A PROVEN CASE OF CONGENITAL TOXOPLASMOSIS

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Congenital toxoplasmosis has been recognized with increasing frequency since it was first described by Wolf, Cowen, and Paige in 1939, and has now been reported from many parts of the world. The first description of a case in this country was given by Jacoby and Sagorin in 1948. Since then a number of others have been reported (Farquhar and Turner, 1949; Wilson and Smith, 1949; Ridley, 1949; Nutt, 1949; Hutchison, 1949; Cathie and Dudgeon, 1949; Wyllie, Fisher, and Cathie, 1950; Farquhar, 1950; Riley and Arneil, 1950; Campbell and Clifton, 1950; Sutton, 1951; Hart, Paulty, Rivers, and Westlake, 1951; Valentine, 1952). Surveys of the incidence of sub-clinical infection by serological methods have also been published (Macdonald, 1950; Fisher, 1951).

The cases so far described in Britain have been diagnosed on clinical and serological grounds, and in 1950 Garnham wrote:

"... It is therefore important to try to clinch the diagnosis in suitable patients by recovering the causative organism either during life (by repeated examinations of body fluids or biopsy material) or post mortem. Serological tests or clinical findings in the present state of knowledge cannot be accepted as final proof."

While few would now regard isolation of the organism as essential for the diagnosis of clinical cases there must still remain an element of doubt unless this can be done. We believe that the case reported here is the first in this country in which the diagnosis has been confirmed by isolation of the organism.

Case History

Mrs. A. W. F., aged 24, was first seen at the Bedford General Hospital in April, 1946, when she was admitted for a miscarriage at about four months. After delivery a fibroid tumour was felt, but no treatment was advised. In 1947 she was delivered of a full-term female infant (birth weight 6 lb. 6 oz.). She was seen again in November, 1949, complaining of a vaginal discharge which had been present since the birth of the baby. In June, 1950, dilatation of the

cervix and curettage of the uterus were carried out. A soft, anteverted and bulky uterus was found. The endometrium was hyperplastic, but no microscopical examination was made. In February, 1951, she was delivered of a healthy, full-term, female infant (birth weight 7 lb. 8 oz.). She attended the orthopaedic department in January, 1952, for spinal curvature. As a child she had worn a spinal brace. An old disc lesion was suspected and manipulation tried without much benefit. She attended the antenatal clinic on September 1, 1952, her expected date of delivery being February 25, 1953. The vaginal discharge had continued despite the dilatation and curettage, and was still present at that time. From the beginning of her pregnancy she had had severe vomiting and diarrhoea with abdominal pain. The pain started in the rectum and radiated to the epigastrium. The only other unusual incident which she could recall was an insect bite on the leg which she received about the second month of pregnancy. This caused a small swelling with a central puncture wound and caused severe irritation. A clear, watery discharge issued from the puncture for about three days. In February, 1953, there was still a reddish-brown mark on the skin. The identity of the insect which caused the bite is not known.

On December 8, 1952, she was admitted to hospital with vaginal bleeding for one day and backache. At this time she also had a cold. It was thought that the abdomen was larger than it should be according to her expected date of delivery. The membranes were intact and there was a brownish vaginal discharge. Her baby was born spontaneously on December 22. The infant was said to have taken a few gasping breaths and died in 15 minutes.

The vaginal discharge present since 1947 cleared up spontaneously shortly after delivery, and two months later she remained quite healthy.

There were a large number of mice in and around the house in which she was living during her pregnancy, and there were also rats in the neighbourhood. A cat had been obtained to keep away the mice, but she denied having nursed or handled it. This cat was subsequently destroyed as too prolific. A male cat was then obtained and is still in good health. During her pregnancy she prepared rabbits for her family, but did not clean or skin them.

