within two hours after infection and was carried out for a period of two to three days only. Table I shows the high degree of protection obtained with relatively small amounts of the ethyl ester.

TABLE I

Total amount of ester in mgs	Dilution of culture (strain $C_{203}Mv$)	Number of mice	Number died (< 48 hrs.)	Number survived (> 7 days)
2.5–4.5	10^{-3}	11		11
	10^{-4}	12		12
	10^{-5}	13	1	12
1.37	10^{-3}	3		3
	10^{-4}	3	1	2
	10^{-5}	3		3
0.6	10^{-3}	3	2	1
	10^{-4}	2	1	1
	10^{-5}	3	1	2
Controls	10^{-6}	12	12	
	10^{-7}	12	12	

A total dose of less than 1.5 mg of the ethyl ester gives complete protection against a 10^{-3} dilution (20,000 to 100,000 lethal doses) of hemolytic streptococci. With the methyl ester, essentially the same results have been obtained, except that a total of at least 2.5 mg was necessary. With penicillin preparations having an activity similar to that of the fractions from which these esters have been made, considerably larger amounts were necessary. The increased stability of the methyl and ethyl esters is illustrated by preliminary experiments indicating that partial protection is obtained by oral administration.

The benzohydryl ester mixture, in contrast to the aliphatic compounds, is hydrolyzed by the test organism. It has a constant *in vitro* activity of 0.3 to 0.6 micrograms per cc which is comparable to the activity of the starting material. The mouse seems to be unable to hydrolyze this compound, however, as no protection was obtained with the dosage employed. The compound is of interest, nevertheless, since it can be split by catalytic hydrogenation with colloidal palladium, giving a highly active acid fraction.

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THE INFLUENCE OF BIOTIN UPON SUSCEPTIBILITY TO MALARIA

IT has long been known that individuals differ in their degree of natural susceptibility to malaria. Almost nothing is known, however, concerning the factors responsible for these differences, nor has it been possible in the past to markedly affect the degree of natural susceptibility to experimental malaria, whether human, simian or avian. Experiments with avian malaria have now shown that the level in the host animal of biotin,[1] an important growth factor, greatly influences the severity of the infection. Also significant is the fact that the concentration of biotin in the blood reaches two or three times its normal value at the peak of an acute experimental malarial infection, and then returns to normal when the infection has subsided.

Most of the work has been done with *Plasmodium lophurae*[2] infections in young chickens and ducks. Chickens or ducks, rendered biotin-deficient by maintenance on an egg-white diet[3] for two or three weeks and subsequently inoculated with large doses of *P. lophurae,* showed peak parasite numbers 50 to 100 per cent. higher than those shown by control animals. Among the biotin-deficient animals, the parasite number persisted at a high level several days longer, and more animals died of the malarial infection than among the controls. The greater susceptibility of the biotin-deficient animals was not directly connected with any general weakness resulting from the biotin deficiency. Chickens or ducks made extremely weak on a pantothenic acid-deficient diet did not develop any heavier infections with *P. lophurae* than did the robust animals which received the same diet supplemented with calcium pantothenate. Moreover, chickens which were provided with just enough biotin so that they grew well and were quite normal, except for a mild scaly dermatitis of the feet, developed more severe infections than chickens provided with more nearly adequate amounts of biotin. Here, in the presence of a small degree of biotin deficiency, the administration of additional biotin acted as a specific therapeutic measure to lessen the severity of the infection. It is also pertinent that older chickens, which are more resistant to *P. lophurae* infection than young chickens,[2] showed a higher level of biotin in the blood.[4]

Chickens kept on egg white diet and infected with *Plasmodium gallinaceum,*[5] either by sporozoites or by blood inoculation, showed higher average peak parasite numbers in the blood than control animals on a similar diet with the egg white replaced by casein. Biotin-deficient ducks infected with *P. cathemerium*[6] did not show higher peak parasite numbers than the non-deficient animals, but their infections persisted at a high level for several days after the blood of the

1 V. du Vigneaud, SCIENCE, 96: 455, 1942.
2 L. T. Coggeshall, *Am. Jour. Hyg.,* 27: 615, 1938.
3 R. E. Eakin, W. A. McKinley and R. J. Williams, SCIENCE, 92: 224, 1940.
4 Total biotin (after acid hydrolysis by the method of J. A. Lampen, G. P. Bahler and W. H. Peterson, *Jour. Nutrition,* 23: 11, 1942) was determined by the microbiological assay method of G. M. Shull, B. L. Hutchings and W. H. Peterson, *Jour. Biol. Chem.,* 142: 913, 1942.
5 The work with *P. gallinaceum* was done at the laboratories of the International Health Division of the Rockefeller Foundation with the generous cooperation of Dr. J. Maier.
6 Duck strain 3 T kindly sent me by Dr. Fruma Wolfson.

controls was virtually free from demonstrable parasites. Several of the biotin-deficient ducks infected with *P. cathemerium* died from the infection.

