ON THE PRACTICAL VALUE OF THE INTRADERMAL REACTION WITH THE TRICHINELLIASIS ANTIGEN FOR THE DIAGNOSIS OF TRICHINELLIASIS IN MAN *

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Owing to the frequency with which trichinelliasis is mistaken for typhoid fever, typhus, rheumatism, grippe, etc., it would seem important that more exact methods of diagnosis should be established. In the hope of accomplishing this, the author made a four-years intensive study of the disease in many patients.

Theoretical Background: In 1931, while studying the clinical aspects of trichinelliasis, the author determined to study the practical value of the intradermal reaction with the specific antigen for the diagnosis of trichinelliasis, following the Casoni method for echinococcosis, the value of this reaction for diagnosis being generally recognized. The idea of the possible application of this reaction to trichinelliasis came from consideration of the fact that all the data then known to us from literature and from personal observations on the pathogenesis, epidemiology, and the clinics, assume that trichinelliasis must be connected with numerous allergical phenomena in the organisms.

In spite of the animal experimentation of Romanovitz and of Flury, it is impossible, even now, to consider it positively proved that trichinellae discharge a toxin. Romanovitz tried the toxicity of serum of animals which he infected with trichinellae upon other animals. The author considers that such a method can not be used as proof of the toxicity of the parasite, since the trichinelliasis toxin is not very likely to be found in the serum of trichinellae-infested animals. Flury did not work with isolated trichinellae, but he treated meat heavily infested with trichinellae in certain ways, and tried out the effect of the mixture of various derivatives upon

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animals. The author considers that the toxicity of his extracts may have been due to protein derivatives and not to trichinellae. The ingestion of meat which contains the killed trichinellae does not cause in human beings any morbid phenomena which would indicate the presence of toxins in the organisms of the trichinellae. The clinical observations of the author as well as those of other workers show that in cases of uncomplicated trichinelliasis in man, the phenomena of general intoxication are relatively insignificant, and seem to favor the theory that trichinellae do not discharge any toxin. In trichinelliasis a constant process of destruction of the young trichinellae (the so-called embryos) is going on, and is followed by their resolution into all the tissues and organs. This destruction and resolution is taking place during the incubation period which often lasts two, three or more weeks, as well as during the course of the disease. It is clear, therefore, that during this time a constant sensitization of the patient's organism by the constituents (proteins) of the organism of the degenerated young trichinellae is taking place.

There are two more facts which speak in favor of the existence of allergic phenomena, namely: 1, the high eosinophilia, which is most common to allergic states, and 2, a skin eruption of an allergic nature often encountered in this disease.

Thus, if a sufficient quantity of the active antigen of trichinellae could be obtained, it would be reasonable to hope on the basis of the considerations given above that an intradermal reaction with this antigen would give positive results in the early stages of the disease, following the type of skin reaction which is already known in echinococcosis, hay fever, bronchial asthma, etc.

History of the Subject: A review of the literature, as far as the material which is available for the author goes, shows that the idea of an intradermal reaction in trichinelliasis was for the first time expressed by Fülleborn in 1926, when he used for this purpose the so-called Voll-antigen, devised by himself. This consisted of from 5 per cent to 10 per cent of dried trichinellae and dried muscle tissue, since Fülleborn did not find the procedure by which trichinellae could be isolated in a pure state, or in an almost pure state, from the

meat. This first method did not emerge from the experimental stages, and was tried out on only four patients.

In 1927 the American investigator, Bachman, developed a method by the application of which it is possible to obtain the isolated trichinellae. Out of these isolated trichinellae Bachman, by a process of drying and extracting, obtained an antigen with the aid of which he proved the formation of precipitins in rabbits' serum after 30 to 40 days following the infection of the animal with trichinelliasis. In 1928 he showed that rabbits and guinea pigs infected with trichinelliasis gave a positive intradermal reaction when his antigen was used. Bachman did not try the effect of his antigen on patients affected with trichinelliasis.

Besides the data given above, we also have the following information about the application of antigen for diagnostic purposes in cases of trichinelliasis in human beings. In 1930, Kovsh and Korjazhnov, working in Romanovitz' laboratory, published a paper on this subject. These authors, however, did not (as far as we know) fully describe the method by which they obtained their antigen; on this account their methods cannot be either adopted or discussed.

In 1933, Maternovskaja from Lvov (Lemberg) published an extensive work from which we assume that she obtained a positive intradermal reaction with her antigen in animals and in 23 patients with trichinelliasis. However, the author is convinced that this was no allergic skin reaction to the antigen, which was introduced intradermally, but a reaction of an inflammatory nature following the injection of the antigen which was used as an emulsion. This emulsion was prepared from trichinellae obtained from the intestines of rats, dried and ground with distilled water. Undoubtedly this was not sterile, and in addition to the components of the trichinellae organisms which were dissolved in the water, it contained insoluble components as, for instance, the membrane of the trichinellae. These insoluble impurities when administered intradermally cause an irritation of the skin. In fact, the reaction of Maternovskaja's antigen was not obtained according to the immediate type as was that obtained by Augustine and Teiler, by McCoy and his collaborators, and also by the author, but according to a delayed type of twelve to twenty-four hours, or even more.

