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CENTRAL INTELLIGENCE AGENCY

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COUNTRY Poland

REPORT

SUBJECT Research on Trichionellosis

DATE DISTR. 1 May 64

NO. PAGES 2

REFERENCES

DATE OF INFO.
PLACE & DATE ACQ.

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annual report (seven pages, English) of a Polish research project entitled "Investigations on Trichinellosis with Special Reference to Epizootiology, Immunology and Pathogenesis." The project, which is being conducted at the Institute of Parasitology at Warsaw, is under the direction of Dr Zbigniew Kozar.

2. The summary of progress:

a. The investigations involve a number of topics, a part of which has been completed or published (five papers), others are being conducted or will be undertaken.

b. Epidemiologic and epizootiologic investigations are designed to establish the incidence of Trichinellaspiralis in people, domestic and wild living animals throughout the country, the effect of contributing factors and to develop control methods.

c. Among the hitherto examined human corpses (more than 2100 cases) from various regions of the country, positive results have been obtained in about four percent. This percentage is lower as compared with that of the US (16 percent), but the differences between individual regions are significant and warrant epidemiologic conclusions. Similar methods (microscopic and digestive) are used to examine dogs, cats and wild living animals. As to the infection in pigs, the analysis of data collected from all over the country will permit the recognition of the influence of cultural and other factors on the incidence of the disease in these animals. The studies are also concerned with the effect of environment, particularly in rural population from certain regions of the country, as assessed by allergic tests.

d. Pathogenesis and therapy of Trichinellosis. To begin with methods of experimenting and studying the effect of invasion in mice have been developed. the critical review is concerned with some reported methods.

e. The pathogenic problem of trichinellosis is extremely complex and should be attacked by possibly all available methods. Some interesting,

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so far unknown data have emerged from the studies on the effect of [redacted] 50X1-HUM
flora in the host's digestive tract on the course of infection [redacted] 50X1-HUM
[redacted] Intravital staining of some host's tissues [redacted] 50X1-HUM
was employed to learn more about host-parasite relationships [redacted]

f. More attention is being paid to alterations which occur in host's 50X1-HUM
tissues. The muscles of infected animals (at various time pi) have 50X1-HUM
been the subject of our hitherto performed studies. Metabolic changes
of the muscles have been examined so far as carbohydrate [redacted] 50X1-HUM
lipids and Krebs cycle are concerned; the results obtained seem to be
of interest. Independently, the activity of some enzymes occurring in
the serum of infected animals and the metabolism of the myocardium are
being investigated. In these investigations, histochemical methods
play a supplementary part. The muscular phase of the invasion has been
the subject of numerous examinations; as to the intestinal phase our
studies have been concerned with the occurrence and distribution of
some compounds (RNA, DRNA etc.) and the enzymatic activity in the pa-
rasite and surrounding host's tissues.

g. As regards the parasite, respiratory and metabolic studies were
made on isolated larvae. Eventual differences in the pathogenicity
of individual parasite strains (eg from Africa and Poland) were also
investigated.

h. The main goal we are aiming at in this field is to obtain an
appropriate therapeutic method. The hitherto applied corticosteroids
are effective clinically, but they result in general, long-lasting,
undesired sequelae. We could demonstrate that a similar clinical effect
but without sequelae is obtainable with plan preparations of azulenes
[redacted] 50X1-HUM

i. In our opinion, trichinellosis of people occurs not only in acute
form (described repeatedly), but also in a chronic one, persisting
occasionally for several years. It is just the latter phase of
trichinellosis which in some countries may become a serious sanitary
problem, that is the main subject of our investigations.

j. In the group of 236 patients, affected by reumatic disease and
subjected to balneologic treatment, 18 cases presented ailments
which apparently could be related to trichinellosis they had years
ago. We also analyzed other post-mortem diagnosed trichinellosis
cases which had reached clinics and autopsy tables with various mis-
diagnosis. Our studies are also concerned with the consequences of
trichinellosis (mostly of the circulatory system) in the group of
patients who had succumbed to the disease a few months ago, as well
as with their treatments.

k. Immunology of trichinellosis. The investigations of this group
aim at the establishment of more detailed information about the host-
parasite system [redacted] 50X1-HUM
[redacted] and at
the development of practical guiding lines for the diagnosis (com-
parison of various reported reactions).

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1. Name of Institution:

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Institute of Parasitology, Pasteura 3, Warszawa
Laboratory of Antropozoonoses, Norwida 29, Wrocław, Poland

2. Name of principal investigator:

Prof.dr Zbigniew K o z a r

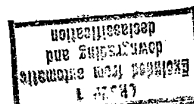
3. Project title:

Investigations on trichinellosis with special reference
to epizootiology, immunology and pathogenesis.

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II. Summary of progress

The investigations involve a number of topics, a part of which has been completed [redacted] others are being conducted or will be undertaken.

