grossly reduced because of the deficiency in the blood of a factor called the Christmas factor.

The Christmas factor can be obtained most readily from serum, and in some features resembles the serum factor VII of Koller *et al.* (1951). It differs greatly from the antihaemophilic globulin, and the blood from patients with true haemophilia (antihaemophilic globulin deficiency) is as effective as is normal blood in correcting the clotting abnormality in the blood or plasma of patients with Christmas disease.

In the treatment of haemorrhage in cases of Christmas disease concentrated preparations of antihaemophilic globulin are ineffective.

Dr. A. S. Douglas thanks the Medical Research Council for a Fellowship in Clinical Research enabling him to take part in this work. Dr. C. Merskey thanks Dr. J. Wolf Rabkin and Dr. P. M. Smythe for assistance and for permission to see Cases 4 and 6. Dr. C. Merskey received a grant from the Staff Research Fund of the University of Capetown. The work at Oxford was financed by a grant from the Medical Research Council and by the Nuffield Haematological Research Fund.

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SUPPRESSION OF MALARIA (P. BERGHEI) BY MILK

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A year ago, during the course of experiments on the metabolism of haemoglobin derivatives in rats infected by blood passage with *P. berghei*, difficulty was experienced in infecting animals which were being maintained on a standard low-iron diet, consisting of milk to which minimal quantities of vitamins B_1 and B_6 and calcium pantothenate had been added (Copp and Greenberg, 1946). It was at first thought that the low iron content of the diet might be responsible for this phenomenon, but the addition of iron in adequate dietary amounts to the milk vitamin was found to have no effect. The results of further preliminary experiments, however, indicated that some degree of suppression of *P. berghei* infection occurred in animals which were infected while on a milk-vitamin diet. Recent experiments carried out under more carefully controlled conditions have confirmed this. Suppression of blood-transmitted *P. berghei* malaria in rats has been obtained in animals fed on diets of cow's milk, reconstituted proprietary dried milks, or human milk. Suppression of infection has also been observed in mice maintained on a diet of cow's milk and vitamins.

Experimental

The Parasite.—The strain of *P. berghei* used in these experiments was obtained originally from the London School of Hygiene and Tropical Medicine, and has been passaged in rats over the last three years by intraperitoneal blood injection. Frequent rapid passage of this strain through rats has provided us with a parasite of high virulence which commonly kills animals living on a normal laboratory diet.

In animals which survive, two waves of parasitaemia are commonly observed. The first wave is usually the more severe, reaching its maximum on about the tenth day after intraperitoneal injection with a standard inoculum of one million infected cells. The second, and usually much milder, wave appears somewhere about the twentieth day after injection.

Method of Infection of Rats.—In our early experiments the infecting dose of parasites was not calculated. Much more consistent results have been recently achieved by controlling the inoculum as follows. Blood is taken from an infected animal in which the parasitaemia is high and rising (usually 30% or more red cells infected). The number of parasites per 500 red cells is estimated and the blood diluted with citrated saline so that 0.2 ml. contains the standard inoculum of one million infected red cells. This volume is immediately injected intraperitoneally into each recipient rat.

Animals.—The rats used in the experiments were albinos bred in our laboratories from a strain obtained 18 months ago from the Sir William Dunn School of Pathology, Oxford. In each experiment the animals were of approximately the same age and weight (usually about 200 g.). In some cases litter mates were used. Before being placed on milk diets rats were maintained on the normal laboratory diet described below.

Diets

The diets referred to in the results were as follows.

(*a*) *Normal Laboratory Diet.*—This consisted of processed material obtained from Lever Bros. Ltd., containing wheat germ, skim-milk powder, dried yeast, fine bran, broad bran, molasses, coconut cake meal, groundnut cake meal, maize, fish meal, dried blood meal, limestone, common salt, bone flour. It was fed in the form of cubes with water *ad lib.* to the animals which served as controls for each experiment.

(*b*) *Cow's Milk.*—Retail whole milk was used. To each kilogram of milk was added 5 mg. each of vitamins B_1 and B_6 and 50 mg. of calcium pantothenate. Each animal was offered 150–160 ml. a day, equivalent to a protein intake of 4–5 g.

(*c*) *Reconstituted Dried Milk.*—Proprietary brands of dried milk were reconstituted according to instructions, and the same quantities of vitamins added per equivalent of reconstituted material. The volume offered was the same as in the cow's-milk diet.

