

Serology in ocular toxoplasmosis

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SUMMARY The diagnostic value of toxoplasma serology in ocular disease was evaluated in the following groups of patients: (I) uveitis cases of various causes (n=291); (II) consecutive posterior and panuveitis patients (n=60); (III) patients with definite congenital and ocular toxoplasmosis (n=8); (IV) cases of clinical ocular toxoplasmosis (n=25); and control patients with uveitis of non-toxoplasma origin (n=12). No relation was observed between the level of the dye test titres and the diagnosis of ocular toxoplasmosis (groups I and II). During the active stages of the disease no typical change of the titres occurred in several longitudinally studied patients with toxoplasmosis. In group III one case was discovered to be negative by the dye test despite active ocular disease; however, IgG antibodies against toxoplasma were detected by the ELISA technique. In group IV, which was investigated by the ELISA technique, 100% of the toxoplasmosis patients were positive for IgG versus 58% of the control patients. Circulating immune complexes containing IgG and toxoplasma antigen were detected in seven of 25 toxoplasmosis patients (28%) and in two of 12 control patients (16%). Our study shows that the definite diagnosis of ocular toxoplasmosis or its exclusion by serological means only is not yet feasible. The possible superiority of the ELISA test to the dye test warrants further investigation.

Toxoplasma retinochoroiditis has been reported as a leading cause of posterior uveitis.¹ Two clinical forms of toxoplasma infection are recognised, namely, a congenital and a postnatally acquired disease. In the case of acquired toxoplasmosis, which rarely causes ocular disease,² the antibody titres are usually very high, and therefore serology for this diagnosis is indispensable.

The diagnosis of ocular toxoplasmosis, which is generally accepted to be a congenital disease,³ may be very difficult. The conclusive diagnosis of active toxoplasmosis depends on the isolation of toxoplasma organisms from the fluid or tissue of the patient suffering from an active form of the disease, but this is rarely possible in ocular disease. The high incidence of IgG antibodies against toxoplasma in the population is mostly due to a past acquired infection; therefore a positive IgG test is not discriminatory for the ocular disease and may not be related to the eye lesion. Most authors agree that a positive test by any

of the accepted serological methods is compatible with the diagnosis of ocular toxoplasmosis.^{2,4,5} A negative test is thought to rule out the diagnosis of ocular toxoplasmosis,⁶ and therefore ophthalmologists have often urged tests to be performed even on undiluted serum. On the other hand cases of histologically proved toxoplasma retinitis have been described with negative serum titres against toxoplasma.⁷

Several authors have suggested that demonstrating the local synthesis of toxoplasma antibodies in the eye is proof of the diagnosis of ocular toxoplasmosis.^{8,10} But this procedure is not yet routinely performed, and the diagnosis of ocular toxoplasmosis is often based on the typical clinical picture in combination with various serological findings.

Since no well documented evidence is available on the value of toxoplasma serology in ocular disease, we have evaluated the serological findings in a large number of patients attending uveitis clinics, as well as in a longitudinal study of children with a definite congenital toxoplasmosis. Special attention was paid to the following points: the diagnostic value of a positive test in case of ocular toxoplasmosis; the

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possible exclusion of this diagnosis by a negative test result; and possible distinctions among several tests in current use, namely, the dye test, complement fixation reaction (CRF), and enzyme linked immunosorbent assays (ELISA) for toxoplasma antibodies, free toxoplasma antigen, and toxoplasma-containing immune complexes.

Patients and methods

Four patient groups with partial overlap were studied for toxoplasma serology.

Group I consisted of 291 uveitis patients attending uveitis clinics of the university eye hospitals in Amsterdam and Rotterdam during 1981 and 1982. These patients suffered from uveitis of various causes. For a more detailed evaluation we selected from group I a subgroup of 60 consecutive patients suffering from posterior uveitis or panuveitis in Amsterdam during 1982 (group II). The third group (III) of patients consisted of eight young adults known to have definite congenital and ocular toxoplasmosis. These eight patients are part of a prospective study based on 1821 pregnant women¹¹ and include all cases of definite congenital and ocular toxoplasmosis, in whom a longitudinal serological follow-up was available. The fourth group (IV) consisted of 25 patients with a clinical diagnosis of ocular toxoplasmosis and 12 control patients with uveitis of non-toxoplasma origin.

