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Studies on Malarial Parasites. V. Effects of Ascorbic Acid on Malaria *(Plasmodium knowlesi)* in Monkeys.*

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Several different investigators[1-3] have reported that in human cases of tertian malaria there was an increased demand for ascorbic acid and the administration of additional vitamin was necessary to prevent a mild state of scurvy. In spite of these observations no one has reported a causal relationship between malaria and ascorbic acid deficiency.

During the course of our malaria studies with *P. knowlesi* in monkeys *(Macaca mulatto.)*[4] certain observations were made which led us to suspect that ascorbic acid might have some influence on the course of parasitemia in the animal host. Using the method of Mindlin and Butler[5] for determining plasma ascorbic acid and of Butler *et al.*[6] for whole blood levels of the vitamin it was found that the average plasma level of ascorbic acid! for 20 parasitized monkeys was only 0.24 mg %. Nonparasitized animals were found to have an average plasma level of 0.56 mg %. Also during the course of our studies on 120 monkeys, 7 animals inoculated with parasites showed no plasma ascorbic acid and less than 0.1 mg % in the whole blood. This condition was present in spite of the fact that the animals were receiving a well balanced diet containing generous daily portions of raw potatoes and oranges. Such a condition has been previously reported in both humans[7] and monkeys.[8] This may be explained on the basis of an abnormal intestinal flora since it has been shown by various workers[9,10] that under certain conditions some intestinal bacteria destroy ascorbic acid and may be responsible for a state of scurvy.

The 7 animals having extremely low blood ascorbic acid values showed abnormal courses of parasitemia. Instead of the usual rapid increases in numbers of parasites and death of the animals within 6 or 7 days after inoculation there was only a slow rise in percentage of parasites, and a gradual spontaneous control of the infection. Into 4 of these animals ascorbic acid was injected intramuscularly on 3 successive days (starting 7, 7, 8 and 9 days following parasite inoculation in the 4 monkeys) and in all cases a significant increase in numbers of circulating parasites was observed. One of these animals (given ascorbic acid 7 days after parasite inoculation) then showed a normal rise in parasite count and died in the usual manner with a high percentage of the erythrocytes parasitized. The other 3 animals (given ascorbic acid 7, 8 and 9 days after parasite inoculation) however, showed a subsequent drop in parasite count with spontaneous control of the infection, presumably due to the development of an immunity prior to the injection of ascorbic acid. The remaining 3 monkeys out of the total 7 were given no vitamin treatment and all 3 completely controlled their infections. In Ta-

* This work was done under a contract recommended by the Committee of Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

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TABLE I
Effect of Ascorbic Acid on Parasitemia of Ascorbic Acid Deficient Monkeys (Spontaneously Appearing).

Days after parasite inoculation	Total parasite count		
	Ascorbic acid deficient animals		
	No. 59	No. 101	No. 109
4	—	0.1	—
5	0.1	—	—
6	0.3	2.4	occasional parasite in thick film
7	0.5	0.8*	—
8	0.5	8.5*	0.004
9	—	20.6*	0.004*
10	2.0	13.5	1.1*
11	2.4	dead	0.05*
12	0.4		—
13	occasional parasite in thick film (chronic)		0.01 (chronic)

* 80-100 mg Merck's crystalline ascorbic acid intramuscularly. All 10 animals referred to were approximately the same size with an average weight of about 2.5 kilos.

ble I are shown the parasite changes of 3 representative animals of the 7.