A similar fallacy was recorded by Killduffe in 1933. He was working not with intestinal but with muscular trichinellae, isolated by Bachman's method. Killduffe, like Maternovskaja, used as an antigen for the intradermal reaction in man an emulsion since, according to his description, his solution of isolated and dried muscle trichinellae mixed in Coca's liquid No. 1 (0.7 per cent NaCl., 0.05 per cent $NaHCO_3$ and 0.1 per cent phenol) was turbid in consistency. As a result of his experiments, Killduffe, like Maternovskaja, obtained in thirty-three cases of trichinelliasis in human beings a reaction after twelve, twenty-four, and even forty-eight hours, i.e. reaction of clearly inflammatory nature.

The work of Augustine and Teiler of Harvard University, which was done in 1932, must be considered as the first work in which the antigen, prepared according to Bachman's method, was correctly applied to man. These investigators worked with a sterile antigen, the methods of preparation of which as well as the character of the reaction (immediate type) will be described later.

In 1933, McCoy, Miller and Friedländer published their findings on the application of the sterilized antigen of trichinellae (Bachman's method) to human beings. These authors allowed, however, some deviations from the classical formula of Bachman, since they used for the extraction of the trichinellae Coca's solution No. 2, namely, a solution consisting or NaCl 0.5 per cent, $NaHPO_4$ 0.143 per cent, KH_2PO_4 0.036 per cent and phenol 0.4 per cent.

Friedländer, in 1934, published a paper in which he describes his experience at the patient's bed-side. This work is a continuation of that which he started in collaboration with McCoy and Miller, and in this description he questions the value of the intradermal reaction for the diagnosis of trichinelliasis in human beings.

The author, late in 1931, started in one of the Kiev laboratories an independent work with view of obtaining an antigen of trichinellae. The works of Fülleborn, Bachman, and others were not known to him at that time, and it turned out that he was pursuing the path which had already been discovered by Bachman in 1927. Therefore, as soon as a reprint of Bachman's work was obtained, in 1933, the author approached Dr. S. V. Ribinsky with the request that in his department of the Microbiological and Epidemiological Insti-

tute of the Ukrainian Academy of Sciences, and under his direction, this antigen should be prepared by Bachman's method, which must be considered as better developed experimentally and as having a more scientific basis than all the other methods. Through direct communication with Bachman the author was fortunate enough to obtain from him dry trichinellae powder, which was used to compare the activities of his antigen and those of the one prepared locally. By the end of 1933 we were able to produce the antigen and were soon able to try it out in the clinic.

Method of Obtaining the Antigen and its Application: In order that the description of our work may be fully comprehended, the procedure of Bachman's method will be reviewed: The meat of the rat or guinea pig which has been infected with trichinelliasis four or five weeks previously is exposed for six hours in the thermostat at 37°C in a solution containing 0.1 per cent of pepsin and 0.3 per cent of hydrochloric acid. The trichinellae settle out after such treatment, and the lower layer of the liquid is then strained off, centrifuged, washed and again centrifuged. By this method pure, or almost pure, trichinellae are obtained.*

These trichinellae are dried at 37°C and then ground in an agate mortar. Each rat gives about 0.04 to 0.08 gms. of the dry trichinellae powder. To this 100 parts of Coca's solution No. 1 is added. After twenty-four hours of extraction the whole mixture is filtered through a Seitz filter and sterilely distributed in ampules, giving 0.1 to 0.2 cc in each ampule. Lately some series of the antigen have been prepared in such a way that the dry powder was placed for twenty-four hours in ether, and after that placed in Coca's solution No. 1. In addition, some series of the antigen have been prepared from the Coca liquid No. 2, as was done by McCoy and his co-workers. We are not yet in a position to say definitely which of these methods is best. The antigen, placed under sterile conditions in the ampules, was kept in the refrigerator and was used as the basic antigen solution. It was conditionally marked as 1:1000, though the real dilution of the antigen was greater than 1:1000, since the larger part of the powder is insoluble in the Coca's solution which was used for extraction, and remained in the filter. It is

* The first series of the antigen were prepared by Ribinsky with the closest participation of the author. The later series were usually prepared by L. M. Zrikina whom the author often assisted.

worth while noticing that the preparation of pollen extract, which is used for the diagnosis and treatment of hay-fever, is carried out by the same method as ours, and about 98 per cent of the initial quantity of pollen remains in the filter. We therefore consider it probable that a similar quantity of trichinellae powder remains in the filter, and that the conditional dilution of the antigen 1:1000 corresponds in reality to a dilution of 1:4000 or 1:5000. Before the antigen is injected, the conditional 1:100 dilution undergoes a further dilution, which will be described later. According to Bachman, his antigen gives typical protein reactions and represents a mixture of protein of which the organism of the trichinellae is composed. This mixture is soluble in Coca's liquid.

The antigen was always administered by us intradermally, usually into the dorsal side of the forearm in doses of 0.05 to 0.1 cc. in a conditional dilution of 1:1000 or 1:2000, i.e. in reality, in a dilution of 1: 50,000 or 1: 100,000. At the start we administered for the first injection antigen in a conditional dilution of 1:10,000 in order to avoid the general reaction of hypersensitivity. This step was recommended by Augustine and Teiler, and also by McCoy and his co-workers. Later, however, we usually began our injections with a conditional dilution of 1:2000 or even 1:1000, because we did not observe in our patients a single case of the general reaction of hypersensitivity. Our experience shows that in the conditions existing in the U.S.S.R. our population shows no general reaction to such a dilution.* As a control measure, Coca's solution No. 1 or No. 2, diluted with physiological saline solution in the same proportion as was the antigen, was injected at a distance of about 20 cm. from the place where the antigen was injected. In repeated tests the reaction was observed on the patient usually with an interval of not less than six days. In repeated administrations of antigen the injections were made alternately in different places on different days, i.e. one day closer to the elbow and the next day in the same forearm, but closer to the wrist in order to avoid local hypersensitivity. In typical positive reactions the following developments took place after three to five minutes: The wheal at the point of injection gradually increases during 2–5 min-

* We sometimes (in four cases) observed in convalescent out-patients a rise of body temperature to 38.5° on the first and second days after the application of the antigen, but we are not sure about the nature of this rise. It is possible that it is due, not to the action of the antigen, but to a peculiar course of the disease with relapses.

