The relationship and significance of antibody titres as determined by various serological methods in glandular and ocular toxoplasmosis

K. A. KARIM AND G. B. LUDLAM

From the Regional Public Health Laboratory, Leeds

SYNOPSIS Three types of antibody curve have been demonstrated by testing sera during the course of acquired toxoplasmosis by six different techniques. These three types are due to cell-wall antibody, (demonstrated by four of the techniques), to antibody to soluble antigen, and to IgM antibody to the cell wall. These findings have been supported by absorption experiments.

A scheme is presented for testing single sera by two or three different tests to indicate the stage and duration of the infection.

Testing for antibody is normally essential in the diagnosis of toxoplasmosis. The method most commonly used in the past has been the dye test of Sabin and Feldman (1948), or some modification of it (Beverley and Beattie, 1952; Frenkel and Jacobs, 1958). Although specific, sensitive, and reproducible (World Health Organization, 1969), it is technically difficult and slow, requires the use of live parasites and suitable fresh human serum as 'accessory factor'. This has led to a search for less exacting but equally effective techniques. Among these have been various types of complement-fixation test (CFT), (Warren and Russ, 1948; Sabin, 1949; Steen and Kass, 1951; Thalhammer, 1956; Fulton and Fulton, 1965), the indirect haemagglutination test (IHAT), (Jacobs and Lund, 1957), the direct agglutination test (DAT), (Fulton and Turk, 1959; Couzineau and Baufine-Ducrocq, 1970), and the immunofluorescent antibody test (IFAT) for detecting either antitoxoplasma IgG/IgM antibodies (Goldman, 1957; Kelen, Ayllon-Leindl, and Labzoffsky, 1962; Fletcher, 1965) or antitoxoplasma IgM antibody alone (Remington, Miller, and Brownlee, 1968; Dropsy, Carquin, and Croix, 1970). In the work reported here, parallel tests have been carried out by these different techniques with sera from large numbers of patients with suspected glandular or ocular toxoplasmosis. In many patients sera were tested at intervals over many months. Comparisons have been made between the antibody titres obtained by the different tests at different stages of the infection.

Materials and Methods

Three hundred and seventy sera from 160 cases of glandular toxoplasmosis and 121 sera from 77 cases of toxoplasma choroidoretinitis were collected over a period of several years and tested by all or some of the methods described. Cases of glandular toxoplasmosis were selected on a combination of clinical history and raised dye test titre. In many positive cases, the diagnosis was supported by histological evidence. The cases with ocular disease were selected by an initial dye test titre of 1:64 or greater together with a supporting clinical diagnosis by an ophthalmologist.

A serum was obtained from each patient together with the clinical history and, with each subsequent specimen, a progress report from the physician-in-charge.

SEROLOGICAL TESTS

Most of the tests described required the initial preparation of suspensions of the parasites as free as possible from host cells. Peritoneal exudates from cotton rats inoculated three days previously with the RH strain of *Toxoplasma gondii* were treated in various ways to produce purified suspensions of toxoplasma and the final method adopted is described. The exudates were collected in veronal-buffered azide saline (VBS, 0·1% sodium azide),

and the volume from each rat made up to 20 ml. Satisfactory washings of the peritoneal cavities containing large numbers of parasites and fewer than 5% of host cells were pooled, washed twice with the buffer, and the sediment was finally re-suspended in 2% formalin in VBS. After 24 hours' fixation in formalin at +4°C, the suspension was centrifuged and the sediments were resuspended in 0·2% formol-saline. After standing for one hour the suspension was removed from particles that had settled out at that time. Exudates that contained more numerous host cells were squirted repeatedly through a fine needle (gauge 25) to break up clumps and disintegrate cells containing parasites. The suspension was then partially purified by slow centrifugation followed by filtration through sintered glass filters (Baird and Tatlock, porosity 20-30 μm). Satisfactory suspensions obtained in this way were then treated by formalin as above.

