THE EFFECT OF NEONATAL THYMECTOMY ON THE SURVIVAL OF GOLDEN HAMSTERS INFECTED WITH *PLASMODIUM BERGHEI*

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CEREBRAL malaria in Africa is predominantly a disease of well nourished children and rarely affects children with marasmus or kwashiorkor (Edington, 1967). One explanation of this observation is that the deficiency of amino acids in these children interferes with parasite multiplication. Children with malnutrition, however, also show impaired delayed hypersensitivity as measured by the tuberculin reaction following vaccination with BCG. (Harland and Brown, 1965; Harland, 1965), and in kwashiorkor an extreme degree of thymic atrophy is an invariable and striking finding at post-mortem examination (Trowell, Davies and Dean, 1954; Vint, 1937). It is possible that immunological deficiencies associated with this thymic atrophy are responsible for the rareity of cerebral malaria in these children. Neonatal thymectomy is effective in suppressing auto-allergic encephalomyelitis in rats (Arnason, Jankovic, Waksman and Wennersten, 1962) and the meningeal lesion of lymphocytic chorio-meningitis in the mouse (Rowe, Black and Levey, 1963), demonstrating the importance of host immune reactions in the pathogenesis of these disease processes. Analogous host parasite interactions might be responsible for the lesions of cerebral malaria, and the impaired immunological response of children with malnutrition might protect them from the consequences of this interaction. To test this hypothesis an investigation was undertaken to determine whether neonatal thymectomy would influence the course of *Plasmodium berghei* infection in hamsters, in particular whether it would protect them from the cerebral manifestations of this disease.

MATERIALS AND METHODS

Hamsters were thymectomised within 24 hr. of birth. Light ether anaesthesia was used and the animals displayed on a dissecting board by means of elastic bands. The sternum was split with fine scissors and each lobe of the thymus carefully identified and removed with cupped forceps. One or two atraumatic braided silk stitches were used to close the skin and the wound painted with celloidin. Sterile instruments and materials were used throughout. The thymectomised animals were returned to their original cage immediately after operation but the mother was not returned for a further 2–3 hr. In order to avoid maternal cannibalism it appeared important to wear rubber gloves while performing the thymectomy and to avoid direct handling of the animals at all stages.

Using this technique the operative mortality was less than 5 per cent and maternal cannabalism was reduced to a very low level. However, a number of animals died, or disappeared, in the first few weeks and the 4 week survival rate was in the region of 60 per cent. Contrary to the findings of Sherman, Adner and Dameshek (1963) the thymectomised hamsters in this laboratory did not show wasting disease. They appeared as healthy as the non-thymectomised animals and, if allowed to, reproduced normally.

