

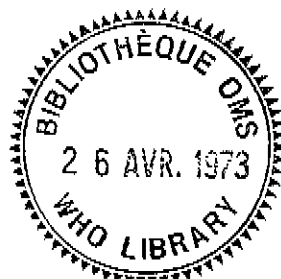


APPLICATION OF THE INDIRECT FLUORESCENT ANTIBODY TEST ON SECTIONS OF ADULT FILARIAE
TO THE SERODIAGNOSIS, EPIDEMIOLOGY AND POST-THERAPEUTIC SURVEILLANCE OF HUMAN FILARIASIS ^a

by

INDEXED

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1.4 Reading the tests

The tests are read with a fluorescence microscope of very low magnification (10 X objective). A bright-field condenser is used with a dry objective and a BG12 (6 mm) emission filter.

If the test is negative, the preparations are coloured a uniform red. On the other hand, if the test is positive, the adult filaria sections display an intense yellow-green fluorescence, localized in the subcuticular region and, to a lesser extent round the edge of the main cavity (Figure 1). The striated muscle surrounding the parasites is always coloured red by the Evans blue. It is therefore a simpler matter to check every section for correct counter-staining.

2. RESULTS AND DISCUSSION

2.1 Comparison of the antigenic properties of *Dirofilaria immitis* and *Dipetalonema viteae*

At the start of our study, we prepared antigen from adult specimens of *Dirofilaria immitis*, a filarial parasite of the dog with many antigenic properties in common with human filarial species. Unfortunately, *Dirofilaria immitis* is becoming increasingly rare, at least in France, and as a result we soon began to prepare our antigen from *Dipetalonema viteae*, which had already been used for that purpose by many authors, among them Duxbury & Sadun, as well as Biguet & Capron.

First of all, it was obviously essential to make sure that these two types of antigen gave the same results. This was confirmed by a study in which 440 specimens of human sera were tested with both *D. immitis* and *D. viteae* sections. In some cases, use was even made of sections from blocks in which filariae of the two species had been placed side by side.

The sera examined were as follows:

288 specimens taken either from healthy subjects or from persons with parasitic infections other than filariasis. All these sera gave negative results at a dilution of 1/20.

152 specimens taken from confirmed cases of filariasis. The results obtained are given in Table 1. At no time were any significant differences noted between the two filarial antigens (i.e. antibody titres differing by two or more steps of the dilution range).

128 (84.2%) of the 152 sera tested were even found to have exactly the same fluorescent antibody titres.

From the practical point of view, these results justified the use of *D. viteae* antigen during the rest of our serological study of human filariasis.

More generally, these observations confirmed that the various species of filaria have a wide range of antigenic properties in common, despite their sometimes quite marked biological differences and fairly remote zoological relationships.

2.2 Specificity

Two complementary methods were used to check the specificity of the test, as follows:

observation of the response to *D. viteae* antigen of sera from healthy subjects or patients with various parasitic or other diseases;

conversely, observation of the response to other parasite antigens of sera from confirmed cases of filariasis.

2.2.1 Specificity of the test against *D. viteae* antigen

Tests were performed on a total of 934 serum specimens, of which 680 were collected in France and 254 overseas (Table 2).

Each serum specimen was tested at dilutions of 1/20, 1/40 and 1/80.

98.2% of the sera collected in Europe gave a negative response to the test at a dilution of 1/20. Only eight sera were found to be positive at dilutions of 1/20 or 1/40, and all came either from subjects with a roundworm infection (*ascariasis*, *strongyloidiasis*) or from patients with parasitic diseases (*intestinal bilharziasis*, *malaria*, *amoebiasis*) which are endemic in areas partially overlapping those where filariasis is endemic. As regards such patients, the possibility of a history of filariasis could therefore not be completely excluded.

In the case of the sera collected overseas, 11.2% gave a positive response to the test at dilutions of 1/20, 1/40 or 1/80. None of the 254 subjects concerned seemed at all likely to be suffering from active filariasis in view of the absence of clinical signs and the negative results of repeated parasitological examinations. The possibility, however, cannot be completely excluded. Moreover, nearly all these patients harboured more than one type of parasite, among them several species of roundworm (*Ascaris*, *Ancylostoma*, *Strongyloides stercoralis*, and in seven cases at least, *Trichostrongylus*).

Finally, on concluding these checks, we adopted a dilution of 1/20 as the specificity threshold for the test. This preliminary dilution probably does not give complete specificity, but the 3.6% of false positives which it may apparently entail is unquestionably an upper limit, for such false positives are only given by subjects with a roundworm infection or by patients for whom a diagnosis of filariasis cannot be completely excluded.

At the present time, in any case, there seems but faint hope that complete specificity could be achieved in serological tests for nematode infections. Because of the ease with which it can be performed, the main advantage of the fluorescent antibody technique in this field is that a very large number of tests can be carried out and the dilution threshold thus determined as accurately as possible.

2.2.2 Specificity of the test with various parasite antigens

Sixty-seven serum specimens from confirmed cases of various types of filariasis were tested by the fluorescent antibody technique against antigen from *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Schistosoma mansoni*, *Fasciola hepatica*, *Echinococcus granulosus* and *Toxoplasma gondii* (Table 3).

Such antigen consisted either of a suspension of the parasite smeared on a slide (*Toxoplasma gondii*) or of frozen sections of the adult worm (*Ascaris lumbricoides*, *Schistosoma mansoni* and *Fasciola hepatica*) or the larva (*Strongyloides stercoralis*, *Echinococcus granulosus*).

At dilutions of 1/20 or 1/40, only the first three antigens gave positive results.

These positives could have occurred for either of the following reasons:

a true cross-reaction between roundworms (i.e. filarial antibody and *Ascaris lumbricoides* or *Strongyloides stercoralis* antigen);

a specific reaction indicating the presence of ascariasis, strongyloidiasis or a former bilharziasis in the seven filariasis patients concerned.

In conclusion, these specificity studies did not seem to indicate any need for revision of the specificity threshold for the test (1/20).

2.3 Usefulness of the test in the serodiagnosis of human filariasis

To evaluate the diagnostic potentialities of the indirect fluorescent antibody test applied to adult filaria sections, we studied 989 serum specimens collected both from patients suffering from parasitologically confirmed filariasis (647 cases) and from subjects with strong epidemiological, clinical or biological evidence of wuchereriasis, loaïasis or onchocerciasis.

In interpreting the results of this study account was taken of the ethnic origin of the patients examined, a factor liable to have a considerable effect on the intensity of filarial infestation and the probability of polyparasitism. A distinction was also made between the sera collected in France and those sent to us from overseas.