Haemagglutination test
The test used was a modification of that described by Jacobs and Lunde (1957). The parasites obtained and purified as described above but without for-malinization were lysed in distilled water, 1 ml of water being added to every 5×10^8 parasites. After 24 hours at +4°C, the suspension was transferred to a Mickle cell, an equal volume of small glass beads (grade 12 ballotini) added and the mixture shaken for 10 minutes on a Mickle disintegrator. The beads were removed by filtration and the filtrate centrifuged at 4000 rpm for 10 minutes. The clear supernatant (soluble antigen) was then stored at $-20°C$ until required. Red blood cells were sensi-tized with this antigen using a modification of the technique described by Jennis (1966). Varying dilutions of the antigen were added to 2% sus-pensions of pyruvic-aldehyde-treated human group 0, rhesus-negative cells, and the coating was carried out at 56°C for one hour in phosphate-buffered saline, pH 6·0. The cells were then washed once with PBS (pH 7·2) and resuspended in the same buffer with 0·1% sodium azide at a final concentration of cells of 1%. The dilution of antigen which gave the the highest titre in the IHAT with a standard positive serum (standardized against the WHO international standard serum) and no agglutination with a negative dye test serum was thus determined. This dilution was used to prepare bulk batches of sensitized cells for use as HA antigen. The use of human erythrocytes instead of sheep cells obviated the need for absorbing sera with unsensitized sheep red cells, to remove heterophile antibody.

The tests were carried out in Microtiter plates, v wells, using 40 μl of test serum, diluted in 2% fetal-calf-serum saline, and 40 μl coated red cells.

The plates were read after two and a half hours and the highest dilution of serum giving an irregular rough button of red cells surrounded by a lawn covering the entire bottom of the well was taken as the endpoint.

Complement-fixation test
Antigen for the CFT was prepared and formalinized from cotton-rats as described above. The optimum dilution of the antigen was determined by the usual chessboard technique and was generally found to be $2-8 \times 10^6$ parasites per ml. The test followed the technique described by Bradstreet and Taylor (1962) for virus CFT, but using Microtiter plates, u wells, and 1½% sensitized sheep red cells and adopting the longer overnight fixation.

Direct agglutination test
The concentration of the formolized toxoplasma suspension obtained above was adjusted to approxi-mately 7×10^7 per ml. The test sera were diluted in 0·4% bovine albumin ,borate saline buffer and serial dilutions made in a Microtitre plate, v wells, 25 μl of the antigen being added to 25 μl of the serum dilution. The plate was shaken in a Luckham shaker for 15 minutes and left overnight at room temperature before reading the test. Agglutination was shown by a diffuse lawn of parasites or an irregularly shaped button, whilst a clearly defined button represented a negative reaction. The highest dilution of the serum producing disruption of the button, with an irregular periphery, was considered the endpoint.

Methylene blue dye test
The dye test technique followed that of Beverley and Beattie (1952) modified from Sabin and Feldman (1948).

Indirect fluorescent test for toxoplasma antibodies
The concentration of the toxoplasma suspension formolized as described above was adjusted to approximately 7×10^6 parasites per ml, and multiple spots of the suspension were placed on a glass slide by the method described by Chessum (1970). The smears, parasites distributed evenly without overlaps, were air dried and stored at $-20°C$ until used. Dilutions of sera were made in a Micro-titer plate and transferred by a template to the smears on the slides. Other reagents were placed on the smears in a similar manner.

For detecting an antitoxoplasma immunoglobulin mixture of IgG/IgM, fluorescein-conjugated anti-human immunoglobulins (Wellcome Reagents) were used at a dilution usually of 1:20. Fluorescein-conjugated antihuman IgM, obtained from Nordic

Diagnostics (batch no.1 071), was used at a dilution of 1:20. After final washing the smears were counterstained with Evans blue (1:5000).

The slides were examined with a Reichert fluorescent microscope with a wide, dark field, immersion condenser, excitation filter UGI/1·5 mm, absorption filter GG13/1 + 3 mm × Wrattenfoil 2A, × 40 objective and × 6·3 binocular eyepieces.

The titre of the test serum was taken as the highest dilution which produced bright complete peripheral fluorescence in the majority of the parasites in several fields of the smear. Incomplete peripheral, or faint staining or fluorescence of only the tips of the parasites was ignored.

ABSORPTION EXPERIMENTS

Using whole parasites

Freshly obtained, washed parasites, both formalin-fixed and unformalinized were used to absorb antitoxoplasma sera. The concentration of the parasites was adjusted to 2×10^8 per ml and 5 ml of the suspension was centrifuged at 3000 rpm for 10 minutes. The pellet was resuspended in a minimum volume of saline and added to serum to give a final dilution of 1:8. This was incubated at 37°C for 30 minutes and then overnight at +4°C. The parasites were then centrifuged out and the serum was tested by the various methods describe above. Control tests were carried out on serum diluted in saline without the antigen.

Using HA antigen

Serum dilutions, 1:4 or 1:8, were made in a 12% suspension of sensitized cells and similar dilutions in 12% unsensitized cells as controls. The reaction mixture was incubated at 37°C for 30 minutes and then overnight at +4°C. The supernatant serum was then collected and examined by the various tests.