The serological tests employed in all four groups included the Sabin-Feldman dye test and complement fixation reaction (CRF). These tests were performed according to standard techniques in the Royal Tropical Institute in Amsterdam.¹² Unfortunately not all our samples were determined by the dye test in very low dilutions or even in undiluted serum. This procedure is not a laboratory routine and is performed only when specially requested. In groups III and IV the enzyme linked immunosorbent assay for IgG and IgM toxoplasma antibodies and tests for detecting free toxoplasma antigen and circulating immune complexes containing toxoplasma antigen were performed as described elsewhere.¹³⁻¹⁵

The serology in all patients of groups I, II, and IV was done during an active stage of their ocular disease, whereas the blood samples in group III were obtained independently of ocular disease.

The clinical diagnosis of ocular toxoplasmosis was based on ophthalmological examination, showing active unilateral focal necrotising retinochoroiditis, often in satellite formation, with associated vitreous inflammation, and also the appearance of pigmented scars after clearing of the vitreous.⁴

The diagnosis of definite congenital toxoplasmosis

in children was based on the following criteria: seroconversion of the mother during pregnancy, and persistence of positive dye test titres beyond the age of 30 months as defined in an earlier study.¹¹

Statistical analysis was performed by the χ^2 test and Kolmogorov-Smirnov test for consistency of frequency distributions.¹⁶

Results

Group I: 291 uveitis patients

To determine whether it is worthwhile to use toxoplasma serology as a screening tool in diagnosing uveitis patients we reviewed the medical records of 291 uveitis patients (group I). The mean age of these patients was 42.4 years (range 9 and 92 years) and the male to female ratio was 1:0.88. The patients were subdivided into three categories according to the localisation of their uveitis: anterior uveitis (n=144), panuveitis (n=71), and posterior uveitis (n=76). The distribution of the dye test titres in all these forms of uveitis was identical (Kolmogorov-Smirnov test) (Fig. 1). Nearly all the patients with clinical ocular toxoplasmosis (n=32) suffered from posterior uveitis.