(*d*) *Human Milk.*—A supply of human milk was obtained from the Liverpool Maternity Hospital. Before use it was stored in a deep freeze unit. No vitamins were added: each animal was offered 150–160 ml. a day, equivalent to a protein intake of approximately 3 g.

Experimental Conditions

All experiments were carried out in the same laboratory, which was controlled within a temperature range of 65–68° F. (18.3–20° C.). Animals were fed from sterilized inverted bottles stoppered by rubber bungs through which a sterilized glass feeding-tube was inserted and passed through the bars of the cage. Sterilization of feeding apparatus before filling was found to be necessary in order to avoid curdling.

In the earlier experiments small groups of animals were fed in the same cage from the same inverted bottles. We

have more recently caged and fed each animal separately, in order to determine its consumption of diet more precisely. The amount of diet consumed is now calculated by measuring the quantity left after a full day's feeding. Milk-bottles are refilled morning and evening.

The animals in most experiments were placed on the respective diets four days or more before being infected. Suppressive activity was sometimes observed in animals infected on the same day as that on which they were placed on the diet, but it was usually advisable to allow a few days before infection for the animals to become accustomed to the diet.

All animals on milk diets suffered some loss of weight. This was most pronounced in the early stages before the animal became accustomed to the changed diet. Animals fed on the normal laboratory diet usually gained weight.

Samples of blood were taken from the tail on the fourth day after infection and subsequently. Thin blood films were prepared and stained with Leishman's stain and examined under the 1/12 oil-immersion objective. The number of parasites present was estimated by counts of the number of infected red cells per oil-immersion field. When the numbers of parasites were high they were represented by one, two, or three "plus" signs. Animals which died or were killed were examined and touch smears made of the spleen and liver. The smears were stained with Leishman's stain and examined for parasites, pigment, and reticulocytes. Histological specimens were prepared in certain cases.

In some experiments subinoculation of blood from animals with milk-suppressed malaria was carried out. Up to 1 ml. of blood from the infected animal was injected intraperitoneally into a non-infected animal of about the same weight; the blood of the recipient was subsequently examined regularly for parasites.

Results

In the examples of experimental results given below the evidence is presented solely in terms of rough parasite counts in individual animals. No indication of the clinical condition of the animals or necropsy findings is given.

Experiment A

Result.—Suppression of primary parasitaemic wave in animals on cow's milk, "ostermilk No. 2," and Australian dried milk. Details shown are of animals on Australian dried milk only. Note the secondary parasitaemia on 17th to 24th day after infection.

Details.—Placed on diets three days before infection. Fed in groups. Arbitrary inoculum on March 28, 1952.

	Normal Diet		Reconstituted Australian Dried Milk		
Rat No.:	853	856	844	846	847
Days after infection and parasitaemia					
3	3/1	5/1	0	0	0
4	3/1	4/1	0	0	0
5	5/1	5/1	0	0	0
6	10/1	10/1	0	0	0
7	20/1	+	0	0	0
8	+	+	0	0	0
9	+	+	0	0	0
10	2/1	+	0	0	0
11	1/20	++	0	0	0
12	1/200	++	0	0	0
13	1/100	++	0	0	0
14	1/100	++	0	0	0
15	1/120	++	0	0	0
16	1/20	++	0	0	0
17	1/20	Killed	0	1/50	1/5
18	1/100		1/1	1/10	15/1
19	0		5/1	5/1	15/1
20	0		5/1	15/1	10/1
21			1/1	10/1	2/1
22			0	1/200	1/1
23			0	—	—
24			0	1/10	1/3
25			0	1/50	0
26			0		
27			0		
28			0		
29			0		

Note.—The animals on ostermilk No. 2 and cow's-milk diets of the standard nature showed similar results. In particular Rat No. 857 remained entirely free of visible parasites for 26 days. On changing its diet to the normal laboratory diet after this length of time and inoculating into a recipient animal (arbitrary inoculum) two days later, parasites appeared in the latter's blood in three days and increased to a count of 5/1, which was maintained for the period 8–12 days after subinoculation.

Experiment B

Result.—Suppression in animals on diluted cow's milk. Failure of suppression on normal diet plus milk *ad lib.*

Details.—Placed on diet six days before infection. Fed in groups. Inoculum of one million cells given on April 28, 1952.

