other things, the data of estimation of the dynamic properties of a person jumping with a closed parachute were presented.

Five reports were devoted to questions of the biomechanics of prosthetics. Yu. V. Kurochkin gave an account of the biomechanical aspects of standing and walking normally and with different pathological deviations, presented biomechanical criteria for the effectiveness of operative treatment of children with congenital dislocation of the femur, and analyzed the characteristics of the statics and kinematics of patients with compression fractures of the vertebral column. V. I. Filatov's report touched on questions of kinematics not only of the upper limbs but also of prosthetics for them. The author presented the theoretical grounds for developing multifunctional prostheses for the hand and gave an evaluation of the use of kineplastic tunnels in control systems for them. In I. A. Mendelevich's report the biomechanical aspects of creation of designs for prostheses and measuring equipment were discussed, including ways of normalizing the functional characteristics of the knee joint of thigh prostheses and questions of the testing interlinking surfaces as a way of reinforcing the joint. V. A. Berdnikov's report was devoted to questions in prosthetics after amputation of the lower limbs and analysis of biomechanical designs for constructing prostheses. I. Sh. Moreynis examined questions of human statics and kinematics after prosthetics, gave an evaluation of the stability of human orthograde posture normally and on a prosthesis and investigated the influence of the normalization of the position of the OTsM [expansion unknown] on the results of prosthetics.

At the end of the conference's work, a meeting of all five sections of the Scientific Council of the USSR AN on problems of biomechanics was held, in which the results of the conference were discussed and proposals were made for improving the conference's work in the future.

In the concluding plenary session Doctor of Medical Science V. S. Gurfinkel', the chairman of the section on the control and regulation of biological systems; Doctor of Technical Science I. V. Knets, the chairman of the section on the biomechanics of biological materials and systems; Associate Member of the USSR AMN [Academy of Medical Sciences] V. K. Kalnberz, the chairman of the section on medical biomechanics; Doctor of Medical Science V. I. Filatov, the chairman of the section on the biomechanics of substitutes for biological tissues, organs and systems, and Doctor of Pediatrics V. T. Nazarov, the chairman of the section on sport biomechanics gave a short evaluation of the works presented on the themes of their sections. A resolution was made concerning wider development of research in the main trends of biomechanics

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SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

RESOLUTION OF THE SECOND ALL-UNION CONFERENCE ON PROBLEMS IN BIOMECHANICS

Riga MEKHANIKA KOMPOZITNYKH MATERIALOV in Russian No 3, 1979, p 563

["Text" of resolution by the Second All-Union Conference on Problems in Biomechanics held in Riga on 18-20 April 1979]

[Text] Three hundred ninety eight specialists took part in the work of the second All-Union Conference on Problems in Biomechanics. Among them were 113 doctors and 176 masters of science. The number of those wishing to participate in the work of the conference was considerably greater. Survey reports represented by the country's most prominent specialists reflected the basic accomplishments of the different branches of biomechanics not only in the world in general but also in our country in particular.

During the period between the First and Second All-Union Conferences on Problems in Biomechanics (October 1975-April 1979) significant success was achieved in the study of the mechanical behavior of biological tissues, systems and fluids, in investigation of the processes of regulation of biomechanical systems, in the biomechanics of artificial tissues, organs and systems, medical biomechanics, danalysis of control of movements in labor and sports. The number of dissertations and publications on the basic problems of biomechanics and the practical significance of proposed scientific developments has increased.

In terms of organization the most significant event was the creation of the Scientific Council of the USSR Academy of Sciences on Problems in Biomechanics which carried out work on determining the basic trends in the development of biomechanics and coordination of theoretical and experimental research. At the present time research in biomechanics is being conducted in more than 30 institutes of the USSR Academy of Sciences, the USSR Academy of Medical Sciences and the union republic academies, including the Ukrainian, Latvian, Georgian and Armenian SSR academies, in 50 universities of the country, 40 scientific research institutes of the USSR and the union republic ministries of health and others.

At the same time the resolution of the First All-Union Conference on Biomechanics on including a "biomechanics" specialty in the List of Specialties

Scientific Workers remains unfulfilled. There is not one single published organ on biomechanics, and specialists are still not being trained widely enough in the higher educational institutions of the country.

