Serology of Toxoplasmosis

SIR,—Dr. J. D. Fulton and Dr. A. Voller compare the titres obtained with the indirect fluorescent antibody titration (I.F.A.T.) with those of other serological methods for the titration of toxoplasma antibodies (7 November, p. 1173). The figures obtained with the I.F.A.T. show a close agreement with those of the dye test throughout the range 1/8 to 1/4,000. I have demonstrated a very similar agreement¹ and the details of the techniques and results are pending publication.²

In the past six months I have used the I.F.A.T. routinely as a diagnostic method. It is simple to perform, reliable, and easy to read with the dark-ground ultra-violet microscope. However, I would disagree with Fulton and Voller that "the reagents are commercially available." Would that this were so. Or, at any rate, suitable reagents. To my mind, the success of any fluorescent antibody technique depends on a complete absence of non-specific staining from the controls. This desirable state is much less likely to be achieved with commercial preparations than with purified fluorescent antibody made in the laboratory. In my experiments Sephadex separation was used to prepare all gammaglobulins, both for primary immunizations of rabbits and for conjugation. Both preparations therefore enjoyed a fairly high degree of immunoelectrophoretic purity.

Manufacturers of fluorescent antibodies rarely state precisely how their products are made. It would seem, however, that salt-fractionation methods are widely used commercially to produce crude globulins for immunization and conjugation.

Since the preparation of a pure fluorescent anti-human gammaglobulin in the laboratory is a time-consuming task, I decided to examine a typical commercial product of British manufacture. The fluorescent antibody, stored according to the maker's instructions, was tested in June 1964, well before its expiry in March 1965. To reduce non-specific staining, absorption with rat-liver powder was used in preference to the more effective, but much less convenient, technique of absorption with toxoplasma suspensions or infected organs. Serial dilutions of the fluorescent antibody were made and each was absorbed separately for two hours with one-third of its volume of moist rat-liver powder. For comparison, the pure preparation was diluted to its known optimum concentration (1/30) and absorbed for 1 hour with rat-liver powder.

Unfixed toxoplasma smears were exposed for consecutive 15-minute periods to (1) patient's serum dilution (or saline control), (2) saline washing with agitation, (3) fluorescent antibody dilutions, and (4) saline washing with agitation. The table below shows the results. The degree of fluorescence was graded and (—) means that

only blue auto-fluorescence with no trace of green is observed.

Although the commercial preparation is often recommended for use neat, it is notable that it has to be diluted to 1/12 before the saline control ceases to fluoresce. Even at this dilution normal serum at dilutions of 1/8 and 1/16 show considerable fluorescence. At higher dilutions aimed at reducing the non-specific staining the sensitivity at limiting dilutions of the strongly positive serum is impaired. The results with pure fluorescent antibody are in marked contrast. Saline controls and both dilutions of normal serum yield only blue autofluorescence: the response at limiting dilutions of the positive serum is good and correlates well with the dye test.

The results quoted are borne out by the impressions of several colleagues who work with different antigens and have experienced similar behaviour with commercial fluorescent rabbit anti-human gammaglobulin.—I am, etc.,

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REFERENCES

- ¹ Fletcher, S., *Proceedings of the Pathological Society*, Birmingham, July 1964.
- ² — *J. clin. Path.*, in press.

Unusual Object

SIR,—Recently I was called to a little girl of 10 months who had a pyrexia of 100° F. (37.8° C.) and had been vomiting for two days. She was a measles contact, which explained the pyrexia, but the vomiting was persistent, and occurred within a short time of ingesting even small quantities of solid or liquid. There was no diarrhoea and she did not appear particularly ill. On the third day the rash appeared, and she vomited with some difficulty a bean-shaped solid 5 cm. long, 15 mm. wide, and 6 mm. thick. It looked, felt, and smelt like cheese, but as the child had eaten no cheese it was assumed to be caseinated milk which had been causing an obstruction in the pylorus (see Fig.).

It would be interesting to know if any of your readers have had similar experiences of milk solidifying into an obstructive foreign body.—I am, etc.,

Clebury Mortimer,
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P. N. STANBURY.

Bruecke Tube for Intestinal Decompression

SIR,—The rubber Bruecke tube for intestinal decompression has been in constant use in this hospital since 1954 and has definite advantages over the rigid tube which was devised by Savage.¹ The end has many perforations, but no balloon (see Figure) like the tube described by Mathieson and Dudley.² It has been used on a large number of patients with intestinal obstruction and has been found to be invaluable, both in facilitating the operation and in making the post-operative course much smoother. The procedure that is adopted is to decompress the distended small intestine, provided there are not extensive adhesions between the loops of bowel, immediately after the abdomen is opened and before proceeding to deal with the cause of the obstruction.

To avoid soiling of the peritoneal cavity while using the tube it is very important to adhere strictly to various details in technique.

(1) The inner plastic tube supplied tends to get blocked and it has been found easier to use the outer rubber tube by itself without the inner tube.

(2) The outer tube is connected, by a large connexion, to the tubing from the theatre suction apparatus. The side tube is clamped with a large pair of artery forceps and allowed to lie in a kidney dish. The dish is left lying on an extra towel placed over the lower half of the operating table. The suction apparatus is not turned on.

(3) The highest available jejunal loop is delivered and the proximal end lightly clamped with an intestinal clamp. About 12 in. (30 cm.) is emptied of its contents and a second clamp is applied distally. A purse-string suture is introduced near the proximal clamp and a half-knot is tied. The bowel on either side of the purse-string suture is held up with Poirier forceps and a small opening is made within the circle of the purse-string suture. The Bruecke tube is introduced through the hole for about 6–8 in. (15–20 cm.) and the purse-string half-knot is pulled tight and held with small artery forceps. The Poirier forceps and intestinal clamps are removed.

(4) The suction is turned on and the tube is slowly advanced until the whole length of the

	Absorbed Dilutions	Initial Exposure to :					
		Saline (Control)	Normal Serum		Serum of Dye Test Titre = 1/4,000		
			1/8	1/16	1/1,000	1/2,000	1/4,000
I. Commercial fluorescent rabbit anti-human gammaglobulin	1/6 1/12 1/24 1/48	++ ++ — —	+++ ++ ± ±	++ ++ — —	++++ ++++ ++ ±	++++ ++ ± —	++ ± — —
II. Purified fluorescent antibody	Absorbed optimal dilution (1/30)	—	—	—	+++	++	±