	Normal Diet		Normal Diet + Cow's Milk			Cow's Milk Diluted with Equal Parts Water		
Rat No.:	875	860	872	877	878	864	873	876
Days after infection and parasitaemia								
1	0	1/100	1/200	0	1/100	0	0	0
2	1/100	0	1/50	0	0	0	1/100	0
3	—	—	—	—	—	0	0	0
4	3/1	3/1	2/1	3/1	3/1	0	0	1/20
5	5/1	5/1	—	1/1	1/1	0	0	1/50
6	—	—	—	—	—	0	0	0
7	10/1	10/1	5/1	10/1	10/1	0	0	0
8	Died	+	20/1	+	+	0	0	0
9		Died	20/1	++	Died	0	0	0
10			Died	Died		0	0	0
11						0	0	0
12						1/100	0	0
13						0	0	0
14						0	0	0
18						0	1/10	0
24						0	1/50	0
25						0	0	0
31						0	0	0
32						Diet changed to normal diet		
41						Occas.	Occas.	1/20
43						0	1/2	1/10
47						0	1/10	1/20
52						0	0	0

Occas. = Occasional parasite.

Experiment C

Result.—Partial suppression in animals on cow's milk and ostermilk No. 2.

Details.—Animals placed on diets on the day of infection. Fed in groups. Inoculum of one million infected cells given on June 16, 1952.

	Normal Diet			Cow's Milk			Reconstituted Ostermilk		
Rat No.:	969	970	971	972	973	974	975	976	977
Days after infection and parasitaemia									
7	10/1	1/1	10/1	0	1/5	1/5	1/3	1/2	1/2
8	7/1	—	3/1	0	1/1	2/1	1/5	1/2	2/1
9	3/1	7/1	2/1	1/10	3/1	1/1	1/2	1/1	4/1
10	15/1	15/1	6/1	1/2	6/1	1/1	1/2	2/1	1/1
11	+	++*	+	1/2	5/1	2/1	1/15	1/1	1/10
12	++	Hb-uria (killed)	10/1	3/1	10/1	2/1	1/10	1/1	1/50
13	—		—	—	—	—	—	—	—
14	+++* (killed)		+++	10/1	7/1	4/1	1/10	1/50	0
15			++* (killed)	5/1	5/1	5/1	0	Occas.	0
16				5/1	2/1	5/1	0	0	0
17				2/1	1/3	8/1	0	0	0
18				1/15	1/10	8/1	0	0	0

* Moribund before being killed. Occas. = Occasional parasite.

Experiment D

Result.—Good suppression of severe infection in animals on cow's milk. Partial suppression in animals on ostermilk.

Details.—Placed on diet of cow's milk 20 days, ostermilk 20 days, before infection. Inoculum of one million infected red cells given on July 21, 1952.

	Normal Diet			Cow's Milk			Ostermilk No. 2		
Rat No.:	982	983	984	985	986	987	988	989	990
Days after infection and parasitaemia									
3	8/1	0	11/1	1/100	0	0	5/1	0	0
4	+	1/100	20/1	1/100	0	0	15/1	0	1/200
5	+	1/100	20/1	0	0	0	20/1	0	0
6	Died	10/1	++	0	0	0	6/1	1/200	1/50
7		15/1	+++	0	0	0	4/1	2/1	1/1
8		+	+++	0	0	0	11/1	5/1	2/1
9		40/1	Died	0	1/50	0	+	15/1	1/20
10		+++		0	3/1	0	2/1	2/1	0
11		Died		0	—	0	—	—	—
12				0	4/1	0	0	0	0
13				0	4/1	0	0	0	0
14				0	1/5	0	0	0	0
15				0	0	0	0	0	0
16				0	0	0	0	0	0
17				0	0	0	0	0	0

Experiment E

Results.—Suppression in animals on human milk and partial suppression on ostermilk. Positive subinoculation from rats on human milk diet.

Details.—Placed on diet of human milk 16 days, of ostermilk 21 days, before infection. Inoculum of one million infected red cells given on November 27, 1952. Fed and housed separately.

