EFFECT OF MILK DIET ON *P. CYNOMOLGI* INFECTIONS IN MONKEYS

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Maegraith, Deegan, and Jones (1952) described the suppressive action of a retail whole-milk diet on blood-induced *Plasmodium berghei* infections in rats, and stated that the true significance of their observations would not be revealed until mosquito-transmitted infections were studied. We now report the results of a few experiments using *Plasmodium cynomolgi*, a monkey parasite closely resembling the human *Plasmodium vivax*; in two animals the infections were produced by inoculation of infective blood, in another two by intravenous inoculation of sporozoites. The object of the work was to see if *P. cynomolgi* behaved like *P. berghei* in animals kept on a milk diet, and to observe the effect (a) on sporogony, and (b) on the pre-erythrocytic cycle.

Details of Experiments

Five rhesus monkeys were used; three (M 121, M 122, M 127) were mature and two (M 120, M 126) were immature, weighing between 2½ and 3½ lb. (1.13 and 1.59 kg.). The animals were maintained on one or other of the following diets:

Normal diet: Biscuits (Spratt's ovals), green vegetables, apples, oranges, carrots, and retail whole milk. The diet was offered *ad lib*.

Milk diet: Retail whole milk to which was added 5 mg. vitamin B₁, 5 mg. vitamin B₆, and 50 mg. calcium pantothenate to a litre of milk. The diet was offered *ad lib*.

Samples of blood were taken daily from all animals. Thick and thin blood films were stained with Giemsa stain. The density of parasites in thin films was estimated and expressed as the number per 1,000 erythrocytes according to the method of Ed. and Et. Sergent (1952). Fifty fields of the 1/7 oil-immersion objective were examined in thick films.

Experiment I. Blood-Induced Infections.—Two monkeys, M 120 and M 121, were placed on the milk diet two days before infection. M 120, M 121, and a control animal, M 122, were infected intraperitoneally with 3 ml. of blood showing a 10% parasitaemia. M 120 remained on the milk diet until 19 days after infection, when the milk was replaced by the ordinary diet. On the thirtieth day after infection M 120 was superinfected intravenously with 2 ml. of blood showing a 5% parasitaemia of a homologous strain. M 120 was splenectomized 14 days after infection whilst

on the milk diet and was returned to the normal diet 26 days after infection. Seventeen *Anopheles maculipennis* var. *atroparvus* were fed on M 121 12 days after infection, when apparently normal gametocytes were present in the blood. Individual mosquitoes were killed at suitable intervals and examined for oökinetes, oöcysts, and sporozoites. M 122 was splenectomized 27 days after infection.

Experiment II. Sporozoite-Induced Infections.—Sporozoites were obtained from infected mosquitoes *A. maculipennis* var. *atroparvus*) in the following manner. Salivary glands, heavily infected with sporozoites, were dissected out into chilled 15% monkey serum in Locke's fluid. These were lightly ground and inoculated intravenously. Two monkeys, M 126 and M 127, were placed on the milk diet two days before being infected in this manner. M 126 received a suspension of 36 infected glands. The animal underwent a liver biopsy by open abdominal operation seven days after infection and was returned to the normal diet 23 days after infection. The piece of liver taken from M 126 was fixed in Carnoy's fluid, sectioned, stained by the Giemsa-colophonium method, and examined for pre-erythrocytic schizonts. M 127 received a suspension of four infected glands and was returned to a normal diet 23 days after infection. As the normal course of sporozoite-induced parasitaemias of *P. cynomolgi* is well known in this laboratory at all levels of sporozoite loading, and as it varies from individual to individual within certain limits, it was not thought necessary to maintain a control in this experiment.

Results

All animals took well to the milk diet and none lost weight. Normal intake of milk was between 1 and 1½ pints (570 and 850 ml.) daily.

FIG. 1.—Blood-induced infections.

FIG. 2.—Sporozoite-induced infections.

Experiment I (see Fig. 1).—Suppression in the immature monkey M 120 was almost complete. Return to normal diet resulted in no recrudescence in M 120, but superinfection with the homologous strain resulted in a heavy parasitaemia. In the mature monkey M 121 there was a minor parasitic break-through at the time of normal primary parasitaemia, but when the suppressive effect of the milk had gained control splenectomy failed to bring about another parasitic break-through. However, return to normal diet resulted in a severe "splenectomy type" recrudescence 31 days after infection. In the mosquitoes fed on M 121, oökinetes were observed 20 hours after feeding. Oöcysts were seen on the seventh, tenth, and fourteenth days, and sporozoites were seen in large numbers in the glands on the tenth and fourteenth days. All stages appeared normal.