Necropsy Report on the Premature Infant

The examination was made 11 hours after delivery. The placenta had been discarded. The body was of a female infant weighing 1,140 g. and 25 cm. from crown to rump. There was generalized cyanosis and very obvious tenseness and prominence of the abdomen, in the skin of which were a few petechial haemorrhages. The peritoneal cavity contained deep yellow, almost gelatinous fluid, but there was none in the pleural cavities, and the pericardium contained only a small amount. Apart from a few petechiae on its external surface the heart was entirely normal. The aorta and great vessels were also healthy and showed no congenital deformities. The tongue and mouth were normal. There was some sticky mucus in the trachea and main bronchi. Both lungs were small and collapsed and selected portions of all lobes sank in water. A rim of possibly aerated lung tissue was present along the anterior margin of both lungs, but even portions of this did not float. The spleen was considerably enlarged, weighing 45 g. It was extremely dark, but showed no external abnormality, and on section was a uniform dark plum colour, with no obvious structure. In the sternum the bone marrow was dark red and appeared normal for a newly born infant; the vertebral marrow was similar. There was no lymph node enlargement. The liver weighed 130 g. and appeared larger than normal. It was very dark in colour and showed a slight unevenness of the capsular surface. When sectioned the structure could not be made out on account of the very dark, brownish-red colour which obscured the normal markings. The gall-bladder and pancreas appeared normal. The stomach showed a few small mucosal haemorrhages, but apart from this the alimentary tract appeared healthy. The kidneys were of normal size and weighed 5 g. each. They were extremely congested apart from a thin rim just beneath the capsule. The renal veins were not thrombosed. The ureters, bladder, and genitalia showed no abnormality. Apart from congestion of the medulla the suprarenals were healthy. The thyroid and thymus were likewise normal. No centre of ossification was found at the lower end of the femur.

The scalp showed little abnormality, but there was some bruising and oedema of the underlying tissues. There was no noticeable hydrocephalus and the skull itself showed no external abnormality. On reflecting the skull bones, however, a stream of deep yellow fluid escaped. It was found that the cerebral hemispheres were virtually non-

existent, being almost completely replaced by yellow fluid contained within a paper-thin membrane over the surface of which ran small blood vessels. It was this fluid which had escaped from an accidental puncture wound made while opening the skull. Scattered irregularly over the surface of the cystic spaces which represented the cerebral hemispheres were numerous yellow flecks of varying size and irregular shape. They measured from less than a millimetre to a centimetre or so across. The brain-stem and cerebellum were relatively well preserved though clearly grossly abnormal. Throughout they showed areas of ochre yellow softening and necrosis with localized areas of haemorrhage and congestion, both externally and on section. The cervical portion of the spinal cord showed similar changes diminishing in intensity from above downwards, and the thoracic and lumbar segments appeared normal. The dura mater of the spinal theca was likewise healthy.

Both eyes showed some yellowness of the sclera and opacity of the lens. On section there was massive haemorrhage into the vitreous chamber on both sides.

Microscopical Examination

Liver.—There was very marked congestion with separation of the liver cords and extensive extensive medullary haemopoiesis throughout. There were a few small areas of haemorrhage and in some places the cellular infiltration contained a considerable number of polymorphs, but neither here nor elsewhere were any *toxoplasma* found.

Kidney.—There was a well-marked neogenic zone and a considerable amount of haemopoietic tissue between the nephrons. There was gross congestion amounting to haemorrhage in places particularly in the pyramids. The tubules and glomeruli appeared normal. No *toxoplasma* were found.

Heart (Left Ventricle).—The muscle was healthy. There was slight infiltration of the epicardial tissues by lymphocytes and plasma cells which were mainly congregated around blood vessels. No *toxoplasma* were found.

Bone Marrow.—The bone marrow was highly cellular and normoblastic. No *toxoplasma* were seen.

Eye.—The sclera showed little abnormality and the conjunctiva was healthy. There was patchy basophilic staining of the lens and the ciliary body showed a moderate infiltration by lymphocytes and plasma cells. The retina had been completely destroyed. Between the choroid and the sclera were a moderate number of plasma

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 1.—Optic cusp of infant showing inflammatory infiltration. Optic nerve cut obliquely top right. Haematoxylin and eosin, $\times 70$.

FIG. 2.—Microglial nodule in brain of infant. Haematoxylin and eosin, $\times 270$.

FIG. 3.—Choroid plexus of infant showing chronic inflammation and hyalinization of capillaries. Nodule of calcium top left. Haematoxylin and eosin, $\times 95$.

FIG. 4.—Proliferative form of *toxoplasma* in infant brain. Azure-eosin, $\times 1,300$.

cells and lymphocytes. Almost the whole of the vitreous chamber was filled with blood and protein-laden fluid. Just behind the lens the blood clot was undergoing organization by fibroblasts and young capillaries. A few granules of calcium were found in this region. Disorganized cells of the choroid were seen streaming out behind the lens into the partially organized blood clot. The optic disc and nerve contained very large numbers of chronic inflammatory cells (Fig. 1). These were mainly lymphocytes and plasma cells and were limited to these two structures. There was practically no infiltration of the surrounding connective tissue. Apart from the inflammatory infiltration the optic nerve appeared normal. Nowhere in the eye or optic nerve could any *toxoplasma* be found.

