duration of their illness, and the duration of treatment. It is concluded that other factors may determine a patient's response to withdrawal of maintenance therapy with these drugs.

We wish to thank Professor E. J. Wayne for the opportunities of studying the cases under his care and the physicians of Sheffield Royal Infirmary for their valuable co-operation. We also wish to thank Dr. A. G. MacGregor for his help and for supplying data on the follow-up of some of the patients, and Mr. Arthur Summerfield for helpful advice on the statistical analysis. Part of the expenses of this research were defrayed by a grant from the Medical Research Council to one of us (A. W.) and to another (H. S.) who is in receipt of a whole-time personal grant.

REFERENCES

- Astwood, E. B. (1949). *Advanc. intern. Med.*, 3, 237.
 Douglas, D., and Kennic, A. T. (1952). *British Medical Journal*, 2, 1387.
 Dunlop, D. M., and Rolland, C. F. (1950). *Proc. roy. Soc. Med.*, 43, 937.
 Himsworth, H. P. (1948). *British Medical Journal*, 2, 61.
 — Morgans, M. E., and Trotter, W. R. (1947). *Lancet*, 1, 241.
 Poate, H. R. G. (1950). *Med. J. Aust.*, 1, 139.
 Trotter, W. R. (1950). *Proc. roy. Soc. Med.*, 43, 945.
 Williams, R. H. (1949). *J. Amer. med. Ass.*, 139, 1064.
 Wilson, A. (1946). *Lancet*, 1, 640.
 — and Goodwin, J. (1947). *Ibid.*, 1, 669.
 Wing, E. S., and Asper, S. P., jun. (1952). *Bull. Johns Hepk. Hosp.*, 90, 201.

MILK, *p*-AMINOBENZOATE, AND MALARIA OF RATS AND MONKEYS

BY

F. HAWKING, D.M., M.R.C.P.

National Institute for Medical Research, Mill Hill,
London, N.W.7

This paper describes investigations concerning the effect of milk diets upon the course of malarial infections, and the supplementation of such diets by *p*-aminobenzoate, which is a growth factor for many species of plasmodia.

In 1952 Maegraith, Deegan, and Jones reported that infections of *Plasmodium berghei* do not develop properly if the hosts (rats) are maintained on a milk diet. A preliminary communication from this laboratory (Hawking, 1953) pointed out that a milk diet was deficient in *p*-aminobenzoate, which is apparently needed by many plasmodia. It was shown that if *p*-aminobenzoate was added to the milk diet the infection of *P. berghei* developed normally, and it was concluded that *p*-aminobenzoate (or some derivative) is a growth factor for *P. berghei*. According to our conception the body fluids of animals usually contain very little *p*-aminobenzoate (otherwise sulphonamides would not be therapeutically effective against so many bacteria); *p*-aminobenzoate is present in many diets; when ingested in the food it raises the blood concentration sufficiently for malaria parasites to grow; and it is rapidly excreted or destroyed, so that constant renewal is necessary. The interrelationship of growth factors and malarial infections raises so many questions, both practical and academic, that several years will be needed to investigate them adequately. As, however, the subject is of interest to other workers, the results obtained to date are presented here.

Plasmodium berghei in Rats

The animals employed were obtained from the strain of piebald rats maintained at this institute. After preliminary experiments it was found that rats weighing 200 g. were

preferable to those of 100 g. or 50 g., because with the smaller weights suppression of the infection by milk diet was more difficult to demonstrate. The normal diet of these rats is a stock laboratory one which has been described by Bruce and Parkes (1949). Briefly, it contains wholemeal flour 46%, Sussex ground oats 40%, white fish meal 8%, dried skimmed milk 3%, dried yeast 1%, cod-liver oil 1%, and salts 1%. The milk employed was obtained from the ordinary dairy, and had been pasteurized. One sample of it was found by bioassay (kindly performed by Dr. A. T. Fuller) to contain 3.1 mμg. free and 19 mμg. total *p*-aminobenzoate per ml. The rats were housed three in a cage over a grid to allow escape of faeces. They were supplied with milk from a bottle. The bottles were changed morning and evening, and they were autoclaved in between each change. The quantity of milk was limited to the amount which the rats would probably consume in four to five hours, and all other possible steps were taken to prevent the milk going sour. The rats were placed on the diets for two to four days before inoculation. The strain of *P. berghei* was the RSVP strain obtained from Professor P. C. C. Garnham. The inoculum usually contained about 2 million parasitized cells per rat, and it was injected intraperitoneally. The degree of infection was expressed as the number of parasitized red blood corpuscles in a thin stained film per microscope field; this usually contained about 200 corpuscles.

