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THE EFFECT OF ANTITHYMOCYTE SERUM ON GOLDEN HAMSTERS AND RATS INFECTED WITH *PLASMODIUM BERGHEI*

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SUMMARY.—Hamsters infected with *Plasmodium berghei* and treated with normal rabbit serum died 6-10 days after infection at a time when they had relatively low parasite rates. This acute death was associated with multiple petechial haemorrhages throughout the brain and was prevented by the administration of antithymocyte serum. Hamsters treated with antithymocyte serum died 12-16 days after infection and did not develop cerebral haemorrhages. It is postulated that the cerebral haemorrhages result from micro-embolisation of capillaries by agglutinated red cells and that antithymocyte serum inhibits or depresses the production of the responsible agglutinin.

Young rats infected with *Plasmodium berghei* died with high parasite rates 2-3 weeks after infection. Antithymocyte serum had no effect on the course of infection in these animals nor on their survival.

The similarity of the cerebral lesions seen in hamsters to the lesions of human cerebral malaria is discussed.

RATS below the age of 8 weeks die with a fulminant parasitaemia 2–3 weeks after infection with *Plasmodium berghei*. Young hamsters on the other hand die approximately 1 week after infection at a time when they have a parasite rate of < 10 per cent. This acute death in hamsters appears to be related to the development of cerebral haemorrhages similar to those seen in human cerebral malaria and is prevented by neonatal thymectomy (Wright, 1968). Thymectomised hamsters respond to *Plasmodium berghei* infection in a similar manner to young rats in that they die 2–3 weeks after infection with very high parasite rates.

Adults rats usually develop immunity to *Plasmodium berghei* with clearance of the parasitaemia approximately 2 weeks after infection. Neonatal thymectomy (Brown, Allison and Taylor, 1968; Stechschultz, 1969) and the administration of antithymocyte serum (Spira, Silverman and Gaines, 1970) appears to impair the immune response of adult rats to *Plasmodium berghei* and results in a high parasitaemia and high mortality. In contrast Sheagren and Monaco (1969) found that antilymphocyte serum prolonged the survival of mice infected with *Plasmodium berghei*. Mice have a similar response to *Plasmodium berghei* to that shown by young rats with a high parasitaemia and high mortality. The experiments reported here were designed to determine whether antithymocyte serum has a similar effect to neonatal thymectomy in protecting hamsters from the cerebral complications of *Plasmodium berghei* infection and also to see whether it has any effect on *Plasmodium berghei* infection in young rats.

MATERIALS AND METHOD

Antithymocyte serum (ATS) was prepared in rabbits using the method described by Turk and Willoughby (1967). Thymus cells were obtained from 4-week-old animals (hamsters or

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rats). Approximately 8×10^7 cells were given into the lateral ear vein on each of 2 days and i.p. on the third day. This regime was repeated after 1 week. A third course was given 1 week later with the i.p. injection preceding the 2 i.v. injections. One week after the last injection the rabbits were bled from the lateral ear vein on each of 3 successive days. The serum was pooled, inactivated at 56° for 20 min. and stored in 5 ml. amounts at −70° until used. Normal rabbit serum (NRS) used for the control animals was inactivated and stored in a similar manner.

Hamsters.—Sixteen 6-week-old animals were infected with *Plasmodium berghei* by the i.p. injection of approximately 6×10^6 parasitised hamster red cells. The animals were divided into 2 groups of 8. One group was given 0·5 ml. rabbit-anti-hamster-thymocyte serum i.p. on the day of infection and on alternate days thereafter. The other group was similarly treated with NRS. The animals were observed at least twice a day. Dead animals were subjected to post-mortem examination and if only recently dead the tissues were examined histologically.

It was considered that to take blood from these animals, by the routes available in the hamster, might adversely affect survival. No attempt was made therefore to follow the parasite rate and haematological changes in these hamsters. In order to observe these changes a further group of animals were bled by cardiac puncture at intervals after infection.

Twenty hamsters were infected with *Plasmodium berghei* as described above. The animals were then divided into 2 groups of 10. One group was treated with 0·5 ml. ATS i.p. on the day of infection and on alternate days thereafter. The other group was similarly treated with NRS. Paired animals from the group treated with NRS were killed on days 3, 6 and 7. Three animals from this group died on day 7 and the one surviving animal was killed on day 9. Paired animals from the ATS treated group were killed on days 3, 6, 7, 9 and 11. The animals were anaesthetised in an ether jar and bled from the heart into a heparinised syringe. A post-mortem examination was then performed and tissues were taken for histology. The spleen and left kidney from each animal were weighed on a torsion balance.

Blood was collected from 2 uninfected 6-week-old hamsters to establish the baseline haematological values.

Rats.—Since blood can readily be obtained from the tail vein of the rat without undue trauma haematological and parasitological changes were followed in the same group of animals that was being used to determine survival. Twenty four 6-week-old inbred Wistar rats were infected with *Plasmodium berghei* by the i.p. injection of $1{\cdot}5 \times 10^6$ parasitised rat red cells.

The animals were divided into 2 groups of 12. One group was treated with 0·5 ml. rabbit-anti-rat-thymocyte serum on the day after infection and on alternate days thereafter. The other group was similarly treated with NRS. The cages were inspected twice daily and dead animals removed and dissected. If the animals had only recently died tissues were taken for histology.

Blood was collected from paired animals in each group before infection and on days 2, 5, 6, 8, 10, 13, 14, 16 after infection. The blood was obtained by slicing off the tip of the tail with a razor blade. All animals were bled once after which the animals to be bled were selected at random.