In both chickens and ducks, whether on a deficient or an adequate diet, the concentration of biotin in the plasma and red blood cells rose during the course of infection with *P. lophurae*. This rise can not be explained solely on the basis of the new red cells formed in response to the anemia produced by the parasites. In ducks, an increased biotin level was already apparent by the fourth day after inoculation, when there was as yet no large proportion of young red cells; the increase appeared in the plasma as well as in the red cells; and both plasma and red cells were back to a normal biotin level by the eighth day after inoculation, when a large proportion of young red cells was still present. Since *P. lophurae* multiplies to a greater extent in animals with a relatively low initial biotin level than in those with a higher initial biotin level, the increase in biotin which occurs during the course of the infection may well be concerned with the elimination of the parasites from the blood.

Whether these findings with avian malaria apply to simian or human malaria can be determined only by extended observations on body biotin levels in these species in relation to the degree of susceptibility to malarial infection. Certainly the results with chickens and ducks would indicate that biotin is one substance of known chemical nature which helps to determine the degree of resistance of the host to infection with malarial parasites. These results are also of interest in that they provide an example, in addition to the very few thus far discovered,[7] of a specific relation between a nutritional deficiency and susceptibility to an infectious disease. The full details of this work are to be published shortly.

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RELATION OF FOOD INTAKE TO RESPONSE OF MICE INOCULATED WITH LANSING STRAIN OF MURINE POLIOMYELITIS VIRUS[1]

IN a recent preliminary communication[2] we reported that mice on a vitamin B_1-deficient diet showed increased resistance, over a period of 30 days, to the Lansing strain of murine poliomyelitis virus. Since then these observations have been confirmed, and in addition we have found that simple restriction of food intake will produce comparable results. In several trials, feeding of about 40 per cent. of the usual daily consumption definitely extended the time before the onset of paralysis and the time of death. To at least the twenty-first day after inoculation there was a statistically significant difference in deaths and cases of paralysis between the restricted groups and those fed ad libitum. This difference had disappeared by the twenty-seventh day.

In one experiment, 176 mice were divided into 6 groups. Group I received a synthetic diet (diet 100), Groups II and III a stock diet (diet 483) and Groups IV, V and VI a synthetic diet in which the relative amounts of all ingredients except carbohydrate were increased at the expense of the latter (diet 515). Groups I, II and IV were fed ad libitum and the other groups were given 1 gm of food per animal per day. On the third day of the experiment, Groups I to V inclusive were injected intracerebrally with a suspension of mouse brain infected with the Lansing strain of murine poliomyelitis virus. This amount of virus corresponded to between 500 and 1,000 fifty-per cent.-mortality doses. Group VI was injected with a suspension of normal brain. The cumulative percentages of animals dying and those showing paralysis by the tenth, fifteenth and twenty-first days after inoculation are given in Table 1. Any animals dying before the third day are not included in the totals.

Increasing the concentration of thiamin in the diet

TABLE 1

Group No.	No. mice 3 days after inoc.	Diet No.	Amt. of diet	Inoculum	Days after inoculation					
					10		15		21	
					Par.[1]	Death	Par.[1]	Death	Par.[1]	Death
					Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
I	16	100	ad lib	virus	88	94	88	100	88	100
II	35	483	ad lib	virus	80	94	80	100	80	100
III	39	483	1 gm	virus	10	10	33	28	56	67
IV	23	515	ad lib	virus	91	87	96	100	96	100
V	25	515	1 gm	virus	20	28	32	48	52	68
VI	23	515	1 gm	normal brain	..	26	..	39	..	44

[1] Paralysis.

[7] "Nutrition and Resistance to Disease," *Nutrition Reviews*, 1: 66, 1943.

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] *Proc. Soc. Exp. Biol. and Med.*, 51: 215, 1942.