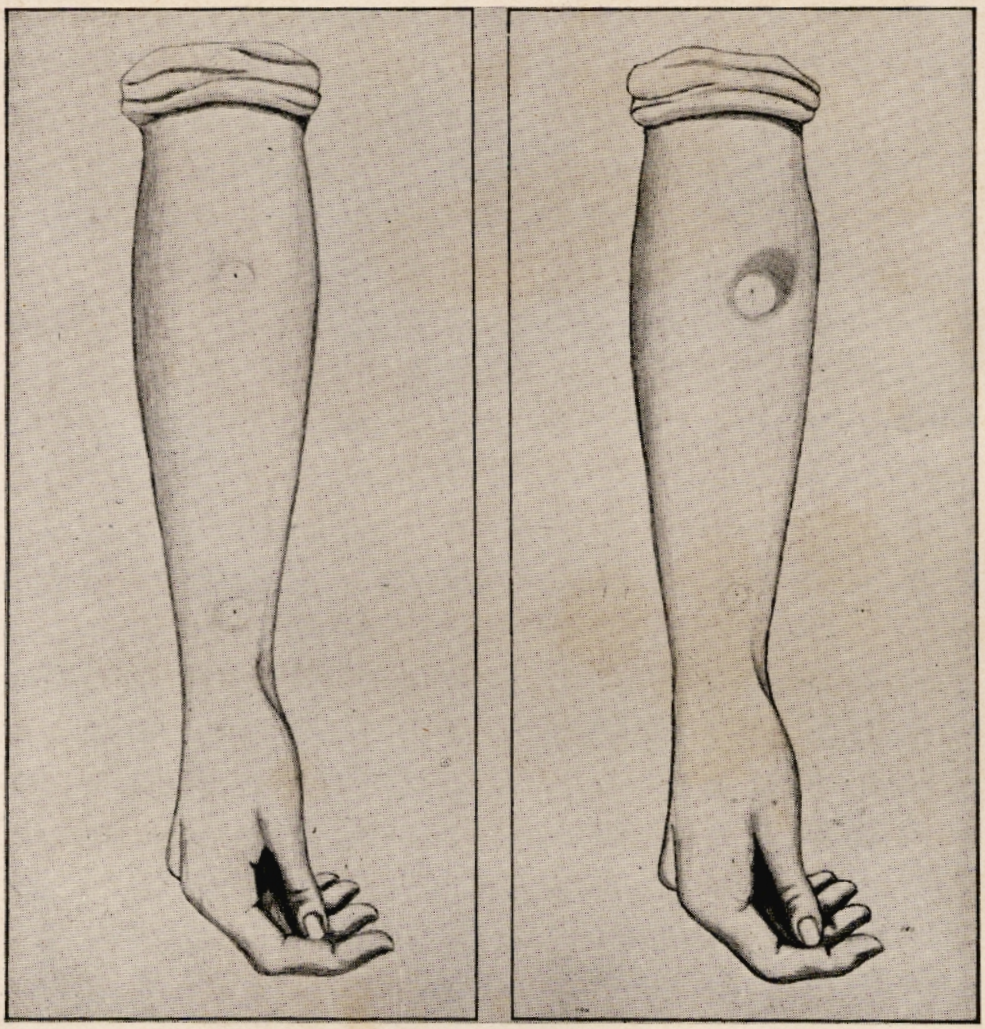
(Figure 1)

A. The forearm skin 30 seconds after injection.

B. The forearm skin 12 minutes after injection.

A. Piel del antebrazo a los 30 segundos después de la inoculación.

B. Piel del antebrazo 12 minutos después de la inoculación.



A B

Kaljus

size, or sometimes remains its initial size. In very rare cases the wheal at the place of the injection of the antigen almost subsides, but quite a large area of red remains around it. It must be remembered that very often the marked pink coloration of the skin is formed after one to two minutes following any intradermal injection as a response to the mechanical irritation of the skin. This irritation caused by the control injection subsides during one to two minutes, while that caused by the antigen begins to develop according to the type described above. Of course, not all patients react with equal intensity. We have evaluated the results of the reaction in such cases and have recorded them as follows:

Size of Wheal	Area of Erythema	Valuation
0.5—0.7	1.5 cm.	+
1 cm.	2 cm.	++
1 cm.	3.5 cm.	+++
1.5 cm.	4.5 cm.	++++
Higher	Higher	+++++

Evidence of the Specificity of the Trichinelliasis Antigen: In order to determine the reliability of this antigen for diagnosis by intradermal reaction, the antigen was tried out by the author 331 times on 66 trichinelliasis patients in the early stages of the disease, on 2 patients of three years standing, on 1 patient of eight months, on 1 patient after five months illness, and on 1 patient after one year following infestation. At the same time as a control measure the antigen was tried out on 109 non trichinellae-infested patients.