	Normal Diet			Ostermilk No. 2			Human Milk		
Rat No.:	227	232	289	245	242	243	231	235	391
Days after infection and parasitaemia									
2	1/20	0	1/100	1/100	0	0	0	0	0
3	1/10	0	0	0	0	0	1/30	0	0
4	4/1	1/2	0	0	0	0	0	0	0
5	5/1	6/1	0	0	0	1/20	0	0	0
6	5/1	10/1	0	0	0	1/5	0	0	0
7	7/1	9/1	1/20	0	0	1/1	0	0	0
8	13/1	6/1	2/1	0	0	7/1	0	0	0
9	Died	16/1	8/1	0	0	9/1	0	0	0
10		12/1	12/1	0	0	4/1	0	0	0
11		5/1	20/1	0	0	1/5	0	0	0
12		11/1	20/1	0	0	1/50	0	0	0

Subinoculations on Day 12

	From Rat 231			From Rat 235			From Rat 391		
Rat No.:	248	249	388	396	394	395	238	239	240
Days after sub-inoculation									
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	1/200	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	1/15	1/8	0	0	0	0	0	0
7	1/100	1/1	1/1	0	0	0	0	0	0

Discussion

The results of the experiments described above and those of other similar experiments not given in detail here indicate that in animals living on a diet of milk plus vitamins there is a suppression of the growth of blood-transmitted *P. berghei*. This is shown in the absence of parasites or the relatively light parasitaemia which develops in animals on the milk diet compared with control animals given a normal laboratory diet.

In most cases there is a considerable suppression of the primary wave of parasitaemia which follows intraperitoneal inoculation of infected blood. In experiments in which the inoculum has been virulent enough to cause early death in control animals some degree of parasitaemia is commonly found for a short time in those animals on milk. This parasitaemia tends to subside and may be followed by a considerable period of quiescence, and sometimes a later milder wave of parasitaemia. Occasionally, however, an animal on milk has died after infection, usually some time after the controls. There may also be some clinical signs of illness, including ruffled fur and listlessness, which may occasionally develop before the parasites appear.

Less virulent inoculations which produce non-fatal attacks in control animals may fail to give rise to any parasitaemia in the animals on milk.

In some cases there may possibly be radical cure or inhibition of the infection, but the evidence suggests that the action of the milk diet is primarily one of suppression. Presumably the multiplication of the parasite is in some way impeded although the organism is not actually destroyed. This is indicated by those experiments in which parasitaemia developed to some extent at some stage, often late, after inoculation, and also by the sometimes successful subinoculation of blood from animals in which malaria has been suppressed by milk to fresh rats. An example of the latter is seen in rat 857 (Experiment A) the blood of which was negative for 26 days after the intraperitoneal administration of a parasite inoculum which killed the control animals. Blood from this rat was inoculated on the 26th day intraperitoneally into a fresh rat, in the blood of which parasites appeared within four days. Similar positive subinoculation occurred in the animals in which malaria was suppressed by human milk.

In some experiments animals in which malaria appeared to be suppressed were restored to the normal laboratory diet. In most of these rats parasites rapidly reappeared in the blood after the change of diet, but the resulting parasitaemia was seldom severe or long-maintained. These results may also be indicative of suppression, although it is possible that the parasitaemia observed might have arisen in some cases spontaneously if the diet had not been changed.

It is interesting to note that the activity of cow's milk in suppressing *P. berghei* was observed in pasteurized and various dried forms.

The human milk was stored before use in a deep freeze in which it was frozen solid. As will be seen from the experiment quoted above (Experiment E) this treatment did not affect its suppressive activity.

Conclusion

So far we have been able to study only blood-transmitted *P. berghei* malaria. The true significance of the suppressive action of milk cannot be assessed until it has been examined in the mosquito-transmitted disease.

Nevertheless, it seems clear that milk contains something that can inhibit or restrict the development of the asexual phase of blood-transmitted *P. berghei* in the rat and the mouse.

We have decided to give this very brief account of the work because we believe this is the first time a dietary factor has been shown to have a suppressive effect on a protozoal infection. Whatever the nature of this suppression, we think its demonstration is likely at least to lead to some further information regarding the metabolism of the parasite.

It is clear also that some attention must now be paid to the effect of milk on human malaria. We feel that it is possible that herein lies the explanation of the common observation that severe malaria is not often seen in very young infants (Bruce-Chwatt, 1952).

Since writing the above note we have been told that Dr. Paul György, of the University of Pennsylvania School of Medicine, Philadelphia, has this month described in a lecture in London experiments indicating the existence of a factor in milk protective against certain virus infections.

We are deeply obliged to all those who have helped us, particularly to Miss E. J. Moss and Miss M. A. L. Hesketh for their technical assistance, and to Professor T. N. A. Jeffcoate and Dr. D. N. Menzies and the Sisters of Liverpool Maternity Hospital for their considerate help in obtaining for us sources of human milk.

This work is part of the research carried out under the recently renewed John Holt Malaria Research Grant, without which it could not have been undertaken.

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