Results

The results of the tests from patients with toxoplasma lymphadenopathy are shown in fig 1 and tables I and III, and from patients with ocular toxoplasmosis in table II.

For each test the results were grouped within the periods of time, shown in table I, from the onset of disease as given by the clinician. These were 0 to one month, one to three months, and so on. Within each period of time the geometric-mean titre, and 95% confidence interval has been calculated.

Table III shows the results for glandular toxoplasmosis of one and a half to three years' duration,
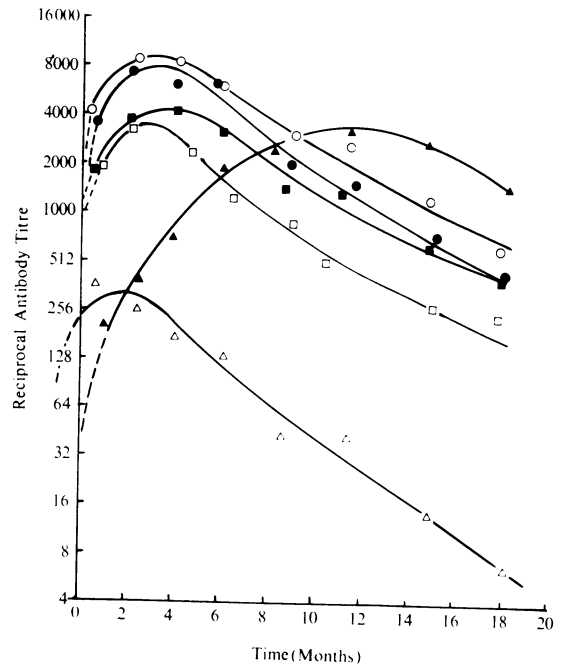

Fig 1 *The antibody curve in glandular toxoplasmosis. The dye test, ●; the direct agglutination test, ○; the IgG/IgM-IFA test, ■; the complement-fixation test, □; the indirect haemagglutination test, ▲; the IgM-IFA test, △.*

the mean titre for each test, the range and the 95% confidence interval.

Tables I, II, and III show the wide range of titres at all stages of the disease. The titre curves of the antibodies (fig 1) in glandular toxoplasmosis when measured by the various tests show that the IgM levels reach their peak first, at one or two months, whereas the titres measured by the dye complement-fixation, direct agglutination, and IgG/IgM-immunofluorescent antibody tests run parallel with one another, all reaching their peaks about two to six months. The titres in the indirect haemagglutination test rise more slowly to reach a maximum in the period of six to 14 months. With the IgM test, there is a fall in antibody titre to become eventually less than 1:8 at any time after 18 months, but with the other tests there is a much slower fall and the results remain positive at higher levels indefinitely. The results of glandular toxoplasmosis one and a half to three years after onset show that the indirect haemagglutination titre is greater than that of the dye test with a low or negative IgM titre, a pattern resembling the results for ocular disease (table II).

Tables IV and V show the titres of antitoxo-

Test	Duration (Months)[1]							
	0-1	*1-3*	*3-5*	*5-7*	*7-10*	*10-13*	*13-17*	*17-21*
Dye (a)	2951	7278	5508	5117	1980	1380	590	388
(b)	1710-5120	5430-9730	4150-7345	3850-6790	1285-2570	1130-2390	464-798	77-1950
Indirect haemagglutination	2200	375	640	1920	2500	3240	2760	1450
	174-355	332-600	470-900	1200-3055	1800-3240	1510-7000	1320-5740	410-5140
Complement-fixation	2290	3236	2270	1270	798	434	496	210
	1183-2570	3083-3396	1521-3396	190-2190	571-1096	178-1052	50-1520	117-326
Direct agglutination	4040	8970	7690	6295	2950	2740	3160	616
	2370-6880	8790-9290	5957-9795	4831-8185	2061-4178	1563-4477	380-3490	299-1276
IgG/IgM-indirect fluorescent Antibody	1884	4325	3126	3100	1320	1350	612	369
	1306-2720	3610-5188	2570-3802	2523-3819	963-1820	1242-1493	362-1038	198-690
IgM indirect fluorescent antibody	370	275	175	128	42	39	14	7
	218-627	210-357	134-230	95-170	30-50	28-56	9-21	4-12

Table I   *Antibody titres at periods after onset of clinical disease in patients suffering from glandular toxoplasmosis*

(a) Geometric mean antibody titre
(b) 95% confidence interval
[1]Durations falling on the boundary between two groups, eg, one month, three months etc, have been divided equally between the two groups.