Taking the above into account, the conference recommends:

1. that research in the basic trends of biomechanics be still further expanded, paying primary attention to the complex solution of the most urgent theoretical problems and practical tasks;

2. that the State Committee of the USSR Council of Ministers on Science and Technology be requested to introduce a "biomechanics" specialty to the list of specialties of scientific workers, a step which would facilitate preparation of highly qualified scientific personnel and at the same time would insure higher quality of the scientific developments accomplished by them;

3. taking into account the acute need for a published organ on biomechanics, that the bureau of the Scientific Council of the USSR AN on Problems in Biomechanics be requested to examine the question of the feasibility of issuing a specialized collection on biomechanics and to negotiate with with the responsible organs for creation of it;

4. that the ministeries of of higher and secondary specialized education and the USSR and union republic ministeries of public health be requested to support the initiative of the VUZ of the country in training specialists in the basic trends of biomechanics.

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PUBLICATIONS

CHEMICAL PREVENTION OF RADIATION CONTAMINATION

Moscow KHIMICHESKAYA PROFILAKTIKA RADIATSIONNYKH PORAZHENIY in Russian 1979 signed to press 24 Jan 79 pp 2,3,4, 190.

[Annotation introduction and table of contents from book by A. S. Mozzhukhin and F. Yu. Rachinskiy, Atomizdat, 2550 copies, 190 pages]

[Text] This book, the first edition of which was issued in 1964, is devoted to medical prevention of acute radiation sickness caused by external x-ray, gamma- and neutron radiation in fatal quantities. The questions of research, experimentation, and the mechanisms of the effect of radioprotectors are examined. Particular attention is devoted to sulphur-bearing compounds, but data are presented on all classes of adequately effective anti-radiation chemical compounds.

The book is intended for radiobiologists and pharmacologists, and for specialists in medical radiology, radiation hygiene and protective medicine.

Eleven drawings, 56 tables, 422 titles in bibliography.

Introduction

At the present time chemical prevention of radiation contamination (chemical protection) is a recognized branch of radiobiology and radiation medicine and no longer needs special justification as it did 10 or 15 years ago [14].

The terms "chemical prevention" and "chemical protection" should not be taken literally. Pharmacological substances that, when introduced before irradiation, completely avert the characteristic changes in the organism known as radiation sickness are still unknown. The terms "chemical prevention" and "chemical protection" should be taken to represent a conception of increased resistance or of decreased sensitivity in animal or human organisms to the harmful effects of ionizing radiation. Future research will demonstrate which of these two concepts most accurately reflects the facts. For the time being an equal sign must be placed between them.

Historically, the first work to show the possibility of chemically preventing radiation damage in mammals was that of Patt [385] and Cronkite [295, 298], who demonstrated in 1949-1951 that cysteine and glutathione, when introduced into the organisms of mice and rats before lethal x-ray irradiation, were capable of preventing death in a significant number of animals. However, the protective effect was not constant, which provoked skepticism about the very fact of protection. The final answer to the question about the possibility of chemical prevention was left to Bacq [279], who showed in 1951 that cysteamine and cystamine, introduced into mice before irradiation, permitted 100% survival of the experimental animals, while the control animals suffered 100% mortality.

Radioprotective compounds were found in various classes of chemical substances, but the most likely ones for future use were aminothioles and their derivatives [18,41]. But the relatively high toxicity and narrow therapeutic range of the aminothioles and most of their derivatives caused doubt as to the possibility of their usefulness in protecting humans from the harmful effects of ionizing radiation [230]. In the succeeding years thousands of new compounds were synthesized and studied in tests on mice, and among these effective radioprotective agents were discovered with a wider therapeutic range than that of cysteamine and cystamine; these finds stimulated interest in the question [181,204,263].

The most thoroughly studied radioprotectants have begun to be used in x-ray therapy and oneological practice.

