

DYNAMICS AND PERSISTENCE OF ANTIBODIES IN TRICHINELLOSIS*

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Immunological tests which play a great role in the diagnosis of trichinellosis have been discussed in numerous publications. Fewer data are available on the dynamics of antibodies and less is known about their persistence and disappearance.

On account of space restrictions we are not able to quote numerous studies of other authors or to discuss extensively particular controversial problems. We wish, however, for better understanding of recent results, to give our opinion about immunological tests in trichinellosis, as derived from examination in our laboratory of thousands of sera from people and animals during a period of about 20 years.

Some years ago the allergic intracutaneous test was the subject of our special studies (Kozar et al., 1958 a). Though we still think it to be comparatively specific (with the use of proper antigen at the dilution of at least of 1:10,000), its practical value in the diagnosis of recent trichinellosis is of a lower order. A positive results occurs comparatively late after infection, the skin hypersensitivity persists for several years, occasionally throughout life, and the intensity of the reaction is not consistent with that of invasion or with the time which elapsed since the infection. On the other hand, the test is relatively easy and convenient for mass epidemiological investigations in various population groups. By means of the test we could detect a number of interesting details, confirmed later by other methods. Examining 2,472 inhabitants of strongly endemic territories we obtained as many as 38% of positive results (Kozar et al., 1958 b), whereas in another region of the country the percentage of positives was only 3.4 out of 1,734 examined cases (Kozar and Kurcio, 1964).

Among the classic serological tests, the complement fixation test (CFT) is still being used as a comparatively sensitive and specific test and because it lends itself

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to the standardization of techniques and evaluation of results (Kozar et al., 1952). Being reproducible, this test has been usually used as a standard in the evaluation of new methods.

We abandoned the tube precipitation ring test, which proved to be of relatively low sensitivity and fairly subjective in the evaluation of results. The same is true for the microprecipitation test with living larvae that we employed in 1946-1952 (Kozar, 1948), which in our opinion is more of theoretical than of practical value (Kozar, 1956). Incontestable results were obtained solely with highly immune sera.

We feel that the greatest practical value can be attributed to current agglutination tests (referred to by some as flocculation tests), particularly with bentonite (A. bent.), latex (A. lat.), cholesterol (A. chol.), and charcoal (A. charc.). The comparative evaluation of these tests was given elsewhere (Kozar, Kozar and Karmańska, 1964; Kozar and Kozar, 1966). We wish to emphasize that in spite of similar theoretical principles, the behaviour of the tests in examined sera is different. The A. bent. test proved to be the most valuable, as it yielded, under our experimental conditions, the greatest number of positive results; the reaction was the earliest one and reached the highest titre (up to 1:20,000). We have found this test to be of high sensitivity and specificity and to show also the most regular pattern.

Comparatively lower titres were obtained in A. lat., its sensitivity may be enhanced by the use of a proper antigen. There were, however, cases in which A. lat. gave a positive result at an earlier date or a higher titre than A. bent. The same can be said about A. chol. in which the antigen used is slightly different from the previous one. A. charc. (known as the charcoal card flocculation test, with the kit of reagents produced by commercial firm Hynson, Westcott and Dunning in USA) was, in our experiments, relatively specific and sensitive, having an additional advantage of being the easiest to perform, even in the most primitive conditions (Kozar and Kozar, 1966). Unfortunately it does not permit the determination of titre and thereby its value is largely qualitative. Our experience being comparatively short, we are not able to express our opinion about the value of the hemagglutination test, which some authors consider to be highly efficient.

Last year we introduced to our studies the indirect fluorescent antibody test (IFA) in our own modification, using the isolated larvae, fixed and counterstained in gentian violet, as antigen (Kozar, Karmańska and Kozar, 1966). In spite of very strict evaluation criteria (a positive result was assumed when the titre amounted at least to 1:500), the test was found to be highly sensitive, specific, reproducible and to yield at times very high titre (up to 1:128,000).

Materials and Methods

The investigations reported here were made on experimentally infected rabbits and with sera of people suffering from trichinellosis or suspected of infection. People who had trichinellosis (acute phase) at varying times in the past were also

included. The most representative groups, among hundreds of examined sera are described; the control groups discussed in other papers dealing with the evaluation of individual tests are omitted.

We have tried to examine each serum in parallel by various methods, on the same day, at the same dilution. We have been concerned with the accurate determination of titre, applying invariably the same evaluation criteria.