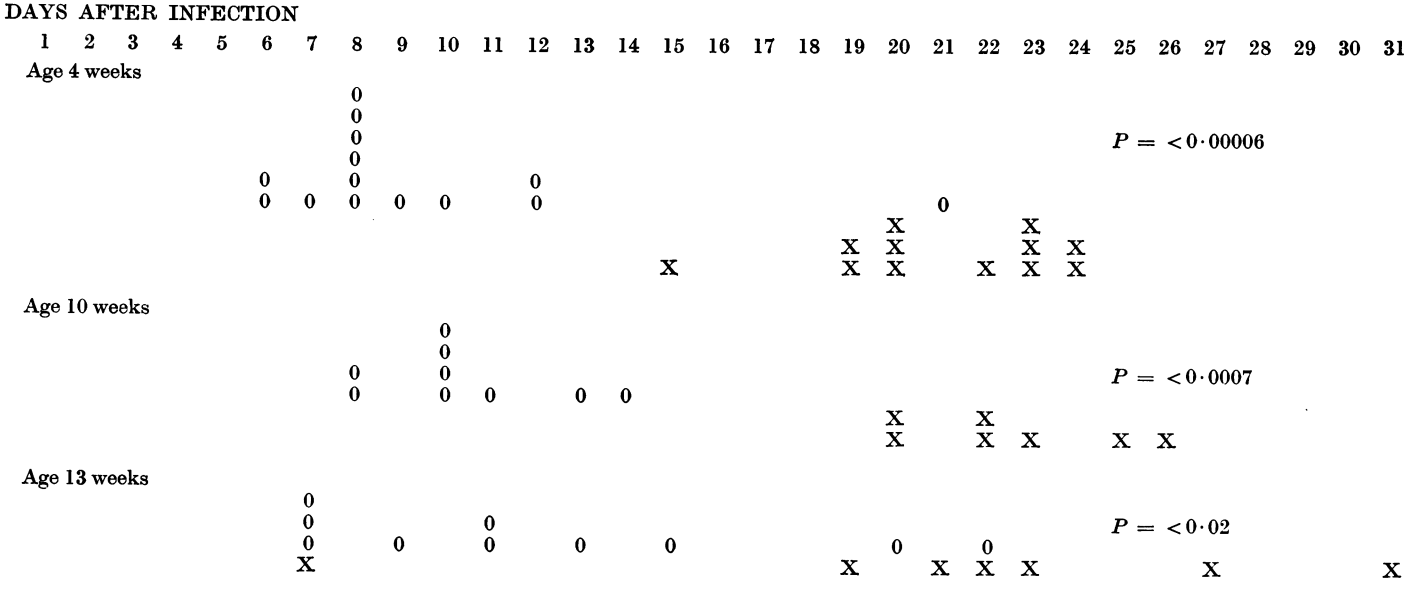

Fig. 1: showing time of death of non-thymectomised 0 and thymectomised X golden hamsters following infection with *Plasmodium berghei*.

Plasmodium berghei was maintained by weekly passage in golden hamsters. Age matched non-thymectomised and neonatally thymectomised animals of both sexes were infected with between 1–1·5 $\times$ 10^7 parasitised RBC by i.p. injection. Three groups of animals aged 4, 10 and 13 weeks were used. All dead animals were dissected and the organs from most of these fixed in 10 per cent formol saline for histological examination. Several thymectomised animals were killed between 6–14 days after infection, at a time when the non-thymectomised animals were dying, for histological comparison with these animals. The mediastinal contents of all thymectomised animals were examined macroscopically and microscopically for residual thymic tissue.

Detailed haematological and parasitological studies were not carried out on these animals since it was considered that repeated handling and bleeding of the animals would influence the time of death. However, blood films were made from the tails of occasional animals in the series at varying times after infection and a few animals were bled from the heart for haemoglobin estimations. None of the animals that were killed are shown in Fig. 1.

RESULTS

The survival times following infection are shown in Fig. 1. There is a highly significant difference in the survival times of the thymectomised and non-thymectomised animals in all the age groups studied. Whereas the majority of non-thymectomised animals died between 6–12 days after infection the majority of the thymectomised animals died between 19–25 days following infection. There also appeared to be a difference in the mode of death between these 2 groups. The non-thymectomised animals fell ill abruptly between the 6–10th day after infection, rapidly sank into a torpor or coma and usually died within 48 hr. of becoming obviously ill. At this stage the thymectomised animals appeared active, bright eyed and outwardly normal. After a further 7–14 days this group of animals became increasingly lethargic, progressed relentlessly downhill and died after a period of obvious ill health, usually lasting for several days.

At the time when most of the non-thymectomised animals were dying they had haemoglobin levels of between 6–8 g. per cent and less than 15 per cent of their RBC parasitised. The thymectomised animals at this stage had similar haemoglobin levels and parasite rates. During the ensuing 2 weeks these hamsters showed a progressive rise in parasite counts and fall in haemoglobin level. In the terminal stages the haemoglobin fell to levels of between 2–3 g. per cent and large numbers of parasitised normoblasts were seen in the blood smear. Thirty to 70 per cent of the circulating RBC were parasitised at this stage and large numbers of extracellular parasites were present in the blood smear.