The results of these investigations were as follows: out of 66 trichinelliasis patients in the early stages of the disease, in 49 (74%) the reaction was positive, while in 17 cases (26%) the reaction was negative. Out of the total number of positive reactions the results were ++++ or over in 33 patients; +++ in 9 patients; ++ in 4 patients and + in 3 patients. In those who gave positive results the reaction was typical and clear-cut. Out of 5 trichinelliasis patients of long standing, 4 gave a positive reaction and one a negative. Out of the 4 positives, 2 gave + after five months and two years illness, respectively, and 2 patients gave ++++ after thirteen and eight months, respectively; the fifth case was negative after four years.

The skin of some trichinelliasis patients did not react to the administration of the antigen at all, although it was repeated eighty-four times on 17 cases with an interval of six days, while those 49 patients whose skin did react usually showed this reaction many times in succession at intervals of six days all through the course of the disease and also in the stages of convalescence. There were, however, cases in which the ability of the skin to react to the antigen was changed or lost when the general condition of the patient improved, but in many, the reaction persisted for a long time after the recovery. The details on the clinical types of the reactions are given in table II,* and also in table V.

The control injection, which was made 20 cm. from the place of the antigen administration, as already described, always gave negative results with the exception of two cases in which the skin reacted equally to the antigen and to the control. Such cases of non-specific hypersensitivity of the skin were considered as giving a negative response, as was a similar result obtained by the author three times in non-trichinelliasis patients. In rare cases (altogether we encountered only six such) it happened, especially in repeated reactions, that the reaction response of the skin changed, or from the beginning of the reaction, after five to fifteen minutes, the control also gave an erythema (which was absent after the first injection) around the wheal, but after ten to fifteen minutes the reaction at the point of the control subsided, while at the place where the antigen was injected the reaction ran a usual course.

Augustine and Teiler, who prepared an antigen similar to ours, obtained a positive intradermal reaction in the first test in 16 out of 18 trichinelliasis cases with an antigen dilution of 1:10,000. McCoy, Miller and Friedländer obtained in their 39 fresh cases of trichinelliasis 70 per cent positive reactions on the first test with an antigen of 1:10,000, and 92 per cent of positive reactions with an antigen of 1:500. These workers obtained on the first test a higher percentage

* In addition to and parallel with the work in the clinic, animal experimentation was also carried on by Ribinsky and Zrikina. The same antigen was tried out intradermally on animals which were preliminarily infected with trichinelliasis. The results of this work will be published in a separate paper. The results on these animals do not agree with those obtained by Bachman, Augustine and Teiler on their animals. These investigators usually obtained positive reactions of the delayed type, while Ribinsky and Zrikina had no positive reactions at all.

of positive reactions than the author, since in later observations, out of a total of 49 patients who gave positive results, only 31 showed these results after the first test (table II).

In 102 of our non-trichinelliasis patients the antigen reaction in a dilution of 1:1000 or 1:2000, and sometimes 1:500, was positive only in 5 patients, i.e. only in 5 per cent of all the cases. But even in these, the reaction was not very striking, and not entirely typical, since the wheal was not of the characteristic flesh color, but small and reddish, and the redness of the skin round it was classified once as + + +, once as + +, and in the rest of the cases as +. Out of this series the + was in a patient whose eosinophilia reached 19 per cent and was of unknown etiology. We must also mention the reaction in the non-trichinelliasis patient, D., who had chronic abscess of the lung. This patient received on March 7th, 1935, an injection into the forearm near the wrist of 0.1 cc. of the antigen in a dilution of 1:1000 and near the elbow as a control, 0.1 cc. of the extract from the semidigested and dried muscles of the rat. At the place of the control there was no reaction at all, while at the place of the antigen injection a redness and swelling accompanied by acute pain developed after several hours; the temperature of this patient was high before the injection of the antigen. On the next day the pain and swelling were still greater, a reddish infiltration formed at the place of the antigen injection and the patient was unable to move the swollen wrist joint on account of acute pain for which morphine had to be administered. The local phenomena progressed; the whole forearm and arm began to swell up, the axillary lymph-glands became painful and a painful infiltration of a dark red with a bluish tint developed at the place of the antigen injection; also the general condition of the patient at this time was serious. Only on March 12th, i. e. on the 6th day, these phenomena began to disappear and within two or three days gradually subsided. In so far as this patient was injected with a protein solution (an extract of the rat's muscle) as a control from which there was no reaction of any kind, the author considers that the symptoms at the place of the antigen injection should be explained, not as a non-specific allergic reaction, shown by an exceptionally sensitive patient, but as an infection acquired in spite of ordinary precaution. We

did not include this case with the positive reactions, since the control should have given an analogous reaction if it had been caused by foreign protein. Such a case might, therefore, present an important complication which might be encountered in practice.

About 95 per cent of our non-trichinellae patients gave a negative reaction which we consider sufficient evidence of the specific action of the trichinelliasis antigen. Our data in this respect disagree to a certain extent with those of Augustine and Teiler, and also with those of McCoy and his co-workers. Augustine and Teiler used a dilution of the antigen proportionately three or four times stronger than ours, i. e. one of 1:500. With this they obtained a positive reaction of 67 per cent in 140 non-infested subjects. The specificity of their antigen was only manifested when they reduced the strength of the antigen to 1:10,000.

McCoy, Miller and Friedländer, using a dilution of 1:500, obtained a positive reaction in 18 per cent of 104 non-infested cases. However, in this same group, 9 per cent of the subjects showed positive reaction when a dilution of 1:10,000 was used. In two other groups composed of 47 and 90 patients respectively, the antigen 1:10,000 gave positive reaction of 4 per cent to 4.5 per cent of all subjects, and this percentage more nearly approaches the findings of the author.