The following tests are considered: — CFT, A. bent., A. lat., A. chol., A. charc. and IFA. Methods for each test above are given in the publications mentioned in the introduction. The antigens used were of our own production (except for A. charc.), and they were whole larval extracts purified through heating at 56°C, centrifuged etc. We were not able to substantiate the statistically significant superiority of the described fractionated antigens (e.g., according to Melcher) over the full one. The values of our antigens varied in preliminary titrations from series to series, depending on modifications in the production technique, which were made during the several years in which the studies were made. It is enough to mention that recently we have used in CFT on the basis of titration an antigen diluted at 1:25,000 of the dry larval weight, whereas those previously employed were diluted at 1:500 to 1:3,000. This did not affect essentially the results obtained, at least in CFT and A. bent., as new antigens are first titrated and their excess removed. Some slight variations were found only in A. lat. In A. chol. other, invariably the same antigens have been used according to the original prescription.

As positive results in CFT, A. bent., A. lat. and A. chol. were assumed those showing a markedly positive reaction (at least ++), obtained the lowest serum dilution in CFT at 1:5 and in 3 subsequent tests at 1:2. In A. charc. we have examined undiluted sera and the evaluation was expressed in "plusses" according to the original suggestions. In IFA the positive evaluation of serum was started from a dilution of 1:500 and that only in the presence of a marked cuticular fluorescence in larvae. Control tests were performed invariably.

Results

The first group to be described comprised 12 rabbits infected with 8,000 larvae each, examined by various tests at short intervals initially and then at least once a month for 1,339 days, e.g., 3 years and 8 months. In spite of the same infecting dose and approximately identical experimental conditions, the rabbits showed various immunological patterns.

Fig. 1 illustrates the time of antibody appearance in individual tests. In one of the rabbits the reaction occurred early, i.e., at the 8th day of infection; in the remainder the antibodies appeared much later, mostly between the 20th and 31st day postinfection. In this case, the A. charc. proved to be a relatively sensitive test; equally satisfactory also were CFT results as compared with other tests; the IFA is not included in the comparison, as it was performed with preserved sera,

Test	Days after infection								
	8	10	12	14	17	20	25	31	38
C. F.T.	•				•	••••	••	••	
A. bent.	•				•	••	••••	••	
A. chol.	•				•		••••	••••	••
A. lat.				•		•	••••	••••	••
A. charc.			•	••••	••		••	•	

Fig. 1. The time of appearance of antibodies in various tests in 12 rabbits infected with *T. spiralis*

maintained in the refrigerator at -15°C for 2 years, and the positive reaction in this test appeared this time later than in the others.

The rate with which the titre increased in individual rabbits varied according to the test used. For economy of space the interesting diagrams for individual rabbits are omitted, the mean values being given for all animals (Fig. 2). The earliest date appearance of the highest titre at 45 days post infection was observed in CFT