Test	No. of Specimens	Mean Titre	Range
Dye	120	294	64-4000
Indirect haemagglutination	121	1091	64-32 000
Complement-fixation	105	139	16-4000
Direct agglutination	110	500	32-8000
IgG/IgM indirect fluorescent	118	280	64-4000
IgM indirect fluorescent	117	5·5	<8-64

Table II   *Antibody titres of patients with ocular toxoplasmosis*

Test	No. of Specimens	Mean Titre	Range	95% Confidence Interval
Dye	80	252	64-4000	208-306
Indirect haemagglutination	78	2477	512-32 000	1980-3133
Complement-fixation	73	104	32-2000	100-200
Direct agglutination	54	250	32-4000	194-328
IgG/IgM indirect fluorescent	75	251	64-4000	210-300
IgM indirect fluorescent	77	3	<8-32	3-5

Table III   *Antibody titres one and a half to three years after the onset of glandular toxoplasmosis*

Serum Test										
	Indirect Haemagglutination		Dye		Complement-fixation		IgG/IgM Indirect Fluorescent Antibody		IgM-immunofluorescence Antibody	
	Unabsorbed	Absorbed	Unabsorbed	Absorbed	Unabsorbed	Absorbed	Unabsorbed	Absorbed	Unabsorbed	Absorbed
1	32 000	16 000	32 000	512	16000	128	16 000	128	512	<8
2	512	512	1024	64	512	64	2048	32	64	<8
3	512	256	512	32	256	32	1024	16	32	<8
4	256	256	1024	<32	512	32	1024	32	16	<8
5	2048	4096	8192	64	4096	16	4096	32	128	<8

Table IV   *Serological tests on antitoxoplasma sera absorbed by washed formolized toxoplasma cells*

Serum	Test							
	Indirect Haemagglutination		Dye		Complement-fixation		IgM-Indirect Fluorescent	
	Unabsorbed	Absorbed	Unabsorbed	Absorbed	Unabsorbed	Absorbed	Unabsorbed	Absorbed
1	32 000	16	32 000	16 000	4096	8192	128	256
2	256	<8	1024	512	512	256	64	64
3	2048	<8	2048	4096	1024	1024	16	32
4	64	<8	128	128	128	64	<8	<8

Table V   *Serological tests on antitoxoplasma sera absorbed by soluble-antigen-sensitized red cells*
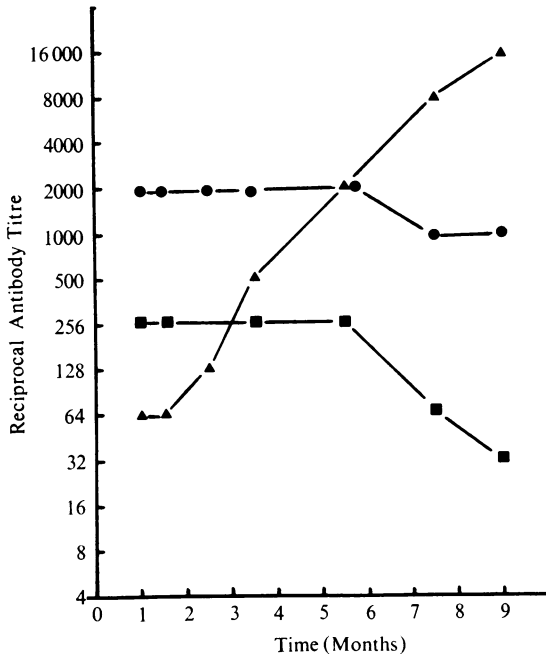
Fig 2a
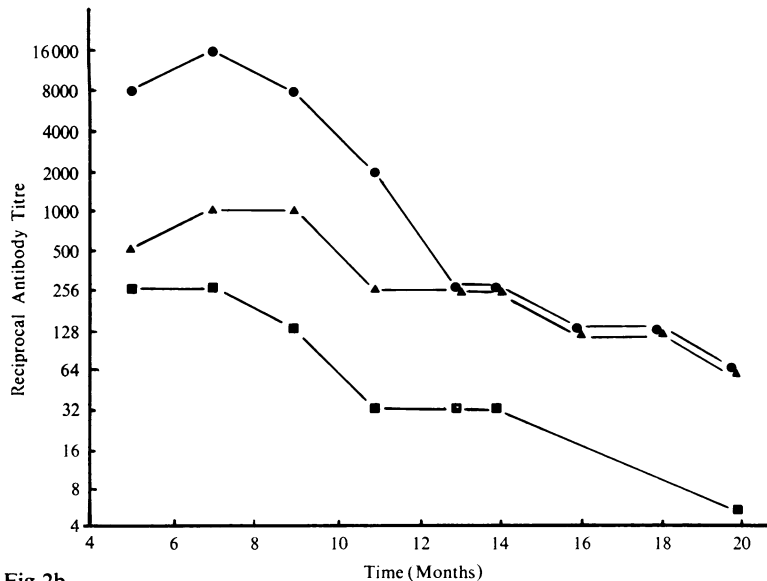
plasma sera before and after absorption with various antigens. Absorption with whole parasites, either formolized or unfixed, resulted in a removal of dye test, complement-fixation, IgG/IgM-immunofluorescent and IgM antibodies whilst the HA antibody was unaffected. When sensitized cells were used for absorbing the sera, HA antibody was removed, the dye test, complement-fixation, IgG/IgM-immunofluorescent, and IgM titres remaining unchanged.