The result of a typical experiment is shown in Fig. 1, in which the intensity of infection in each cage of three rats is shown as the mean of the logarithms of the number of

FIG. 1.—The development of *P. berghei* in rats on milk diet, milk plus *p*-aminobenzoate (P.A.B.) 1/40,000, and stock diet. Each line represents the mean of the logarithms of three rats, the infections being expressed as the number of parasitized cells per 20,000 erythrocytes. Inoculation intraperitoneally on Day 0 with 100,000 parasitized cells per rat. Weight of rats, 190–220 g.

parasitized erythrocytes per 20,000 cells in a thin blood film. (For this calculation, animals which showed no parasites were deemed to have 1 per 20,000 cells; animals dying of the infection towards the end of the experiment were deemed to have 8,000 parasites per 20,000.) This figure shows that the infection of *P. berghei* does not develop in rats on a milk diet (confirming the report of Maegraith *et al.*), but that if *p*-aminobenzoate is added to the milk the infection develops as well as in the control rats on the complete laboratory diet.

In order to obtain suppression of the infection on milk diet various precautions are necessary. Thus the rats employed should be fully adult—that is, weighing 200 g.—in younger rats the suppression is more difficult to obtain. The

milk must not be allowed to become sour; presumably souring is accompanied by increase of the *p*-aminobenzoate content. Prolongation (beyond three to four days) of the period on the diet before inoculation did not seem greatly to improve the results.

Probably this suppression of infections of *P. berghei* by milk diet is a marginal phenomenon, and a small alteration of the conditions may make the difference between success and failure. The composition of milk, including its content of *p*-aminobenzoate, is not uniform, but will vary according to the season and diet of the cows; at some seasons it was difficult to demonstrate suppression of parasitaemia by milk diet. It was sometimes observed that in two of a group of rats on milk there was complete suppression of parasitaemia, while in the third rat the infection was as great as in the controls; such a result suggests some individual factor present in certain rats, possibly different bacterial flora or syntheses in the intestine.

In some experiments chloramphenicol 1/20,000 was added to the milk in the hope of reducing bacterial growth in the intestine and thus diminishing a factor which might interfere with the effect of the milk diet; in such experiments, however, the malarial infection developed almost as well as in the control rats on a complete diet—that is, the suppression of the malaria by milk diet was reversed. This result was surprising, until it was learnt that chloramphenicol can be decomposed by *Bact. coli* and other bacteria into various derivatives which include *p*-aminobenzoate (Smith, 1953).

The minimum concentration of *p*-aminobenzoate in milk required to permit growth of *P. berghei* as good as in the controls seemed to be about 1/40,000; concentrations of 1/100,000 and 1/1,000,000 were sometimes effective and sometimes not. Assuming that each 200-g. rat consumes 150 ml. of milk a day, a concentration of 1/40,000 corresponds to a daily intake of approximately 20 mg. of *p*-aminobenzoate per kg. body weight.

Rats on a milk diet sometimes get diarrhoea, indicating modification of the intestinal flora. Rats on the control diet lost on an average 5% of weight in two weeks during the experiments, presumably owing to the malaria; on a milk diet they lost 2–3%. Pteroylglutamic (folic) acid 1/10,000 added to the milk promoted the growth of *P. berghei* as well as an equimolar solution of *p*-aminobenzoate—that is, 1/40,000; accordingly, these two compounds seem to be interchangeable, but it is not clear which of them is converted into the other. The suppression of *P. berghei* by milk diets could not be reversed by adding methionine (1/2,000) or *p*-hydroxybenzoate (1/3,000) to the milk or by injecting vitamin B₁₂ (150 µg. per kg. daily).

Infection by *P. berghei* could also be prevented from developing by placing the rats on other deficient diets—for example, a grossly deficient one consisting only of 10% glucose. Rats live well on this diet for over two weeks (end of experiment), with a loss of weight averaging 6–24% in different experiments. Addition of *p*-aminobenzoate 1/1,000 plus methionine 1/500 to this diet was not enough to allow the infection to develop.