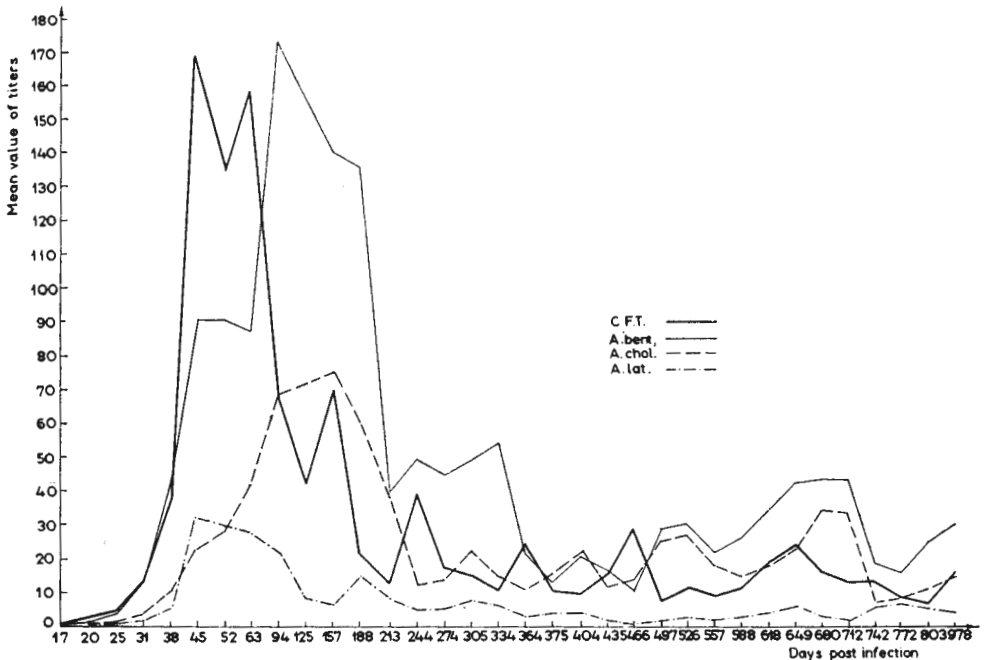


Fig. 2. The behaviour of the mean titres in various tests in 12 rabbits investigated through 2 years and 9 months

and *A. lat.*; somewhat later (at 94 days post infection) there was a peak titre by *A. bent.* and still later (at 157 days post infection) by *A. chol.*

The fall in the antibody level was not simultaneous in all tests and all animals; appreciable individual variations were seen. Certain increases in the titre were observed when green foodstuff was administered for instance, in spring and summer time.

TABLE I

Titres on sera from rabbits 3 years and 8 months (1339 days)
after infection with 8,000 larvae

Rabbit No.	CFT	<i>A. bent.</i>	<i>A. chol.</i>	<i>A. lat.</i>	<i>A. charc.</i>	I.F.A.
1	20	20	10	5	++	4000
2	40	40	10	2	++	1000
3	10	10	10	2	+	4000
4	40	40	10	5	++	2000
5	20	40	10	2	++	1000
6	5	2	2	0	+	1000
7	20	40	20	2	++	2000
8	80	80	40	10	++	8000
9	20	20	10	5	++	2000
10	20	40	10	5	++	4000
11	20	40	40	5	++	1000
12	40	20	10	5	++	2000

The figures designate the titers (highest serum dilution with positive result). According to the original instruction the evaluation in *A. charc.* is given in +, ++, +++ . 0 = negative result.

It is of interest that even in the last examination, i.e., 1339 days after infection (Table I) all animals except one (No. 6) by the *A. lat.*, showed a positive reaction. The antibody titre in IFA was quite high. This indicates a long duration of antibodies or a longstanding action of antigenic stimulators. Positive serological tests were also obtained in another rabbit infected 5 years ago. After its death most larvae in the muscles were dead, some of them showing complete calcification.

In people, such long-term studies are difficult for various reasons. This will be exemplified by human groups examined at varying post-infection periods.

The first group of 23 persons is derived from the epidemic in Cieplice (Feb. 1966), where the infection source was meat from uncontrolled home slaughter and the infection time of individual persons could be established. Unfortunately, the immunological studies were started too late and failed to detect the first appearance of antibodies in all tests and persons. The material under examination was divided into 2 subgroups: A—13 hospitalized persons with typical clinical symptoms and a rather slight or moderate course of the disease; B—10 persons with oligosymptomatic trichinellosis, or only suspected of infection (they ate infected meat in various forms and amounts) without clear-cut clinical manifestations. In the latter subgroup it

might have been assumed that in some persons the infection was only slight or even absent.

The time at which the first positive reaction appeared by individual tests in persons from subgroup A is given in Fig. 3. The earliest time of antibody appearance,

Test	Days after infection				Negative results
	19 - 24	32 - 36	52 - 54	85 - 87	
C.F.T.		•• ••	•• ••	••	••••
A. bent.	••••• •••••	•• ••			
A. chol.	•• ••	••••• •••••	•		•
A. lat.	••••	••••• •••••	••••		
A. charc.		••••• •••••	••••		••
J. F. A.	••••• •••••	••••	•	•	

Fig. 3. The appearance of antibodies in various tests in a subgroup A of 13 patients with clinical symptoms of trichinellosis

i.e., between the 19th and 24th day postinfection was detected in A. bent. and IFA. In other tests, positive reactions were mostly found only in the second examination (at 32-36 days of infection) or later, but in CFT (3 cases), A. charc. (2 cases) and A. chol. (1 case) results were invariably negative in five examinations (only 4 are given in the table).