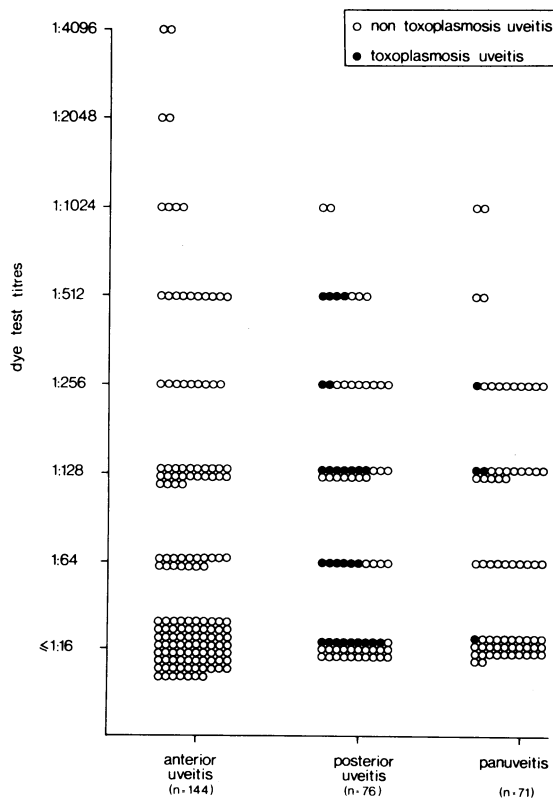


Fig. 1 *Toxoplasma serology in uveitis patients (group I).*

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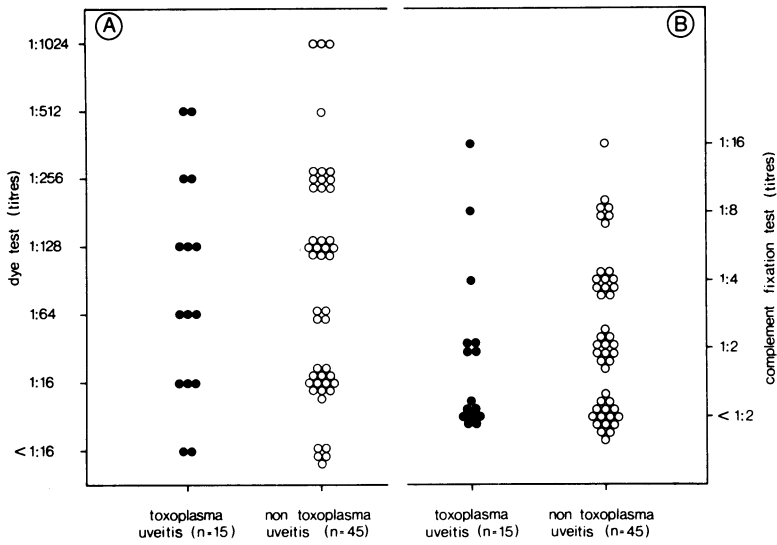


Fig. 2 *Toxoplasma* serology in posterior uveitis and panuveitis patients (group II). A: dye test; B: complement fixation test.

In the posterior uveitis group a similar distribution of the dye test titres was found in the toxoplasmosis and the non-toxoplasmosis patients. No correlation was found between the level of the titre and the clinical diagnosis.

Group II: 60 consecutive posterior uveitis and panuveitis patients

To discover whether toxoplasma serology correlates with the clinical diagnosis and therefore could be of diagnostic value in a more restricted group of patients we analysed 60 consecutive patients with posterior uveitis and panuveitis in more detail (group II). This group included 15 clinical cases of ocular toxoplasmosis. The titre distribution of the dye test and complement fixation reaction in the toxoplasmosis and the non-toxoplasmosis groups showed a similar pattern (no significant difference found by Kolmogorov-Smirnov test) (Fig. 2). The toxoplasmosis patients were aged 10 to 40 years. We therefore studied the relationship between the dye test, complement fixation reaction titres, and the age of the patient (Fig. 3) to determine whether serology could be more valuable in a certain age group. Even in the young patients no correlation was found between the level of the titre and the clinical diagnosis of toxoplasmosis. Out of the six patients with a raised dye test titre ($\geq 1:512$) two had ocular toxoplasmosis, while two more out of nine patients with a raised complement fixation reaction ($\geq 1:8$) suffered from this disease.

In several patients of group II the dye test titres were monitored with an interval of three weeks to see whether a change of titre could be found, and, if it was, to see whether a rise of the titre could be found

only in the toxoplasmosis patients. The longitudinal follow-up during the course of the uveitis, however,