McCoy, Miller and Friedländer's findings about the carriers of *Trichuris trichiura* are of importance. These authors obtained positive reactions with an antigen of 1:10,000 dilution in 18 per cent of all the cases on 92 patients who were carriers of *Trichuris trichiura* while with an antigen dilution of 1:500 they obtained positive results in 62 per cent of the same patients. The infestation of *Trichuris trichiura,* according to these authors, quite often gives a false reaction to the trichinella antigen. However, Augustine and Teiler on the basis of their investigations on 140 non-trichinelliasis and 18 trichinelliasis patients came to the conclusion that infestation by other parasites does not affect in any way the percentage of positive reactions in trichinelliasis patients.

In table I are given the data on the worm carriers among those trichinelliasis and non-trichinelliasis patients (altogether 86 people) to whom the intradermal reaction was applied, and whose feces were simultaneously investigated

for the presence of ova *. The result shows that the influence of trichuris or other worms upon specific or non-specific reactions with the trichinelliasis antigen is doubtful. Out of the 49 trichinelliasis patients who reacted positively, 25 patients had their feces examined for the presence of ova. Out of 18 trichinelliasis patients who did not react to the antigen, 8 patients had their feces examined, and out of 102 non-trichinelliasis patients 53 people had their feces examined for the presence of ova. Thus, out of a group of 102 non-trichinelliasis patients, 53 were examined for worm carrying, and 22 of this number were carriers of *Trichuris trichiura.*

TABLE I

THE FREQUENCY OF SPECIFIC AND OF NON-SPECIFIC REACTIONS TO THE TRICHINELLA ANTIGEN IN WORM-CARRIERS AND IN NON-CARRIERS*

Group of Patients	Ova of Ascaris	Ova of Trichuris	Ova of Ascaris and of Trichuris	Non-carriers	Total
Trichinelliasis patients with a positive response to the antigen.	8	7	5	5	25
Trichinelliasis patients with a negative response to the antigen.	5	1	0	2	8
Non-trichinelliasis patients with negative response to the antigen.	8	16	6	23	53

* The figures indicate the number of patients.

We mentioned already that among the 102 non-trichinelliasis patients only 5 gave a faint positive reaction to the trichinelliasis antigen. Out of these 5, 2 were carriers of *Trichuris trichiura,* 2 did not have any ova in their feces, and the fifth was not examined. In the group of 49 of our trichinelliasis patients who reacted positively to the trichinellae antigen, out of the 25 who were examined for worm carrying, 12 proved to be carriers of trichuris.

Among the five allergic cases * four of whom gave a positive reaction to the trichinellae antigen, only one was a carrier of trichuris, one had the ova of ascaris, while the other two

* The analyses of the feces were done in the laboratory of our clinic by Dr. K. M. Hatzkevitz, to whom acknowledgment is hereby made by the author.

* These included those four cases of bronchial asthma and one of eczema which are mentioned later.

showed no ova in the feces at all. One must bear in mind that in our locality trichuris invasion is very frequent, and that the methods that are usually used for the examination of feces is inadequate. When this is considered in relation to the data given in table I, it is possible to state with assurance that the carrying of *Trichuris trichiura* does not influence, or influences very little, the frequency of positive reactions in trichinelliasis patients.

The correctness of this conclusion becomes clear when the data given above on the frequency of the positive reactions (74%) in 66 trichinelliasis patients and the frequency of the reactions (only 5%) in 102 non-trichinelliasis patients are compared. There is no doubt that the living conditions of the two groups of patients—66 with trichinelliasis and 102 without—permitted an equal risk of worm infestation. At the same time the results of the reaction with the trichinelliasis antigen in these patients (in the 2 groups) are such that the specificity of the trichinelliasis antigen in trichinelliasis is proved beyond any doubt, in spite of the possibility of false reactions occurring in rare cases. The phenomena of false reactions may be met with in other immuno-biological reactions too, but this does not discredit the specificity of the antigen.

It is interesting that in the two of our patients with echinococcus of the liver (both cases were confirmed on operation) the echinococcus antigen gave a clear-cut positive reaction of the immediate type, while the trichinelliasis antigen as well as a physiological solution of sodium chloride in the same patients and at the same time did not give any reaction whatsoever. These two cases of echinococcus confirm the specificity of the two antigens.

We also tried out simultaneously with the trichinelliasis antigen and the ordinary control the echinococcus antigen on 21 patients. The results showed (see Summary Record table V) that out of this group (21 people) 11 patients usually gave a positive reaction to the trichinelliasis antigen; 8 out of these 11 did not react to the echinococcus antigen while the other 3 did, but in a somewhat weaker manner than to the trichinelliasis antigen. The other 10 (out of 31) trichi-

nelliasis patients gave reaction. Thus, in spite of the fact that the echinococcus antigen sometimes gives a false reaction in trichinelliasis patients, we consider the specificity of both antigens confirmed.

In two cases of taeniasis we obtained a negative reaction with the trichinelliasis antigen, while McCoy and his coworkers obtained a positive reaction with the trichinelliasis antigen in two cases of taenia patients out of eight cases.