Epidemiologic and epizootologic investigations are designed to establish the incidence of trichinella spiralis in people, domestic and wild living animals throughout the country, the effect of contributing factors and to develop control methods.

Among the hitherto examined human corpses (more than 2100 cases) from various regions of the country, positive result have been obtained in about 4%. This percentage is lower as compared with that of USA (16%), but the differences between individual regions are significant and warrant epidemiologic conclusions. Similar methods (microscopic and digestive) are used to examine dogs, cats and wild living animals. As to the infection in pigs, the analysis of data collected from all-over the country will permit the recognition the influence of cultural and other factors on the incidence of the parasite in these animals. The studies are also concerned with the effect of environment, particularly in rural population from certain regions of the country, as assessed by allergic tests.

Pathogenesis and therapy of Trichinellosis. To begin with methods of experimenting and studying the effect of invasion in mice have been developed [redacted] the critical review is concerned with some reported methods. 50X1-HUM

The pathogenic problem of trichinellosis is extremely complex and should be attacked by possibly all available methods. Some interesting, so far unknown data have emerged from the studies on the effect of flora in the host's digestive tract on the course of infection. 50X1-HUM

[redacted] Intravital staining of some host's tissues was employed to learn more about host-parasite relationships. 50X1-HUM

More attention is being paid to alterations which occur in host's tissues. The muscles of infected animals (at various time p.i.) have been the subject of our hitherto performed studies. Metabolic changes of the muscles have been examined so far as carbohydrate [redacted] lipids and Krebs cycle are concerned; the results obtained seem to be of interest. Independently, the activity of some enzymes occurring in the serum of infected animals and the metabolism of the myocardium are being investigated. In these investigations, histochemical methods play a supplementary part. The muscular phase of the invasion has been the subject of numerous examinations; as to the intestinal phase our studies have been concerned with the occurrence and distribution of some compounds (RNA, DRNA ect.) and the enzymatic activity in the parasite and surrounding host's tissues.

As regards the parasite, respiratory and metabolic studies were made on isolated larvae. eventual differences in the pathogenicity of individual parasite strains (e.g. from Africa and Poland) were also investigated.

The main goal we are aiming at in this field is to obtain an appropriate therapeutic method. The hitherto applied corticosteroids are effective clinically, but they result in general, long-lasting, undesired sequelae. We could demonstrate that a similar clinical effect but without sequelae is obtainable with plan preparations of azulenes [redacted] 50X1-HUM

In our opinion, trichinellosis of people occurs not only in acute form (described repeatedly), but also in a chronic one, persisting occasionally for several years. It is just the latter phase of

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trichinellosis which in some countries may become a serious sanitary problem, that is the main subject of our investigations.

In the group of 236 patients, affected by rheumatic disease and subjected to balneologic treatment, 18 cases presented ailments which apparently could be related to trichinellosis they had years ago. We also analyze other post-mortem diagnosed trichinellosis cases which had reached clinics and autopsy tables with various misdiagnosis. Our studies are also concerned with the consequences of trichinellosis (mostly of the circulatory system) in the group of patients who had succumbed to the disease a few months ago, as well as with their treatment.

I m m u n o l o g y o f t r i c h i n e l l o s i s . The investigations of this group aim at the establishment of more detailed information about the host-parasite system (e.g. published paper on the incidence of antigen in the blood and urine depending on the post-infection period) and at the development of practical guiding lines for the diagnosis (comparison of various reported reactions).

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III. Detailed Report

The investigations undertaken by a team of specialists (biologists, epidemiologists, clinicians, veterinarians, biochemists etc) have been designed to contribute some new data about trichinellosis, particularly about the aspects which, in the present state of the knowledge, require some explanations, mostly for the treatment and control of the disease. Several interrelated problems, completing one another are being studied independently, which are expected to yield in the end-phase some general conclusions. Some topics are prompted only in the course of investigations; particular attention is being paid to the 3 above given trends: epidemiology and epizootiology, pathogenesis and therapy, and immunology.

The investigations concerning the first problem are being led all over the country according to the current concept postulating a circulation of the parasite in domestic environment and among the wild living animals. The hitherto obtained data from Polish territories are fragmentary and rather local ones. We intend to complete and extend them in order to draw definite conclusions for the complex control of the parasite, conforming to the instructions of the International Commission on trichinellosis. We feel that such measures should be practiced in all countries, where trichinellosis is a sanitary problem.

Pathogenetic problems are being dealt with by means of all available for us methods (biologic, biochemic, histochemic) according to our assumption that trichinellosis brings about a general shift in the organism, and is a disease with prevailing allergic symptoms. In the current studies on the host-parasite relationships, attention has been mostly paid to the parasite: its growth and behaviour under various conditions. What we intend is to extend their scope by studying changes occurring in the host, particularly in the striated muscles and myocardium. It is assumed on the basis of our present observations that trichinellosis occurs in people not only in the acute but also in chronic form which occasionally persists for years and is characterized by certain complaints which the clinicians mostly fail to relate to the proper cause. The importance of this problem is stressed, including also the therapeutic aspect.