After the release of the first edition a great deal of material was published on new radioprotective agents and the mechanisms of their pharmacological and protective action [18, 70, 103, 173, 183, 184, 256]. This spared the authors of this book the need to systematically set forth the problem of chemical prevention of radiation contamination. In the present edition, as previously, the superior radioprotective effect of aminothioles and their derivatives is examined, as well as their conversion in the human and animal organism and their influence on the condition of the organism. The authors have had much personal experience in the synthesis and analysis of these compounds. They also considered it essential to compare the radioprotective effect of aminothioles with that of other radioprotective compounds, and to present new data on the effectiveness of radioprotectants under different conditions of the action not only of x- and γ-ray irradiation, but also of neutrons and protons, and data on the effectiveness of radioprotectants in combination. At the same time the authors have shortened or deleted several chapters and sections whose contents fell outside the framework of analysis, testing and evaluation of radioprotectants.

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PUBLICATIONS

CHEMICAL AND BIOLOGICAL AGENTS FOR PLANT PROTECTION: A SHORT HANDBOOK

Moscow KHIMICHESKIYE I BIOLOGICHESKIYE SREDSTVA ZASHCHITY RASTENIY (Kratkiy Spravochnik) in Russian 1978 signed to press 7 Jul 78 pp 2, 4-6, 207

[Annotation, introduction and table of contents from book by N. V. Sazonova, "Kolos" Publishers, 100,000 copies, 207 pages]

[Text] This book contains short items about preparations recommended for practical use and experimental production. Recommendations are given for the use of chemical and biological protective agents for agricultural crops against pests and disease, and the conditions and basic regulations for their application are indicated. A list of preparations for protecting plants in collective and individual gardens, and the rules for their use, is presented. Safety measures for working with pesticides are set forth.

The book is intended for plant protection specialists, agronomists, and sookhoz and kolkhoz directors.

Introduction

In view of the further intensification of agriculture, protecting plants for pests, disease and weeds is an integral part of a progressive technology of crop cultivation.

The most important tasks involved in perfecting and reinforcing plant protection in this country were determined in the basic directives for national economic development in the USSR for 1976-1980, accepted by the 25th CPSU Congress: further expansion of pesticide production, with deliveries to agriculture of up to 628,000 tons (in conditional units) was called for, as was the need to protect the biosphere from pollution. The July (1978) Plenum of the CC CPSU also devoted considerable attention to the protection of plants.

The importance of the chemical method of protecting harvests from plant pests and disease lies in its high effectiveness, return for money expended, and accessibility for use. For example, in 1975, thanks to the use of pesticides to combat pests, disease and weeds in the main agricultural crops, an additional 16.5 million tons of grain, 11.7 million tons of vegetables and potatoes, 16 million tons of sugar beets, 2.2 million tons of raw cotton, 3.6 million tons of fruit, 2.3 million tons grapes and other products, were harvested for a total of 7.8 billion rubles.

The chemical method is now being perfected with the aim of reducing the danger of these preparations to humans, useful organisms, and nature as a whole. To this end persistent preparations are being replaced by less resistant chemicals, quick to break down without toxic residue as well as by less toxic substances. Preparations containing arsenic, mercury and fluorine derivatives are being almost totally eliminated from the assortment of chemicals; new chemical substances harmless to humans and useful animals are being introduced, and the forms and methods of their preparation are being perfected.

In recent years definite success has been observed in decreasing pesticide toxicity to warm-blooded organisms. A large number of new pesticides have been studied in detail, permitting fundamental revision and renewal of the available assortment of pesticides, thanks to the efforts of the All-Union scientific research institute for hygiene and toxicology of pesticides, polymers and plastics, the All-Union scientific reserach institute for chemical means of plant protection, and other scientific and production establishments of the Ministry of Agriculture, the Ministry of Chemical Industry and the Ministry of Health. In 1965 the average toxicity of insectoacaricides for warm-blooded organisms was 200 mg/kg, and in 1975, 980 mg/kg, so the toxicity of the preparations has been decreased almost five-fold. During this period all preparations that are highly toxic for warm-blooded animals were eliminated, as were resistant cumulative substances that can cause chronic poisoning. As replacements for DDT and other chlorine organic compounds, phosphororganic compounds of medium or low toxicity for warm-blooded animals, which break down in one vegetation period to nontoxic metabolites, were adopted.