Haematology and parasite count.—Total white cell counts were performed on the blood samples from each animal using a counting chamber. Reticulocyte counts were performed using cresyl violet stained cells. Thin blood smears were stained by May–Grunwald–Giemsa stain for parasite counts and differential white blood cell counts. All counts were made in duplicate by 2 separate observers on each of the paired animals. At least 400 cells were counted by each observer to determine the parasite rate, reticulocyte count and differential white cell count. The results are expressed as the average of these observations. The haematocrit was estimated using a microhaematocrit technique.

RESULTS

Hamsters

Survival

The survival following infection with *Plasmodium berghei* is shown in Fig. 1. Hamsters treated with NRS became listless and ataxic between 4–5 days after

infection. All the animals in this group died between 5–10 days after infection. The ATS treated animals appeared externally normal and behaved normally until 10 days after infection. They then exhibited progressive listlessness, and died between 12–17 days following infection. The difference in survival between these 2 groups is highly significant ($P > 0{\cdot}0001$).

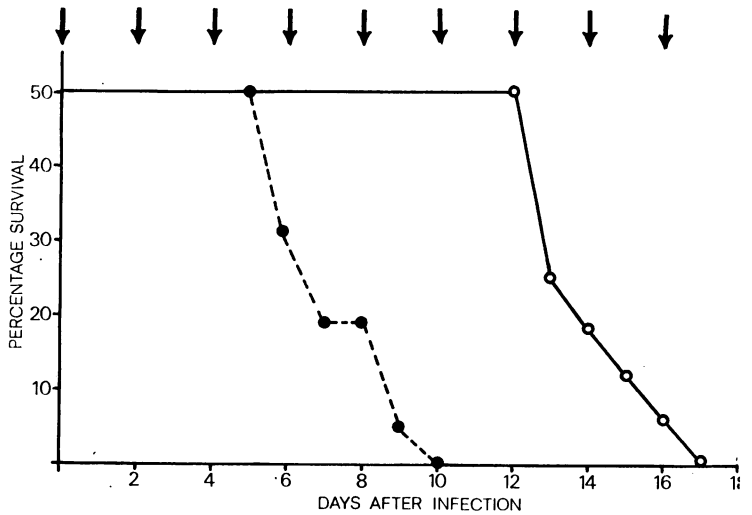


FIG. 1.—Percentage survival of hamsters infected with *Plasmodium berghei*. ●- - - -● NRS treated. ○———○ ATS treated. Arrows indicate injection of 0·5 ml. serum i.p.

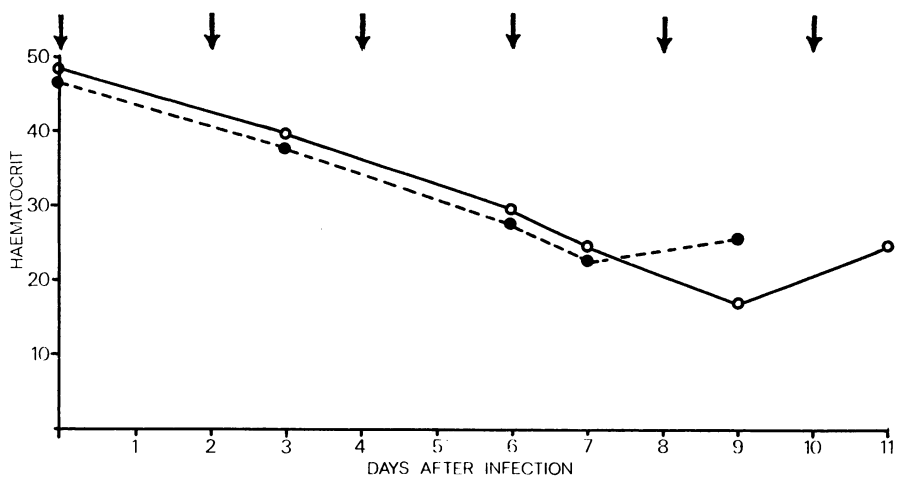


FIG. 2.—Haematocrit values in hamsters infected with *Plasmodium berghei*. ●- - - -● NRS treated. ○———○ ATS treated. Arrows indicate injection of 0·5 ml. serum i.p.

Haematology and parasite rate

The haematocrit values are shown in Fig. 2, the reticulocyte counts in Fig. 3 and the parasite rates in Fig. 4. There is no significant difference in the findings between the 2 groups of animals up to day 9 after which all the NRS treated group

had died. The parasite rate and the reticulocyte count continued to rise in the ATS treated animals up to day 11 when the last animals were killed. Terminally both groups showed a slight rise in the haematocrit value. This may have been due to dehydration, the animals being too lethargic to drink.