Suppression of *P. berghei* infection by milk diet could also be demonstrated in mice, but often the suppression was less complete than that observed in rats.

Plasmodium knowlesi in Monkeys

The suppression of *P. berghei* infections by the administration of a *p*-aminobenzoate-deficient diet—for example, milk—can be correlated with the great susceptibility of this parasite to sulphonamides (which antagonize *p*-aminobenzoate). Accordingly experiments were made to ascertain whether the same phenomenon (suppression by milk diet and reversal by *p*-aminobenzoate) could be demonstrated with other malarial infections known to be sensitive to sulphonamides. The first study was made with *P. knowlesi*, which is known to respond readily to sulphonamide treatment. The strain was derived from the new and virulent strain recently isolated

by Edeson (Edeson and Davey, 1953). Four small *Macaca mulatta* monkeys weighing approximately 1.5 kg. were placed as follows:

Monkey 224 on the normal complete laboratory diet, as used for rats.

Monkey 225 on milk.

Monkey 226 on milk plus *p*-aminobenzoate 1/10,000.

Monkey 227 on milk plus *p*-aminobenzoate 1/1,000,000.

After three days, each of them was inoculated intramuscularly and intraperitoneally with 5×10^7 parasitized blood corpuscles. The course of the resultant infections is shown in Fig. 2. (The infection in Monkey 226 was indistinguishable from that in Monkeys 224 and 227 and has been omitted.)

Briefly the control Monkey 224 showed parasites in the blood five days after inoculation, and the next day it was

treated with mepacrine and sulphadiazine to save its life. Monkey 225 (on milk) never showed any parasites during five weeks while it was fed only on milk; four days after it had been transferred to a normal diet its blood contained one parasite in every microscope field. Clearly the infection had been kept at a level too low for recognition while the monkey was on the milk diet, but the parasites multiplied rapidly when a complete diet was given. Monkey 227 (on milk plus *p*-aminobenzoate 1/1,000,000) and also Monkey 226 (milk plus *p*-aminobenzoate 1/10,000) showed the development of parasitaemia indistinguishable from that of the control monkey (224). Monkey 227, weight 2 kg., consumed about 1 litre of milk a day, so that it received about 0.5 mg. of *p*-aminobenzoate per kg. daily; this small amount was apparently enough to make it fully susceptible to infection with *P. knowlesi*. As the supply of monkeys was limited and the results formed such a striking confirmation of expectations, the experiment was not repeated. Possibly with a larger group of monkeys on milk diet the suppression of parasitaemia would not always have been so complete.

Similar experiments were made with *P. cynomolgi* in monkeys and yielded similar results; they are described later in this paper. Preliminary attempts were made to keep chickens on diets of liquid milk or dry milk so as to test *P. gallinaceum* (which is also sensitive to sulphonamides). The chickens did not take well to the diets, and the effects upon the infection were not clear. Further experiments with *P. gallinaceum* were postponed.

Malaria in Baby Animals

As pointed out by Maeraith *et al.*, the suppression of malaria infections in laboratory animals by milk diet raises the interesting question whether a similar suppression may occur in human babies in the Tropics while fed exclusively on breast milk. Certainly *P. falciparum* and *P. vivax* are sensitive to sulphonamides, and so presumably they require *p*-aminobenzoate. Model experiments were made on young laboratory animals.

FIG. 2.—The development of *P. knowlesi* in monkeys on milk diet, milk plus *p*-aminobenzoate 1/1,000,000, and stock diet. The infection is expressed as the number of parasites per 100 microscope fields each containing approximately 200 erythrocytes.