Much poorer results were obtained in subgroup B (Fig. 4). A. bent. and IFA

Test	Days after infection				Negative results
	19 - 24	32 - 36	52 - 54	85 - 87	
C.F.T.					••••• •••••
A. bent.	••••	•	•		••••
A. chol.		••			••••• •••••
A. lat.	•	••	••		•••• ••••
A. charc.					••••• •••••
J. F. A.	••••		••••	••	••

Fig. 4. The appearance of antibodies in various tests in a subgroup B of 10 persons with oligo-symptomatic trichinellosis or only suspected of infection

proved once more to be the most sensitive tests as compared with CFT and A. charc., which were negative in all cases.

A fairly regular increase in the antibody titre in 11 patients of subgroup A is illustrated in Table 2. The frequency of negative results in CFT is striking as compared with those in rabbits, suggesting a temporary inhibition of reaction, in spite of high titre detected by other tests (cases 4, 6, 9, 11); This phenomenon which is encountered with increasing frequency in the diagnosis of trichinellosis, may be accounted for, on the one hand, by the lower sensitivity of the test in comparison with the others, particularly pronounced in cases with slight infection, and on the other hand, by the effect of drugs that are being used recently (probably of the corticosteroid group). Moreover, some sera showed in CFT the inhibition of reaction at low dilutions and positive results were obtained only at higher ones.

The highest titre was in 5 cases consistent in all tests simultaneously and coincided with the end of the second (cases Nos. 1, 3, 5) or even the third (cases No. 7 and 10) postinfection month. In the remaining patients there were some time differences in the appearance of the peak titre for individual tests, but they were not so characteristic as those observed in rabbits. It is to be stressed that as late as in the sixth examination, i.e., about 8.5 months after infection, all examined persons showed positive results, occasionally even with high titre. Negative results were obtained only 4 times in CFT, and twice in A. lat.

Less regular and often disappointing were the results in group B (6 examples are given in Table 3); if we assume that the persons had a light infection since only some of them showed a few symptoms, the sensitivity of the tests used can be given in decreasing order as follows: A. bent., IFA, A. lat., and A. chol.; CFT and A. charc. were of no help in the diagnosis of such cases, as they have invariably failed in all examined persons.

In other groups, the examined persons had contracted clinical trichinellosis (confirmed), mostly with a severe course, at varying times points in the past. The studies were designed to establish the duration of antibodies subsequent to infection.

The results of examinations in 19 persons who had trichinellosis in 1959 (epidemic in Bydgoszcz) i.e. 7 years previously, are shown in Table 4. Only in 3 persons were negative results obtained by all tests, in the remaining 16 positive results were yielded, mostly by A. bent. and to a lesser extent to A. chol., A. lat. and IFA. The CFT and A. charc. were invariably negative; the titres were low in the agglutination tests (1:10) and in the IFA (1:1000).

The results from a similar group of 21 persons who contracted the disease in 1957 (outbreak in Bydgoszcz), i.e., 9 years previously are presented in Table 5. Ten individuals showed negative results, and positive results were obtained in the remaining.

The results of more remote examination period after the regression of acute

TABLE 2

Titres detected in individual tests at varying post infection periods in 11 persons with trichinellosis (subgroup A — clinical cases)

Case No.	Test	Time of investigation (days p.i.)					
		19-24	32-36	52-54	85-87	132-135	252-254
1	CFT	0	80	320	160	20	0
	A. bent.	5	160	2560	1280	640	320
	A. chol.	2	160	2560	1280	320	320
	A. lat.	0	20	640	320	80	20
	A. charc.	0	+++	+++	+++	+++	+++
	IFA	4000	8000	8000	8000	4000	2000
2	CFT	0	10	10	20	2	0
	A. bent.	0	80	160	160	40	5
	A. chol.	0	40	40	40	20	10
	A. lat.	0	5	5	160	2	0
	A. charc.	0	+	++	++	++	+
	IFA	1000	8000	2000	2000	1000	500
3	CFT	0	40	320	160	40	20
	A. bent.	0	80	2560	1280	320	80
	A. chol.	0	160	1280	1280	160	20
	A. lat.	0	10	320	160	20	10
	A. charc.	0	++	+++	+++	+++	+++
	IFA	500	8000	128000	16000	4000	2000
4	CFT	0	0	160	160	80	20
	A. bent.	5	320	1280	1280	320	320
	A. chol.	0	80	640	640	640	160
	A. lat.	0	20	80	160	160	40
	A. charc.	0	+++	+++	+++	+++	+++
	IFA	0	4000	8000	8000	4000	2000
5	CFT	0	--	160	80	40	10
	A. bent.	40	--	640	640	320	40
	A. chol.	2	--	320	320	80	40
	A. lat.	2	--	320	80	40	5
	A. charc.	0	--	+++	+++	+++	++
	IFA	500	--	16000	8000	4000	2000
6	CFT	0	0	320	320	160	80
	A. bent.	1280	2560	2560	5120	2560	320
	A. chol.	2	40	640	640	640	160
	A. lat.	40	40	640	640	640	40
	A. charc.	0	++	+++	+++	+++	+++
	IFA	2000	8000	32000	16000	16000	4000

