

do so they are often a late manifestation of the disease and the organs most commonly involved are the liver, lungs, bones and bowel (Arneson and Williams, 1960, Herbut, 1953). The most common histological type is the squamous celled tumour which occurred in nearly 80% of the series of cases reviewed by Blaikley, Lederman and O'Connor (1962).

In reviewing post-mortem findings in 108 autopsies on patients who had had carcinoma of the cervix, Sotto, Graham, and Pickren (1960) found that, of those in which the pelvis was clear, only 9% had extrapelvic metastases. In 3% of this series deposits occurred in the pancreas but it is not stated whether these occurred with or without deposits elsewhere or whether residual growth was present in the pelvis. It was also noted that of 100 patients dying of the treatment or complications of the disease only 8% did so in the 5th to 10th post treatment year.

The incidence of both pancreatic deposits and extra-pelvic deposits with a clear pelvis was slightly higher in a series of autopsies performed on patients who underwent radical surgery for cancer of the cervix reviewed by Kelly, Parson, Friedell and Summers, (1960). 16% had deposits elsewhere but not in the pelvis, and 9% of all the patients in the series had deposits in the pancreas.

Late recurrence of this cancer has been reported in a number of sites; thus Hawkins and Andres (1935) described a patient who developed a recurrence on the cervix itself which occurred 30 years after treatment with radium. Von Capellar and Cummings (1957) reported a patient who had a solitary duodenal metastasis occurring eight years after treatment of a Stage I carcinoma. They point out, however, that in their patient the deposit may have arisen in the para-aortic lymph nodes and secondarily invaded the duodenum.

The patient reported presents, therefore, a combination of unusual features and the pathology appears to be unique.

### Summary

A patient is described who had a solitary secondary deposit from a neoplasm of the cervix treated by irradiation five years previously, in the head of the pancreas producing obstructive jaundice. In the discussion the exceptional rarity of this case is pointed out.

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## CHRONIC TOXOPLASMOSIS WITH NEGATIVE DYE TEST?

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ALTHOUGH high or rising antibody titres are needed to support a clinical diagnosis of acute toxoplasmosis, low titres to the dye test are believed to indicate past infection. Since the ocular manifestations of toxoplasmosis usually result from long standing chronic infections it is of importance in ophthalmic practice to determine

whether or not a patient has been infected. It has been the custom to assume that patients who are negative to the dye test at a titre of 1:4 do not suffer from chronic toxoplasmosis.

Recent evidence now suggests that infection with *Toxoplasma* does not always provoke a dye-test response. Engelbrecht and Franceschetti (1963)

found parasites in material aspirated from retinal lesions of a patient who was negative to the dye and haemagglutination tests. Four out of 23 women from whose aborted foetus' or body discharges Langer (1963) isolated parasites were serologically negative. Walton and Walls (1964) in their survey of wild animals isolated parasites from a racoon and a squirrel which had negative dye tests, and during mouse passage testing three serologically negative mice were found to have toxoplasmic cysts in their brains.

### Case Report

Mrs. H, a woman of 29 who after a successful pregnancy in 1954 had aborted in 1959 and 1960, had a premature baby which died soon after birth in 1961, aborted again in 1962, and had yet another abortion, in the 21st week of her pregnancy on 20.9.63. After removing a kidney for sectioning I sent this last foetus and its placenta to Dr. Ludlam at Leeds, and he inoculated suspensions of brain and of ground-up placenta each into 3 mice. All six mice sickened and died of acute toxoplasmosis on the 8th day. Further mice inoculated with exudate from these mice died after only four days.

A specimen of serum taken at the time of the abortion was weakly positive at a titre of 1:8 when first tested, (as quoted by Beattie, 1964) but the same serum retested the next day was negative at 1:8. Further specimens taken on 8.10.63 and on 7.10.64 were both negative at a titre of 1:8, and portions of this last serum were sent to Dr. Desmonts in Paris and Dr. Fulton in London for confirmatory tests. Although Desmonts' *Toxoplasma* Lysis test, (Desmonts and Cousin, 1963) commonly yields a slightly higher titre than does the classical Sabin Feldman test this serum was negative to his test even at a dilution of 1:2. Fulton's direct agglutination test was positive at a titre of 1:40, a level which he does not consider significant.

On two occasions, once a month after the abortion and again 6 months later, the patient was found to be negative to the *Toxoplasmin* skin test. At no time during this period had she exhibited glandular enlargement or symptoms suggestive of active toxoplasmic infection.

### Discussion

In view of the high virulence of the strain of *Toxoplasma* isolated from this patient the possibility of contamination of the specimen or the mice with the R.H. strain used in the laboratory for dye tests was considered. The passage testing had not however been carried out in the laboratory which undertakes the *Toxoplasma* serology, but had been done in the virology laboratory in a different part of the building. Only the bottle of penicillin and streptomycin used to protect the mice from bacterial infection had come from the laboratory which deals with *Toxoplasma* serology. Apart from the remote possibility that this had somehow become contaminated, and that parasites had remained alive in it for many hours there seems no conceivable route for such contamination, and one is forced to the conclusion that despite her persistently negative

dye test result this patient had a symptomless infection from which the highly virulent strain isolated from her foetus was derived.

This case, like that reported by Remington, Newell and Cavanaugh, (1964) lends some support to the hypothesis of Langer and of Werner, Schmidtke and Thomascheck (1963) that *Toxoplasma* infection is a cause of repeated abortion. It also supports the observations of Langer that chronic infection causing repeated abortion may not be detectable by a routine dye test.