(a) Suckling Rats Inoculated with *P. berghei*

Litters of rats about one week old were taken when the baby rats weighed about 10–15 g., and were tested as shown in the Table. In this experiment the babies (eight in each cage) were inoculated intraperitoneally each with 1 million parasitized cells (Day 0). The mothers were placed on a

Development of *P. berghei* in Suckling Rats Receiving *p*-aminobenzoate (P.A.B.) by Different Routes

Cage	Contents	Mean Parasitaemia			
		4 Days	5 Days	6 Days	7 Days
1	Controls	4.6 (3/8)	13 (4/8)	51 (6/8)	126 (8/8)
2	Babies inoculated with 0.1 mg. P.A.B. i.p.	2,000 (8/8)	3,400 (8/8)	—	2,800 (8/8)
3	Mother smeared with P.A.B.	1,250 (8/8)	2,400 (8/8)	—	3,100 (8/8)
4	Mother inoculated with 10 mg. P.A.B. i.p.	2,000 (8/8)	4,000 (8/8)	—	3,500 (8/8)
5	Young rats (50 g.) on stock diet	—	1,000 (4/4)	3,000 (4/4)	—

Mean parasitaemia is expressed as the geometric mean of the number of parasitized erythrocytes per 20,000 cells. The figures in brackets indicate the proportion of animals showing parasites.

diet of 4 parts starch plus 1 part casein, beginning on the day before the experiments in order to diminish the amount of *p*-aminobenzoate in their diet. In cage 2 the baby rats were inoculated intraperitoneally twice daily with 0.1 mg. of *p*-aminobenzoate each; in cage 3 a mixture of 10% glucose plus 1% *p*-aminobenzoate was smeared on the nipples of the mother twice daily, so that the babies would receive the compound when they sucked; in cage 4, 10 mg. of *p*-aminobenzoate was injected intraperitoneally twice daily into the mother (200 g. weight) with the expectation that some would be excreted in the milk. Cage 5 contained four young rats (50 g.) on stock diet as an extra control. When the blood of these baby rats was first examined four days after inoculation, only three out of the eight controls (cage 1) showed scanty parasites. By seven days all the controls showed parasites, but their numbers were few. Cage 5 (weaned rats on stock diet) showed the usual acute infection developing. In all the three litters which received *p*-aminobenzoate by any route (cages 2, 3, and 4), the infection was already almost at its maximum level even on the fourth day. The fact that this occurred even in the babies of the mother receiving *p*-aminobenzoate by injection (cage 4) confirms the expectation that *p*-aminobenzoate (or a derivative) is excreted in the milk.

This experiment (which is typical of others) shows that infections of *P. berghei* in suckling rats develop much more rapidly and intensely if *p*-aminobenzoate is supplied by some route or other; from which it may be concluded that in normal circumstances the malarial infection is held back by a deficiency of this subject. Theoretically, suckling rats on a diet completely deficient in *p*-aminobenzoate would be insusceptible to infection; in practice suckling rats probably receive a small amount of *p*-aminobenzoate in their mother's milk, derived from her diet or perhaps from her intestinal bacteria, and this is sufficient to account for the low-grade infection which develops. The more stringent the precautions taken to exclude *p*-aminobenzoate from the diet of the mothers, the greater the suppression of parasitaemia in the babies. Thus in some experiments the mother rats were placed on *p*-aminobenzoate-deficient diet (starch 80%, casein 20%), beginning three days before parturition, and the babies were inoculated with malaria three days after they were born; babies of mothers which received *p*-aminobenzoate by twice-daily injection developed a parasitaemia of about 1% in five days, and the parasitaemia rapidly increased; babies of mothers not receiving *p*-aminobenzoate remained free from parasitaemia for 13 days and many of them never developed infection. If, on the other hand, the laboratory diet of the mother rats was rich in *p*-aminobenzoate, it might probably be impossible to demonstrate the above insusceptibility of sucklings to malaria.

(b) Baby Monkeys and *P. cynomolgi*

A few young *M. mulatta* monkeys were available from breeding at this institute. Two of these were about 1 month old, receiving milk from the breast or a bottle, and the others were 5 to 14 months old and had been weaned on to ordinary diet.

In the first experiment there were four monkeys as follows:

Monkey 217, weighing 3.8 kg., fed on the normal laboratory diet (food cubes as for rats and also occasional cabbage).

Monkey 221, weighing 3.5 kg., on milk diet.

Monkey 222, over 1 year old, weighing 2.5 kg., placed on a diet of milk plus *p*-aminobenzoate 1/1,000.

Monkey 223, 4 weeks old, weighing 0.75 kg. It had been brought up on milk from a bottle because its mother had refused to feed it. It had also received 9 ml. daily of a vitamin preparation called "haliborange." (This contains 75 m μ g. of *p*-aminobenzoate per ml.; Dr. A. T. Fuller.)