TABLE 2 (continued)

Case No.	Test	Time of investigation (days p.i.)					
		19-24	32-36	52-54	85-87	132-135	252-254
7	CFT	0	0	20	320	80	20
	A. bent.	5	40	160	10240	640	640
	A. chol.	0	0	160	1280	640	320
	A. lat.	0	0	160	640	320	40
	A. charc.	0	0	+++	+++	+++	+++
	IFA	500	500	2000	16000	4000	1000
8	CFT	0	10	160	320	80	80
	A. bent.	20	320	2560	10240	1280	640
	A. chol.	2	160	1280	1280	640	320
	A. lat.	5	10	1280	1280	160	40
	A. charc.	0	+++	+++	+++	+++	+++
	IFA	500	1000	16000	8000	16000	4000
9	CFT	0	0	0	320	5	2
	A. bent.	160	320	5120	20480	1280	1280
	A. chol.	0	160	2560	1280	1280	320
	A. lat.	0	20	640	1280	320	40
	A. charc.	0	+++	+++	+++	+++	+++
	IFA	1000	8000	32000	32000	2000	8000
10	CFT	0	0	0	80	10	0
	A. bent.	2	5	10	320	320	160
	A. chol.	0	2	5	160	160	80
	A. lat.	0	0	2	160	160	20
	A. charc.	0	+	+	+++	+++	+++
	IFA	0	0	500	16000	4000	2000
11	CFT	0	0	0	0	0	0
	A. bent.	0	80	80	160	20	5
	A. chol.	0	40	80	80	20	5
	A. lat.	0	5	20	20	10	0
	A. charc.	0	++	+++	+++	++	+
	IFA	0	2000	2000	2000	1000	500

Explanations as in the table 1.

- = serum not examined at the time.

trichinellosis are given in Table 6; 19 persons with the disease history from 1947, i.e., 19 years previously (outbreak in Racibórz) were examined. As compared with the preceding group, a greater number of positive results was obtained, and a titre of 1:20 was detected 3 times by A. bent. and twice by A. chol.; an IFA titre of 1:2000 was detected twice.

TABLE 3

Titers detected with various tests at varying intervals after the consumption of infected meat in some persons of subgroup B (oligosymptomatic or subclinical cases)

Case No.	Test	Time of investigation (days p.i.)				
		19-24	32-36	52-54	85-87	132-135
1	CFT	—	0	0	0	—
	A. bent.	—	20	10	10	—
	A. chol.	—	5	2	5	—
	A. lat.	—	0	0	2	—
	A. charc.	—	0	0	0	—
	IFA	—	0	500	0	—
2	CFT	0	0	0	0	0
	A. bent.	5	5	2	5	0
	A. chol.	0	0	0	0	0
	A. lat.	0	2	2	2	0
	A. charc.	0	0	0	0	0
	IFA	500	0	0	1000	(500±)
3	CFT	0	0	—	0	0
	A. bent.	5	5	—	5	0
	A. chol.	0	0	—	0	0
	A. lat.	10	5	—	2	0
	A. charc.	0	0	—	0	0
	IFA	0	0	—	0	(500±)
4	CFT	0	0	0	—	—
	A. bent.	2	2	5	—	—
	A. chol.	0	0	0	—	—
	A. lat.	0	0	10	—	—
	A. charc.	0	0	0	—	—
	IFA	0	0	0	—	—
5	CFT	0	0	0	—	—
	A. bent.	5	5	5	—	—
	A. chol.	0	0	0	—	—
	A. lat.	0	2	5	—	—
	A. charc.	0	0	0	—	—
	IFA	0	0	500	—	—
6	CFT	0	0	0	0	0
	A. bent.	5	5	5	10	0
	A. chol.	0	2	2	0	0
	A. lat.	0	0	0	0	0
	A. charc.	0	0	0	0	0
	IFA	500	0	500	0	0