Figures 2a and 2b show antibody curves from individual cases selected from the series; fig 2a shows representative curves and fig 2b is less typical in that the indirect haemagglutination test curve never rises to a higher level than that of the dye test.

Discussion

A satisfactory serological diagnosis of toxoplasmosis requires the demonstration of a four-fold or greater change in antibody titre. However, as shown in fig 1, the titres obtained in the majority of tests have already reached high levels by about two months, and high titres may persist for months or

Fig 2b

Fig 2   *Antibody curves in two cases of glandular toxoplasmosis Dye test, ●; haemagglutination test, ▲; the IgM-IFA test, ■.*
a *typical relationship*
b *atypical relationship.*

years. As it is unusual to obtain sera before this time, it is often difficult to demonstrate a rise in titre. There is therefore a special need to make the maximum use of a single serum and this can be done by carrying out two or more tests giving different types of information. The frequency with which toxoplasma antibodies are found in the general population, about 30% in England and the USA (Beattie, 1957; Feldman and Miller, 1956), also creates a need for tests which distinguish between these antibodies and those produced as a result of recent infection. Sabin, for example, as early as 1949 suggested that the complement-fixation test (Warren and Russ, 1948) could be a valuable additional test for proof of active infection, since the complement-fixation test (using soluble antigen) reverted to negative with the subsidence of the infection.

The characteristics of the curves in fig 1 suggest three different types of antigen-antibody reaction in the serological methods used in the present series. The indirect haemagglutination curve is distinct from the curves of the complement-fixation, dye test, direct haemagglutination, and IgG/IgM-immunofluorescent antibody tests, all of which run closely parallel and appear to measure the same antibody. The third type of reaction, the IgM-immunofluorescent antibody test, differs from others in that a particular class of antibody (IgM) is involved.

Absorption of high-titre antitoxoplasma sera (table IV) by intact parasites indicates that the surface or cell-wall antigen combines with the antibody reacting in the complement-fixation, direct agglutination, dye, and immunofluorescent antibody tests, but not with HA antibody. Fleck (1961), using a toxoplasma-infected mouse peritoneal exudate, removed complement-fixation and dye test *and* HA antibodies. The presence or absence of HA antigen on the intact cell wall of the parasite has important implications in toxoplasma serology as will be discussed later, but the peritoneal exudate in Fleck's experiment may well have contained soluble antigen. Red cells sensitized with such an antigen are capable of absorbing HA antibody (as was shown by Fleck), the dye, complement-fixation, and IgM-immunofluorescent antibody tests being unaffected (table V).

Further evidence for the presence of two antigens came from Lunde and Jacobs (1967). Predominantly indirect haemagglutination test antibodies resulted following immunization of a rabbit with a 40 000 rpm supernatant fraction and predominantly dye test antibodies from immunization of a rabbit with toxoplasma 'ghosts' after cell sap had been extracted by lysing the parasites in distilled water. The

concept, therefore, of cell-wall antibody and HA antibody corresponding to the two antigens may be used in discussing the toxoplasma antibody response. Several reports comparing the haemagglutination and dye test titres have appeared in the literature, some claiming a close correlation between the two (Jacobs and Lunde, 1957; Knierim, Niedmann, and Thiermann, 1960; Thorburn and Williams, 1972; Tönder, Closs, and Digraves, 1974), others reporting varying degree of discrepancy (Mitchell and Green, 1960; Lewis and Kessel, 1961; Chordi, Walls, and Kagan, 1964). Both groups of workers can justify their conclusions but the reported studies probably reflect the limitations imposed by their choice of material and may at times be misleading. Although two different antibodies are being measured by the tests, a large number of cases fall in the group in which the cell-wall antibody runs parallel with and more or less equal to the indirect haemagglutination test, but our work has shown that differences between the two tests occur mainly early in the infection and at a later stage, both periods when sera are less commonly tested.