For each of these categories, the percentage of positive tests was calculated as well as the mean fluorescent antibody titre (GMRT) determined.

2.3.1 Results given by the indirect fluorescent antibody test with 647 serum samples from parasitologically confirmed cases of filariasis

In the case of 632 of these patients, filariasis had been confirmed and its type determined by the detection of microfilariae in the blood (wuchereriasis, loaïasis, infection with *Dipetalonema perstans*) or in the skin fluid (onchocerciasis). In 15 cases diagnosis was confirmed by the discovery of adult filariae in the subcutaneous cellular tissue (the six dracunculiasis cases) or under the ocular conjunctiva (9 of the 180 loaïasis).

The results of the fluorescent antibody tests are given in Table 4.

In all cases where there were enough results to be significant, 84 to 95% of the fluorescent antibody tests were positive. These figures are at least comparable, if not superior, to any that we have so far been able to obtain with other serological tests performed under the most favourable conditions.

It is, moreover, remarkable how similar the responses of the various types of filariasis to serological tests are in spite of their differences from the clinical and parasitological point of view. Each type of filariasis gives largely the same percentage of positive tests and comparable mean fluorescent antibody titres.

Lastly, if account is taken of the ethnic origin of the subjects examined, the results given by Europeans who have become infected overseas are less often positive and, in particular, less intensely positive, in the fluorescent antibody test than indigenous inhabitants of regions where filariasis is endemic. These results unquestionably reflect a difference in the intensity of parasitic infection and also, perhaps, in the frequency of reinfestation.

On the other hand, in the case of patients indigenous to regions where filariasis is endemic, no significant differences were observed between sera collected in Europe and those sent us from overseas.

2.3.2 Comparison of the results of the indirect fluorescent antibody test with data provided by parasitological examination

Serological diagnosis is obviously only of interest when the search for microfilariae has been negative. To assess the usefulness of the fluorescent antibody test, we studied 342 sera from subjects who, although parasitological proof was lacking, showed strong epidemiological, clinical or biological evidence of filarial infection by Wuchereria bancrofti, Loa loa or Onchocerca volvulus. The results in each case were compared with those obtained earlier with parasitologically confirmed cases of the type of filariasis concerned (Tables 5, 6 and 7).

With each type of filariasis studied, the intensity of the response to the indirect fluorescent antibody test appears to be inversely proportional to the number of microfilariae present, for the percentage of positive tests and, more particularly, the mean fluorescent antibody titres were much higher for cases where no microfilariae could be found than for patients with parasitologically confirmed filariasis.

This result is completely in line with the findings of Capron, Gentilini and Vernes in their application of immunoelectrophoresis to the study of sera from patients or from experimental animals.⁸ To conclude from this that the circulating antibody has a specific microfilaricidal action would clearly be premature. However, in most if not all cases, such antibody does appear to be directed against the larval stages of the filariae.

From the practical point of view, these facts give clear proof of the usefulness of serodiagnosis since the probability of a positive result increases the less chance there is of finding microfilariae. In all three types of filariasis considered, diagnosis followed from parasitological examination in only 58% of cases, while the indirect fluorescent antibody test was positive in nearly 90% of cases (Table 8).

2.3.3 Results obtained with specific antigen

For the total cases studied, the indirect fluorescent antibody test provided a serological method for the diagnosis of filariasis with a probability of error in the region of 10% of false negatives and 3.6% or less of false positives (cross-reactions).

However, this test merely indicates the presence of a filarial infection without specifying the type concerned. Non-pathogenic parasites, such as Dipetalonema perstans, give almost as high a proportion of positive responses to the test as pathogenic filariae (Table 4). This is all the more unfortunate in that the treatment of filariasis with diethylcarbamazine has a number of side effects. Such treatment is therefore only indicated after comparison of the results of the serological test with the clinical and epidemiological data.

In some ways immunoelectrophoresis ensures greater diagnostic accuracy, for even if a group antigen such as Dipetalonema viteae is used the position of the major precipitin arc will vary with the type of filariasis concerned (Capron et al., 1968).⁸

An effort was made to improve the diagnostic potential of the fluorescent antibody test by using specific filarial antigen. Our experience in this context is still very limited and comprises only the study of 97 sera, which were tested both against the corresponding antigen and against D. viteae antigen (Table 9). Twenty-nine of these sera were tested against the following four antigens: D. viteae, W. bancrofti, L. loa and O. volvulus (Table 10). The comparative study of each serum or group of sera was carried out in a single series of tests to give greater accuracy in ascertaining variations, however slight, in the fluorescent antibody titre.

The use of specific antigen improves the results of the technique (percentage of positive reactions and mean fluorescent antibody titre). To a certain extent, precise diagnosis of a specific type of filariasis can therefore be reached by comparing the results obtained with the four antigens mentioned. In the case of loiasis, for example, the response is more strongly positive when the fluorescent antibody test is carried out with Loa loa antigen than with D. viteae, W. bancrofti or O. volvulus antigen. However, such behaviour is not invariable and it often happens that the four sets of results are either identical or very close to one another. Possible reasons for this are:

- mixed infection;
- infection with non-pathogenic filariae;
- a true group reaction, indicating the presence of antinematelminthic antibody; this last is the most probable cause when a serum gives a weakly positive response to all the filarial antigens.

2.3.4 Application of the indirect fluorescent antibody test to the epidemiology of filariasis

Serological methods can be extremely useful for evaluating the endemic level of the various types of human filariasis (onchocerciasis in particular), in so far as field work can be limited to the collection of serum specimens and the tests themselves carried out later.

In a preliminary study, we examined 258 sera collected in Africa from onchocerciasis cases in three population groups living in hypo- meso- and hyperendemic areas, respectively.^a All these sera were tested against D. viteae antigen (Table 11). There seems to be good correspondence between the results given by the fluorescent antibody test and the endemic level of onchocerciasis. This is clearly shown by the percentage of positive tests: 89.6% for villages with a hyperendemic level and 78% and 66% respectively, for villages with meso- and hypoendemic levels. It is the quantitative results, however, that bring out the serological differences between the three areas most sharply, for the mean fluorescent antibody titres are 52.7, 25.9 and 10.3 respectively.

These data indicate that the technique would make a very useful epidemiological survey tool and we hope shortly to be able to confirm this on a more extensive scale. It should be noted, moreover, that in comparison with other methods the fluorescent antibody technique has the great practical advantage of requiring only micro-samples of blood collected by finger puncture.³ This considerably facilitates the collection of blood samples, their storage and their subsequent dispatch to the laboratory where the serological tests will be carried out.

