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.,

STEWART FLETCHER.

Pathology Department,
University of Glasgow,
Glasgow W.1.

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,
Nr. Kidderminster,
Worcs.

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)	—	—	—	+++	++	±

small intestine is pulled over it. If the mucosa is sucked into the holes of the tubing the negative pressure is relieved by releasing the artery forceps on the side tubing. The presence of the kidney dish described above avoids soiling of the towelling by any small amount of intestinal contents which might occasionally come out of the side tubing. When the whole length of small intestine is decompressed the intestine is then pulled off the tubing, and later the tube itself is slowly withdrawn from the purse-string opening. During the withdrawal of the tube the assistant is very careful to handle the tube only and he becomes unsterile, whereas the surgeon handles the intestines only and remains sterile. As the last 6 in. (15 cm.) of tubing is withdrawn it is important that there should be a negative pressure in the tubing to avoid spillage from either the intestine or tube itself. As the terminal part is withdrawn the artery forceps holding the half-knot of the purse-string are lifted up so that the purse-string tightens as the tube is removed. Poirier forceps are then applied on either side of the purse-string suture, the artery forceps are removed, the mucosa is invaginated with fine dissecting forceps, and the purse-string is tied.

The tube is available in various sizes so that it can be used for children or infants as well. It is manufactured by Willy Rusch, K.G., of Rommelshausen bei Stuttgart, Germany.—I am, etc.,

Mary Calvert Holdsworth FRANK I. TOVEY.
Memorial Hospital,
Mysore City, S. India.

REFERENCES

- ¹ Savage, P. T., *Brit. J. Surg.*, 1960, 47, 643.
- ² Mathieson, N. A., and Dudley, H. A. F., *Lancet*, 1963, 1, 695.

New Look at Toxaemia

SIR,—Your recent editorial, "A New Look at Toxaemia of Pregnancy" (22 August, p. 459) touched on certain researches by Berger^{1,2} that purported to show that the aetiological factor in this state was derived from the ischaemic placenta. The same author collaborated in another study³ that claimed that placenta and myometrium were responsible for the production of renin, a claim that I showed could not be substantiated.⁴ Neither does it appear that his thesis of a placental ischaemic factor in the aetiology of pre-eclampsia is valid, and that it can replace the utero-renal reflex in the causation of the disease. By overstretching the non-pregnant uterus renal ischaemia was evoked.⁵ Amoroso,⁶ by compressing the conceptus between finger and thumb, produced the same change contemporaneously and in proportion to the pressure exerted. Likewise Berger, repeating Amoroso's experiments, caused a rise of blood-pressure during the manoeuvre.⁷ None of these changes could be brought about if the kidney had been previously denervated. It was obvious therefore that in the normal animal—i.e., with kidneys *in situ* and with their innervation intact—blood-pressure changes were initiated akin to those in pregnancy nephropathy by nociceptive stimulation. These findings help to explain the clinical accompaniments of the tense, tender uterus of concealed accidental haemorrhage, that, giving rise to nociceptive stimuli, cause the renal artery to constrict⁸ and on occasion produce significant vasospasm in the peripheral limb vessels⁹—changes that yield to denervation also.

Berger, by applying "Z" sutures to the myometrium at the placental site (and in-

cluding the placenta), provoked a considerable nociceptive stimulation conducted along the nerve sheaths of the innumerable blood-vessels in that area—a stimulus that exceeded a threshold and caused a rise of blood-pressure. (Outside the placental area, because of the limited vascularity, the stimulus was not thus effective.) Z sutures produce nociceptive compression, whose effects have already been noted. The extent of the pressure they exert is to be gauged by their multiple application to 3 to 10 conceptuses and the fact that they reduce the foetal blood supply by 30%—a no mean application of force. They are reflections, therefore, though by employing exaggerated means, of the clinical findings in concealed accidental haemorrhage. These changes can be brought about in an animal deprived of its kidneys and therefore without the antihypertensive protection of the renal medulla⁹ which could destroy hypertensive material evoked at the termination of the axonus on sympathetic nociceptive stimulation. Hickler¹⁰ has shown that the hypertensive response evoked by tilting occurs only if there is an excess of norepinephrine or epinephrine in the circulation of the subject even if previously adrenalectomized, presumably due to discharge of these catecholamines at the effector site of the sympathetic nervous system and is a generalized reaction. He has thus provided the explanation of the hypertension elicited in Berger's experiments where sympathetic stimulation had been overtly applied.