The third type of antibody, the IgM determined by FAT using whole formalin-fixed parasites as antigen, is one produced against the surface or cell-wall antigen and not the soluble antigen (table V). The titre of this antibody remains raised for a number of months and then gradually falls to become negative in 18 months or less in the majority of cases. Since this antibody persists beyond the period of illness, as was also found by Remington *et al* (1968) and Thiermann and Stagno (1971), its value is somewhat diminished. Nevertheless, especially used in combination with other tests, it is valuable in that a higher titre, 1:64 or greater, suggests a recent infection and a negative result a past infection, probably one that occurred at least 18 months before (tables I and III).

A low indirect haemagglutination test titre combined with a cell-wall antibody titre at least eightfold higher and a high level of IgM indicates a relatively recent infection, of less than four months' duration (fig 1). Later, at about six to eight months the indirect haemagglutination test titre becomes equal to or greater than the cell-wall antibody titre and at the same time, there is a fall in the IgM antibody. It has been found possible in practice to predict the approximate onset of the disease by comparing the titres obtained in the various tests on a single serum. The antibody curves for individual cases of glandular toxoplasmosis illustrate the validity of the interrelationship and its usefulness, but inevitably in some the antibody curve will not follow the usual course (fig 2b). However, for all

3

glandular cases, the HA to cell-wall antibody titre difference early in the disease has been a constant finding. Fairchild, Greenwald, and Decker (1967), in an evaluation of the indirect haemagglutination as a test for toxoplasmosis, noted a similar discrepancy between the antibody titres in the dye and indirect haemagglutination tests in the early stages of glandular toxoplasmosis. These differences can also be shown in the early stages of accidental laboratory infections (Kayhoe, Jacobs, Beye, and McCullough, 1957; Frenkel, Weber, and Lunde, 1960). Even negative HA results have been reported on rare occasions in the presence of a high dye test titres (Jacobs and Lunde, 1957; Fairchild et al, 1967; Miller and Brown, 1969; Karim and Ludlam, 1973). Thorburn and Williams (1972) and Tönder et al (1974) seem to regard a positive indirect haemagglutination test as due mainly to IgM antibody. However, as we have found high HA titres in old cases (tables II and III) when IgM antibody was not to be expected (and indeed when cell-wall IgM was virtually absent), and, as high titre HA antibody can be found not only in the mother but also in her uninfected infant (to be published), it would appear that the IHAT is also a sensitive indicator of IgG antibody.

The absence of HA antigen on the cell surface of the toxoplasma and its location in the cytoplasm may partly explain the observed sequence of antibody production in toxoplasmosis.

Antibody tests in ocular toxoplasmosis (table II) normally showed an IHAT titre equal to or greater than cell-wall antibody and an IgM titre low or negative even in the acute episodes, findings resembling those in patients recovered from glandular toxoplasmosis (table III). This supports the idea that ocular toxoplasmosis in adults is not normally a new infection (Jacobs, Cook, and Wilder, 1954;

Frenkel and Jacobs, 1958). In eye infections there appears to be no case for a combination of tests and a single test either for IHA or cell-wall antibody should be sufficient.

In considering the relative merits of these tests the most useful for routine purposes are the ones measuring cell-wall antibody, since this develops at an earlier stage in the disease and to a higher titre. There is the choice of the dye, complement-fixation, direct agglutination, and immunofluorescent antibody tests.

The dye test has its well known defects, but is reliable in experienced hands and has the support of many years of use. However, the complement-fixation, direct agglutination, and IgG/IgM-immunofluorescent antibody tests have the advantage that the antigen can be stored and if necessary provided from a central source. The complement-fixation test used, essentially that of Fulton and Fulton (1965), differs from many used in the past in that it consists of whole parasites and tests for cell-wall antibody rather than of an antigen consisting solely or largely of soluble antigen. It is a test that could be readily carried out in laboratories where viral complement-fixation tests are done routinely by microtitre techniques. The direct agglutination test has the merit of great simplicity, but the requirements for antigenic purity are more demanding and the greater quantity of antigen needed may be a practical problem. The IgG/IgM-immunofluorescent antibody test is useful when not many tests are carried out and when there is already the experience and equipment for this type of technique. The choice of method will therefore vary from laboratory to laboratory.