TABLE 4
Serological studies performed in 19 persons 7 years after the regression of acute trichinellosis
(an outbreak in B., in 1959)

No. of cases	CFT	A. bent.	A. chol.	A. lat.	A. charc.	IFA	
16	5	—	+	+	+	—	+
	4	—	—	+	+	—	—
	1	—	+	+	—	—	+
	1	—	—	—	—	—	+
	5	—	+	—	—	—	—
	3	—	—	—	—	—	—
	19	0	16	10	9	0	7
+ positive result		— negative result					

TABLE 5
Serological studies performed in 21 persons 9 years after the regression of acute trichinellosis
(an outbreak in B. in 1957)

No. of cases	CFT	A. bent.	A. chol.	A. lat.	A. charc.	IFA	
11	1	—	+	+	+	—	+
	1	—	+	+	+	+	—
	6	—	+	—	—	—	—
	1	—	—	—	+	—	—
	2	—	—	—	—	—	—
	10	—	—	—	—	—	—
21	0	8	2	3	1	3	
+ positive result		— negative result					

TABLE 6
Serological studies performed in 19 persons 19 years after the regression of acute trichinellosis
(an outbreak in R. in 1947)

No. of cases	CFT	A. bent.	A. chol.	A. lat.	A. charc.	IFA	
15	2	+	+	+	+	+	+
	3	—	+	+	+	+	+
	2	—	+	+	—	+	+
	1	—	+	+	+	—	+
	1	—	+	+	+	—	—
	2	—	+	+	—	—	+
	2	—	+	+	—	—	—
	2	—	+	—	—	—	—
	4	—	—	—	—	—	—
19	2	15	13	7	7	10	
+ positive result		— negative result					

Discussion and Conclusions

The present studies imply that the individual serological tests are characterized by a varying degree of sensitivity. It may be assumed that they are capable of detecting certain antibodies but not necessarily the same in each test. This is possible as from the theoretical point of view, various antigens or their complexes are active in trichinellosis in various periods of infection. The most sensitive in the diagnosis of human trichinellosis proved to be A. bent. and IFA, less so were A. chol. and A. lat., and CFT and A. charc. were least sensitive. This is not consistent with the results in rabbits, in which the two latter tests were the first to give positive results. It seems that the main difference consisted in the intensity of infection. Though it has not been proved, it may be assumed that the rabbits in the experiments which were described were more heavily infected than the people under examination, and perhaps different results would have been obtained if the human infections had been more acute. The course of the disease in the patients listed in Table 2 was, as a rule, slight or moderate. The effect of therapy on the appearance of antibodies, especially in CFT, can not be ruled out. This fact stressed the necessity of performing concomitantly as many serological tests as possible, in order the results obtained might confirm or complement one another.

The rate of antibody appearance and the height of titres depend to some degree on the intensity of infection, but certainly more on the individual properties of the host, which could be demonstrated in rabbits infected with the same doses.

Both in people and animals the first antibodies were detected about the end of the 3rd postinfection week. They occurred only exceptionally at an earlier, but frequently at a later, date. The increase in antibody titre was usually gradual, reaching its peak at various postinfection periods, mostly by the 3rd month. In rabbits there were certain differences in the time at which the highest titres appeared in various agglutination tests and in the CFT, which favours a hypothesis that various antigen complexes and antibodies take part in these reactions. This could not be confirmed in people, perhaps because the material was not uniform with respect to the degree of infection.

It has been found that sensitive tests permit the detection of antibodies for a long time after infection. Most authors feel it is feasible only for a few months. In rabbits antibodies have been detected for 3 years and 8 month, and the observations are not yet completed. It is known that in these animals all the *T. spiralis* larvae do not survive in muscles such a long time, some of them dying and undergoing calcification. Apparently, even the parasites disintegrating in the host are able to stimulate antibody production.

Individual animals showed incises and falls in antibody levels which suggested dynamic changes in titre and active antibody production, in spite of the parasite's encystment. No other explanation of the persistence of antibodies seems more plausible. The living sited in the muscle larvae are known to take their food from

the host through the capsule, and it is conceivable that they also eliminate metabolic products which act as antigens. Additional stimuli may be involved which contribute to an exacerbation of the process and increase the antibody titre, as, for instance, the condition of the host, other diseases, etc.