In general, Augustine and Teiler, and also McCoy, Miller and Friedländer, obtained non-specific reactions to the administration of the trichinelliasis antigen to non-trichinelliasis patients more often than did the author. This discrepancy may be due to the fact that in the U.S.A. trichinellae infestation is much more frequent than in our country (according to the latest data of certain American authors, trichinellae are found in 18 per cent of all autopsies). It must be pointed out, however, that Augustine and Teiler also found non-specific reactions to the trichinelliasis antigen in the inhabitants of Colombia, where trichinelliasis is practically nonexistent. The positive reaction, however, was obtained in that country with an antigen of high concentration (1:500). It is possible that the frequency of the non-specific reactions in some non-trichinelliasis patients depends largely upon the fact that the population of America, for some reason, is especially sensitive to allergic diseases. More than 1 per cent (Hoffman, Prausnitz and Küster) of the population of America is affected with hay-fever, and the percentage of other allergic affections is also very high (7% —Cooke); while in our country, hay-fever is practically never encountered and the allergic diseases are met with less frequently than in the States. In addition to the 102 non-trichinelliasis patients the reaction was also tried for in 5 allergic patients, out of which number 4 were asthma cases and one was affected with diabetes and eczema. The 3 asthmatic patients gave a clear-cut positive reaction, the fourth asthmatic patient gave a negative reaction, while the eczema patient gave a + + + reaction. Our data differ in this respect also from those of McCoy, Miller and Friedländer, who obtained a positive reaction to the antigen in a 1:10,000 dilution only in 4 out of 38 allergic patients. We applied the

antigen in dilutions of 1:1000 or 1:2000. Thus, according to our experiments, patients who are affected with allergic diseases are not suitable subjects for the test of the intradermal reaction with trichinelliasis antigen.

We noticed that if the material from which the isolated dried trichinellae were prepared contained noticeable impurities, such as undigested muscle tissue, its effect would be weaker than that of a purer type.* In Fülleborn and Luger's experiences the preparations of the muscle of heteregenous animals also did not give any positive reactions when applied as a control to trichinelliasis patients on the skin or intrademally.

From our experiments, observations and tests, we have obtained conclusive evidence that the trichinelliasis antigen prepared by Bachman's method, and administered intradermally, possesses a clearly expressed specificity and in the majority of patients causes a positive reaction.

Value of the Reaction for the Diagnosis of Trichinellae in Man: The general estimate of the results of the reaction and the types of the reaction on the repeated injections of the antigen in 66 trichinelliasis patients in the early stages of the disease are given in table II. (See also table V.)

TABLE II

A General Estimate of the Results of the Reaction	Trichinelliasis Patients, Number and Percentage	No. of Repeated Reactions	Notes
Negative Reaction	17(26%)	84	The reaction was always negative on repeated tests.
Positive Reaction	49(74%)	247 (The reaction was positive 170 times while 77 times it was negative)	The patients who reacted positively gave on repeated reactions a response according to one of four types.

* The four described types of reactions should be understood conditionally.

The types of the reactions were as follows:

First: In 23 patients the reaction was positive on the first and all subsequent tests. Altogether 113 reactions were seen in these patients.

Second: In 8 patients the first, and often the second and third, tests were positive, but after that they became negative. Altogether this group of patients gave twenty-four positive reactions and twenty-six negatives.

Third: In 10 patients the reaction was negative for one or more times, but after, there were one or more clear-cut positive responses. Such an occurrance never having been encountered in repeated injections into non-trichinelliasis patients, we definitely classified these 10 patients with those that gave a positive response. We consider that the conclusion may be drawn from these facts that during the course of the disease or during the period of convalescence the skin may change its degree of sensitivity. Altogether these patients gave 20 positive and 30 negative reactions.

Fourth: In 8 patients the reactions on repeated tests were sometimes positive and sometimes negative, but in each individual patient there was at least one definite positive reaction. Altogether these patients gave thirteen positive and twenty-one negative reactions.

The following conclusions may be drawn from this table: a negative result of the reaction does not indicate freedom from trichinelliasis. In our experience, 26 per cent of the trichinelliasis patients gave a negative response, and on this account we disagree with Friedländer who obtained, similarly to other American authors, a very great percentage of positive results (over 90%) and who assigns, therefore, a very great importance to negative reactions, considering them as evidence of absence of trichinelliasis. As to the value of the positive reaction for the diagnosis of trichinelliasis, it must be considered that a positive response to the antigen cannot confirm diagnosis, since there is the possibility of false reactions in non-trichinelliasis patients, though such cases do happen very rarely. But a persistent response (in non-allergic patients) to repeated tests of the reaction with the antigen in dilutions of 1:1000 to 1:2000 indicates a strong probability of trichinelliasis.

How early in the course of the disease the reaction began to give general positive results in our subjects, is seen from the following table:

TABLE III

No. of Cases	Number of days between onset of disease and First Positive Response	Number of days between invasion and First Positive Response
1	3	20
1	4	?
3	9	23, 32, ?
2	10	32, 33
5	11	33, 42, 32, 29
1	12	?
3	13	32, 25, ?
2	14	21, 26
1	15	31
1	16	31
2	17	28, 28
3	18	?, 40, 40
2	19	32, 36
1	22	55
1	23	37
1	25	40
1	27	43
2	29	?, 40
1	30	?
1	31	36
1	32	38
2	38	56, 53
1	43	?
1	46	?
1	252	264
1	120	?
1	380	?

The earliest reaction that gave a positive result in these cases was on the 3rd day of the symptoms or the 20th day after the infestation. It is impossible as yet to determine the exact day when the reaction begins to give a positive response after the infestation. It is possible that the sensitivity of the skin changes on various days of the disease, and in the first period of convalescence. All this limits to a certain extent the value of the reaction as an early diagnostic sign, but the auxiliary value of the reaction remains incontestable.

When the antigen prepared by us was compared with the one which we received from Bachman there was, in general, no difference. Whatever there was, could be explained most likely by the fact that there is as yet no method for the exact standardization of the antigen.