The State Commission for Chemical Substances for Combating Pests, Plant Disease and Weeds recommended for agricultural use organic phosphorus compounds ([antio], basudin, [bromophos], [gardona], [volathon], DDVP, [amiphos], metathion, [phosalon], [B1-58], [phthalophos] and others), carbamic acid esters (sevin, [pirimor]), organic halogen compounds ([dilor], [tedion], keltan, gamma-isomer HCCH and others), nitro derivatives (acrex) and others. About a third of the recommended preparations are selective insecticides and acaricides with a lethal dosage (causing over 50 percent mortality in test animals) of over 1,000 mg/kg. Specifically, this figure is 1,900-5,000 mg/kg for [gardona], 5,000-9,000 mg/kg for [dilor], 5,000-10,000 mg/kg for [tedion] and so forth. The use of these preparations permits control of the chemical method and decreased danger to useful members of the agroceuosis. For example, specific acaricides and aphicides (acrex, [tedion], keltan, [plictran, pirimor] and others) are 500-1,000 times more toxic to pests than to their main enemies (chrysopids, coccinellidae, predatory bugs, flies, etc.). Insecticides such as [gardona, dilar, amiphos] are 100-500 times more dangerous for harmful insects than for entomophages.

New forms of preparations and methods of their application have been worked out to further increase the efficiency and safety of chemical substances. Preparations in granular form are highly effective for combating pests (wireworm, grain ground beetle, weevil, cutworm, nematodes); they are also significantly less dangerous to useful organisms and contribute much less to

the contamination of the environment. For widespread use granulated preparations based on the gamma-isomer HCCH, basudin [valekson], methaldehyde, [phosphamid], chlorophos, etc., are recommended. Aerial ultra-low volume spraying is being tested for use in combating harmful locusts, stink bugs, and grain cutworm using special forms of these preparation.

The assortment of fungicides and seed mordants has been improved and updated. On green plants, instead of Bordeaux liquid, low-toxic preparations related to the thiocarbamates and phthalimides (zineb, [polycarbazine], [polymarzine], [polyhom], [ditan M-45], caprozan, captan, [phthalan]) are used against false mildew fungi and leaf spot. To successfully combat powdery mildew, which affects various agricultural crops, [caratan], acrex, and [morestan] are recommended in addition to colloidal sulphur. In recent years systemic fungicides have come widely into use that are effective simultaneously against several diseases which may develop on the same culture (benomil, [uzgen], [topsin-M], etc.). It has also been shown that such systemic fungicides as benomil, [topsin-M] and [uzgen] may also be used a mordants for seeds and soil preparations.

Great achievements have also been made in the search for non-mercury bearing mordants. Among the effective replacements for granosan, mercurhexane, and mercurbenezene are benomil, [vitarax], hexathuiran, [tigam], [EP-2] and others.

Various biological preparations based on bacteria and fungi are a part of the broad practice of crop protection.

The use of selectively toxic rodenticides has noticeably increased. In field conditions the Soviet preparation gliftor, which is practically harmless to birds, is widely used, and synthetic blood anticoagulants are highly effective in combating rats and mice.

The entire contemporary arsenal of chemical substances for protecting plants has been thoroughly studied at the corresponding scientific establishments of the USSR Ministry of Public Health, and based on this study standards have been determined for the the use of these preparations: optimal norms for expenditure of pesticides; permissible maximum residue in food products; permissible amount of the preparations in the air of work zones; waiting period from last processing to harvest.

Successful solutions to the problems of regulating the chemical method and eliminating the possible negative consequences to the use of pesticides (such as poisoning of humans and domestic animals, of bees, which pollinate plants, and of entomophages; formation of resistant populations of harmful insects, acauds, and pathogen; desposit of preparation residues in harvested products, and pollution of the environment with these residues) may be reached not only by selecting new preparations, but by rational and expert inclusion of chemical methods in the zone system of protecting agricultural

crops, the foundation of which should be agrotechnical prophylactic methodology. A thorough analysis of all biological peculiarities of the protected crop's agrocenosis is essential, and must take into account the economic threshold of harmfulness for every pest species within the zonal sector.

Branch and zone institutes are studying and refining the optimum standards, time periods and application methods for the recommended preparations. Knowledge and observance of the regulations established for use of chemical substances by workers engaged in plant protection and by agrochemical kolkhoz and sookhoz personnel should permit all positive aspects of active chemical protection of plants to be maximized, and to allow the elimination of many undesirable consequences.

The present handbook is a practical guide to the existing regulations for the application of contemporary pesticides and their effective and safe use.