After Monkeys 217, 222, and 223 had been on their diets for one week, and 223 was 4 weeks old, all were inoculated intraperitoneally and intramuscularly with blood containing

about 2×10^7 parasites of *P. cynomolgi*.

The infections in 217, 221, and 222 are shown in Fig. 3. Monkey 217 (control on full diet) showed a typical infection with this parasite.

Monkey 221 (on milk diet) showed no parasites for 10 days; it was then placed on milk plus *p*-aminobenzoate 1/1,000, but no parasites appeared during the next nine days; it was then inoculated with blood containing *P. cynomolgi* (to demonstrate its susceptibility), and a typical infection developed. Monkey 222 (on milk plus *p*-aminobenzoate 1/1,000) developed a typical infection, similar to that of the control (217); but rather more severe. Monkey 223 showed no parasites until the

fourteenth day, when two rings were discovered in 50 fields; on the fifteenth day the infection was six per 50 fields; on the sixteenth day it was three per 50 fields, and on the seventeenth it was one per 100 fields. On the fifteenth day it was discovered that the monkey had received 9 ml. of haliborange daily from the sixth day onward, which would contribute 0.6 μ g. of *p*-aminobenzoate per day; this was stopped. On the nineteenth day one parasite was seen in 50 fields. The monkey ceased to gain weight, and put its hand to its head as though it had headache. On the twenty-second day it was put on chloroquine treatment (15 mg. per kg. daily intramuscularly for three days) and no further evidence of malaria has been seen.

The results with Monkeys 217, 221, and 222 show that a milk diet suppresses blood-induced infections of *P. cynomolgi* and that this suppression is reversed by *p*-amino-

FIG. 3.—The development of *P. cynomolgi* after inoculation with infected blood of monkeys on various diets, as described in the text. The infection is expressed as the number of parasites per 100 microscope fields, each containing 200 erythrocytes; for numbers above 100 the scale is greatly reduced for convenience.

parasites until the fourteenth day, when two rings were discovered in 50 fields; on the fifteenth day the infection was six per 50 fields; on the sixteenth day it was three per 50 fields, and on the seventeenth it was one per 100 fields. On the fifteenth day it was discovered that the monkey had received 9 ml. of haliborange daily from the sixth day onward, which would contribute 0.6 μ g. of *p*-aminobenzoate per day; this was stopped. On the nineteenth day one parasite was seen in 50 fields. The monkey ceased to gain weight, and put its hand to its head as though it had headache. On the twenty-second day it was put on chloroquine treatment (15 mg. per kg. daily intramuscularly for three days) and no further evidence of malaria has been seen.

The results with Monkeys 217, 221, and 222 show that a milk diet suppresses blood-induced infections of *P. cynomolgi* and that this suppression is reversed by *p*-amino-

benzoate. In the baby monkey (223) suppression was incomplete, although the parasitaemia was very much later and lower than in the control (217). This incompleteness of suppression may have been due to variation between different animals, or it may have been due to the halib-orange preparation.

Fig. 3 also shows an experiment with another monkey (228) which was performed on a later occasion but which is added here for ease of comparison. This was a baby rhesus monkey, 10 weeks old, which was being nursed on its mother's breast. (In a previous experiment—see below—it had been inoculated twice with sporozoites of *P. cynomolgi* and no infection had developed.) The mother was inoculated intramuscularly twice daily with *p*-aminobenzoate, 0.03 g. per kg.

After this had been done for six days the baby (228) was inoculated intraperitoneally with approximately 3×10^6 parasites of *P. cynomolgi*. Parasites appeared in the blood after four days and reached the stage of two parasites per field seven days after inoculation (after which the monkey was treated). As shown in Fig. 3, the development of infection in this monkey was similar to that of the previous control monkey (217) and of the monkey receiving *p*-aminobenzoate as well as milk (222). This experiment confirms the previous experiments with rats, demonstrating that when *p*-aminobenzoate is administered to nursing mothers it (or a derivative) passes via the milk to the offspring and renders them susceptible to malarial infection.

In the second type of experiment, infection was induced by means of sporozoites of *P. cynomolgi*. There were four *M. mulatta* monkeys:

Monkey 218 was kept on normal diet as a control.

Monkey 229, weight 3.8 kg., was placed on milk diet.