The ATS treated animals showed a lower WBC, absolute lymphocyte count

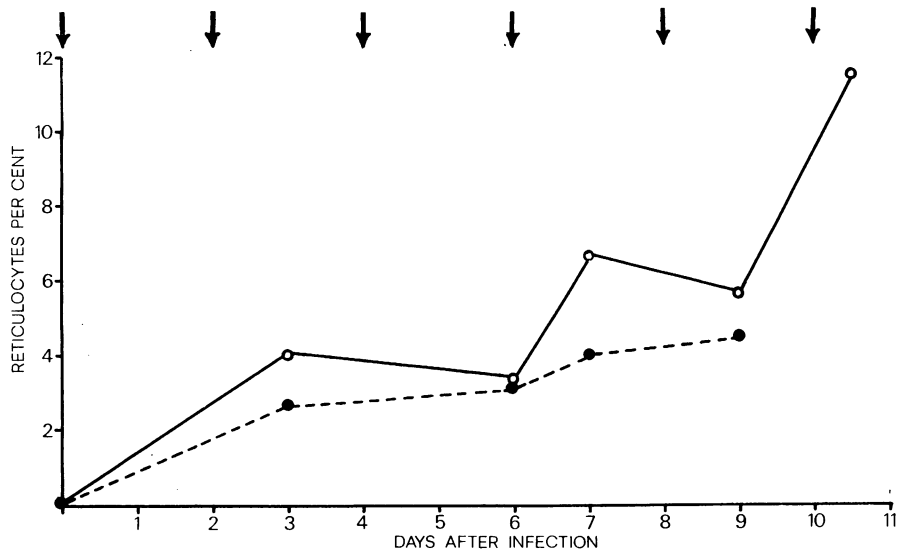


FIG. 3.—Reticulocyte count in hamsters infected with *Plasmodium berghei*. ●----● NRS treated. ○——○ ATS treated. Arrows indicate injection of 0·5 ml. serum i.p.

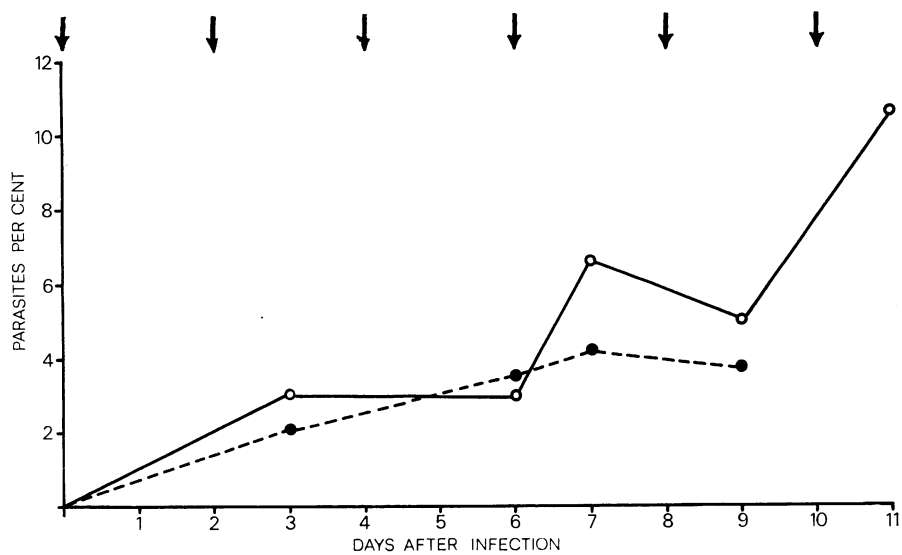


FIG. 4.—Percentage parasite rate in hamsters infected with *Plasmodium berghei*. ●----● NRS treated. ○——○ ATS treated. Arrows indicate injection of 0·5 ml. serum i.p.

and absolute monocyte count than the NRS treated hamsters. The NRS treated animals showed a peak WBC, lymphocyte count and monocyte count 7 days after infection. The ATS treated group showed a lower peak 9 days after infection (Table I).

TABLE I.—*Total WBC per mm³ and Absolute Lymphocyte and Monocyte Counts per mm³ in ATS Treated and NRS Treated Hamsters Following Infection with* Plasmodium berghei

	ATS treated			NRS treated		
Day	WBC ($\times 10^3$)	Lymphocyte count ($\times 10^3$)	Monocyte count ($\times 10^3$)	WBC ($\times 10^3$)	Lymphocyte count ($\times 10^3$)	Monocyts count ($\times 10^3$)
3	1·7	1·5	0·94	3·5	0·8	1·8
6	2·4	3·75	1·6	4·4	1·4	3·2
7	5·8	3·75	4·0	13·0	3·6	7·4
9	8·2	7·0	5·1	7·0	1·5	5·6
11	1·8	0·5	1·4	—	—	—

Uninfected untreated hamster controls:

WBC	$5{\cdot}4 \times 10^3$ per mm³
Lymphocyte count	$1{\cdot}8 \times 10^3$ per mm³
Monocyte count	$3{\cdot}0 \times 10^3$ per mm³

Gross findings

The weights of the spleen and left kidney were not significantly different in the 2 groups (Table II). Both organs increased in weight up to 7 days after infection and then decreased in weight. This decrease in weight was particularly marked in the NRS treated animals and was probably related to tissue catabolism, dehydration and haemoconcentration in the late stages of infection.

TABLE II.—*Weights of Spleen and Kidney in mg. of ATS Treated and NRS Treated Hamsters Infected with* Plasmodium berghei

	ATS treated		NRS treated	
Day	Spleen	Kidney	Spleen	Kidney
3	170·5	355·5	178·5	358·5
6	556	451·5	408	415
7	879	510	930	524
9	699·5	359	534	285
11	776	402	—	—

The gross appearance of the brain showed some difference in the 2 groups. The NRS treated animals had swollen tense brains and small petechial haemorrhages were visible with the aid of a hand lens both on the meningeal surface and on the cut surface. These features were not seen in the brains of ATS treated animals.

Histology

NRS treated animals that died.—The brains of these animals showed widespread petechial haemorrhages more marked in the white matter than in the grey matter and more numerous in the cerebellum than in other parts of the brain. The haemorrhages closely resembled the ring haemorrhages seen in human cerebral

malaria (Figs. 5, 6, 7). A variable proportion of the red cells in the haemorrhages were parasitised. The cerebral capillaries appeared congested and some contained rounded aggregates of parasitised and non-parasitised red cells (Fig. 5). Staining with phosphotungstic acid-haematoxylin revealed fibrin strands in some vessels.