The interrelation between the reaction and the degree of eosinophilia is recorded in its different aspects in table

V. The eosinophilia may be noted before, after, or at the time of the intradermal reaction.

As an example of the value of the intradermal reaction for the correct diagnosis in some cases, and to show that there may be no parallelism between the eosinophilia and the intradermal reaction, we record here the abbreviated case history of the patient No. 42, Zh-M: (see also table V, fig. 1, and fig. 2).

He became ill on October 2nd, bed-ridden on October 5th, and entered our clinic on October 25th, 1934. Until the latter date he had been treated for rheumatism and other illnesses, and only for three days before his entering the clinic was trichinelliasis suspected. The first blood analysis was made on October 25th, and gave 7 per cent eosinophiles; then, on October 31st, 2 per cent; November 1st, 1 per cent; November 8th, 2 per cent; November 13th, 1 per cent; November 25th, 3 per cent; November 27th, 3 per cent; November 30th, 3 per cent; December 9th, 15 per cent, and on December 22nd, 7 per cent; *i. e.* the eosinophilia was within normal limits during the height of the disease. The muscles, on the other hand, showed a typical picture of a very serious case of trichinelliasis; there was contraction of the extremities, pain and swelling in the muscles, trismus and a false Kernig's sign. We see that the eosinophilia was within the normal limits and the diagnosis remained doubtful. The intradermal reaction, however, which was carried out during the period when the eosinophilia was normal, gave a striking positive reaction (++++). During this period the reaction was shown three times—on October 28th, Nov. 3rd, and on November 8th. On November 3rd a biopsy of the calf muscle of this patient was made and this showed two or three trichinellae in every field of vision. The microphotogram of this condition is given in fig. 2; the reaction of this patient is shown in fig. 1. We see that in this case the intradermal reaction facilitated the correct diagnosis before the biopsy was made.

Similarly in the case of the patient No. 43, Zh-B, the intradermal reaction was very striking and constant, while the eosinophilia was only moderately increased.

We encountered, however, a light case of trichinelliasis (No. 6) which came us with a normal temperature and with considerable eosinophilia; the intradermal reaction was repeated on this patient four times, and only once was the response slightly positive, while the eosinophilia showed very high figures on repeated analyses; the high eosinophilia together with the clinical picture (edema of the lids and face) was beyond any doubt in favor of trichinelliasis.

Since the intradermal reaction can not be substituted by the eosinophilia test or by any other clinical signs, it must be considered only as an important auxiliary method for diagnosis.

The following table shows the interrelation between the severity of the disease on the one side and the intradermal reaction on the other:

TABLE IV

	In Mild Cases	In Cases of Average Severity	In Severe Cases	In Fatal Cases
Positively responded	25	18	5	1
Negatively responded	8	9	0	0

This table shows that the severity of the disease does not have any particular influence upon the frequency of the ocurrence of the positive reaction, although in severe cases we always obtained a positive response.

McCoy, Miller and Friedländer obtained in some severe cases a negative response and in some which were mild, a positive one.

It is important to note that in one case (No. 12), which had a fatal outcome, the patient was sick for about a month and was always under our observation; the intradermal reaction was repeated on this patient three times with six-day intervals, and it was always clearly positive up to the last record, six days before his death. It is also interesting to note that on the seventeenth day of the disease this patient's eosinophilia reached 87 per cent; this is the highest percentage which the author ever encountered in his practice. On the day the patient died the eosinophilia dropped to 1 per cent.

The patient No. 42, Zh-M. (See table V), was a very severe case; this patient gave three times in succession a positive intradermal reaction during the most serious period of the disease; when he improved, the sensitivity of his skin to the antigen was lost, and the injections which were administered to this patient during convalescence eleven times with an interval of six days or more between, gave persistently negative results. These two last examples tend to show that a positive intradermal reaction can be of slight value for the prognosis, since several days before death Case No. 12,

* We consider a case as being mild if the patient is bed-ridden for four to eight days; as of average severity, for nine to fifteen days. All other cases were considered as severe.

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FIGURE 2

Biospy of Patient No. 42.

Preparación anatomopatológica de un tejido. Enfermo No. 42.

It is important to note that in one case (No. 12), which had a fatal outcome, the patient was sick for about a month and was under observation; the intradermal reaction was repeated on this patient three times with six-day intervals, and it was always clearly positive up to the last record, six days before his death. It is also interesting to note that on the seventeenth day of the disease this patient's eosinophilia reached 87 per cent; this is the highest percentage which the author ever encountered in his practice. On the day the patient died the eosinophilia dropped to 1 per cent.

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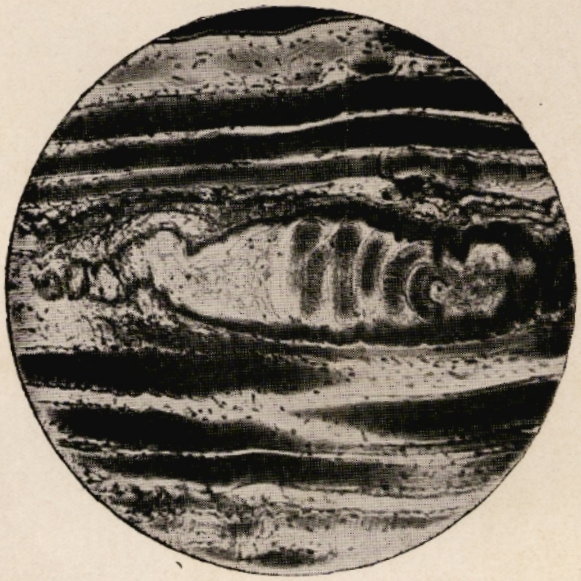

Kaljus

and for several days before his improvement Case No. 42, gave similar reactions (positive).