In the field of immunology, we wish to elucidate some points which might shed light on host-parasite relationships and to prepare material for standardisation of mostly used diagnostic methods.

The list of topics terminated in 1963 or conducted currently is given below:

A. Epidemiologic and epizootologic investigations.

1. Mass post mortem examinations of human corpses. The supply of the material from various localities of the country has been secured. By means of the two methods (microscopic and digestive) about 2100 samples were investigated with positive results in about 4 per cent of the cases. Great differences were found in the distribution of the positive cases on the terrain of the country. The completion of sampling and elaboration of the paper are anticipated for summer 1964.

2. Epidemiologic investigations by means of intradermal test in Trichinella free regions. In connection with our previous investigations by the use of the allergic test on strongly endemic terrains and on the basis of very high positive results we have undertaken similar tests on terrains on which for

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many years the disease was found neither in the man nor in animals (province Cracow). The data have been obtained from 951 adults (rural population). In most parts there were negative results but in some localities there emerged unexpected positive results which could be later explained in the light of a detailed epidemiologic and epizootiologic analysis. This fact is an additional support for the value of the test in mass investigations and confirms our older view on the applicability of the allergic test to mass orientation in epidemiological situation.

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3. **Epizootiology of trichinellosis in swine.** By way of personal enquiries and contacts detailed data were collected on the occurrence of trichinellosis in pigs in the individual regions of the whole country. Endemic centres were found where cases of infection occur every year and areas where cases of trichinellosis are not recorded for many years. For analysis are submitted the various epizootiological and other factors. The analysis of the data will be terminated in the next months.
 4. **Incidence of *Trichinella spiralis* in animals.** By means of the two methods (as in 1) samples from about 1000 dogs and cats and more than 200 wild animals were investigated. The material was collected from various provinces of the country. Attention is drawn also to larvae of other nematodes found in muscles, which may be erroneously taken for the *Trichinella* larvae. Especially if wild animals material is coming at irregular intervals these studies must be prolonged for the next year.
- B. Pathogenesis and therapy of trichinellosis.**
5. **Influence of *B. mesentericus* on the development of trichinellae in mice.** The experiments were conducted on mice, males (in 3 series). They were given per os during 6 days fresh culture of *B. mesentericus*. The first series exhibited 3,46 ($\pm 0,8$) intestinal trichinellae, in controls were found 11,71 ($\pm 1,17$) trichinellae. Series II (*B. mesentericus* during the first 6 days) exhibited on average 896,1 ($\pm 289,4$) muscle larvae. In 59 controls there were on the average 1824 larvae ($\pm 250,4$). Series III. (*B. mesentericus* 6-12 days p.i.), the number of muscle larvae 1309 ($\pm 330,8$). In 27 control - 1863 ($\pm 369,2$) larvae. It was proved that the feeding of the infected mice with the culture of *B. mesentericus* decreases by several times the number of the developing intestinal trichinellae ($P < 0,01$). There were also less muscle trichinellae (II series) in the experimental mice as compared with the controls. The administration of bacteria after 6 days post infection (III series) exerts no major influence on the invasion. It appears that the intestinal flora plays a certain role in the establishment of the trichinellae in the alimentary tract of the host.
6. **Supravital staining of the intestine and muscle tissue during infection with trichinellae.** Infected with trichinellae mice were given at various periods of time 1 per cent solution of trypan blue, which is normally taken up by cells of mesenchymal origin, mainly by cell of the RES.