Around some vessels and sometimes within the haemorrhages were eosinophilic PAS and PTAH positive droplets varying in size from approximately 1–20 μm. These bodies had the appearance and staining properties of protein droplets.

Associated with the haemorrhages and occluded capillaries were areas of neuronal degeneration and necrosis.

The macrophages of the lymph nodes and spleen and the Kupffer cells of the liver showed malarial pigmentation. There was marked plasma cell proliferation in the medulla of the lymph nodes and accumulations of pyroninophilic cells in the portal areas of the liver and the sinusoids of the spleen. Probable erythrophagocytosis was seen in the liver, spleen and lymph nodes. The thymus showed only slight atrophic changes. Pulmonary oedema was present in several animals.

ATS treated animals that died.—Very occasional haemorrhages and pericapillary protein droplets were seen in the brains of these animals. Many of the cerebral capillaries appeared distended by pigmented monocytes but no red cell aggregates were seen. There was no evidence of neuronal necrosis.

The lymph nodes showed depletion of the parafollicular areas, and there was a marked proliferation of plasma cells around the follicles and in the medulla. Many nodes contained isolated Langhans type giant cells some of which were laden with malarial pigment. No epithelioid cells or areas of necrosis were seen. The thymuses of this group were more atrophic than those of the NRS treated animals and the spleens and livers contained fewer lymphoid cells but showed much more marked pigmentation of the macrophages and Kupffer cells. This was presumably related to the longer survival of these animals and the high parasitaemia at death. Centrilobular fatty vacuolation of liver parenchymal cells was prominent in the animals of this group.

NRS treated animals that were killed

These animals showed progressively more pigmentation of the lymphoreticular system with increasing duration of the infection. There was also a progressive increase in pyroninophilic cells and plasma cells in the lymph nodes and to a lesser extent in the spleen and portal areas of the liver.

Brain haemorrhages were not seen in animals killed 3 and 6 days following infection. One of the animals killed 7 days after infection and the one surviving animal killed 9 days after infection showed extensive brain haemorrhages.

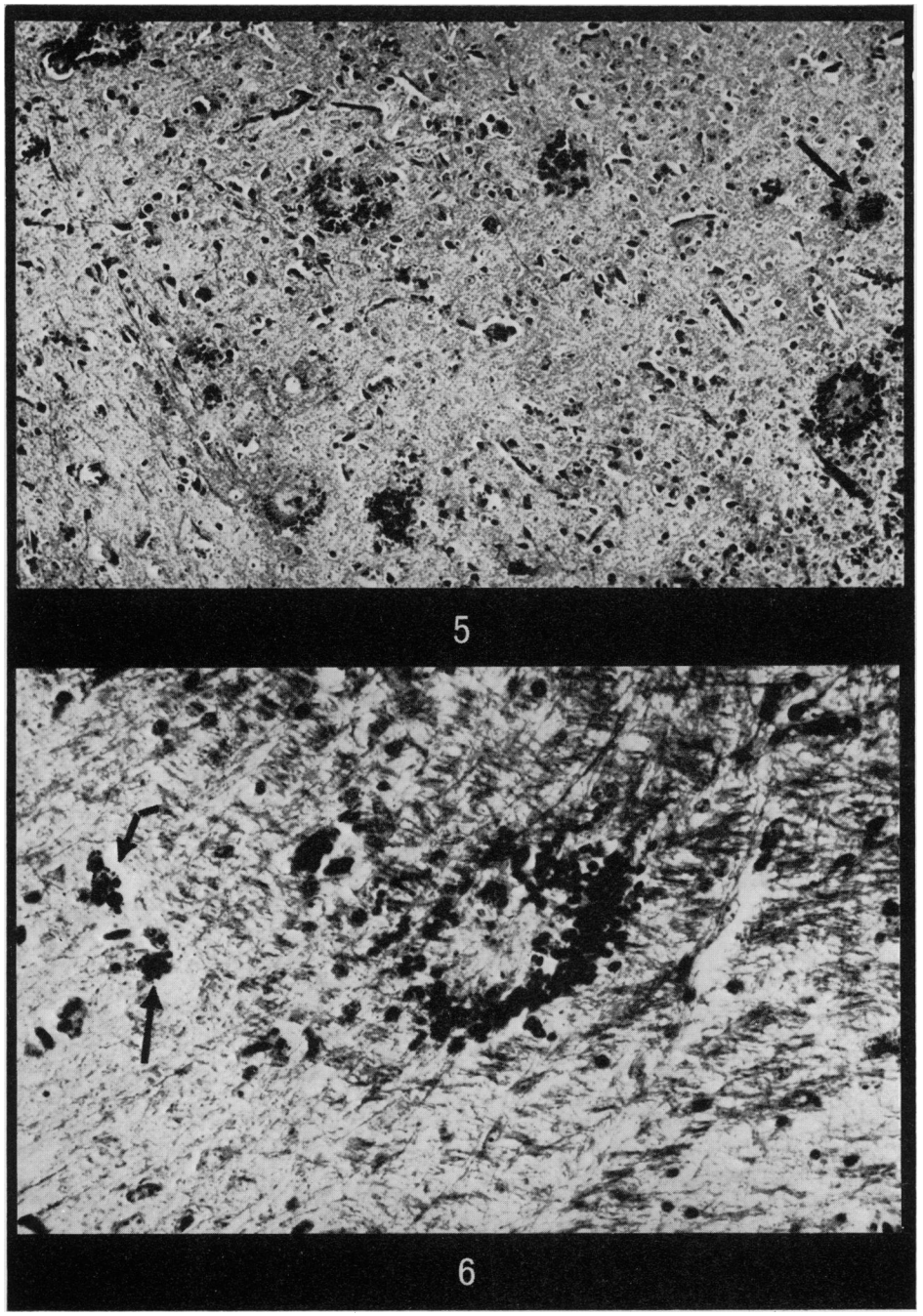
EXPLANATION OF PLATES

FIG. 5.—Section of brain from NRS treated hamster showing multiple ring haemorrhages. Arrow indicates capillary occluded by agglutinated red cells. Phospho-tungstic acid-haematoxylin stain. PTAH. ×130.