2.4 Value of the fluorescent antibody test in the surveillance of filariasis after treatment

We have carried out serological tests on a large number of patients treated for various filarial infections. However, we shall consider here only the 27 cases that were adequately documented and for which at least three fluorescent antibody tests were performed, one of them before the start of treatment.

All these patients had filariasis of the Loa loa type and were treated with diethyl-carbamazine (Notazine*) administered in very gradually increasing doses as follows: 1/4 of a tablet a day for two days, followed by 1/2 a tablet a day for the next two days, after which the dose was increased by half a tablet a day each day for the next 10 days. The course of treatment lasted 26 days in all. Twenty-three patients followed a single course of treatment (Table 12, Figs. 2 and 3) but four had to repeat the course (Case Nos. 29, 32, 43 and 77; Figs. 4 and 5).

^a These sera were kindly provided by Professor André Capron, Lille.

In order to decrease the intensity of allergic response during treatment with diethylcarbamazine, most patients were given antihistamines (buclizine hydrochloride (Aphilan R*), three tablets a day for the first eight days; promethazine syrup (Phenergan*), one teaspoon at night before retiring or corticoids, 15 to 20 mg a day of prednisone (Cortancyl*)).

The overall test results show that treatment was followed by a distinct increase in fluorescent antibody titre. Not until between the fortieth and seventieth days did a gradual decline in titre take place, while the test became negative only after a fairly long period of time had elapsed (Fig. 2). We have observed a similar peak in antibody titre following treatment of other types of helminthiasis (bilharziasis in particular).⁴

In filariasis, however, in contrast to other verminoses, the pattern of variation in antibody titre after treatment is much more variable, and the following types of behaviour have been observed:

an immediate fall in antibody titre following treatment (Case No. 3, Fig. 3);
constant values of antibody titre followed by a very gradual decline (Case No. 23, Fig. 3);
finally, a fairly distinct antibody titre peak (Case No. 30, Fig. 3).

A number of explanations can be advanced for these differences:

(a) The increase in antibody titre observed in helminthiasis cases after treatment is probably connected with the destruction of the worms and the release of intraparasitic antigen into the circulation. As helminths have a large volume in proportion to their surface area, there will be a greater quantity of intraparasitic antigen than of surface antigen and its appearance will boost the production of antibody. Now, in the case of filariasis, diethylcarbamazine attacks mainly the microfilariae and has only an indirect effect on the adult worms.

Microfilariae are very small and their volume to surface ratio is much lower than in the case of parasites such as Schistosoma mansoni. It would therefore be logical to expect the destruction of the microfilariae and the release of their somatic antigen to cause only minor changes in antibody titre. In point of fact, this hypothesis is contradicted to some extent by the allergic response provoked by the treatment of filariasis and corresponding to the destruction of the microfilariae (Mazotti test). Two other possible explanations must therefore be considered.

(b) For the express purpose of preventing allergic disorders due to lysis of microfilariae, the dosage in diethylcarbamazine treatment is increased very slowly. This ensures a gradual release of microfilarial somatic antigen, which naturally partially masks the resultant serological phenomena. Conversely, if the antiparasite treatment is more brutal, there is a well marked peak in antibody titre. We observed this phenomenon a few years ago in the case of four patients who were straightaway given a daily dose of four diethylcarbamazine tablets.

(c) Lastly, and again in order to reduce the intensity of the allergic response, diethylcarbamazine treatment is generally associated with the administration of antihistamines and especially corticoids which are able to modify the intensity of the immunological phenomena following treatment.

These are possible explanations for the fact that the rise in fluorescent antibody titre following treatment is less constant and less marked in the case of filariasis than in the other helminthiasis.

From the practical point of view, this is extremely unfortunate since in cases where a verminosis is strongly suspected on clinical and epidemiological grounds, but where parasitological evidence is lacking and the serological test weakly positive, the diagnosis is frequently confirmed by the appearance of a peak in the antibody titres after treatment. We regularly apply this post-therapeutic test to bilharziasis. However, this method of confirming diagnosis a posteriori appears to be unreliable in the case of filariasis.

Finally, the time taken for the test results to become negative varies within wide limits and may exceed eight months. It is not therefore possible, at the present stage of our knowledge, to assess the precise reliability of the indirect fluorescent antibody test for determining recovery from filariasis.

3. CONCLUSION

The indirect fluorescent antibody test carried out on frozen sections of Dipetalonema viteae adults seems to be of considerable value in the diagnosis of human filariasis, for it gives positive results in nearly 90% of cases. However, the use of a group antigen permits only a general diagnosis of filariasis and cannot determine whether pathogenic or non-pathogenic filariae are involved. Such additional information can in many cases be obtained by the use of specific antigen, which gives higher fluorescent antibody titres than those given by other filarial antigens. Such, at least, is the conclusion of a preliminary study carried out with D. viteae, W. bancrofti, L. loa and O. volvulus antigens. The main advantage of the technique is the ease with which such antigens can be used. Since the test is carried out on frozen sections 5 μ thick, a very few adult parasites will provide sufficient antigen for several thousand tests.

The use of the indirect fluorescent antibody test in epidemiological surveys has been considered. From the results given by 250 serum specimens collected in West African villages with hyper-, meso- and hypo-endemic levels of onchocerciasis, it was clear that the technique gave an accurate picture of the situation as regards filariasis infection since the preparation of antigen requires very little parasite material and as the test can be carried out on a micro-sample of blood obtained by pricking a finger, the indirect fluorescent antibody test is ideally suited to the large-scale survey, currently in progress.

Finally, diethylcarbamazine treatment of filariasis appears to cause temporary, and sometimes large, increases in fluorescent antibody titre. A similar effect is noted in the case of other helminthic infections. In filariasis, however, this increase does not occur invariably and the presence of a peak in the antibody titre cannot be used as a measure of the efficacy of a drug or as a a posteriori confirmation of diagnosis.

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SUMMARY

The indirect fluorescent antibody test using frozen sections of adult filariae has great practical advantages. Since the sections are only 5 μ thick, a very small amount of parasite material will suffice for the preparation of antigen. A single adult filaria thus provides enough antigen for several thousand tests. The test can also be carried out with specific antigen prepared from filariae pathogenic to man.

This test has been in regular use since its development in 1968, and the results obtained with 2611 human serum specimens are given. Initially, the group antigens were prepared from Dirofilaria immitis but, after comparison of the characteristics of this antigen with those of Dipetalonema viteae, the latter was used as group antigen for the rest of the study.