Histopathological examination of animals dying between 7–10 days after infection showed marked pigment deposition in the splenic histiocytes, Kupffer cells and portal histiocytes of the liver. Varying degrees of extra medullary erythropoiesis were also found in these organs. The feature that differentiated the non-thymectomised hamsters from the thymectomised hamsters killed at the same time was the presence of many acute haemorrhages in the white matter of the brain in the former animals (Fig. 2, 3 and 4). These haemorrhages usually surrounded small vessels containing many parasitised RBC and fibrin plugs. Only very occasional haemorrhages were found in the brains of thymectomised animals. The thymectomised animals also showed depletion of the lymphoid tissue in the liver, spleen, intestine and lungs. Those animals surviving beyond 2 weeks after infection showed increasing fatty change in the liver and kidneys and increasing degrees of extramedullary haematopoiesis in the liver and spleen.

Marked pigment deposition occurred in histiocytes in all organs. Brain haemorrhages and capillary fibrin plugs were not seen in these animals.

DISCUSSION

The course of infection with *Plasmodium berghei* can be materially altered by the age, sex and strain of host animal. The parasitaemia may be affected by the presence of other blood parasites and also by the diet on which the animal is fed. Garnham (1966) has warned that the variable behaviour of this parasite under different circumstances must always be borne in mind or anomalous results may be misinterpreted. The observations reported here that thymectomised hamsters between 4–13 weeks of age survive longer than control animals when infected with large numbers of parasitised RBC is open to a number of different interpretations. It is possible that the acute deaths of the non-thymectomised animals was not due to *Plasmodium berghei* but to another agent transmitted with the infected blood. Mercado (1965) observed paralysis and acute deaths in rats, associated with brain haemorrhages, following infection with a strain of *Plasmodium berghei* that had been passed through mice. At the time of death the animals had parasitaemias of 8–10 per cent and were not anaemic. Mercado was unable to transmit the paralysis with cell free extracts of blood or tissues but was able to make other strains of *Plasmodium berghei* " paralytic " by simultaneously injecting them with these extracts into susceptible rats. She concluded that the pathological effects were brought about either as a result of modification of the malaria strain or the interaction of a filterable factor and the malarial parasite.

If a filterable agent is responsible for the brain haemorrhages and acute deaths of non-thymectomised hamsters in the experiments reported here, thymectomy must presumably protect against this agent. The nature of the brain haemorrhages in these animals, surrounding small blood vessels occluded by parasitised RBC and fibrin plugs is however, very reminiscent of cerebral malaria in human patients and suggests that *Plasmodium berghei* is the cause.

Kreier, Shapiro, Dilley, Szilvassy and Ristic (1966) have recently shown that rats infected with *Plasmodium berghei* develop an agglutinin to trypsinised autologous and homologous parasitised and non parasitised RBC. This agglutinin is mercapto-ethanol sensitive indicating that it is a macroglobulin. It is possible that the pathogenesis of the disease observed in hamsters in the experiments reported here was due to the production of an RBC agglutinin resulting in the micro- embolisation of cerebral capillaries followed by diffuse pericapillary haemorrhages. Neonatal thymectomy by inhibiting or delaying the production of the agglutinin would prevent the acute death due to capillary embolisation. It is