FIG. 6.—Section of brain from NRS treated hamster showing a ring haemorrhage. Arrows indicate protein droplets around capillaries. PTAH. ×330.

FIG. 7.—Section of brain from NRS treated hamster showing a ring haemorrhage. The mixture of parasitised and non-parasitised red cells in the haemorrhage can be seen; also the leakage of red cells into the subarachnoid space. H. and E. ×520.

FIG. 8.—Section of brain from NRS treated hamster showing PAS positive protein droplets around capillaries. PAS photographed with green filter. ×330.



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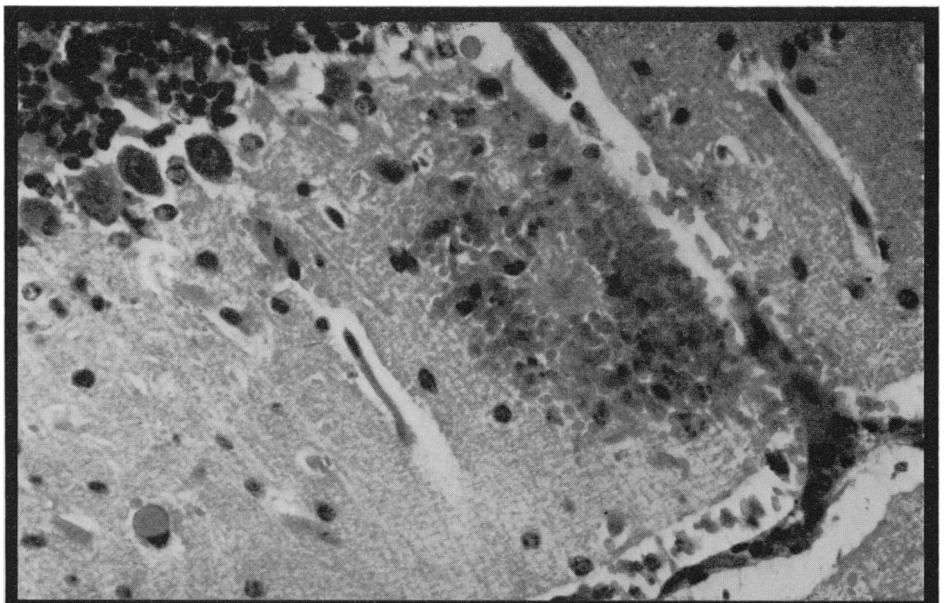

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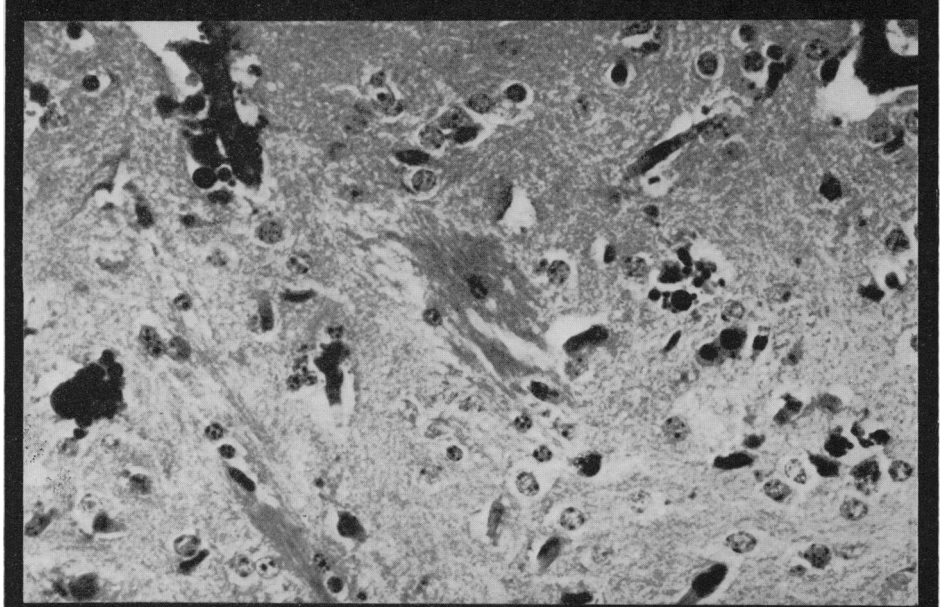

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ATS treated animals that were killed

Brain haemorrhages were not seen in these animals. The thymuses of this group appeared slightly more atrophic than those of the NRS treated group and there was atrophy of the para-follicular areas of the lymph nodes although the actual follicles were hyperplastic. There was a well developed plasma cell proliferation in these nodes. Many of the nodes contained isolated pigmented Langhans type giant cells. One such giant cell was also seen in the thymus of an animal killed 9 days after infection.