Experiment II (see Fig. 2).—Suppression in the mature monkey M 127 was almost complete, though the milk diet was unable to cope with the primary merozoite wave released from the pre-erythrocytic phase which resulted in a mild parasitaemia for three days. Return to normal diet caused a mild recrudescence. In the immature monkey M 126 a primary parasitic break-through of merozoites from the pre-erythrocytic phase of considerable proportions occurred over two days. This was quite rapidly brought under control by the milk diet. A mild parasitaemia was evident on the eighteenth to twentieth days after infection, which coincides with a merozoite release from the presumed secondary tissue phase. Sections of the liver of M 126 showed large numbers of pre-erythrocytic schizonts developing normally in the parenchyma. Return to normal diet resulted in a mild recrudescence. The course of a typical sporozoite-induced parasitaemia of *P. cynomolgi* is shown in Fig. 2 for comparison.

Discussion

The suppressive effect of a milk diet was observed in our four experimental monkeys. It was not unlike that recorded by Maegraith *et al.* in *P. berghei* infections in rats. In no animals was suppression complete, but (except temporarily in one instance) parasitaemia was at a low level, and all four infections were quite different from those seen in monkeys on a normal diet. Sporozoite-induced infections behaved much the same as blood-induced infections; however, in monkey 126, which had received the heavier dose of sporozoites, a break-through occurred on the tenth and eleventh days after inoculation, when the blood became flooded with young rings; the density of parasites did not then increase, but on the contrary dropped to a negligible level. The liver biopsy of this animal had revealed numerous pre-erythrocytic schizonts of normal morphology. These apparently reached maturity, ruptured, and their products entered the circulation unharmed by the "milk factor." In other words the latter acted neither on the pre-erythrocytic schizont nor on the free merozoite, and it is thought that the adverse effect must be upon the immature blood schizont—probably at the stage of active nuclear division when desoxyribonucleic acid becomes essential (Bray and Garnham, 1953)—in fact such schizonts seen on the eleventh day were smaller than normal and irregular in formation. The observation that sporogony in the mosquito followed the normal course indicated that the "milk factor" was unable to harm the gametocyte stage.

A milk diet was powerful enough to prevent a break-through after splenectomy (see monkey M 121); but within a few days of return to a normal diet a severe recrudescence took place. Superinfection with a homologous strain had the same effect once the milk diet had been stopped. With no interference (as in the sporozoite-induced infections) the infections became slightly heightened after return to a normal diet. These results suggest that little or no premunition developed during the time that the animals had been exposed to a low parasitaemia—that is, for four weeks.

Summary

Two monkeys infected with *P. cynomolgi* by blood inoculation developed a low-grade parasitaemia only while on a milk diet.

One of two monkeys infected with *P. cynomolgi* by sporozoite inoculation and also on a milk diet showed an equally slight response; the second had a rather heavier infection for two days while the pre-erythrocytic schizonts were rupturing in the liver.

Pre-erythrocytic schizonts, sporogonic stages, and merozoites all appeared to be unaffected by the milk.

The suppressive action seemed to be upon the immature schizonts in the blood, probably during active division of the nucleus.

These low-grade parasitaemias were unable to produce premunition in the host animals.

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MILK DIET, *p*-AMINOBENZOIC ACID, AND MALARIA (*P. BERGHEI*)

PRELIMINARY COMMUNICATION

BY

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It has been reported by Maegraith, Deegan, and Jones (1952) that infections of *Plasmodium berghei* do not develop properly in rats if the animals are maintained on a milk diet. These authors conclude that "milk contains something that can inhibit or restrict the development of the asexual phase of blood-transmitted *P. berghei* in the rat." Experiments have confirmed their finding that infections of *P. berghei* are wholly or partially suppressed if the animals are maintained on a milk diet. It seemed possible that the failure of malaria parasites to develop in these circumstances might be due to lack of something in the milk diet, a hypothesis which was also considered by the above workers. Fresh milk is known to contain very little *p*-aminobenzoic acid (P.A.B.), not more than 0.004 part per million; but this substance is much more plentiful in some of the constituents of a good laboratory diet—for example, dried yeast may contain 6–60 parts per million (Lewis, 1942). *P. knowlesi* requires P.A.B. for satisfactory growth *in vitro* (Anfinson *et al.*, 1946). Also, most mammalian malaria parasites are sensitive to sulphonamides (which inhibit the utilization of P.A.B.); and *P. berghei* is particularly sensitive to these drugs.

Experiments were undertaken to investigate this possibility. It was found that if P.A.B. is added to the milk the infection develops with its usual virulence. This is illustrated by the extract (see Table) from the protocol of one of our experiments. The rats weighed approximately 100 g. They were placed on the diets two days before inoculation. The normal diet was the stock cube diet for this institute, containing all the requirements for the health and growth of rats. The milk was ordinary milk from a dairy. The rats were inoculated intraperitoneally with two million