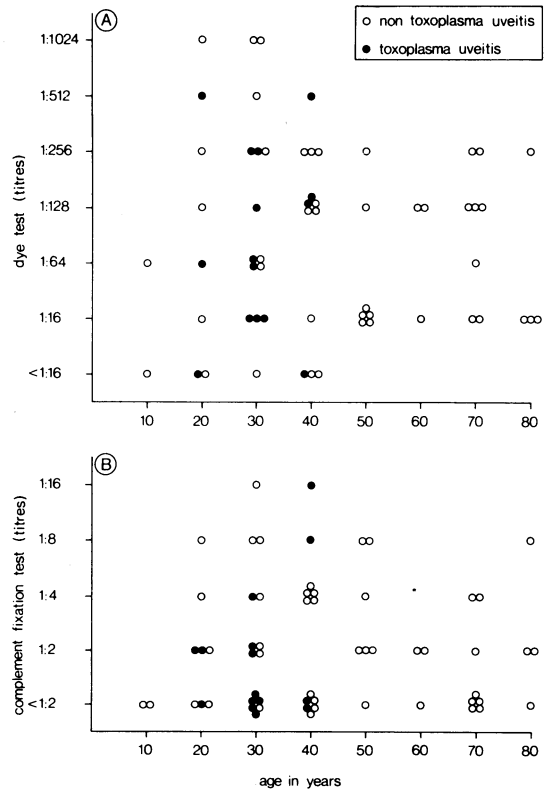
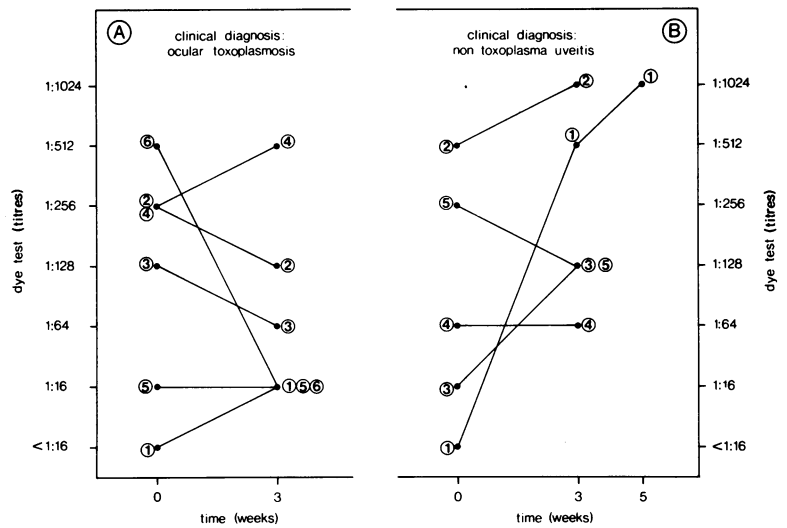


Fig. 3 Relation between toxoplasma serology and age in posterior uveitis and panuveitis patients (group II). A: dye test; B: complement fixation test.

Fig. 4 Longitudinal follow-up of toxoplasma antibodies in uveitis patients (group II). The numbers indicate individual patients.



showed an extremely variable course of the dye test titre in both the toxoplasmosis and the non-toxoplasmosis groups (Fig. 4). Patient no. 1 of the non-toxoplasmosis group was the only patient who showed an evident rise in titre. He had probably been infected or reinfected owing to his poor general condition. Ophthalmologically he presented with bilateral retinitis during subacute sclerosing panencephalitis; there was no clinical resemblance to ocular toxoplasmosis. Patient no. 6 of the toxoplasmosis group showed a sharp decline of his dye test titre.

Group III: eight cases of definite ocular toxoplasmosis (longitudinal study)

To ascertain whether the absence of toxoplasma antibodies indicates that an ocular toxoplasma infection is highly improbable, we reviewed the dye test titres in eight young adult patients with known

congenital and ocular toxoplasmosis. These patients are a part of a big prospective study of children born from mothers with a seroconversion during pregnancy.¹¹ Most children were followed up annually until the age of 20 years by an ophthalmological as well as a laboratory investigation. The fluctuation of their titres is shown in Fig. 5. There was one boy (case no. 7) with ocular toxoplasmosis and a negative dye test titre. A more detailed laboratory investigation was performed when the children were 18 years old (Table 1). At that age all the patients had positive IgG antibodies against toxoplasma as measured by an ELISA technique, though in one case no antibodies were detectable by the Sabin-Feldman dye test.

Group IV: 25 cases of clinical ocular toxoplasmosis and 12 control patients with non-toxoplasma uveitis
To investigate whether the newer, currently applied serological methods have the same limitations as the

Table 1 *Toxoplasma serology in eight cases of congenital and ocular toxoplasmosis at the age of 18 years (group III)*

Pat. no.	Dye test	CFR	ELISA				
			IgG	IgM	Circ. Ag	CIC cont. IgG	CIC cont. IgM
1	1:4096	1:32	+	-	-	-	-
2	1:4	negative	+	-	-	-	-
3	1:64	negative	+	-	-	-	-
4	1:128	1:2	+	-	-	-	-
5	1:64	1:2	+	-	-	-	-
6	1:16	negative	+	-	-	-	-
7	negative	negative	+	-	-	-	-
8	1:64	negative	+	-	-	-	-

CFR=complement fixation reaction; circ. Ag=circulating antigen; CIC cont. IgG=circulating immune complexes containing toxoplasma antigen and IgG; CIC cont. IgM=circulating immune complexes containing toxoplasma antigen and IgM.