Rats

Survival

There appeared to be no difference in the survival of the ATS treated and NRS treated animals. One ATS treated animal survived the experiment with clearance

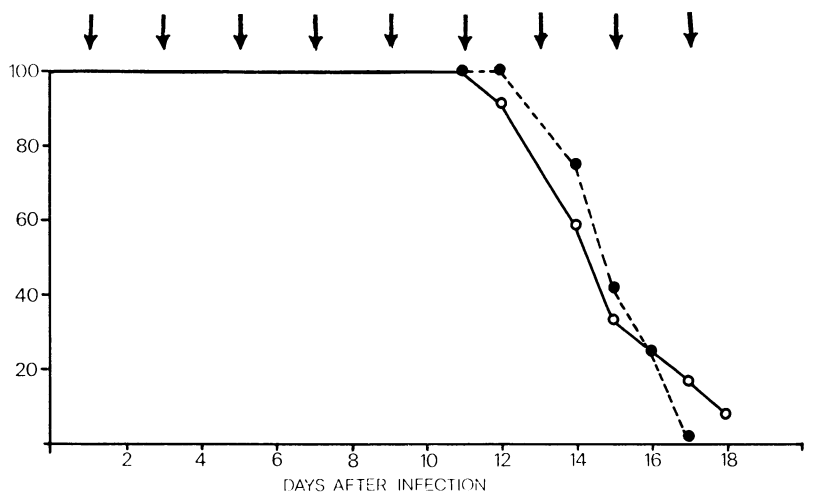

FIG. 9.—Percentage survival of rats infected with *Plasmodium berghei*. ●– – – –● NRS treated. ○————○ ATS treated. Arrows indicate injection of 0·5 ml. serum i.p.

of the parasitaemia. This animal lived for several months until it was killed. All other animals in both groups died between 12–17 days following infection (Fig. 9).

Haematology and parasite rate

The percentage of parasitised red cells rose in a similar manner in both groups of animals to reach a peak of 55 per cent at day 14 (Fig. 10). Thereafter there was a slight fall in both groups. The rather greater fall in the ATS treated group was probably related to the one animal that survived the experiment with clearance of its parasitaemia.

The reticulocyte count closely parallelled the parasite rate and was similar in both groups reaching a peak of 55 per cent at day 14 and falling thereafter (Fig. 11). The haematocrit was also similar in both groups apart from one discordant result in the NRS treated group on day 13 (Fig. 12).

The total WBC never rose above the control value in the ATS treated animals

throughout the infection. In contrast the NRS treated group showed a terminal leucocytosis. This was in part due to an increase in circulating monocytes. The monocyte count in the ATS treated animals was raised above the control values

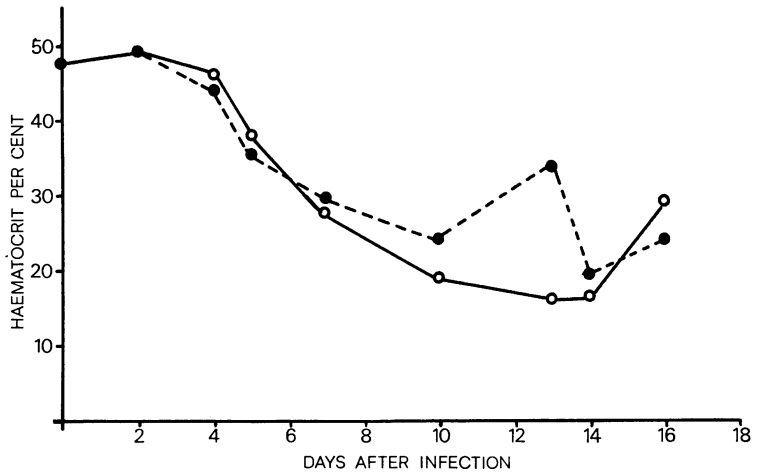

FIG. 10.—Haematocrit values in rats infected with *Plasmodium berghei*.
●----● NRS treated. ○——○ ATS treated.

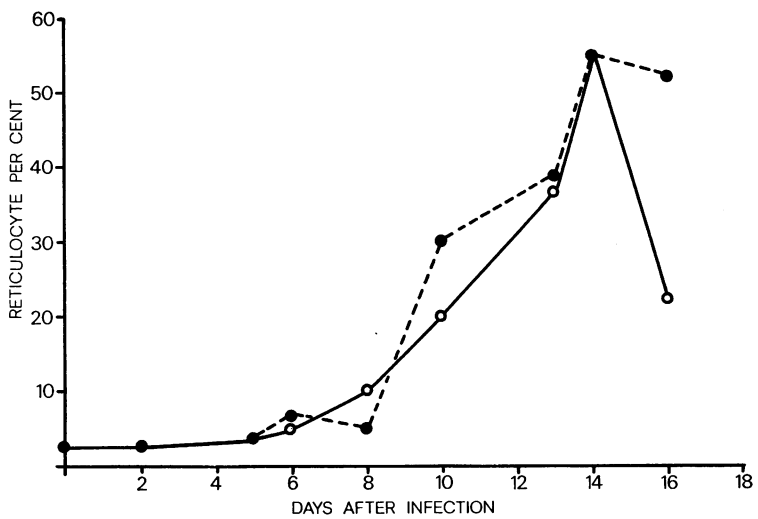

FIG. 11.—Reticulocyte count in rats infected with *Plasmodium berghei*.
●----● NRS treated. ○——○ ATS treated.

but was consistently lower than in the NRS treated group. Both groups showed a fall in the lymphocyte count below the control values. This was most marked in ATS treated animals except in the late stages of the infection when the count in the NRS treated animals fell to its lowest value (Table III).