The specificity of the test was checked by testing this antigen against 934 specimens of sera from healthy people or from patients with various parasitic and other diseases. At the same time, 67 specimens of sera from filariasis patients were tested against antigen from other parasites. These checks showed that the limiting specificity of the test, when this was carried out under carefully defined conditions, corresponded to a dilution of 1/20.

The diagnostic value of the test was assessed in a study of 647 serum specimens from patients with parasitologically confirmed filariasis and 342 serum specimens from patients strongly suspected to be suffering from wuchereriosis, loiasis or onchocerciasis. The test proved to be positive in 84 to 95% of cases of the various types of filariasis. However, the results obtained with the group antigen gave no information as to whether or not the patient was harbouring a really pathogenic filaria. In order to clarify this point, 97 serum specimens from cases of wuchereriosis, loiasis or onchocerciasis were tested against both the corresponding specific antigen and D. viteae antigen. In many instances, significantly higher fluorescent antibody titres were obtained with the specific antigen than with the other antigens. The use of specific antigen can thus considerably enhance the value of the test as a serodiagnostic tool and, in particular, give better grounds for deciding on treatment.

Two hundred and fifty-eight serum specimens collected in West Africa from regions with different endemic levels of onchocerciasis were studied by the indirect fluorescent antibody technique. The test results appear to give an accurate picture of the epidemiological situation and indicate that the test could provide much useful information of an epidemiological nature. The test is particularly well suited to such work since it is simple to apply on a large scale and can be performed on micro-samples of blood, whose collection in the field entails far less difficulties than does venous puncture.

Finally, the antibody titres of 27 patients with loiasis were kept under observation before and after a course of treatment with diethylcarbamazine. Four of these patients underwent two courses of treatment. Treatment was generally followed by a temporary rise in the fluorescent antibody titre, but this increase did not appear as invariably as it did in the case of other forms of tissue helminthiasis. This phenomenon is no doubt due to the manner in which diethylcarbamazine is administered (i.e. very gradually increasing doses) and to the simultaneous administration of antihistamines or corticoids for the purpose of reducing the allergic response frequently associated with the treatment of filariasis.

TABLE 1. SERODIAGNOSIS OF FILARIASIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST.
 COMPARISON OF DIROFILARIA IMMITIS AND DIPETALONEMA VITEAE ANTIGENS
 IN A STUDY OF SERA FROM 152 FILARIASIS CASES

Dipetalomea viteae antigen

Dirofilaria
immitis
antigen

	<1/20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	TOTAL
<1/20	14	2	0	0	0	0	0	0	16
1/20	3	28	2	0	0	0	0	0	33
1/40	0	4	19	3	0	0	0	0	26
1/80	0	0	1	34	0	0	0		35
1/160	0	0	0	1	14	4	0		19
1/320	0	0	0	0	1	16	0		17
1/640	0	0	0	0	2	1	2		5
1/1280	0	0	0	0	0	0	0	1	1
TOTAL	17	34	22	38	17	21	2	1	152

TABLE 2. SERODIAGNOSIS OF FILARIASIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST SPECIFICITY STUDIES (D. viteae antigen)

Source of control sera	No. of sera	Fluorescent antibody titres			
		<1/20	1/20	1/40	1/80
I. Sera collected in Europe					
Healthy subjects	80	80	0	0	0
Ascariasis	54	53	1	0	0
Trichuriasis	18	18	0	0	0
Ancylostomiasis	14	14	0	0	0
Strongyloidiasis	39	37	2	0	0
Trichostrongylosis	2	2	0	0	0
Fascioliasis	42	42	0	0	0
Urinary bilharziasis	39	39	0	0	0
Intestinal bilharziasis	43	41	1	1	0
Cysticercosis	2	2	0	0	0
Hydatid cyst	29	29	0	0	0
Alveolar hydatid disease	7	7	0	0	0
Taeniasis (<u>T. saginata</u>)	18	18	0	0	0
Malaria	74	73	1	0	0
Hepatic amoebiasis	38	37	1	0	0
Toxoplasmosis	100	100	0	0	0
Kala-Azar	4	3	1	0	0
Lambliasis	7	7	0	0	0
Candidiasis	17	17	0	0	0
Aspergillosis	19	19	0	0	0
Syphilis	29	29	0	0	0
Yaws	5	5	0	0	0
<u>TOTAL</u>	680	672 98.2%	7 (1%) 1.5%	1 0.02%	0
II. Sera collected overseas					
Tunisia	106	97	3	5	1
Central African Republic	31	27	2	1	1
Sudan	85	78	0	6	1
Colombia	32	26	0	5	1
<u>TOTAL</u>	254	228 (89.8%)	5 (2%)	17 (6.7%)	4 (1.5%)
III. Overall total	934	900 (96.4%)	6 (0.6%)	24 (2.6%)	4 (0.4%)

TABLE 3. SERODIAGNOSIS OF FILARIASIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST
 SPECIFICITY STUDIES. SERA FROM CASES OF FILARIASIS TESTED AGAINST VARIOUS PARASITE ANTIGENS

Type of serum	No.	Antigens and fluorescent antibody titres														
		Ascaris lumbricoidea		Strongyloides stercoralis		Schistosoma mansoni		Fasciola hepatica		Echinococcus granulosus		Toxoplasma gondii				
		<1/20	1/20	1/40	<1/20	1/20	1/40	<1/20	1/20	1/40	<1/20	1/20	1/40	<1/20	1/20	1/40
Dracunculiasis	3	2	1	0	3	0	0	3	0	0	0	3	0	0	0	0
Mucheriasis	14	12	2	0	13	1	0	14	0	0	0	14	0	0	0	0
Loiasis	22	20	1	1	21	1	0	22	0	0	0	22	0	0	0	0
Oncocerciasis	19	17	2	0	18	1	0	19	0	0	0	19	0	0	0	0
Filariasis with D. perstans	9	8	1	0	8	1	0	9	0	0	0	9	0	0	0	0
TOTAL	67	59 (88.1) %	7 (10.4) %	1 (1.5) %	63 (94) %	4 (6) %	0	66 (98.5) %	1 (1.5) %	0	0	67 (100) %	0	0	67 (100) %	0

TABLE 4. SERODIAGNOSIS OF FILARIASIS BY THE FLUORESCENT ANTIBODY TEST.
 STUDY OF 647 SERA FROM PARASITOLOGICALLY CONFIRMED CASES OF
 FILARIASIS (D. VITAEAE ANTIGEN)