Brain-stem and Fourth Ventricle.—Throughout the section there was considerable chronic inflammatory exudate consisting of lymphocytes, plasma cells, and microglial cells with occasional polymorphs. There were small foci (microglial nodules of Frenkel) in which the cellular exudate was more dense (Fig. 2). These foci were often related to blood vessels and sometimes perivascular collections of inflammatory cells could be seen. Over the external surface of the brain the cellular exudate was extremely intense and was related to a zone of necrosis of varying depth involving the superficial portion of the brain. In this necrotic zone the brain tissue had been largely destroyed, and there was much fibrinoid material between the necrotic cells. Only the blood vessels remained relatively normal. Also found in the necrotic zone was a fine dusting of calcium particles. Beneath the floor of the fourth ventricle a much deeper zone of necrotic brain tissue was found. The appearance of this zone suggested that it was due to infarction and in this instance the blood vessels were also seriously affected, showing fibrinoid necrosis of their walls. Within the fourth ventricle itself, in addition to the choroid plexus, which was in places normal, there was much chronic inflammation and granulation tissue formation. There was an abundant fibrin exudate and large numbers of red cells. The choroid plexus was in most places heavily involved in the chronic inflammation, and a striking feature was the occasional very marked hyalinization of the capillary walls (Fig. 3). The lumen of the small vessels was greatly reduced thereby and in places almost obliterated. The cellular exudate in the choroid plexus consisted predominantly of plasma cells and lymphocytes. Portions of the cerebellar peduncles also included in the section showed gross necrosis, cellular infiltration, and fine

calcification similar to that within the brain-stem. There were also a few much larger patches of calcification.

Numerous collections of *toxoplasma* were found in this section. They were readily visible in the ordinary haematoxylin and eosin section (Helly fixation) and appeared for the most part as round or oval collections of parasites approximately $15 \times 9 \mu$ across. Between 10 and 20 parasites could be counted in these aggregations (Fig. 4). The individual parasites were round or oval, occasionally semilunar with a central haematoxylin-staining nucleus surrounded by eosinophilic cytoplasm. The distinction between nucleus and cytoplasm was more readily appreciated by the use of one of the Romanowsky staining methods, but could be seen quite clearly in the routine haematoxylin and eosin preparation. In none of these collections could anything in the nature of a capsule be found either by the periodic-acid-Schiff or Wilder's silver techniques. In addition to these localized collections numerous intracellular forms could be found, the parasites being present singly or up to six in a cell (Fig. 5). In these

FIG. 5.—Free and intracellular forms of *toxoplasma* in infant's brain. A collection of free forms to left of mid-line and three cells containing parasites to right of mid-line. Azure-eosin, $\times 1,300$.

instances the characteristic crescentic or semilunar shape was more obvious. In places where the parasites were numerous one could feel fairly certain that free forms were present in the surrounding tissue. These individual parasites measured $4-5 \mu$ in length and $2-3 \mu$ in breadth.

Animal Inoculations

A provisional diagnosis of congenital toxoplasmosis was made and portions of the brain were ground in a Griffiths tube to make an approximately 10% w/v emulsion with broth. Two mic

were inoculated with this suspension on December 23, 1952. After an interval of about 10 days the animals became sluggish in movement and showed roughening of their coats. On January 5, 1953, they were killed and portions of brain emulsified and injected intracerebrally into two further mice and intraperitoneally into three mice. Sections of the brains were also made and showed encephalitis; *toxoplasma* were found. On January 16 the two mice inoculated intracerebrally were showing symptoms similar to those noticed in the first-passage mice, and in order to increase the chances of establishing the strain one of these mice was

changes. These took the form of perivascular infiltration by lymphocytes and plasma cells. There were also small localized areas of microglial proliferation. Scattered throughout the section could be found numerous collections of *toxoplasma*. They were much more numerous in the brains of the second-passage mice than in those of the first, in which they were found with difficulty. With repeated passage the organisms became more and more numerous and the inflammatory reaction more acute. The animals died more rapidly with the repeated passages. After the eighth passage death occurred regularly in six

sent with the permission of the Home Secretary to Sheffield for further passage. From this stage animal passage was continued independently in both Sheffield and Bedford. A diagram showing the direct line of passage omitting numerous side lines is seen above. At a later date infected mouse brain was inoculated into fertile hens' eggs previously incubated for 10 days. After a further five days' incubation the chorio-allantoic membrane showed small white nodules which contained numerous *toxoplasma* in the free form.