When a high borderline titre is obtained with one of these tests it may be useful to perform the indirect haemagglutination and/or IgM-immunofluorescent
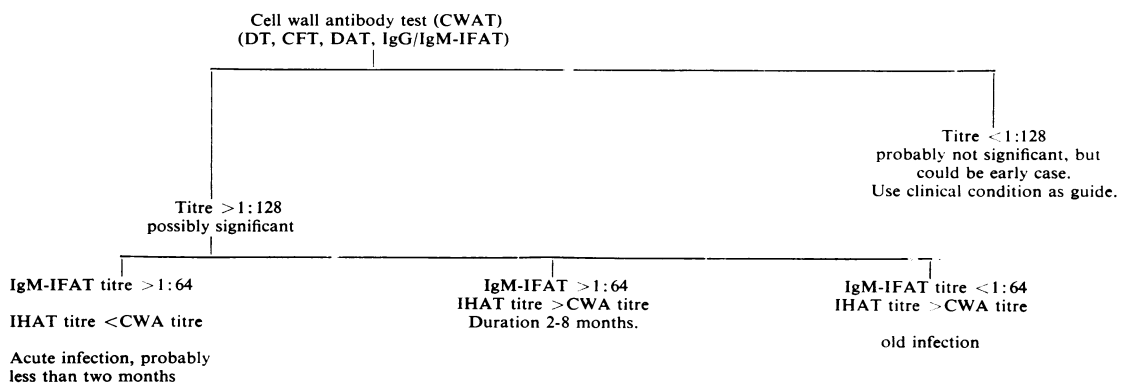

Fig 3 *Scheme for determining the stage of disease in toxoplasmosis by different serological tests on a single serum.*

antibody tests to determine whether the cell-wall antibody level is due to a current, recently past, or old infection. For example, where the cell-wall antibody titre is borderline, eg, 1:256, a positive IgM titre of, eg, 1:128, would suggest an early or current infection, a negative IgM test a past infection, unrelated to the patient's current condition. A low indirect haemagglutination test titre would suggest an early infection, a high titre one of longer duration. Figure 3 summarizes the use and interpretation of the tests. The titres mentioned are rather arbitrary, but have proved useful in this laboratory and of course the clinical condition must often influence the choice of tests and the interpretation of the results.

We believe that the methods described here and the interpretation of the results of tests carried out on single sera will provide useful information regarding the stage and duration of infection in toxoplasmosis and will therefore be of value in the diagnosis of the disease.

References

Beattie, C. P. (1957). Clinical and epedemilogical aspects of Toxoplasmosis. *Trans. roy. Soc. Trop. Med. and Hyg.* 51, 96-103.

Beverley, J. K. A., and Beattie, C. P. (1952). Standardization of the dye test for toxoplasmosis. *J. clin. Path.*, 5, 350-353.

Bradstreet, C. M. P., and Taylor, C. E. D. (1962). Technique of complement-fixation test applicable to the diagnosis of virus diseases. *Mth. Bull. Minist. Hlth*, 21, 96-104.

Chessum, B. S. (1970). Examination of sera for toxoplasmosis antibody using immunofluorescence. *J. med. Lab. Technol.*, 27, 49-54.

Chordi, A., Walls, K. W., and Kagan, I. G. (1964). Studies on the specificity of the indirect haemagglutination test for toxoplasmosis. *J. Immunol.*, 93, 1024-1033.

Couzineau, P., and Baufine-Ducroq, H. (1970). Agglutination directe des toxoplasmes. *Ann. Biol. clin.*, 28, 411-415.

Dropsy, G., Carquin, J., and Croix, J.-C. (1971). Techniques de mise en évidence des anticorps de type IgM dans les infections congénitales. *Ann. Biol. clin.*, 29, 67-73.

Fairchild, G. A., Greenwald, P., and Decker, H. A. (1967). An evaluation of the indirect haemagglutination test for toxoplasmosis. *Amer. J. trop. Med. Hyg.*, 16, 278-283.

Feldman, H. A., and Miller, L. T. (1956). Serological study of toxoplasmosis prevalence. *Amer. J. Hyg.*, 64, 320-335.

Fleck, D. G. (1961). Serological tests for toxoplasmosis. *Nature (Lond.)*, 190, 1018-1019.

Fletcher, S. (1965). Indirect fluorescent antibody technique in the serology of *Toxoplasma gondii. J. clin. Path.*, 18, 193-199.

Frenkel, J. K., and Jacobs, L. (1958). Ocular toxoplasmosis, pathogenises, ciagnosis, and treatment. *Amer. med. Ass. Arch. Ophthal.*, 59, 260-279.

Frenkel, J. K., Weber, R. W., and Lunde, M. N. (1960). Acute toxoplasmosis. *J. Amer. med. Ass.*, 173, 1471-1476.