Type of serum	No.	Fluorescent antibody titres								Percentage of positives test	GMRT	
		1/20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280			
<u>DRACUNCULIASIS</u>												
E.R. Aut.	6	0	1*	0	0	2	2	1	0	-	-	
<u>WUCHERERIASIS</u>												
E. Eur.	6	1	0	3	0	1	1	0	0	-	-	
Aut.	3	0	1	0	1	0	0	1	0	-	-	
E.R. Aut.	94	14	16	19	20	5	17	1	2	85.0	41.3	
Total	103	15	17	22	21	6	18	2	2	85.4	42.2	
<u>LOAIASIS</u>												
E. Eur.	34	5	8	7	5	7	1	1	0	85.3	33.5	
Aut.	37	5	6	7	4	9	3	1	2	86.5	50.4	
E.R. Aut.	109	12	7	12	28	16	22	5	7	89.0	80.6	
Total	180	22	21	26	37	32	26	7	9	87.8	61.8	
<u>ONCHOCERCIASIS</u>												
E. Eur.	2	0	1	1	0	0	0	0	0	-	-	
Aut.	6	0	1	3	0	1	1	0	0	-	-	
E.R. Aut.	189	18	26	41	55	29	9	8	3	90.5	50.6	
Total	197	18	28	45	55	30	10	8	3	94.7	52.1	
<u>LOAIASIS & ONCHOCERCIASIS</u>												
E. Aut.	3	0	1	0	1	1	0	0	0	-	-	
E.R. Aut.	19	1	6	6	3	1	2	0	0	94.8	39.4	
Total	22	1	7	6	4	2	2	0	0	95.4	42.1	
<u>LOAIASIS & FILARIASIS with D. perstans</u>												
E. Eur.	5	0	2	1	0	1	1	0	0	-	-	
Aut.	7	1	0	3	1	0	1	1	0	-	-	
E.R. Aut.	14	1	2	4	1	3	0	2	1	92.8	74.1	
Total	26	2	4	8	2	4	2	3	1	92.3	65.2	
<u>FILARIASIS with D. perstans</u>												
E. Eur.	3	1	0	1	1	0	0	0	0	-	-	
Aut.	7	2	2	1	0	2	0	0	0	-	-	
E.R. Aut.	103	15	32	30	17	5	3	1	0	85.4	24.6	
Total	113	18	34	32	18	7	3	1	0	84.0	23.7	
TOTAL												
E. Eur.	50	7	11	13	6	9	3	1	0	84.0	34.2	
Aut.	63	8	11	14	7	13	5	3	2	87.3	47.9	
E.R. Aut.	534	61	90	112	124	61	55	18	13	88.6	47.3	
OVERALL TOTAL	647	76	112	139	137	83	63	22	15	88.2	40.1	

* Calcified filaria. E = sera collected in Europe. Eur. = Europeans. Aut. = subjects indigenous to regions where filariasis is endemic E.R. = sera collected in an endemic region.

TABLE 5. SERODIAGNOSIS OF WUCHERERIASIS. COMPARISON OF THE RESULTS OF THE INDIRECT FLUORESCENT ANTIBODY TEST (D.VITEAE ANTIGEN) WITH DATA ON THE PRESENCE (P+) OR ABSENCE (P-) OF PARASITES IN THE BLOOD
 (See Table 4 for abbreviations)

Place of serum collection and categories of patients examined	No. of sera	Fluorescent antibody titres								Percentage of positives	GMRT
		1/20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280		
E. Abt.	P +	0	1	0	1	0	0	1	0	-	-
	P -	0	5	2	2	0	0	0	0	-	-
E. R. Aut.	P +	14	16	19	20	5	17	1	2	85.0%	41.3
	P -	7	14	15	17	7	14	1	2	90.9%	55
Total	P +	14	17	19	21	5	17	2	2	85.6%	42.5
Total	P -	7	19	17	19	7	14	1	2	91.9%	52

TABLE 6. SERODIAGNOSIS OF LOIASIS. COMPARISON OF THE RESULTS OF THE INDIRECT
 FLUORESCENT ANTIBODY TEST (D. VITAEAE ANTIGEN) WITH DATA ON THE PRESENCE (P+)
 OR ABSENCE (P-) OF PARASITES IN THE BLOOD
 (See Table 4 for abbreviations)

Place of serum collection and categories of patients examined	No. of sera	Fluorescent antibody titres								Percentage of positives	GMRT
		Fluorescent antibody titres									
		1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/1280		
E	P +	5	7	5	7	1	1	0		85.3%	33.5
	Eur	7	11	17	8	3	1	1		87.7%	42.4
E	P +	5	7	4	9	3	1	2		86.5%	50.4
	Aut	4	7	3	10	4	2	1		88.8%	60.7
E. R.	P +	12	12	28	16	22	5	7		89.0%	80.6
	Aut	6	14	19	14	21	6	5		93.6%	64.0
TOTAL P +	180	22	26	37	32	26	7	9		87.8%	61.8
TOTAL P -	187	17	32	39	32	28	9	7		90.9%	68.3

TABLE 7. SERODIAGNOSIS OF ONCHOCERCIASIS. COMPARISON OF THE RESULTS OF THE INDIRECT FLUORESCENT ANTIBODY TEST (D. VITAEAE ANTIGEN) WITH DATA ON THE PRESENCE (P+) OR ABSENCE (P-) OF PARASITES IN THE SKIN
 (See Table 4 for abbreviations)

Place of serum collection and categories of patients examined	No. of sera	Fluorescent antibody titres								Percentage of positives	GMRT
		1/20	1/20	1/40	1/80	1/160	1/320	1/640	1/1280		
E	P +	0	1	3	0	1	1	0	0	-	-
	Aut	1	3	2	0	0	1	1	0	-	-
E. R.	P +	18	26	41	55	29	9	8	3	90.5%	50.6
	Aut	4	14	7	11	17	2	5	1	93.4%	60.2
TOTAL P +	195	18	27	44	55	30	10	8	3	90.8%	42.0
TOTAL P -	69	5	17	9	11	17	3	6	1	92.7%	56.7

TABLE 8. BIOLOGICAL DIAGNOSIS OF FILARIASIS CAUSED BY W. BANCROFTI, LOA LOA OR O. VOLVULUS.
 COMPARISON OF THE RESULTS OF THE INDIRECT FLUORESCENT ANTIBODY TEST
 USING D. VITEAE ANTIGEN WITH THE RESULTS OF A SEARCH FOR MICROFILARIAE