TABLE III.—*Total WBC per mm³ and Absolute Lymphocyte and Monocyte Counts per mm³ in ATS treated and NRS Treated Rats Following Infection with* Plasmodium berghei

	ATS treated			NRS treated		
Day	WBC ($\times 10^3$)	Lymphocyte count ($\times 10^3$)	Monocyte count ($\times 10^3$)	WBC ($\times 10^3$)	Lymphocyte count ($\times 10^3$)	Monocyte count ($\times 10^3$)
2	7·6	4·6	1·6	11·5	7·9	2·1
5	12·0	4·0	7·7	15·9	6·5	9·2
6	10·9	2·8	8·9	13·7	6·5	10·0
8	9·7	3·5	3·3	16·4	4·6	10·2
10	10·0	2·8	4·6	15·7	6·5	9·0
13	12·3	5·4	4·8	16·7	6·0	7·8
14	11·0	3·0	6·5	28·0	5·6	7·2
16	9·5	3·5	3·2	19·6	2·3	10·6

Uninfected untreated rat controls:

WBC	$14·0 \times 10^3$ per mm³
Lymphocyte count	$10·8 \times 10^3$ per mm³
Monocyte count	$7·7 \times 10^2$ per mm³

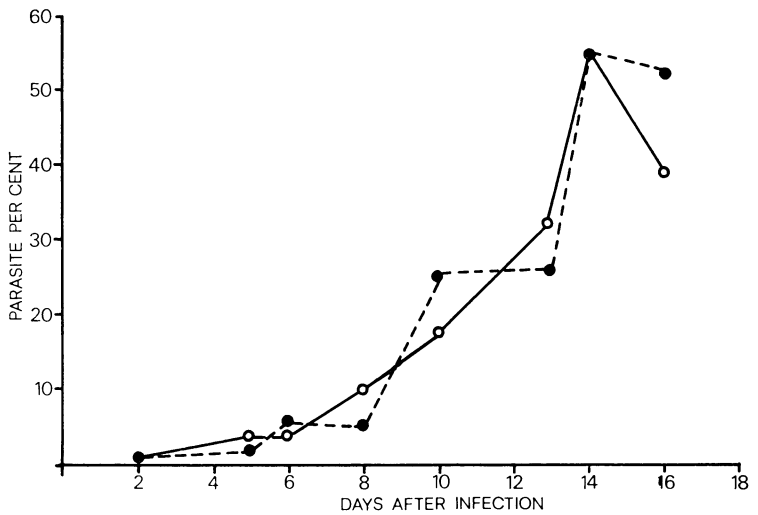

FIG. 12.—Percentage parasite rate in rats infected with *Plasmodium berghei*.
●- - - -● NRS treated. ○———○ ATS treatod.

Gross findings

Many of the animals died overnight and were either autolysed or partly eaten. No gross differences were seen in the 2 groups of animals.

Histological findings

Neither the ATS treated nor the NRS treated animals showed brain haemorrhages though several animals exhibited marked congestion of the brain capillaries. The liver, spleen and lymph nodes showed marked malarial pigmentation. There was no obvious difference in the spleen sections between the 2 groups, both showed marked extramedullary haematopoiesis a feature that was not seen in the hamster spleens. The ATS treated animals exhibited an extreme degree of thymic and

lymph node atrophy with depletion of small lymphocytes. There was moderate thymic atrophy in the NRS treated animals. Both groups showed large numbers of plasma cells in the medulla of the lymph nodes.

DISCUSSION

Anti thymocyte serum has a similar dramatic effect on *Plasmodium berghei* infection in the hamster as neonatal thymectomy. Six-week-old hamsters treated with normal rabbit serum died abruptly between 5–10 days after infection in a similar manner to that previously reported in untreated hamsters infected with *Plasmodium berghei* (Wright, 1968). They rapidly became drowsy and ataxic and usually died within 24–48 hr of becoming ill. At this time the ATS treated animals were bright eyed and active. Both groups had parasite rates between 2–6 per cent and haematocrits between 20–30 per cent. The ATS treated animals died between 12–17 days after infection. Their mode of death was different from that of the NRS treated animals in that they did not show ataxia but became progressively more lethargic with rapid respirations and died after several days of apparent illness.

In these experiments the parasite rate was not measured in the ATS treated animals beyond the eleventh day after infection when it had reached 10 per cent. In neonatally thymectomised hamsters the parasite rate reaches 50 per cent or more before death (Wright, 1968).

In contrast to the hamsters ATS had no effect on the survival of rats infected with *Plasmodium berghei*. Animals in both the ATS treated and the NRS treated groups died between the 11–18th days after infection with parasite rates ranging from 25–50 per cent. The mode of death in both groups was similar, with progressive lethargy and apparent dyspnoea for several days before death. Although the ATS was not tested for activity by skin grafting or *in vitro* tests it did have an apparent effect in causing marked thymic and lymph node atrophy and in lowering the blood lymphocyte count. The lowered monocyte count in the ATS treated rats relative to the NRS treated animals was presumably also brought about by the ATS.

Rats below the age of 8 weeks usually die with fulminating parasitaemia following infection with *Plasmodium berghei* whereas most adult rats develop immunity with clearance of the parasitaemia after about 10 days. Brown *et al.*, (1968) showed that neonatally thymectomised Sprague-Dawley rats infected with *Plasmodium berghei* at 13 weeks had a high parasitaemia and 50 per cent death rate whereas intact animals of the same age had a lower parasitaemia and only 2 per cent of the animals died. They suggest that a thymus-dependent immune reaction is involved in the resolution of the malarial infection. Stechschultz (1969) similarly showed that neonatally thymectomised rats had a higher percentage parasitaemia and higher mortality than sham-operated animals. He found no impairment of carbon clearance and of antibody response as measured by indirect haemagglutination and fluorescence antibody titres in the thymectomised animals and concluded that these experiments support the concept that cellular immunity participates in the development of acquired resistance to *Plasmodium berghei* in rats. Spira *et al.* (1970) demonstrated that antithymocyte serum had a similar effect to neonatal thymectomy. Using 6–7-week-old Lewis rats all the ATS treated animals died whereas only 50 per cent of the NRS treated animals