Monkey 230, weight 2.2 kg., was placed on milk plus *p*-aminobenzoate 1/5,000.

Monkey 228, a baby on the breast (mentioned above), was 4 weeks old when inoculated.

After 229 and 230 had been on their diets for five days all four monkeys were inoculated intraperitoneally with a suspension containing the glands of one infected mosquito—that is, each monkey received one-quarter of this amount; 20 days later this inoculation was repeated for each monkey. The results with three of the monkeys are shown in Fig. 4. Briefly, 218 first showed parasites 14 days after the first inoculation, and the infection reached a peak of 10 parasites per field on the twenty-second day; this is a typical infection following the inoculation of small numbers of sporozoites of *P. cynomolgi*. Monkey 228 (breast-fed baby) showed no parasites during eight weeks from inoculation, after which it was used for another experiment. Monkey 229 (on milk diet from a bottle) showed no parasites for five weeks. It was then placed on the normal diet again. Eighteen days after this change it began to show parasites, and mild parasitaemia (maximum 19 parasites per 50 fields) persisted for five days. (As the graph of this monkey was indistinguishable from

FIG. 4.—The development of *P. cynomolgi* after inoculation of sporozoites (on two occasions) in monkeys on diets of breast milk, cow's milk plus *p*-aminobenzoate 1/5,000, and stock food cubes. The infection is expressed as in Fig. 3.

that of 228 it has been omitted from Fig. 4.) Monkey 230 (milk plus *p*-aminobenzoate) showed parasites 14 days after the first inoculation and experienced an infection indistinguishable from that of the control (218).

This work shows that infections of *P. cynomolgi* induced by sporozoites are suppressed by a milk diet, and that again this suppression is reversed by *p*-aminobenzoate. Bray and Garnham (1953) have previously shown that the pre-erythrocytic forms are not abolished by the milk diet; they persist in a latent form, and the infection breaks out again when the animal is returned to a normal diet. The absence of parasitaemia in the breast-fed baby (228) is particularly suggestive as an explanation of the partial insusceptibility of human infants to malaria.

Discussion

In the above work and the papers already quoted it is shown that milk (and other) diets render rats and monkeys insusceptible to infection with malaria, and that this insusceptibility may be due to a deficiency of *p*-aminobenzoate (sometimes together with other factors), which is apparently a growth factor for these species of plasmodia. It has long been known that the course of infections can be modified by the diet of the host. According to a review by McKee (1951), the growth of various plasmodia has been reported at different times to be depressed by feeding the host on diets deficient in *p*-aminobenzoate, methionine, ascorbic acid, vitamin A, riboflavin, or pantothenate, but to be increased by diets deficient in biotin, folic acid, nicotinic acid, choline, or protein.

In particular, Geiman and McKee (1948) found that if monkeys were starved they were not susceptible to infection with *P. knowlesi*, but they became susceptible if given *p*-aminobenzoate or methionine. There is a striking parallelism between their experiment and the ones described above, in which monkeys were not susceptible to this infection when kept on a milk diet, but became susceptible if given *p*-aminobenzoate. Previously, workers of their group had shown that *p*-aminobenzoate was necessary for the growth of *P. knowlesi* *in vitro* (Anfinsen *et al.*, 1946).

Further evidence for the relation of this compound to the growth of malaria parasites is given by the sensitivity of many species of plasmodia to sulphonamides (which antagonize *p*-aminobenzoate). Thus *P. knowlesi* and *P. berghei* are very sensitive to sulphonamides (and their growth is prevented by a milk diet but promoted by *p*-aminobenzoate); *P. gallinaceum*, *P. cynomolgi*, *P. falciparum*, and *P. vivax* are moderately sensitive to sulphonamides (the growth of *P. cynomolgi* is inhibited by a milk diet, etc.); while *P. relictum* is insensitive to sulphonamides (its response to a *p*-aminobenzoate-free diet has not yet been elucidated). It may be noted, however, that the amount of *p*-aminobenzoate which has usually to be added to the milk diet of rats to promote the growth of *P. berghei*—namely, the equivalent of 20 mg. per kg. of rat per day—is much greater than the amount which is probably provided by the normal stock diet.