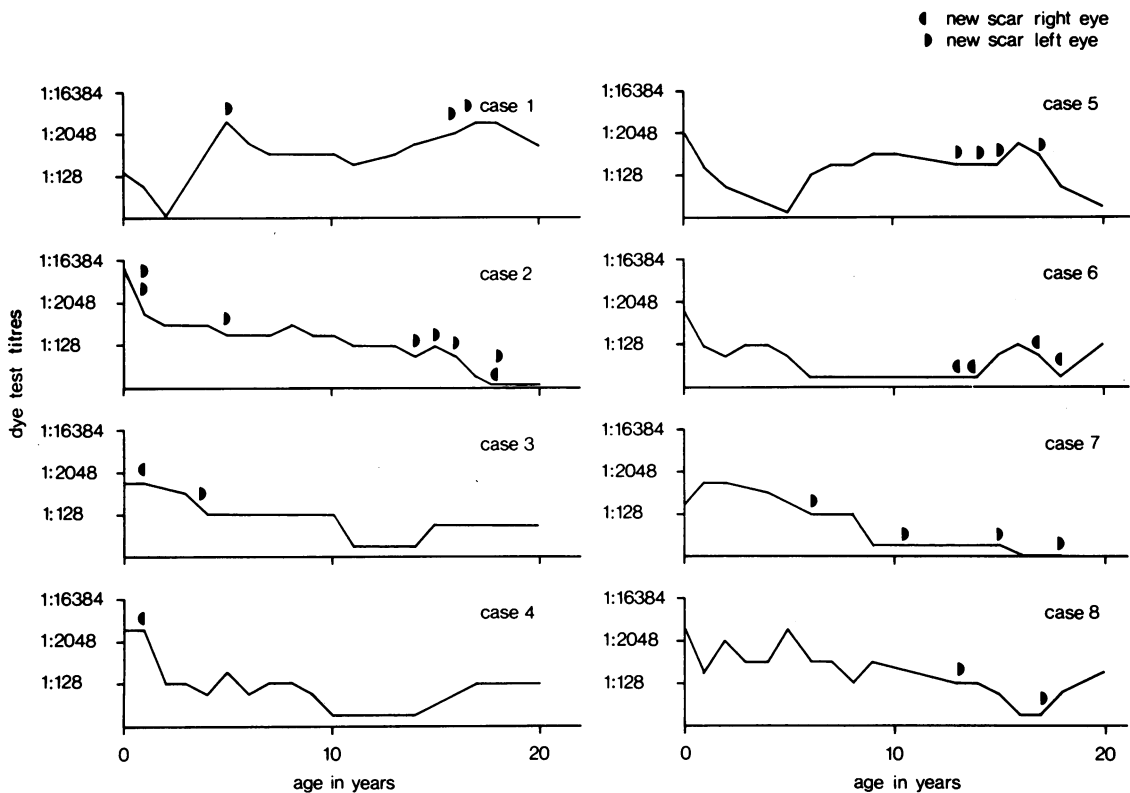


Fig. 5 The course of the dye test titre in eight patients with definite congenital and ocular toxoplasmosis (group III).

dye test we compared serological data obtained by the ELISA technique from 25 patients with ocular toxoplasmosis and 12 controls with non-toxoplasma uveitis. The mean age in the toxoplasmosis group was 29.2 years (range 16 and 47 years) and the male to female ratio was 1:1.18. In the control group the mean age was 31.9 years (range 16 to 47 years) and the male to female ratio was 1:0.7.

All patients in the toxoplasmosis group were positive for IgG antibodies by the ELISA technique, which was in accordance with their dye test titres (Table 2). There were four cases, however, in which a comparison was impossible because the dye test titres were not determined in undiluted serum.