died. In 10-week-old rats the NRS treated animals had a low parasitaemia and all survived whereas the ATS treated animals had a 40–60 per cent parasitaemia and 7 out of 8 died. They showed that humoral antibodies as measured by agar-gel double diffusion were present in the ATS treated animals as well as those receiving NRS and they conclude that cell-mediated immunity is substantially involved in resistance to *Plasmodium berghei*. While the effects of neonatal thymectomy and ATS are consistent with the hypothesis that acquired immunity to *Plasmodium berghei* is mediated by cellular mechanisms it does not exclude the possibility that a thymus-dependent humoral reaction is responsible for this immunity.

The rats used in the experiments reported here were all under 6 weeks old and therefore at an age when their immune reaction appears to be incapable of overcoming the malarial infection. It is not surprising therefore that further depression of the immune reaction by ATS has no apparent effect. Neonatal thymectomy similarly has no apparent effect on the course of *Plasmodium berghei* infection in rats at 6 weeks of age (Wright and Pike, unpublished). It should be noted, however, that neonatal thymectomy and ATS have no protective effect in the rat as they do in the hamster.

Sheagren and Monaco (1969) demonstrated a protective effect of antilymphocyte serum on mice infected with *Plasmodium berghei* with a significant delay in mortality. They suggest that impairment of the immune response permits longer circulation of parasitised red cells and therefore longer survival of the ALS treated animals. Their results, are, however, confused by the observation that the ALS treated mice had a lower parasitaemia than the NRS treated animals and they suggest that ALS may have an intrinsic anti-parasitic effect. No anti-parasitic effect of ATS was found in hamsters or rats in these experiments nor by Spira *et al* in rats.

It is apparent that the mechanism of death in hamsters infected with *Plasmodium berghei* is different from that in rats. The latter develop a high parasitaemia and die 2–3 weeks after infection. Hamsters die only 5–10 days after infection with a parasitaemia of less than 10 per cent. If hamsters are treated with ATS or have been subjected to neonatal thymectomy their infection follows a similar course to that seen in young rats with death occurring 2–3 weeks after infection and parasite rates of 50 per cent or more.

The ATS and NRS treated hamsters show striking differences in survival despite similar parasite rates and haematocrit values at the time when the NRS treated animals are dying. The NRS treated hamsters show neurological changes before death and at post-mortem examination are found to have petechial haemorrhages in the brain. Microscopically these haemorrhages often surround capillaries that appear to be plugged by agglutinated red cells, appearances that are similar to those seen in human cases of cerebral malaria. Neonatal thymectomy and ATS appear to exert their protective effect in hamsters infected with *Plasmodium berghei* by preventing these cerebral haemorrhages. The haemorrhages occur between the 5–6th day following infection and are not associated with cellular infiltrates but are associated with red cell agglutinates. It would seem reasonable to suggest that the infected animals produce an agglutinin, probably an IgM antibody, that results in the agglutination of parasitised and possibly non-parasitised red cells and the micro-embolisation of cerebral capillaries. Occlusion of capillaries results in ischaemic damage to brain tissue and in damage to vessel walls with the escape of protein and red cells from the vessels. The

production of this agglutinin is presumably thymic dependent and is inhibited or delayed by neonatal thymectomy or ATS. Experiments are now in progress to substantiate this hypothesis.

The reason why rats do not develop brain haemorrhages is not clear but it could be due to many factors including a different time scale of antibody response or to different red cell antigens. In these experiments the NRS treated hamsters developed a relative lymphocytosis during the course of the infection whereas the lymphocyte count in the NRS treated rats was initially high but fell as the infection progressed. Conversely the rats showed a much more marked monocyte response, relative to the control levels, than the hamsters.

Kreier, Shapiro, Dillen, Szilvassy and Ristic (1966) have shown that rats infected with *Plasmodium berghei* develop a mercapto-ethanol sensitive agglutinin to trypsinised autologous and homologous parasitised and non-parasitised red cells. They did not report petechial haemorrhages in their animals. Mercado (1965), however, 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 severely anaemic. She was able to make other strains of *Plasmodium berghei* " paralytic " by injecting them with cell-free extracts of blood or tissues from affected rats, although cell free extracts alone produced no paralytic effects. She concluded that the pathological changes were brought about either as the result of the modification of the malarial parasite or by the interaction of a filterable agent and the malarial parasite. The response of the rats in these experiments appears to be similar to that of hamsters infected with *Plasmodium berghei* and the significance of the apparent modification of parasite behaviour by cell free blood or tissue extracts is not clear. The development of brain haemorrhages in hamsters infected with *Plasmodium berghei* has been observed in Kampala and Birmingham (Williams and Wright, unpublished) using different colonies of hamsters and different sources of *Plasmodium berghei* indicating that these observations are not due to local peculiarities. Also the rapid death of hamsters infected with larger doses of *Plasmodium berghei* has been recognised for many years (Adler, Yoeli and Zuckerman, 1950; Yoeli and Most, 1964) although the mechanism of death of these animals appears to have been ignored.

The mode of death and pathological changes in the brain of hamsters infected with *Plasmodium berghei* has many features in common with human cerebral malaria and may form a valuable model for the study of this condition. It has previously been noted that children with kwashiorkor are less susceptible to cerebral malaria than well nourished children. It may be that the extreme thymic atrophy and immunological impairment of children with kwashiorkor protects them from this most serious complication of malaria.

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