It is known that larvae encysted in human muscle generally die later than those in animals. They have been found to persist for 40 years, though such lengthy periods are not indisputable. Distinct antibodies, though at low titres, were found in our experiments as late as 7, 9 and 19 years after infection. These findings refute current views in the duration of antibodies.

Before all, positive results were obtained only by sensitive tests which have not so far been used routinely. Some positive reactions in *A. charc.*, which proved to be less sensitive than others when applied in the early — postinfection phase are not fully understood. This test yielded positive results in as many as 7 out of 19 persons examined after 19 years. A clear-cut positive result was also found in the case of a physician who had had trichinellosis 20 years previously and who since that time presented complaints of the chronic phase.

The fact that the incidence of positive reactions was higher after the lapse of 19 than of 9 years since the infection is also puzzling. The persons included in the last 3 groups did not live in the endemic areas and intermittent reinfection seems hardly probable. This is particularly true of the last group examined after 19 years who came from areas where, apart from an outbreak in 1947, no epidemics were registered. Only a small group of persons was affected in 1961 after the consumption of boar meat. Trichinellosis of pigs in this territory was exceptional (no case was recorded in the last four years among 1, 191, 870 examined animals). It should be mentioned that the persons included in Table 6 had had severe trichinellosis 19 years earlier, and the number of such cases in the two previous groups (Tables 4 and 5) was comparatively lower. Our results in the last group may be due to chance selection of patients or to hitherto unidentified strain differences in the immunological properties of *T. spiralis* in infected persons as compared with those of the worms used for antigen production.

There remains still the problem of chronic trichinellosis with persisting complaints after the regression of the acute phase, which we regard as an allergic process. In many persons under examination the persistence of such complaints, mostly of pains in the muscles and cardiac region, was also noted. No relation could, however, be established between the presence of detected antibodies and the persistence of the disease, or between the severity of acute phase trichinellosis and the incidence of reaction a few or more years later.

The results of other authors are not discussed here since they have been considered elsewhere. In spite of great concern with immuno-diagnostic problems in trichinellosis, much remains to be explained. The most essential requirement is perhaps the isolation of the simplest, immunologically active antigens to be used in various tests. However, it is not certain whether the same antigens are equally active in

all persons and at different postinfection periods. Another problem is that of the persistence of various antibodies in the organism, or their production under the influence of new antigenic stimuli. A number of interesting general, immunologic aspects remain to be explored.

Summary

The time of antibody appearance in 6 different serological tests and the dynamic pattern of titre were observed for 3 years and 8 month in a group of 12 experimentally infected rabbits. A similar follow-up as much for more than 8 months in a group of 13 cases with clinical and in 10 cases with subclinical trichinellosis (suspected infection). The time at which the first positive reaction appeared depended on the intensity of infection, individual properties and the test used. This appeared mostly at the end of the 3rd postinfection week, sometimes earlier but more often later. The increase of antibody titre was usually gradual reaching its peak at various periods, mostly by the 3rd month. There were interesting differences in various tests in rabbits. Antibodies have been detected in rabbits for at least 3 years and 8 month, in a group of clinical cases for 8 and half months. To prove the persistence of antibodies in man, 3 groups of patients who had had trichinellosis 7 years (19 cases), 9 years (21 cases) and 19 years (19 cases) previously were examined by various tests. Antibodies were detected even in the last group, though at lower titre and only by more sensitive tests. The persistence or rather the production of antibodies during the prolonged postinfection period is discussed against the background of chronic trichinellosis.

On the basis of almost 20 years' experience and the present investigations, an evaluation of various immunological diagnostic tests in trichinellosis is given. The flocculation test with bentonite is referred to as the most simple, sensitive and specific one. The indirect fluorescent antibody test is also efficient, but more difficult to perform. The complement fixation test is less sensitive and has been found to fail many cases, probably under the inhibiting effect of drugs being used. As in the initial phase of trichinellosis positive results are not obtained simultaneously by all tests and are not predictable in individual cases, the concomitant use of several tests is recommended.