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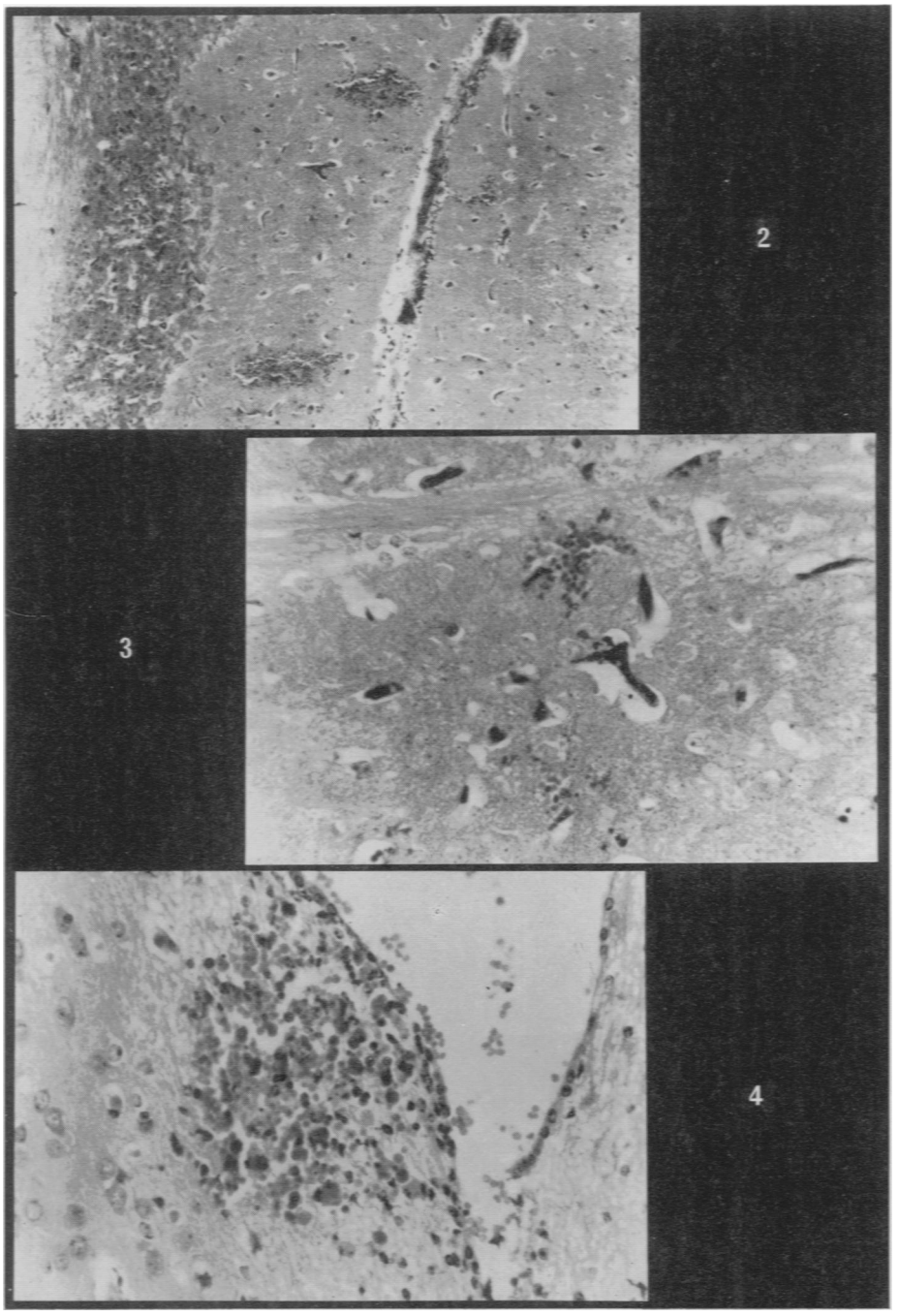
EXPLANATION OF PLATE

FIG. 2.—Photomicrograph of brain of non-thymectomised hamster infected with *Plasmodium berghei*. Multiple small haemorrhages are present in the white matter of the brain. Phosphotungstic acid haematoxylin stain × 190.

FIG. 3.—Higher power photomicrograph of Fig. 2 showing fibrin plugs in capillaries and small haemorrhages into brain substance.
Phosphotungstic acid haematoxylin stain × 350.

FIG. 4.—Photomicrograph of brain from non-thymectomised hamster infected with *Plasmodium berghei* showing presence of parasitised red cells in an area of sub-ependymal haemorrhage. H and E × 350.