Type of filariasis	Place of serum collection	Subjects examined	No. of cases	No. of cases in which microfilariae were found	No. of positive indirect fluorescent antibody tests
WUCHERERIASIS	E	Aut	12	3 (25.0%)	12 (100%)
	E.Z.	Aut	171	94 (54.9%)	151 (88.3%)
	Total		183	97 (53.0%)	163 (83.0%)
LOAIASIS	E	Eur	91	34 (37.4%)	79 (89.1%)
		Aut	73	37 (50.7%)	64 (87.7%)
	E.Z.	Aut	203	109 (53.7%)	185 (91.1%)
	Total		367	180 (49.0%)	328 (89.4%)
ONCHOCERCIASIS	E	Aut	14	6 (42.8%)	13 (92.8%)
	E.Z.	Aut	250	189 (75.6%)	228 (91.2%)
	Total		264	195 (73.9%)	241 (91.3%)
TOTAL	E		190	80 (42.1%)	168 (88.4%)
	E.Z.		624	392 (62.9%)	564 (90.4%)
OVERALL TOTAL			814	472 (58.0%)	732 (89.9%)

TABLE 9. SERODIAGNOSIS OF FILARIASIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST.
 STUDY OF 97 SERA FROM CONFIRMED CASES OF FILARIASIS
 RESULTS OBTAINED AGAINST THE SPECIFIC ANTIGEN CONCERNED AND AGAINST D. VITAEAE ANTIGEN

Type of filariasis	No. of sera	Specific antigen fluorescent antibody titres						Total positive results	GMRT	D. vitaeae antigen fluorescent antibody titres						Total positive results	GMRT	
		Fluorescent antibody titres								Fluorescent antibody titres								
		1/20	1/40	1/80	1/160	1/320	1/640			1/20	1/40	1/80	1/160	1/320	1/640			
BUCHERINIASIS	31	2	7	7	9	4	1	29 (93.5%)	47.1	4	9	9	5	3	1	0	27 (83.1%)	27.7
LOAIASIS	14	0	1	5	1	2	3	14 (100%)	125	0	3	5	3	2	1	0	14 (100%)	56.5
ONCHOCERCIASIS	52	0	0	4	9	16	14	52 (100%)	222	1	2	9	8	14	11	7	51 (98.1%)	145
TOTAL	97	2	8	16	19	22	18	95 (97.9%)	126	5	14	23	16	19	13	7	92 (94.8%)	74.3

TABLE 10. SERODIAGNOSIS OF FILARIASIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST
STUDY OF 29 SERA FROM CONFIRMED FILARIASIS CASES TESTED AGAINST
D. VITEAE, W. BANCROFTI, L. LOA AND O. VOLVULUS ANTIGENS

Sera	Antigens			
	<u>D. viteae</u>	<u>W. bancrofti</u>	<u>L. loa</u>	<u>O. volvulus</u>
	Reciprocals of the fluorescent antibody titres			
WUCHERERIASIS	0	20	0	0
	20	20	20	20
	40	80	40	40
	40	80	40	40
	80	320	40	80
	80	160	40	40
	160	160	NP	80
	160	640	160	160
	160	320	NP	80
LOAIASIS	20	20	80	40
	40	0	40	20
	40	20	80	20
	40	40	640	20
	40	40	40	20
	80	80	320	160
	80	40	80	80
ONCHOCERCIASIS	0	20	0	20
	40	20	0	80
	40	0	20	40
	40	40	20	320
	40	20	40	40
	80	40	NP	160
	80	40	NP	160
	160	80	160	640
	160	80	80	320
	160	80	NP	320
	320	160	160	640
FILARIASIS CAUSED BY <u>D. PERSTANS</u>	40	40	80	40
	80	80	80	80

TABLE 11. INDIRECT FLUORESCENT ANTIBODY TEST FOR FILARIASIS (D. VITRAE ANTIGEN)
SERA COLLECTED IN AFRICAN VILLAGES WITH VARYING ENDEMIC LEVELS OF ONCHOCERCIASIS

Endemic level of onchocerciasis in the villages studied	No. of sera	Fluorescent antibody titres						Total positive tests	GMRT
		1/20	1/40	1/80	1/160	1/320			
Hyperendemic	97	10	22	30	20	3	87 (89.6%)	52.7	
Mesoendemic	134	28	31	26	17	3	105 (78.0%)	25.9	
Hypoendemic	27	9	4	3	1	0	18 (66.0%)	10.3	

TABLE 12. CHANGE IN FLUORESCENT ANTIBODY TITRE AND THE BLOOD/EOSINOPHIL COUNT (FIGURES IN BRACKETS) AMONG FILARIASIS (LOA LOA) PATIENTS TREATED WITH DIETHYLCARBAMAZINE

Case No.	DAYS											
	0	10	20	30	40	70	100	120	130	160	190	240
3	40 (24)	-	20 (10)	20 (15)	20 (12)	-	0 (6)	-	-	-	-	-
5	40 (26)	-	-	20 (17)	20 (15)			-	40 (15)	-	-	-
10	40 (30)	-	-	160 (41)	-	40 (21)	0 (12)	-	-	20 (7)	-	-
12	40 (18)	80 (21)	160 (47)	-	-	0 (10)	0 (9)	0 (6)	-	-	0 (4)	0 (4)
14	20 (16)	40 (19)	-	20 (15)	20 (12)	-	-	-	-	-	-	-
15	40 (30)	-	40 (27)	-	-	-	-	20 (9)	-	0 (4)	-	-
23	80 (16)	-	-	80 (42)	-	40 (25)	-	-	-	20 (4)	-	-
24	20 (21)	80 (20)	-		-	-	20 (7)	0 (6)	-	0 (3)	20 (7)	-
25	40 (15)	-	-	40 (20)	-	-		-	0 (2)	-	-	
27	20 (15)	20 (40)	160 (36)	-	-	40 (20)	-	-	-	-	-	-
28	40 (21)	-	-	80 (29)	-	-	-	40 (26)	-	-	-	-
30	80 (54)	-	160 (41)	320 (32)	-	80 (15)	-	-	40 (8)	-	-	20 (2)
31	40 (18)	-	-	160 (21)	-	-	-	-	-	20 (2)	-	-
44	40 (18)	80 (50)	-	-	40 (17)	-	-	40 (12)	-	-	-	-
52	80 (16)	-	40 (18)	80 (21)	80 (17)	-	20 (11)	-	-	-	20 (9)	20 (6)
54	0 (17)	80 (29)	-	-	-		-	-	0 (6)	-	-	-

TABLE 12. CHANGE IN FLUORESCENT ANTIBODY TITRE AND THE BLOOD/EOSINOPHIL COUNT (FIGURES IN BRACKETS) AMONG FILARIASIS (LOA LOA) PATIENTS TREATED WITH DIETHYLCARBAMAZINE (continued)