By cross-circulation experiments Berger has proved their presence. The data provided, though of much interest, provide no aetiological evidence in pre-eclampsia. Renin (of renal ischaemic origin) is present in the uterus and placenta in pregnant animals⁴ and renal cortical extract is capable on its own, when injected into renoprival animals, of producing all the signs of the toxaemic syndrome.¹⁰⁻¹² There is no need to invoke a non-renal cause to explain the aetiology, especially when it is realized that these are physiologically explicable as based on nervous and not on primary humoral mechanisms exaggerated in the absence of the kidney.—I am, etc.,

Worthing, Sussex.

JOHN SOPHIAN.

REFERENCES

- ¹ Berger, M., and Cavanagh, D., *Amer. J. Obstet. Gynec.*, 1963, 87, 293.
- ² — and Boucek, R. J., *ibid.*, 1964, 89, 230.
- ³ Gross, F., Schaechtelin, G., Ziegler, M., and Berger, M., *Lancet*, 1964, 1, 914.
- ⁴ Sophian, J., *ibid.*, 1964, 1, 1445.
- ⁵ — *Toxaemias of Pregnancy*, 1953. Butterworth, London.
- ⁶ Amoroso, cited by Sophian, J., *ibid.*, p. 45.
- ⁷ Louw, J., personal communication, 1954.
- ⁸ Combrink, P. B., *J. Obstet. Gynaec. Brit. Cweth*, 1964, 71, 461.
- ⁹ Muirhead, E. E., *Hypertension*, 1959, 8, 21.
- ¹⁰ Ascher, A. W., and Anson, S. G., *Nature (Lond.)*, 1963, 198, 1097.
- ¹¹ Nairn, R. C., Masson, G. M. C., and Corcoran, A. C., *J. Path. Bact.*, 1956, 71, 155.
- ¹² Giese, J., *Acta path. microbiol. scand.*, 1963, 59, 417.
- ¹³ Hickler, R. B., Wells, R. E., jun., Tyler, H. R., and Hamlin, J. T., *Amer. J. Med.*, 1959, 26, 410.

Postgraduate Diplomas

SIR,—Sir Charles Illingworth's excellent letter (5 December, p. 1458) about the "F.R.C.S." makes welcome reading. The multiplicity as well as the proliferation of postgraduate medical diplomas in this country threatens the future of postgraduate

education and brings the good name of British medicine into disrepute.

A blueprint for a Joint Examination Board already exists in the present Conjoint Examination Board for England and Wales. Surely it is not beyond our powers or even our prestige to devise some means of either extending its present usefulness or reconstituting it altogether to include *all* postgraduate medical and surgical diplomas—M.R.C.P. and F.R.C.S. alike. A new board should also consider the drastic pruning of further diplomas, which seem to sprout with the formation of each new "college."

At present certain specific postgraduate qualifications are registerable with the G.M.C. If *all* future approved diplomas were to be made compulsorily registerable, this might also act as growth-deterrent.

Freedom to seek admission to the college of their choice could still remain the prerogative of prospective candidates. Removing their examination powers with their profit-making and prestige-promoting bias must surely enhance the standing of all our Colleges still further.—I am, etc.,

Chadwell Health Hospital, I. M. LIBRACH.
Romford, Essex.

SIR,—As a young Scottish doctor about to emigrate, I would like to comment upon the arrangements for the new examination for Membership of the Royal College of Physicians of London.

The new London examination seems to be a compromise between the old one and the better one of Edinburgh, and I think it has no advantages over the old examination. The statement issued by the London College on new arrangements for the examination for Membership (*B.M.J.*, 16 May, p. 1306) stated: "It is thought that these changes are sufficiently comprehensive to bring the examination up to date and to remove any hardship which may at present exist in its make-up." In a spasm of misguided enthusiasm for degree hunting (a pastime detrimental to original thought in medicine but encouraged by the profusion of Colleges, diplomas, and degrees), I obtained the regulations for the new examination. What irony there was in the phrase "... remove any hardship ..." ! The price of passing had been increased to 65 or 70 guineas (exclusive of travel and London accommodation) and the price of failing to 10 or 40 guineas according to the point of failure. It remains to be seen whether the majority of candidates fail at the 40- or the 10-guinea stage. If the latter, then some hardship may have been removed, but in general I consider that the fees for this examination—which should be taken 18 months after qualification and which is considered essential to consultant training by all save the income-tax authorities—are excessive. I estimate that in the recent past £35,000 per annum was paid to the College by successful and unsuccessful candidates. Is it necessary for such a large part of the College's income to be obtained at the expense of candidates, 90% of whom will never use its facilities? To the impecunious young married doctor with a family the increase in fees for the new examination, particularly if an annual subscription is to be levied, is an inexplicable hardship. Perhaps the College would publish in detail the accounts for the examination?