Three monkeys were then placed on a synthetic diet deficient in ascorbic acid similar to that of Shaw and coworkers.[11] This diet which was freshly mixed every 5 or 6 days and stored in the cold room at +2°C consisted of the following percentages of ingredients: sucrose 65, casein IS, Phillips and Hart IV salts 4, corn oil 4, cod liver oil 2, and yeast 10.† Vitamins were supplemented in the indicated numbers of milligrams per kilogram of diet: biotin 0.1, thiamine 5, riboflavin 5, pyridoxine 5, calcium pantothenate 15, nicotinic acid 25, choline 250, inositol 500, and paraaminobenzoic acid 500. When early deficiency symptoms appeared (disappearance of plasma and whole blood ascorbic acid, slight loss of body weight and increased capillary fragility), the animals were inoculated intravenously with 6 million parasites per kg of body weight. A control animal on a normal diet containing adequate ascorbic acid was likewise inoculated with the same dosage of parasites. To the 3 experimental animals, No. 120, 119 and 117, a solution of crystalline ascorbic acid was injected, as shown in Table II, starting, respectively, immediately before, 4 days after and 14 days after parasite inoculation. The control and the animal No. 120 showed immediate parasite increases with normal courses of parasitemia and deaths on the 7th day with, in each case, 31% of the erythrocytes parasitized. Animal No. 119 showed no parasite increase until after ascorbic acid was given but then had a normal course of parasitemia, with death on the 6th day following vitamin administration. Animal No. 117 showed only a small parasite increase prior to ascorbic acid administration, but died 2 days after receiving vitamin with only *8%* of the erythrocytes parasitized. This animal did not die of the malaria infection alone, but manifested a complicating anemia perhaps due to the more advanced state of the vitamin deficiency and a greatly lowered food intake during the last week of the animal's life. (There appeared also to be some resistance to parasite increase indicative of an immune reaction).

Whether this action of ascorbic acid administration to the vitamin-deficient animals is a direct effect on the parasites or if it is an indirect one through the animal host we do not know. We suspect that it may be the latter since attempts to demonstrate the need of ascorbic acid for the *in vitro* growth and multiplication of *P. knowlesi* in

11 Shaw, J. H., Phillips. P. H., and Elvehjem, C. A., *J. Nutrition,* 1945, **29,** 365.

† Generous quantities of Strain K Dried Brewer's Yeast were kindly supplied by Anhauser-Busch, Inc., St. Louis, Mo.

TABLE II.
Effect of Ascorbic Acid on Parasitemia of Ascorbic Acid Deficient Monkeys (Produced by Dietary Deficiency).

Days after parasite inoculation	Total parasite count: Control animal (normal diet)	Ascorbic acid deficient animals (Vitamin C deficient diet): No. 120	No. 119	No. 117
0	—	—‡	—	—
3	0.3%	0.3%‡	350/mm³	50/mm³
4	2.0 ''	2.7 ''‡	270/ ''‡	120/ ''
5	10.0 ''	8.0 ''‡	0.2 %‡	30/ ''
6	12.6 ''	14.9 ''‡	0.15 ''‡	150/ ''
7	31.3 ''*	31.0 ''*	0.8 ''‡	100/ ''
8			3.2 ''‡	300/ ''
9			26.0 ''	580/ ''
10			144.7 ''*†	2000/ ''
11				0.5%
12				1.2 ''
13				0.9 ''
14				5.5 ''‡
15				8.8 ''*‡

† 84% of the red cells contained parasites with many double and triple infections per cell.
* Animal died within 18 hours following this parasite count,
‡ 80-100 mg Merck's crystalline ascorbic acid intramuscularly.

24-hour cultures have so far been inconclusive, although in the absence of the vitamin the usual protoplasmic mass is not achieved. This growth deficiency and the degenerate appearance of the parasites are also very striking in the vitamin-deficient host. These studies on the effect of ascorbic acid and of ascorbic acid analogs are being continued with simian and human plasmodia.

Conclusions and Summary. 1. Plasma and whole blood ascorbic acid levels were significantly lower in parasitized monkeys than in normal animals.

2. There was an abnormal course of parasitemia in 7 of our monkeys which showed spontaneous ascorbic acid deficiency and in 2 other animals which were made ascorbic acid deficient by removing the vitamin from their diet.

3. Following the administration of the pure ascorbic acid, there was a normal course of parasitemia in one of the monkeys spontaneously ascorbic acid deficient and in those made ascorbic acid deficient by dietary means.

Withholding ascorbic acid therapy for more than 7 days appeared to give the spontaneously deficient monkeys time to produce an immunity and to control the infections. If ascorbic acid treatment was withheld from the animals made vitamin C deficient there was a prolongation of the course of infection but eventually the parasitemia overwhelmed the animal.

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OCTOBER-DECEMBER, 1946 (INCLUSIVE)

VOLUME 63

NEW YORK