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7. Changes of the metabolism of the skeletal muscles of guinea pigs caused by *Trichinella spiralis* invasion. I. Influence of the invasion on the carbohydrate metabolism of muscles. It was found that following the invasion changes in the muscle metabolism take place. They are expressed by a decreased intensity of respiration and a decrease of RQ. These changes depend on the period of infection. In the first period (up to the 18-20th day p.i.) the breakdown of sugars is decreased and the synthesis of glycogen increased. This is accompanied by decreased quantities of phosphorylated sugar metabolites increase in the concentration of glycogen and an increase in the metabolic processes of fats. During the second period (from about 21th day p.i.) the breakdown of glycogen is increased, and thus increased is the synthesis of fats. This is manifested by decreased level of glycogen and increase in RQ above 1.0.
8. Changes of the metabolism of the skeletal muscles of guinea pigs caused by *Trichinella spiralis* invasion. II. Activity of enzymes of the Krebs cycle and fat metabolism. The results obtained point to metabolic changes occurring at various time following the infection in host's muscles. Computations and detailed analysis of the results are just being conducted.
9. Studies on respiration and metabolism of *T. spiralis* larvae. The main subject was concerned with the role of carbohydrate metabolism in the parasite's energetics; new, hitherto unpublished data about some enzymatic systems operating in aerobic sugar metabolism of the parasite became available.
10. Enzymatic activity in the serum of rabbits infected with *T. spiralis*. The hitherto completed first part of the experiments was dealing with the behaviour of aspartate aminotransferase, alanine amino-transferase and l-lactate oxidoreductase activity, depending on the time lapse after infection and the intensity thereof. These studies are being continued.
11. The course of experimental trichinellosis in the mouse. Methods of infection and the influence of various doses on the mortality and weight of animals. The methods of experimental infection of mice were elaborated and evidence is presented proving false results after the use of other methods cited sometimes in the literature. Criteria were determined which allow to trace the course of trichinellosis in mice. LD₅₀ = 801 larvae per mouse. Both the mortality rate and survival times depend on the level of the infection dose. The curve illustrating the mean weight of the infected mice had a characteristic course with the first infliction (about 15th day p.i.), a following increase and a second infliction (about the 25th day p.i.). The mice are very suitable for experimental studies of trichinellosis and the described methods may find various application.

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12. Comparative studies on pathogenicity of various *T. spiralis* strains. Using the method given above comparative studies were performed with 3 *T. spiralis* strains: freshly isolated from man, that derived from Kenya and a laboratory strain passaged for about 15 years on mice. The experiments are being conducted.
13. Histochemical studies on the intestinal phase of trichinellosis in mouse. The content of nucleinic acids (RNA and DNA), glycogen, mucopolysaccharides, lipids and of fatty acids was examined; the examinations were also concerned with the distribution and activity of alkaline and acid phosphatase, succinic acid dehydrogenase, cytochrome oxidase and gamma-glutamyl-transpeptidase (GGTP). The results obtained imply some differences between intestinal and muscular trichinellae.
14. Studies on the possibility of utilization of pharmacological properties of Azulenes in the treatment of acute and chronic trichinellosis. It was proved experimentally and clinically that cortisone preparations can be replaced by preparations Azulen SE/III in the treatment of trichinellosis. This drug, in contrast to cortisone, does not increase the intensity of invasion but possesses also antiallergic and anti-inflammatory properties. Also in the course of the use of methods described in the paper No. 11 it was proved in mice the more beneficial influence of Azulen as compared with cortisone. The results of treatment by intravenous injections of Azulen in 18 cases of acute trichinellosis and 6 cases of chronic trichinellosis in human beings were good and encouraging further studies on the use of Azulenes which are chemical compounds of plant origin (*Chamomilla vulgaris*).
15. Clinical problems of chronic Trichinellosis in man. I. Examinations in the clinic Rheumatologic Centre in Busk. In 236 patients receiving balneologic treatment because of chronic rheumatic disease allergic studies were carried out with Trichinella antigen. A positive result was obtained in 18 subjects, who, when examined thoroughly, were found to have had trichinellosis previously; they often showed a reaction to baths. Cardiac alterations were studied in detail.
16. Clinical problems of chronic Trichinellosis in man. II. Analysis of autopsy and clinical diagnoses in subjects with established presence of Trichinella larvae. Mass post-mortem examinations disclosed some cases of severe *T. spiralis* infection although neither case history nor post-mortem protocol contained any mention about the disease. The material being collected from various centres will permit to establish an eventual correlation between the previous trichinellosis and the later symptoms and course of the disease leading to death.

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17. Clinical problems of chronic trichinellosis in man. III. Periodical examination of patients with history of acute trichinellosis. Detailed examinations (particularly of the circulatory system) were performed in 35 persons who had acute trichinellosis 9 months ago. All of them presented complains, occasionally severe ones, which are undoubtedly due to chronic trichinellosis. Treating procedures are attempted.

C. Immunology of trichinellosis.

18. The occurrence of antigen in the blood and urine of rabbits in the course of experimental infection with *Trichinella spiralis*. The antigen in the blood serum could be demonstrated at the earliest on the 3rd day p.i. It was present in the serum 34-54 days p.i. In the urine, the antigen was demonstrated on the 3rd day and was present during 30-57 days p.i. This latter was detectable later and demonstrated for a shorter period of time, than the antigen in the serum. The antigenic substance recovered from the urine shows an increase after the infection reaching its peak level on the 14th day and next its slow decrease is observed.

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19. The comparative value of various immunodiagnostic methods in trichinellosis. Sera of infected animals and human beings are examined in comparative studies on the value of the agglutination reactions especially with cholesterol, latex and bentonite. The work are continued.

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Wroclaw, December 31th, 1963

Principal investigator

Z. Kozar

Prof. dr Zbigniew Kozar

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