Relation to Proguanil and Pyrimethamine

The recognition that *p*-aminobenzoate is a growth factor for the malaria parasites helps to elucidate the antimalarial action of proguanil and pyrimethamine, since (1) under suitable conditions the antimalarial action of proguanil and pyrimethamine upon *P. berghei* can be antagonized by *p*-aminobenzoate (Thurston, 1950, 1954); therefore these drugs probably interfere with the utilization of *p*-aminobenzoate (or some derivative like folic acid); (2) there is often cross-resistance between proguanil and pyrimethamine on the one hand and sulphonamides (which antagonize *p*-aminobenzoate) on the other; (3) pyrimethamine inhibits the growth of *Lactobacillus casei* and of *Str. faecalis* *in vitro*, and this inhibition can be overcome by folic or folinic acid (Hitchings, 1952).

Functions of *p*-aminobenzoate

The need of many malaria parasites for *p*-aminobenzoate is of particular interest because so much work has been done by microbiologists in elucidating the role of this compound in cell metabolism. Attention was first drawn to it because it antagonized the antibacterial action of sulphonamides (Woods, 1940). The exact details of its action are still the subject of controversy; but, briefly, (1) much (but not all) of the *p*-aminobenzoate absorbed by bacterial cells is built up into pteroylglutamic acid (folic acid), folic acid, or some similar compound; (2) *p*-aminobenzoate and/or a folic acid derivative acts as a catalyst in various important synthetic reactions, by which (usually) an extra carbon atom is added to some molecule such as an amino-acid; thus homocysteine is converted into methionine, glycine into serine, etc.; (3) *p*-aminobenzoate or its derivative is necessary as a catalyst for the synthesis of purine and pyrimidine rings (probably by the addition of another carbon atom); these are fundamental for the synthesis of nucleic acids and nucleoprotein, and so for the growth and multiplication of nuclei and cells.

In the case of the malaria parasite, morphological evidence for this relation between the supply of *p*-aminobenzoate and the division of the nucleus is provided by the following facts. The utilization of *p*-aminobenzoate by the parasite is antagonized by sulphonamides, proguanil, and pyrimethamine. If malaria parasites—for example, *P. falciparum*, *P. cynomolgi*, or *P. berghei*—are exposed *in vivo* to the action of one of these drugs the early development of the parasite inside the erythrocyte—that is, from ring to trophozoite—proceeds apparently unaffected. But when the chromatin (nucleus) begins to divide to form the schizont, morphological evidence of profound disturbance becomes manifest; division of the chromatin is arrested or takes place abnormally; and then the parasite gradually degenerates and disintegrates (Black, 1946; McFadzean, 1951; Thurston, 1951). Thus interference with the utilization of *p*-aminobenzoate prevents normal division of the nucleus (chromatin), perhaps because the synthesis of new nucleoprotein has been interrupted. The development of the gametocytes does not involve the need for so much new nucleoprotein, and it is not noticeably disturbed by sulphonamides and similar drugs. However, there is disturbance and suppression of the subsequent development of the gametes after conjugation—that is, the development of the oocyst—during which much nuclear division normally takes place.

Human Malaria

Finally, the application of this work to human malaria may be considered. It is said that there is a widespread belief in Arab countries that a milk diet protects against attack by malaria; on the supposition that this milk diet was not supplemented by other foods containing *p*-aminobenzoate, this belief would seem to possess biochemical justification. Further information of such folk-beliefs relating to milk and malaria would be welcome, even though such situations are of interest rather than of importance. Much more practical is the question of babies and malaria. It has long been recognized that babies in West Africa and similar hyperendemic regions do not contract malaria as early or as intensely as would have been expected from the degree of their exposure to infected mosquitoes (cf. Bruce-Chwatt, 1952; Reed, 1952). It has previously been considered that such early insusceptibility might be due to immunity inherited or acquired from the mother. In view of Maegraith's discovery and of the work reported above, it seems quite possible that their insusceptibility might rather be due to their milk diet—that is, their deficiency of *p*-aminobenzoate. The common human malaria parasites, *P. falciparum* and *P. vivax*, are susceptible to sulphonamides, and therefore they presumably require *p*-aminobenzoate as a growth factor.