Whether this is due to a complete failure of the host to produce specific antibody or merely to a lack of sensitivity of current serological tests is uncertain. In the case of Mrs. H, the fact that on one occasion, when the tests at Leeds appeared to be slightly more sensitive than usual her serum was reported positive, and the low titre to Fulton's direct agglutination test suggest that specific antibody may have been present in small amounts. More sensitive tests might detect such infections. Many tests have been developed. Tests dependent on agglutination of sensitised particles tend to be less sensitive than the dye test (Lunde and Jacobs, 1963; Garin, 1960) as are the fluorescence inhibition tests (Goldman, Gordon and Carver, 1962; Garin, and Ambrose-Thomas, 1963; Kelen, Ayllon-Leindl and Labzoffsky, 1962). Dye or Lysis tests can be modified to increase their sensitivity by using washed parasites (Goldman, 1956) and could be done with undiluted serum. Fulton and Turk (1959) direct agglutination test shows that nearly all sera have some agglutinating power. It would however be extremely difficult to prove that positive results at low titres to any of these tests, which could not be confirmed by another test, were specific and indicative of *Toxoplasma* infection.

Alternatively it is possible that some or all of the patients or animals whose infections have been found associated with negative serological tests have indeed no specific antibody. Since most of these hosts have enjoyed reasonably good health it is not likely that they have agammaglobulinaemia. The possibility that as a result of exposure to infection very early in foetal life the development of those "clones" which would produce the antibody specific for *Toxoplasma* has been inhibited, may be considered. Although Burnet (1959, 1962) has described such a mechanism there appears to be no evidence of such immune tolerance to infective agents. The high dye-test titres found in cases of congenital toxoplasmosis, and in mice even when the mother's infection was congenital, (Beverley, 1959) suggests that such a mechanism seldom if ever functions between either man or mouse and *Toxoplasma*.

One way in which the case of Mrs. H differed from other published incidents of infection without detectable antibody, is that the strain derived from her foetus was of very high virulence, while in the other recorded instances the parasites have been initially of low virulence.

It has long been known that different strains of toxoplasma vary widely in virulence. Strains isolated from cysts in muscle or brain are usually of very low virulence when first isolated, and when maintained in the laboratory by injecting cysts from one host into another, as with the "Beverley" strain, no increase in virulence occurs. Strains isolated from cases of acute disease are more commonly found as trophozoites. These tend to be of higher virulence, and with repeated intraperitoneal passage at short intervals this virulence becomes augmented until they commonly kill their new host before any cysts have developed. Occasionally, as in the instance reported by Nakayama and Matsubayashi (1961), when a mouse became infected by nibbling at the body of another dead of a virulent infection, a stable avirulent strain may be derived from a stable virulent strain. A hypothesis which accounts for these observations is that virulence is genetically determined, depending upon the tendency of the parasite to proliferate, infect and destroy new cells; strains which tend to multiply within one cell and then form a cyst being of low virulence; and that either as a result of mutation or of redistribution of genes in sexual reproduction, variation occurs. Modes of transmission giving a survival advantage to cyst-forming deviants would then tend to produce avirulent strains, and modes of transmission giving the advantage to trophozoites would lead to the evolution of strains of high virulence.

Langer isolated parasites from his cases of repeated abortion by re-passing blind through six batches of mice at eight-day intervals, and seldom found any evidence of disease before the second or third re-passage. The original strains were so avirulent that no disease was produced in the mice. By re-passing at short intervals when trophozoites would still be present he "selected" in favour of the most rapidly multiplying deviants. In the incident described by Nakayama and Matsubayashi the infection of a mouse by ingestion would select in favour of parasites in cystic phase, as trophozoites are destroyed by peptic digestion whereas parasites in cysts are resistant.

There is a considerable body of evidence in favour of this hypothesis which accounts for many of the observed facts about toxoplasmosis, and particularly for the much higher virulence of the congenital infection than of the postnatally acquired form. If the mode of transmission common in nature favoured the cysts the extreme avirulence of most natural infections becomes understandable, and the selection in favour of trophozoites due to the greater probability of their passing the placental barrier accounts for the high virulence of the congenital disease which results when primary maternal infection occurs during gestation. If, as is suspected, the low antibody level in Mrs. H was due to the low antigenicity of cysts, and her previous abortions and infant death were due to toxoplasma in-

fection, one would not expect to find trophozoites in her blood. The evolution of a strain of high virulence in her foetus can only be reconciled with this hypothesis if it is assumed either that a gross mutation occurred, that a cyst ruptured liberating trophozoites or that this patient in fact experienced her primary infection during her last pregnancy, and her previous abortions were due to other and unknown causes. If the extremely low antibody levels in this case are an indication of long standing infection and due to the low antigenicity of cysts this incident seems inexplicable except on the basis of mutation.

#### Summary

Reports of isolations of toxoplasma from chronically infected hosts with negative dye tests are reviewed. The case is described of a woman with a history of repeated abortion, whose dye test was, apart from one dubious test, negative at a titre of 1:8 but from whose aborted foetus a highly virulent strain of *Toxoplasma* appeared to have been isolated. This case is compared with some described by Langer. Possible explanations of chronic infections without positive dye test are discussed.

An evolutionary theory to explain the variations in virulence of strains of toxoplasma is outlined and an attempt is made to reconcile the case described with this hypothesis.

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