The brains of the affected mice when examined microscopically showed very marked inflammatory

to seven days. In the second-passage mice some difficulty was experienced in deciding on the precise nature of the toxoplasmic aggregations, for they did not exactly correspond with the descriptions given by Frenkel of either the proliferative or pseudocyst forms. They contained many more organisms than were present in the proliferative forms as found in the brain of the infant. Many contained 50 or more parasites but measured only 20 to 30 μ across. Furthermore they were often seen in close association with areas of inflammation as well as in apparently normal brain tissue. Several showed quite definite argyrophil

"capsules" by Wilder's method (Fig. 6). An enclosing membrane could also be demonstrated by the periodic-acid-Schiff technique. Parasites could not be seen in the livers or spleens of several

FIG. 6.—Pseudocyst in mouse brain. Wilder's silver method, $\times 950$.

mice which had abundant *toxoplasma* in their peritoneal fluid, although there was well-marked round cell infiltration of the portal tracts of the liver. Two mice which were inoculated intracerebrally with spleen and liver of the infant remained healthy and were killed on February 18, 1953. The brains of both these animals were macroscopically and microscopically normal and did not show any *toxoplasma*.

Serological Investigations

Serum from the mother and father and from their two surviving children were examined by Sabin and Feldman's "dye" test as modified by Beverley and Beattie (1952) and by the complement fixation test using infected egg antigen. The results of these investigations are set out in Table I.

TABLE I
RESULTS OF SEROLOGICAL INVESTIGATIONS

	Dye Test	Complement Fixation Test
Mrs. F.: 6/1/53 11/2/53	Positive 1/7 000 " 1/2,000	Positive 1/32 " 1/80 (partial 1/160)
Mr. F.: 11/2/53 J. F., aged 5: 11/2/53 P. F., aged 2: 11/2/53	" 1/14 Negative "	Negative " "

Discussion

The naked eye and microscopical changes in congenital toxoplasmosis have been described in considerable detail by Frenkel and Friedlander (1951) and the findings in our case were substantially similar. In common with many previously reported cases the changes were largely confined

to the central nervous system and the eye. Minor and non-specific abnormalities were found in the other organs.

The main interest in this case lies in the demonstration of the infecting parasite in sections of the brain and in its transmission to laboratory animals. Thus there has been established for the first time a strain of *toxoplasma* derived from a naturally occurring infection in Great Britain.

The parasites were readily visible in routine haematoxylin-and-eosin sections and no great difficulty was experienced in finding them. They were most easily seen in the proliferative form in clusters of 10 to 20. Frankel and Friedlander have drawn attention to the distinction between the proliferative form and the pseudocyst. The main distinctions are that the pseudocyst is considerably larger than the proliferative form, contains many more organisms, and has a limiting membrane which is argyrophilic and positive to the periodic acid-Schiff test. Frenkel and Friedlander insist that care should be taken not to use the term pseudocyst except in the strict sense which they define. The pseudocyst is usually found in apparently normal brain tissue and is characteristic of the chronic stage of the disease. We have found structures resembling the pseudocyst in mouse brain of the second passage with quite considerable inflammatory reaction around them.

Two of us had no previous experience with these organisms and we found it easier to recognize the parasites in sections of the brain than in smears of brain tissue. Free forms do not appear in the peritoneal fluid in the early passages. Despite our inexperience we had no great difficulty in isolating the parasite, but in this it is probable that we were extremely fortunate. It is likely that our success was due to the fact that the infection in the infant was in an acute phase with large numbers of still viable parasites present. In many cases the disease process is arrested *in utero* by the transfer of maternal antibodies to the foetus, and this accounts for the failure to isolate the parasite in such instances. Had the infant not been born prematurely a similar state of affairs might have resulted in our case.

Owing to the small numbers of mice available it was not possible to follow the technique suggested by Frenkel (1949), who recommends the use of two groups of four to six mice each. One of these groups should be inoculated intracerebrally and the other intraperitoneally. Successive passages are then made from these groups. In the case of the cerebral route control is maintained by histological examination. The intraperitoneal route

remains "blind" until the infection is established, when the organisms are readily visible in the peritoneal fluid. The presence of the infection can, however, be demonstrated by the injection of the peritoneal fluid into the brain of other animals. When the peritoneal route is used subinoculations are made every four to six days, at about which time antibodies begin to appear. One advantage of the intraperitoneal route is that larger quantities of material can be used and contaminating bacteria can be destroyed by mixing the material with antibiotics. The peritoneal route is also the most convenient method of keeping a strain of *Toxoplasma* in the laboratory, and forms the source of the suspension used in the dye test. If initial isolation has been by the intracerebral route it should be possible to adapt the parasite to the peritoneal cavity. This may take time, and Gard (personal communication) mentioned one case in which it took six months.