Case No.	DAYS											
	0	10	20	30	40	70	100	120	130	160	190	240
56	0 (18)	-	20 (16)	-	-	-	-	-	20 (9)	-	0 (2)	-
57	20 (17)	-	80 (20)	-	80 (14)	-	20 (8)	-	0 (9)	-	-	-
63	40 (18)	-	-	160 (23)	-	-	20 (11)	-	-	-	0 (9)	0 (3)
64	80 (21)	-	80 (16)	-	40 (10)	-	-	0 (6)	-	-	-	-
65	0 (16)	-	20 (21)	-	-	0 (8)	-	-	-	-	-	-
67	80 (17)	-	80 (22)	-	-	-	-	-	-	20 (4)	-	-
68	40 (32)	-	-	80 (34)	-	20 (10)	-	0 (8)	-	-	-	0 (3)
TOTAL	23	6	11	11	7	7	6	7	6	6	5	5
GMRT	25.5	56.4	58.4	71.2	61.2	13.9	7.4	4.4	5.6	7.4	3.3	3.3

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Fig. 1

Fluorescent antibody test for filariasis. Positive result. The characteristic yellow-green fluorescence (not very clear on a black and white photograph) is located in the subcuticular region.

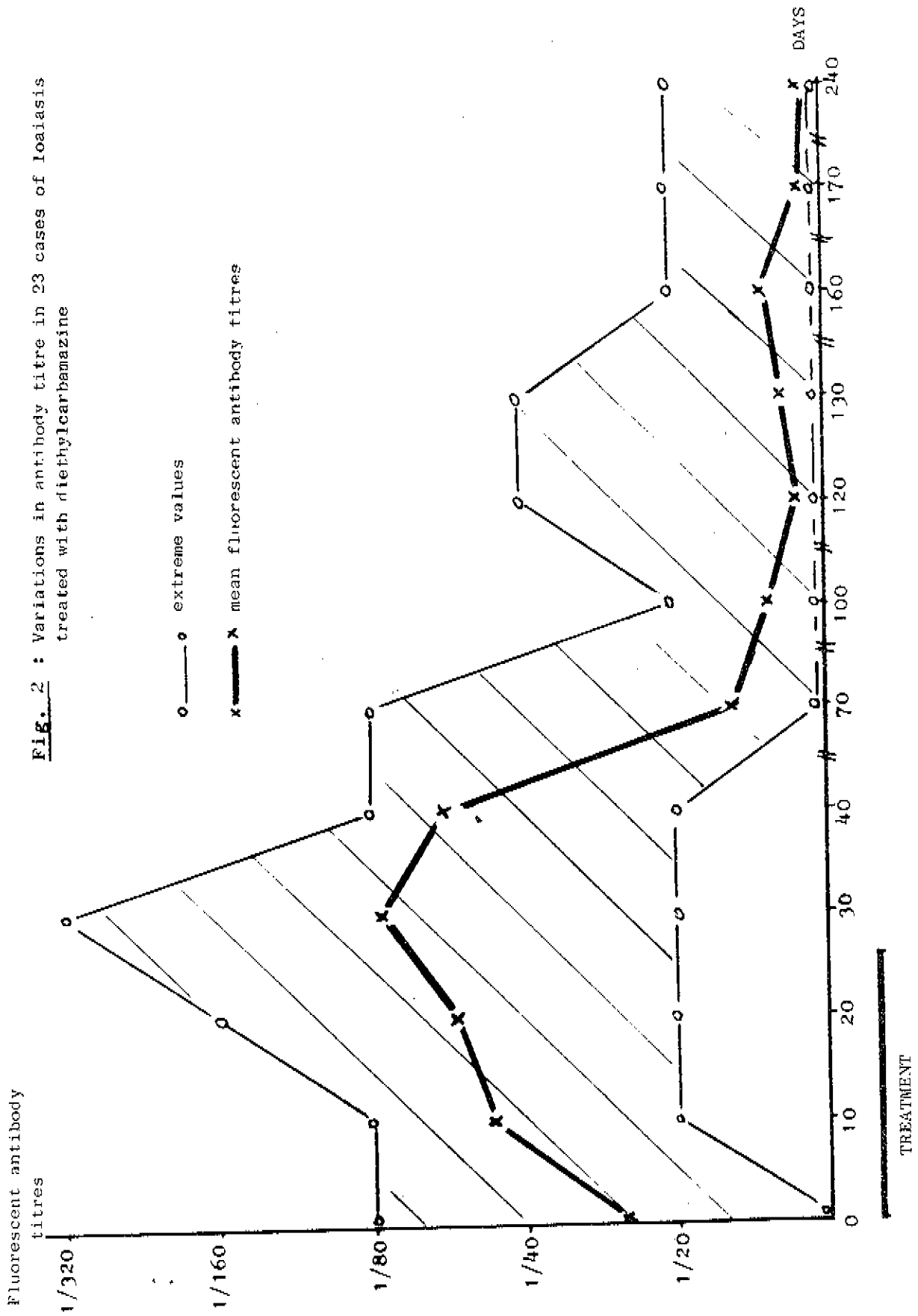


Fig. 2 : Variations in antibody titre in 23 cases of loiasis treated with diethylcarbamazine

Fig. 3 : Different patterns of change in antibody titre in loiasis cases treated with diethylcarbamazine