In the non-toxoplasmosis group only seven patients (58%) were positive for IgG antibodies by ELISA. Of these seven patients six had positive dye test titres; the remaining patient was not examined by this technique. None of the patients in either group had detectable IgM antibodies or free toxoplasma antigen in the circulation. Circulating immune complexes containing IgG antibodies were found in seven toxoplasmosis patients (28%) and in two control cases (16%). These two patients were diagnosed as having heterochromic cyclitis of Fuchs and bilateral retinal

vasculitis of unknown origin respectively. Circulating immune complexes containing IgM antibodies were found in the patient with heterochromic cyclitis.

Discussion

From the data presented in this study it can be concluded that classical serology (dye test) is of little value when screening uveitis patients. A positive test (independent of the titre) does not prove the diagnosis and a negative result may not exclude it.

The clinical value of a positive test result is dependent on the prevalence of positive tests in the population, the age of the patient, and the specificity and sensitivity of the test employed.² In the Netherlands more than 60% of the population in the third and fourth decades have been shown to be positive for antitoxoplasma antibodies.¹⁷ It is not possible to determine whether the antibodies found are related to the eye lesion or whether they are a coincidental finding.⁹ In the clinical situation the presence of antibodies indicates only that the patient has been infected by toxoplasma organisms. Nearly all toxoplasmosis patients in our study had low positive dye test titres, and no correlation was found between the

Table 2 *Toxoplasma* serology by ELISA in 25 cases of clinical ocular toxoplasmosis and 12 control cases (group IV)

Case no.	Sex, age	Dye test	ELISA				
			IgG	IgM	circ.Ag	CIC cont.IgG	CIC cont.IgM
<i>Toxoplasmosis patients</i>							
1	F39	1:512	+	-	-	-	-
2	M19	1:512	+	-	-	+	-
3	F19	1:256	+	-	-	+	-
4	F24	1:256	+	-	-	-	-
5	F40	1:256	+	-	-	-	-
6	M28	1:256	+	-	-	+	-
7	F25	1:128	+	-	-	-	-
8	F37	1:128	+	-	-	-	-
9	F38	1:128	+	-	-	-	-
10	M25	1:128	+	-	-	-	-
11	M32	1:128	+	-	-	+	-
12	M38	1:128	+	-	-	-	-
13	F18	1:64	+	-	-	-	-
14	F30	1:64	+	-	-	-	-
15	F32	1:64	+	-	-	-	-
16	M19	1:64	+	-	-	-	-
17	F29	1:16	+	-	-	+	-
18	F30	1:16	+	-	-	-	-
19	F37	1:16	+	-	-	+	-
20	M16	1:16	+	-	-	+	-
21	M47	1:16	+	-	-	-	-
22	F23	<1:128	+	-	-	-	-
23	F29	<1:16	+	-	-	-	-
24	F34	<1:16	+	-	-	-	-
25	M23	<1:16	+	-	-	-	-
<i>Control patients</i>							
1	M29	1:512	+	-	-	+	-
2	M31	1:256	+	-	-	-	-
3	F41	1:128	+	-	-	-	-
4	M38	1:64	+	-	-	-	-
5	M47	1:64	+	-	-	-	-
6	F28	1:16	+	-	-	-	-
7	M16	<1:16	-	-	-	-	-
8	M31	not done	-	-	-	-	-
9	F31	not done	-	-	-	-	-
10	F32	not done	-	-	-	-	-
11	F35	not done	+	-	-	+	+
12	M24	not done	-	-	-	-	-

circ.Ag=circulating antigen; CIC cont.IgG=circulating immune complexes containing toxoplasma antigen and IgG; CIC cont.IgM=circulating immune complexes containing toxoplasma antigen and IgM.

level of the titre and the clinical diagnosis. These findings are in agreement with those of other authors.^{2,6}