In order to exclude the possibility that the manipulations are exciting a latent infection in stock animals Frenkel suggests the "blind passage" of brain or other organs of the stock animals. The mice in the Bedford laboratory are all obtained from the same breeder, and three blind passages of this type using the intracerebral route failed to produce any infection in the mice used, three mice being used for each passage.

We noticed that in sections of the infant's brain the organisms were more plentiful in the brain tissue at the edges of the necrotic zones and that no organisms could be found within areas of necrosis. We therefore suggest that when selecting brain tissue for animal inoculation completely necrotic areas should be avoided.

In common with other published cases there is little in the mother's history to suggest at what stage of the pregnancy or by what route infection was acquired. There are, however, two features of possible importance in the history. The first is the insect bite received in early pregnancy. The work of Piekarski (1949), van Thiel (1949), Laven and Westphal (1950), and Blanc, Bruneau, and Chabaud (1950) suggests that the parasites may be transmitted by blood-sucking insects such as fleas, lice, ticks, bed bugs, and blood-sucking flies. Such insects are known to become infected when they feed on infected mice. Furthermore, mice may be infected by inoculating them with the ground-up bodies of such insects or by feeding infected animals to mice. The parasites have been found in the body of a tick on a dog belonging to an infected patient (Giroud, Jadin, and Reizes, 1951), but transmission by the bite of such insects has so far not been proved.

It is not uncommon for a history of an insect bite to be obtained in cases of toxoplasmosis, but the danger of extracting such information by the use of leading questions must not be forgotten. There can be no doubt that the mother of this stillborn infant did in fact suffer such a bite, as the mark still remained many months later. In spite of the unusual severity of the bite, she had, however, failed to mention it in her unaided statement. It is therefore more than probable that less severe bites may have passed unnoticed in the published reports of cases in which no mention of them is made. It would seem important, therefore, while recognizing the danger of leading questions, to inquire in subsequent cases about insect bites and when positive answers are obtained to give some idea as to how the information was acquired.

We would also urge the recording in the fullest detail of all incidents which the mother can remember, however trivial they may appear. In this way some common factor may be found which would provide a clue to the method of infection in this mysterious disease. It is for this reason that we draw attention to the second incident in the pregnancy—namely, the cramp-like pains in the abdomen. We did not at first pay much attention to them, although it is clear that the mother herself, who had had two previous pregnancies, regarded them as abnormal. Moreover, a number of cases in the literature have given similar histories (Gard, Magnusson, Wahlgren, and Gille, 1949; Lelong, Le Tan Vinh, Desmonts, and Dupré-Bouteloup, 1951; Mellgren, Alm, and Kjessler, 1952). Since it has been shown (van Thiel) that mice can be infected orally, although with difficulty, it seems possible that these pains and diarrhoea may be of importance. On the other hand an investigation of mothers of normal infants might show a similar incidence of abdominal disturbances. The opportunity for oral infection certainly exists by the contamination of food by the faeces or urine of animals. *Toxoplasma* have been found in the excretions of dogs and cats and also in the tissues of rats (Perrin, Brigham, and Pickens, 1943; Eyles, 1952). Laboratory mice have also been found to harbour the parasite in Switzerland (Mooser, 1950). Pigeons are commonly infected, and Beattie and Beverley (1953) have recently suggested that rabbits may be a source of infection. A low titre of antibodies is found in 28% of the adult population, 35% of slaughterhouse workers, but 65% of rabbit handlers. This may be a false correlation in that rabbit handlers may come into greater contact with other animals such as rats or mice or be more frequently subject to insect bites.

Hares, too, have been shown to have a high incidence of *Toxoplasma* antibodies in Denmark (Christiansen and Siim, 1951). The mother of our case had ample opportunity for contact with animals—two cats, many mice, and possibly rats. Although she bought rabbit for her family on a number of occasions during her pregnancy it had always been cleaned and skinned before purchase.

Summary

The pathological findings in a newly born premature infant with congenital toxoplasmosis are described.

The infecting parasite has been demonstrated in sections of the infant's brain.

Toxoplasma have been transmitted from the brain of this case to laboratory animals. A brief outline of the methods used is given.

Attention is drawn to an insect bite and cramp-like abdominal pains during the pregnancy. It is suggested that full details of even apparently trivial incidents should be recorded in future cases in the hope that they may provide a clue as to the source of infection in this disease.

Some possible sources of infection are briefly discussed.

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