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PUBLICATIONS

CLINICAL METHODS, DIAGNOSIS AND TREATMENT FOR ATTACKS BY TOXIC CHEMICAL AGENTS

Moscow KLINIKA DIAGNOSTIKA I LECHENIYE PORAZHENIY OTRAXLYAYUSHCHIMI VESHCHESTVAMI in Russian 1978 signed to press 26 May 78 pp 2-5, 176

[Annotation, Introduction and Table of Contents from book by Yu. N. Stroykov, Meditsina Publishers, 110,000 copies, 176 pages]

[Text] This book is devoted to questions of clinical procedure, diagnosis and treatment for patients affected by chemical agents in the event of mass contamination of given areas by chemical weaponry. A short description of chemical weaponry and its features is given and the range of medical assistance, both in the center of chemical contamination and at the stage of medical evacuation, is set forth, as applied to the medical service system of Civil Defense. The principles of antidote therapy which is of great importance in the treatment of contamination by toxic chemical agents, are presented. Methods of self- and mutual assistance, which to a significant degree determine the course and outcome of contamination, are examined, and the basic methods used to restore respiratory function are elucidated, as are means of individual and collective antichemical defense.

The book is intended for middle medical workers; it contains 17 drawings, and 23 titles are included in the biography.

The high contaminating effect of toxic chemicals /OV/ was irrefutably demonstrated during the First World War when the use of chemical weaponry caused the deaths of huge numbers of people and serious damage to Army personnel subjected to such attacks. In the following years it was found that people who had been seriously affected by OV frequently became invalids requiring constant medical observation or long-term treatment.

At the Genoa Conference in 1922 the question of forbidding chemical warfare was brought up on the initiative of the Soviet Union, but the representatives of the imperialist states declined to discuss the issue. In Geneva in 1925 an agreement was worked out forbidding the use in war of "...asphixiating, poisonous or similar gases and bacteriological substances." Although the Soviet Union was among the first to ratify this agreement, a number of countries, including the USA and Japan, refused ratification. In 1952 on

the initiative of the Soviet Union the United Nations called for the ratification of the Geneva protocol by all countries. Only in 1970 did the U.S. government propose partial recognition of the Geneva protocol, keeping for itself the right to use OV not mentioned in the document.

After World War II a continual search went on in the U.S. and the NATO countries for new OV, distinguished by highly contaminatory features. Some of these were used by the U.S. army in the war against the Vietnamese people.

The armies of imperialist states are equipped with extremely toxic OV, which can be categorized in the following basic groups: fatal OV (FPS, yperites); those causing temporary incapacity (psychomimetic, some irritants and tear-gases); and those causing short-term incapacity. Foreign military specialists continually stress that the great effectiveness of OV is manifested particularly clearly in sudden attacks and when the population is inadequately trained in antichemical defense. There is no doubt whatever that the effectiveness of chemical weaponry is sharply decreased when preventive and treatment measures are taken correctly and in time against OV affection.

The threat of mass contamination of the population in the event that an aggressor employs chemical weaponry forces the Civil Defense (CD) medical services to face a number of tasks, of which the most important are: a) implementing all-round preventive measures with the aim of avoiding OV contamination; b) taking correct and timely measures to provide self- and mutual assistance, and CD assistance in the chemically contaminated zone; c) to warn of the later development of after-effects characteristic of serious damage by OV. Although these tasks are faced in the event of a threat, not only of chemical, but of nuclear and bacteriological warfare, the scope and nature of the medical measures taken in a zone of chemical contamination are unique and specific. We have devoted much attention in this publication to explaining the peculiarities of damage caused by chemical weaponry.

The fulfillment of the tasks set before the CD medical services requires of the middle medical workers a clear understanding of the nature of the medical measures taken under the threat of a chemical attack, in the zone of chemical contamination and during medical evacuation.

The history of the use of chemical weaponry shows convincingly that the greatest damage is caused by OV when the army and population are not prepared to combat the later consequences of a chemical attack. A high level of preparedness in the population, all CD services and particularly the medical service is a sure guarantee of keeping total personnel losses to a minimum, in particular by reducing the number of irretrievable losses and changing the balance of injuries in favor of light and semi-serious cases.

The material presented below has been arranged to correspond to the training level of middle medical workers in problems of antichemical defense.

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