In the adults with clinical ocular toxoplasmosis (group II), who were followed up longitudinally, as well as in the congenitally infected children with ocular toxoplasmosis (group III) no correlation was found between the toxoplasma antibody titre and the activity of the ocular disease, which confirms previous observations.^{4,5} A decline in the dye test titre during acute ocular disease was observed in one patient (case no. 6, toxoplasmosis patients, group II). This change could be explained by the formation of immune complexes containing toxoplasma antibodies. It is not possible to determine whether the changes in the titre are due to ocular disease or to the

basic fluctuations of the titre, or whether they are related to the activity of the disease elsewhere in the body.¹⁸ The antibody titre in serum is dependent on antigenic stimulation in the body as a whole, and this can be very low or even absent in cases of ocular inflammation.^{6,8,11} It is evident that in the case of ocular toxoplasmosis the level of the titre has no diagnostic value. On the other hand Vadot¹⁹ has recently reported that the presence of low dye test titres in patients with toxoplasma retinochoroiditis was associated with a higher recurrence rate when compared with cases of high dye test titres. The clinical relevance of these findings deserves further study.

Our data on positive dye test titres show that the

only important result of a test in cases of ocular toxoplasmosis in adults should be the negative one. The clinical relevance of the negative antibody test is related to the sensitivity of the test used. Several cases of histologically proved ocular toxoplasmosis with a negative dye test have been reported. The two classical examples of a negative dye test despite proved ocular toxoplasmosis^{20,21} were in reality cases with unknown but certainly low dye test titres. In a case described by Zscheille²⁰ the patient's dye test was negative at a dilution of 1:16 and Franceschetti and Englebrecht's²¹ patient had a negative dye test at a dilution of 1:25. Blood samples from neither of these patients were tested in lower dilutions. Ikuy *et al.*⁷ isolated toxoplasma organisms from the cerebrospinal fluid of a patient with clinical ocular toxoplasmosis and found his dye test negative with undiluted serum. Our own case of a negative dye test with undiluted serum was observed in a boy with definite congenital and ocular toxoplasmosis at the age of 16 years (case no. 7, group III). The dye test of this patient remained negative despite the reactivation of his ocular disease. However, he was found to be positive for IgG antitoxoplasma antibodies when tested by ELISA.

The Sabin-Feldman dye test was the first reliable test developed and was a standard by which other tests were judged. However, live toxoplasma organisms are employed, and special laboratory precautions must be taken to perform this test. For this reason the dye test became unpopular^{5,6} and other tests were developed.

Our finding shows that the dye test may be negative in ocular toxoplasmosis. This limitation does not seem to apply to the ELISA technique. With ELISA all our clinical toxoplasmosis cases (group III and IV) were positive for IgG antitoxoplasma antibodies, whereas the control patients (group IV) were found to be positive in 58%, which difference is highly significant ($p < 0.01$). Our findings suggest that the ELISA is more sensitive than the dye test, which is in agreement with several other studies, where the sensitivity of the ELISA is higher, especially when dealing with low positive titres.^{22,23} Since our patients were more extensively studied by means of the dye test, this observation needs further investigation.

The detection of circulating antigen or circulating immune complexes containing toxoplasma antigens which can be found for a short time during a fresh infection or reinfection is promising.^{13,15} However, in our study of 25 patients with ocular toxoplasmosis we could not find any free circulating antigen. Circulating immune complexes containing IgG antibodies were found in 28% of ocular toxoplasmosis patients and in 16% of controls. This difference is not statistically significant, though it should be remem-

bered that the number of patients was very small and that active toxoplasma infection in immune complex positive control patients cannot be excluded.

Such a serological diagnosis of active toxoplasma infection was made in one patient with heterochromic cyclitis of Fuchs. This patient also had an old retinal toxoplasmosis scar, which was certainly not active. The association between toxoplasmosis and heterochromic cyclitis has already been noted.^{24,25} Whether the serological findings in the patient presented here are coincidental or are evidence of a relationship between active toxoplasmosis and heterochromic cyclitis deserves further investigation.

We conclude that a definite diagnosis of ocular toxoplasmosis by serological means only is not yet feasible. The possible superiority of the ELISA test to the dye test warrants further investigation. In cases of doubt other diagnostic tests, such as detection of local antibody production, should be used. The final conclusion of this study is that a negative test result with undiluted serum indicates that ocular toxoplasmosis is highly improbable

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