The course of the reaction in those trichinelliasis patients in which the disease runs with a rash on the skin is of special interest with respect to the interpretation of the pathogenesis of the reaction. Patient No. 12, M., had all through the course of the disease a petechial rash and a positive reaction. The patient No. 16, M. had in the beginning of the disease a skin rash of the urticarial type and the reaction was also clearly positive, although at the time when this patient came under our observation the rash had subsided. In addition to these two patients, three more trichinelliasis cases came to us in May, 1934—a man and wife, Nos. 21 and 22, and No. 23, M., who became ill at the same time and from the same source; in these 3 patients the illness also started with an urticarial rash, which had disappeared by the time they consulted us, although a slight itching of the skin remained. Out of these 3 patients No. 22 many times gave a marked positive reaction to the antigen in dilutions of 1:10,000 and 1:5000; No. 23 gave a positive reaction to the antigen in dilutions of 1:4000 and 1:2000, while No. 21 gave no positive reaction to the antigen even in a dilution of 1:500. Thus, trichinelliasis accompanied by a rash may or may not give a positive reaction.

RÉSUMÉ

1. The trichinelliasis antigen, obtained by the author by Bachman's method, gave a positive intradermal reaction in 74 per cent of people affected with trichinelliasis (in 49 out of 66 cases).

2. The same antigen administered to 102 non-trichinelliasis patients at the author's clinic gave only in 5 cases (about 5%) a positive reaction, 95 per cent of the cases being negative.

3. Two patients with echinococcus of the liver gave a marked positive to the echinococcus antigen and simultaneously a negative reaction to the trichinelliasis antigen.

4. On the basis of our data there is no reason to consider that the worm invasion of *Trichuris trichiura*, or any of the infestations which we usually encounter, influence the frequency of occurrence of the positive results in trichinelliasis patients.

5. A conditional dilution of the antigen of 1:1000 or 1:2000 gives a specific reaction on the intradermal administration to trichinelliasis patients in a considerable majority of the cases.

6. The typical intradermal reaction with the trichinelliasis antigen does not always appear in the early stages of the diseases.

7. An increased eosinophilia is usually observed more often and earlier than the intradermal reaction, but there are cases in which the increased eosinophilia of the blood in acute cases of trichinelliasis is mildly manifested or even not manifested at all, while at the same time the intradermal reaction is strikingly positive.

8. The intradermal reaction cannot serve for prognostic purpose, since it may be either positive or negative shortly before death or convalescence.

9. A negative reaction was obtained by the author in 26 per cent of all the cases (in 17 out of 66 trichinelliasis patients). Therefore, a negative reaction does not signify absence of trichinelliasis.

10. A positive intradermal reaction cannot have an absolute value for the correct diagnosis of trichinelliasis, since it may, although in rare cases, be positive in non-trichinelliasis patients too.

11. In allergic patients suspected of trichinelliasis, a positive reaction has no diagnostic value, since in such patients a non-specific reaction with the antigen is very often encountered.

12. A positive intradermal reaction with trichinelliasis antigen may serve with other clinical signs as an auxiliary method for the correct diagnosis of trichinelliasis in approximately 74 per cent of all the cases.

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TABLE V

EXTRACT OF THE SUMMARY RECORD TABLE

Patient	Date of infestation	Time of onset of disease	Eosinophilia		Reaction and its results			Characteristic of the case and notes	To this type no reaction
			Date	Percentage	Date	Dilution	Result		
					1934				
43. Zh-ky W. (brother No. 42)	About 9|26|34	10|9|34	10|18|34	14	10|28	1:1000	++++	Mild case. Ascaris ova in feces.....	23 cases (1st type)
			11| 9|34	10					
					11|10	1:1000	++++		
					11|17	1:1000	++++		
					11|22	1:1000	+++++		
					11|27	1:3000	+++++		
					12|4	1:1000	+++++		
					12|10	1:1500	++++		
					12|10	echinoc. ant.	Neg.....		
					12|16	1:1000	++++		
					12|16	echinoc. ant.	Neg.....		
					12|22	1:1000	++++		
					12|28	1:1000	++++		
				(1935)	2|10	1:1000	++		
42. Zh-ky M.	About 9|26|34	10|2|34	10|18|34	7	10|28	1:1000	++++	Very severe case. Patient bedridden for 3 weeks, sick for several months; diagnosis rheumatism. Eosinophilia normal while in clinic. First 3 intradermal reactions positive. On Nov. 3 biopsy revealed trichinellae in muscles. Improvement began Nov. 12. Reaction negative. Ascaris and rarely trichuris ova found in feces.	8 cases (2nd type)
			10|28|34	2	11|4	1:1000	++++		
			10|31|34	1	11|8	1:1000	++++		
			11| 8|34	1					
			11|25|34	3	11|17	.....	Neg.....		
			11|27|34	3	11|22	.....	Neg.....		
			11|30|34	3	11|28	.....	Neg.....		
			12| 9|34	15	12	.....	Neg.....		
			12|22|34	7	12|10	.....	Neg.....		
					12|16	.....	Neg.....		
					12|22	.....	Neg.....		
					12|28	.....	Neg.....		
				(1935)	2|10	.....	Neg.....		
					3|25	.....	Neg.....		
				(1934)	12|10	echinoc.....	Neg.....		
					12|16	echinoc.....	Neg.....		