Babies in Africa, and presumably most other parts of the Tropics, are at first fed exclusively on the breast; during this period, which may last for five or more months, they

would theoretically be insusceptible to malaria. After this period their diet is supplemented with various pastes or porridges of ground grain, etc.; if these contain *p*-aminobenzoate (as will often be the case) the increased malaria rate in babies at this age group is easily explained. Some babies, however, do get malaria at 3 months or earlier, when it is improbable that their diet is supplemented by solid food. In such cases it is possible that the mother may have absorbed increased quantities of *p*-aminobenzoate (either from her own diet or from unusual bacterial synthesis in the intestine) and she may have passed it via the milk to the baby. All these hypotheses are well supported by the experiments with suckling rats and monkeys described above, but extensive and circumspect observation in the field will be required before they can be proved or disproved.

Summary

Rats fed on a milk diet are insusceptible (or only slightly susceptible) to infection with *Plasmodium berghei* (Maegraith, Deegan, and Jones, 1952). This insusceptibility can be reversed by the addition to the diet of *p*-aminobenzoate, which seems to be a growth factor for many species of malaria parasite. The insusceptibility can also be produced by maintaining rats on other diets deficient in *p*-aminobenzoate.

The addition to the milk diet of 20 mg. of *p*-aminobenzoate per kg. of body weight per day was sufficient to permit normal multiplication of the malaria parasite.

The deficiency in the milk could also be corrected by adding folic acid (in molar amounts corresponding to the amount of *p*-aminobenzoate needed), but not by adding methionine, vitamin B₁₂, or *p*-hydroxybenzoate.

Monkeys maintained on a milk diet were insusceptible to infection by *P. knowlesi* or *P. cynomolgi*, but they became fully susceptible when *p*-aminobenzoate was added to the milk in amounts of 0.5 mg. per kg. of body weight per day.

Baby rats and baby monkeys, while exclusively breast-fed from their mothers, are (almost or completely) insusceptible to infection with *P. berghei* or *P. cynomolgi* respectively, provided that their mothers are fed on a diet deficient in *p*-aminobenzoate. If this substance is administered either to them or to their mothers they become fully susceptible.

It is suggested that the relative immunity to malaria shown by infants under 6 months in many parts of the Tropics may be due to a deficiency of *p*-aminobenzoate in their diet of mother's milk.

Grateful acknowledgments are due to Dr. A. T. Fuller for the estimations of *p*-aminobenzoate by bioassay, and to Mr. K. Broomfield and Miss J. P. Selby for invaluable technical assistance throughout these experiments.

REFERENCES

- Anfinson, C. B., Geiman, Q. M., McKee, R. W., Ormsbee, R. A., and Ball, E. G. (1946). *J. exp. Med.*, **84**, 607.
 Black, R. H. (1946). *Trans. roy. Soc. trop. Med. Hyg.*, **40**, 163.
 Bray, R. S., and Garnham, P. C. C. (1953). *British Medical Journal*, **1**, 1200.
 Bruce, H. M., and Parkes, A. S. (1949). *J. Hyg., Camb.*, **47**, 202.
 Bruce-Chwatt, L. J. (1952). *Ann. trop. Med. Parasit.*, **46**, 173.
 Edeson, J. F. B., and Davey, D. G. (1953). *Trans. roy. Soc. trop. Med. Hyg.*, **47**, 259.
 Geiman, Q. M., and McKee, R. W. (1948). *Sci. Monthly, Wash.*, **67**, 217.
 Hawkins, F. (1953). *British Medical Journal*, **1**, 1201.
 Hitchings, G. H. (1952). *Trans. roy. Soc. trop. Med. Hyg.*, **46**, 467.
 McFadzean, J. A. (1951). *Ibid.*, **44**, 707.
 McKee, R. W. (1951). In *Biochemistry and Physiology of Protozoa*, edited by A. Lwoff, p. 251. New York.
 Maegraith, B. G., Deegan, T., and Jones, E. S. (1952). *British Medical Journal*, **2**, 1382.
 Reed, F. S. (1952). *W. Afr. med. J.*, **1**, 127.
 Smith, G. N. (1953). *Bact. Rev.*, **17**, 19.
 Thurston, J. P. (1950). *Lancet*, **2**, 438.
 — (1951). *Trans. roy. Soc. trop. Med. Hyg.*, **44**, 703.
 — (1954). *Parasitology*. In press.
 Woods, D. D. (1940). *Brit. J. exp. Path.*, **21**, 74.