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evident that neither the anaemia nor the height of the parasitaemia is directly responsible for the death of the non-thymectomised hamsters since the thymectomised animals with the same haemoglobin levels and parasite counts appear in normal health. These latter animals develop severe anaemias and parasitaemias before they die and it is probable that death in their case is directly attributable to these 2 factors. Brain haemorrhages do not occur in this group of animals.

If an agglutinin is produced against parasitised or normal RBC in *Plasmodium berghei* infections the development of micro-emboli in the brain capillaries with acute death is probably dependent on the relationship of the rise in antibody response to the rise parasitaemia. Adler, Yoeli and Zuckerman (1950) reported that *Plasmodium berghei* infections were fatal in hamsters after a period of 8–29 days. They did not however report the mode of death of their animals or note any difference between the pathology of those dying early and those dying late. Yoeli and Most (1964) have noted that whereas all hamsters infected with large doses of *Plasmodium berghei* die a rapid death, with smaller infecting doses, an increasing proportion of the animals survive. Similar observations have been made in this laboratory where it has also been noted that the length of survival increases with increasing age of the animal. The nature of the host and the strain of the parasite is probably also important in the development of the acute disease.

A further possible explanation of the pathogenesis of the lesions seen in these hamsters is that an antibody produced in response to the parasite results in an intravascular antigen antibody reaction that damages the endothelium of the cerebral capillaries resulting in intravascular fibrin thrombi and extravasation of red cells through the damaged capillary wall. The absence of any cellular infiltrate around the cerebral capillaries suggests that cell mediated immunity is not responsible for the cerebral haemorrhages.

It has recently been postulated that certain aspects of the pathology of malaria may be autoimmune in nature (Cox, 1964; McGhee, 1964; Zuckerman, 1964). These publications have been mainly concerned with the observations that the anaemia developing during the course of malarial infections often appears to be of greater severity than could be expected on the basis of the destruction of parasitized RBC alone. It is suggested that the lesions observed in hamsters in the experiments reported here are also auto-immune in nature and that neonatal thymectomy protects against these as it does against some other auto-immune phenomena.

The lesions seen in the brains of the non-thymectomised hamsters dying with *Plasmodium berghei* infection are similar to those seen in the brains of humans dying of cerebral malaria. The findings of this investigation support the hypothesis that it is the extreme thymic atrophy and impaired immunity of children with malnutrition that protects them from cerebral malaria. Further studies will be needed to determine the exact pathogenesis of the cerebral lesions seen in animals dying with *Plasmodium berghei* infection and the lesions found in human cerebral malaria. Experiments are now in progress in this laboratory to determine the effect of anti-lymphocyte serum on *Plasmodium berghei* infection in rodents.

SUMMARY

Normal and neonatally thymectomised hamsters between 4–13 weeks of age were infected with *Plasmodium berghei*. The non-thymectomised animals died

within 1–2 weeks of infection at a time when their haemoglobin levels were between 6–8 g. per cent and less than 15 per cent of their circulating RBC were parasitised. Death in these animals appeared to be due to multiple small haemorrhages in the brain. The thymectomised hamsters at this stage had similar haemoglobin and parasite levels to the non-thymectomised animals but appeared outwardly in normal health. They subsequently became progressively more anaemic with haemoglobin levels falling to 2–3 g. per cent and parasite rates of up to 70 per cent. Death occurred between 3–4 weeks after infection and appeared to be attributable to the anaemia and parasitaemia. Cerebral haemorrhages were not found in these animals. It is postulated that the non-thymectomised animals develop an agglutinin, in response to the malarial infection, that causes microembolisation of the cerebral capillaries with agglutinated parasitised RBC, and that neonatal thymectomy inhibits or delays the production of this agglutinin. The significance of these findings to the pathogenesis of human cerebral malaria is discussed.

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