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LITERATURE

1. Kozar, M., Kozar, Z., Karmańska, K.: The comparative evaluation of some agglutination tests in the diagnosis of trichinellosis. — *Wiad. Parazytol.*, 10:717-737, 1964.
2. Kozar, Z.: Immunology of trichinellosis in the light of recent investigations. — *Med. Wet.*, 4:26-30, 1948.
3. Kozar, Z.: The immunology of trichinellosis. — *Postępy Hig. i Med. Doświad.*, 10:329-358, 1956.
4. Kozar, Z., Karmańska, K., Kozar, M.: Indirect fluorescent antibody test with isolated larvae of *Trichinella spiralis*. — *Wiad. Parazytol.*, 12:637-642, 1966.
5. Kozar, Z., Kołoto, B., Warda, L.: Untersuchungen über die Trichinellose mittels des Intradermaltestes. I. Bedeutung des Intradermaltestes bei frischen und älteren Infektionen. — *Zentralbl. Bakt.*, I Orig., 172:164-174, 1958 a.
6. Kozar, Z., Kołoto, B., Warda, L.: Untersuchungen über die Trichinellose mittels des Intradermaltestes. II. Epidemiologische Untersuchungen im Gebiete von Białystok. — *Zentralbl. Bakt.*, I Orig., 172:175-183, 1958 b.

7. Kozar, Z., Kozar, M.: Evaluation of charcoal card test as compared with other tests in the diagnosis of trichinellosis. — *Wiad. Parazytol.*, 12:629-635, 1966.
8. Kozar, Z., Kurcio, W.: Epidemiologic investigations on trichinellosis by means of allergic test in Miechów district, Cracov voivodeship. — *Wiad. Parazytol.*, 10:739-746, 1964.
9. Kozar, Z., Wysocka, F., Bławat, F.: Evaluation of diagnosis methods in symptomatic and latent trichinellosis in human beings on the basis of 97 observed cases. — *Biul. Inst. Med. Morsk.*, Gdańsk, 4:361-378, 1952.

DYNAMIKA I TRWAŁOŚĆ PRZECIWCIAŁ W WŁOŚNICY

Z. KOZAR, M. KOZAR

Za pomocą 6 odczynów serologicznych badano czas pojawiania się przeciwciał i dynamikę zmian w ciągu 3 lat i 8 miesięcy w grupie 12 królików zarażonych doświadczalnie. Podobne badania prowadzono przez więcej niż 8 miesięcy w grupie 13 przypadków włośnicy klinicznej i 10 przypadków z włośnicą podkliniczną (podejrzanych o zarażenie). Czas pojawiania się pierwszych odczynów dodatnich zależy od intensywności zarażenia, właściwości osobniczych i stosowanego odczynu. Przeciwciała stwierdzano na ogół pod koniec 3 tygodnia po zarażeniu, czasem wcześniej, częściej jednak później. Wzrost miana przeciwciał był zwykle stopniowy, osiągając szczyt w różnych okresach, przeważnie w 3 mies. po zarażeniu. Wystąpiły tu u królików interesujące różnice między poszczególnymi odczynami. Przeciwciała wykrywano u królików przez co najmniej 3 lata i 8 mies. (najdłuższy okres badania), w grupie zaś przypadków klinicznych przez co najmniej 8 i pół miesiąca.

Dla stwierdzenia trwałości przeciwciał u człowieka zbadano różnymi próbami 3 grupy osób, które przebyły włośnicę kliniczną przed 7 laty (19 osób), 9 laty (21 osób) i 19 laty (19 osób). Nawet w ostatniej grupie wykryto jeszcze przeciwciała u niektórych osób, choć z niskim mianem i tylko przy pomocy bardziej czułych prób. Dyskutuje się w świetle włośnicy przewlekłej trwałość lub raczej wytwarzanie przeciwciał w czasie tak długiego okresu po zarażeniu.

W oparciu o blisko 20-letnie doświadczenia autorów i obecne badania podano ogólną ocenę różnych odczynów immunologicznych we włośnicy. Odczyn aglutynacji z bentonitem jest spośród najprostszych najbardziej czuły i swoisty. Odczyn fluorescencyjny pośredni jest nie mniej dobry, ale trudniejszy do wykonania w rutynowej diagnostyce. Odczyn wiązania dopełniacza uważamy za mniej czuły od poprzednich i zawodny w niektórych przypadkach, szczególnie leczonych kortykosteroidami. Ponieważ w początkowej fazie włośnicy nie uzyskuje się wyników dodatnich równocześnie przy pomocy wszystkich prób i trudno przewidywać, która próba, w którym przypadku wypadnie wcześniej dodatnio, zaleca się stosowanie możliwie kilku prób jednocześnie.