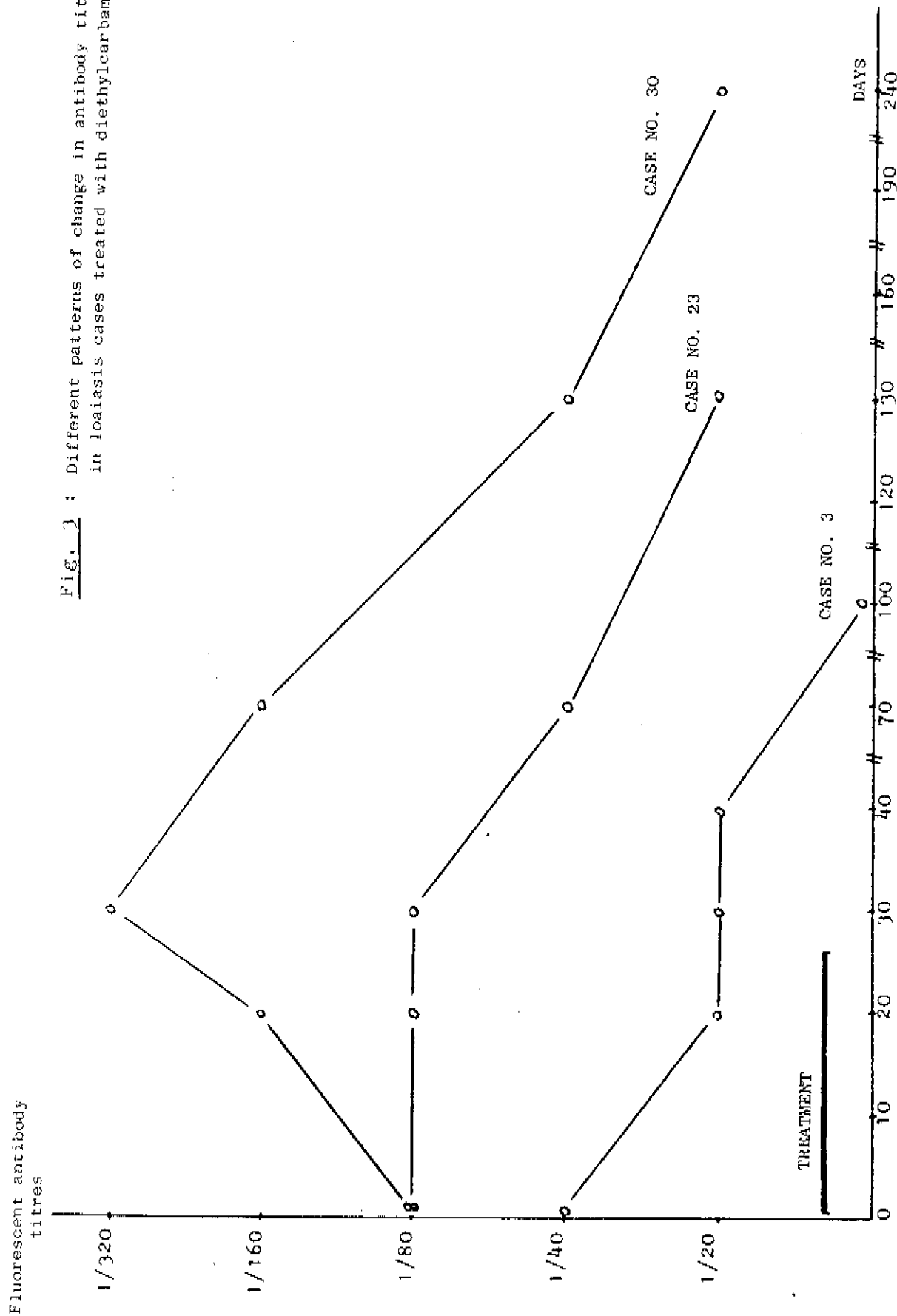


Fig 4: Change in antibody titres in cases of loiasis
Given two courses of treatment with
diethylcarbamazine (cases Nos. 32 and 43)

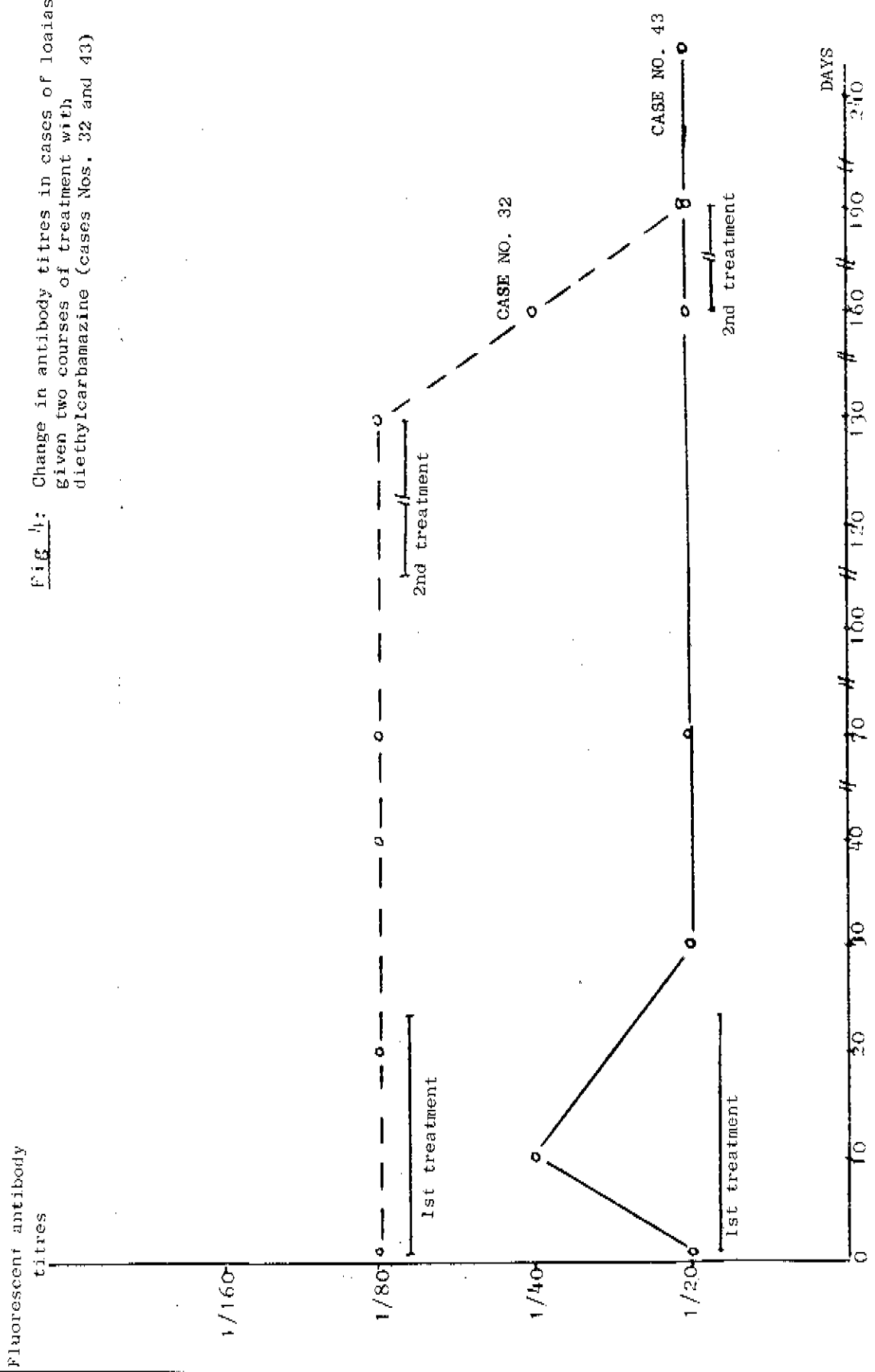


Fig. 5 : Change in antibody titres in cases of loiasis given two courses of treatment with diethylcarbamazine (cases nos. 29 and 77)