Fulton, J. D., and Fulton, F. (1965). Complement fixation tests in toxoplasmosis with purified antigen. *Nature (Lond.)*, 205, 776-778.

Fulton, J. D., and Turk, J. L. (1959). A direct agglutination test for *Toxoplasma gondii. Lancet*, 2, 1068-1069.

Goldman, M. (1957). Staining *Toxoplasma gondii* with fluorescein-labelled antibody. II. A new serological test for antobodies to toxoplasma based upon inhibition of specific staining. *J. exp. Med.*, 105, 557-573.

Jacobs, L., Cook, M. K., and Wilder, H. C. (1954). Serologic data on adults with histologically diagnosed toxoplasmic chorioretinitis. *Trans. Amer. Acad. Ophthal.*, 58, 193-200.

Jacobs, L., and Lunde, M. N. (1957). A haemagglutination test for toxoplasmosis. *J. Parasit.*, 43, 308-314.

Jennis, F. (1966). A simplified haemagglutination test for toxoplasmosis using pyruvic aldehyde treated cells. *Aust. J. exp. Biol. med. Sci.*, 44, 317-322.

Karim, K. A., and Ludlam, G. B. (1973). Haemagglutination test for toxoplasmosis. (Letter) *J. clin. Path.*, 26, 162.

Kayhoe, D. E., Jacobs, L., Beye, H. K., and McCullough, N. B. (1957). Acquired toxoplasmosis: observations on two parasitologically proved cases treated with pyrimethamine and triple sulphonamides. *New Engl. J. Med.*, 257, 1247-1254.

Kelen, A. E., Ayllon-Leindl, L., and Labzoffsky, N. A. (1962). Indirect fluorescent antibody method in serodiagnosis of toxoplasmosis. *Canad. J. Microbiol.*, 8, 545-554.

Knierim, F., Niedmann, G., and Thiermann, E. (1960). La reacción de hemaglutinacion aplicada al diagnóstico serológico de la toxoplasmosis. *Biol. Chil. Parasit.*, 15, 48-50.

Lewis, W. P., and Kessel, J. F. (1961). Hemagglutination in the diagnosis of toxoplasmosis and amebiasis. *Arch. Ophthal.*, 66, 471-476.

Lunde, M. N., and Jacobs, L. (1967). Differences in toxoplasma dye test and hemagglutination antibodies shown by antigen fractionation. *Amer. J. trop. Med. Hyg.*, 16, 26-30.

Miller, L. H., and Brown, H. W. (1969). The serologic diagnosis of parasitic infections in medical practice. *Ann. intern. Med.*, 71, 983-992.

Mitchell, R. G., and Green, C. A. (1960). The haemagglutination test for toxoplasma antibodies. *J. clin. Path.*, 13, 331-335.

Remington, J. S., Miller, M. J., and Brownlee, I. (1968). IgM antibodies in acute toxoplasmosis. II. Prevalence and significance in acquired cases. *J. Lab. clin. Med.*, 71, 855-866.

Sabin, A. B. (1949). Complement fixation test in toxoplasmosis and persistence of the antibody in human beings. *Pediatrics*, 4, 443-453.

Sabin, A. B., and Feldman, H. A. (1948). Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (Toxoplasma). *Science*, 108, 660-663.

Steen, E., and Kåss, E. (1951). A new toxoplasma antigen for complement fixation test. *Acta. path. microbiol. scand.*, 28, 36-39.

Thalhammer, O. (1956). Über ein neues, haltbares Antigen für KBR und Hauttest auf Toxoplasmose. *Mschr. Kinderheilk.* 104, 110-112.

Thiermann, E., and Stagno, S. (1971). Demonstración de anticuerpos IgM en casos de toxoplasmosis aguda y crónica. *Bol. Chil. Parasit.*, 26, 55-56.

Thörburn, H., and Williams, H. (1972). A stable haemagglutinating antigen for detecting toxoplasma antibodies. *J. clin. Path.*, 25, 762-767.

Tönder, O., Closs, O., and Digranes, A. (1974). Comparison of the Indirect Haemagglutination and Dye Test for Detection of Antibodies to *Toxoplasma gondii. Scand. J. infect. Dis.*, 6, 63-68.

Warren, J., and Russ, S. B. (1948). Cultivation of Toxoplasma in embryonated egg: an antigen derived from chorioallantoic membrane. *Proc. Soc. exp. Biol. (N.Y.)*, 67, 85-89.

World Health Organization (1969). Toxoplasmosis. *WHO Techn. Rep. Ser.*, 431, 18-19. HMSO, London.