TABLE I.
Effect of Subcutaneous Vaccination upon Febrile Response of Human Subjects to Induced Infection with Influenza Virus, Type B.

Vaccination record	No. of subjects	Highest Temperature <99 No.	%	99+ No.	%	100+ No.	%	101+ No.	%	102+ No.	%
1. Unvaccinated	27	5	19	22	81	11	41	6	22	2	7
2. 4½ mos. before	27	12	44	15	56	2	7	0	0	0	0
3. 4½ mos. and 4 wks. before	19	4	21	15	79	2	11	0	0	0	0
4. 4 wks. before	23	12	52	11	48	3	13	0	0	0	0

induced infection with Type B influenza virus for at least one to 4 months. These data together with those in the preceding paper show that the same mixed vaccine induced an increased resistance of human individuals to infection with virus of Type A or Type B.

Further details will be reported subsequently.

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Effect of Biotin Deficiency on the Course of *Plasmodium lophuræ* Infection in Chicks.

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It has generally been assumed that malnutrition decreases the resistance of the organism to malaria. The first experimental evidence to support this view is Trager's[1,2] recently published observation suggesting that biotin deficiency increased the severity of certain avian malaria infections. Similarly, Caldwell and György have reported that biotin deficiency will prolong *Trypanosoma lewisi* infections in the rat.[3]

Since we had found, in experiments to be reported soon, that a deficiency in certain dietary factors which have not yet been chemically characterized has a pronounced effect on the course of *Plasmodium lophuræ* infections in chicks, we were interested in determining whether or not the effect of biotin deficiency on avian malaria was specific. In this communication we are reporting experiments on the relationship of biotin deficiency to the severity of *P. lophuræ* infections which, although the diets and experimental procedures used differed from those of Trager, confirm his findings on the specific effect of biotin.

Experimental. While *P. lophuræ* infections are more severe in the duck than in the chick the latter species was chosen for the following experiments because much more is known about its nutritional requirements. Because of simplicity and convenience it was decided to produce biotin deficiency for this experiment by adding dried raw egg white to a commercial chick ration. In order to be certain that the addition of egg white proteins did not in itself influence the course of the malaria, a control diet was used in which steamed egg white replaced the raw egg white. To further demonstrate whether or not biotin was the specific factor involved, 0.0001% of crystalline biotin was added to the diet containing dried raw egg white.

Twenty-five 9-day-old S.C.W. Leghorn male chicks were set out on each of the following diets:

1 Trager, W., *Science*, 1943, **97**, 206.

2 Trager, W., *J. Exp. Med.*, 1943, **77**, 557.

3 Caldwell, F. E., and György, P., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 116.

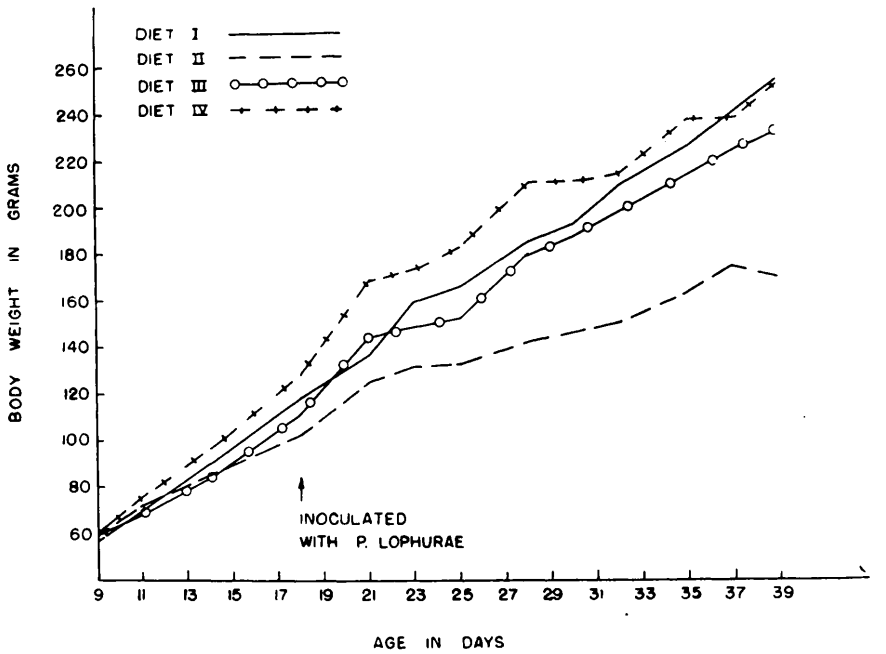

FIG. 1.

Growth curves of chicks infected with *P. lophuræ* and receiving diets adequate (I, III, IV) or deficient (II) in biotin.

TABLE 1.
Percent Erythrocytes Parasitized by *P. lophuræ*.

Days after inoculation	Diets I Mean ± S.E.	II Mean ± S.E.	III Mean ± S.E.	IV Mean ± S.E.	Least significant difference*
3	0.72 ± 0.09	0.78 ± 0.07	0.53 ± 0.06	0.54 ± 0.06	NS
5	1.76 ± 0.23	3.30 ± 0.41	2.24 ± 0.38	1.64 ± 0.21	0.93
7	0.66 ± 0.30	1.87 ± 0.89	0.30 ± 0.10	0.29 ± 0.08	NS
9	0.09 ± 0.05	0.14 ± 0.10	0.02 ± 0.01	0.003	NS

* Probability = 5%; NS = differences between means are not significant.

I. Startena.*
II. 85% Startena + 15% dried raw egg white.
III. 85% Startena + 15% steamed egg white.
IV. Diet II + .0001% crystalline biotin.

The dried raw egg white was obtained on the market and the steamed egg white was prepared by exposing this material to moist heat of 100°C for one hour. Crystalline biotin was obtained through the courtesy of Dr. Folkers of Merck & Co.

After the chicks had been on the above diets for 9 days, when the growth retardation of the birds on the biotin-deficient diet (Diet II) became apparent, 15 of each diet group were inoculated intravenously with a saline suspension of 10,000,000 erythrocytes parasitized by *P. lophuræ* per 50 grams of body weight. Beginning on the third day after inoculation, blood smears were made every 48 hours and the number of parasitized erythrocytes per 10,000 erythrocytes was counted.

Results. During the course of the experiment the rate of growth of the biotin-deficient birds (Diet II) was poor (Fig. 1). Lesions on the legs and beaks, characteristic of biotin deficiency, were first noticed on the third day after inoculation. There was no difference in the growth rate and the extent of the lesions

* Trade name for a chick starting mash manufactured by the Ralston Purina Company, St. Louis, Missouri.

between the infected and the uninfected birds. None of the birds on Diets I, III, and IV showed any gross evidence of deficiency disease.

Mean parasite counts (Table I) in the biotin-deficient birds (Diet II) at the peak of their infections were approximately twice as high as those of the birds whose diets contained a presumably adequate amount of biotin (Diets (I, III, and IV). It is evident from the above results that the peak parasite counts in *P. lophuræ* infections are much greater in chicks on a diet deficient in biotin than in chicks on a diet which is identical except that it contains an adequate amount of biotin. It is not know whether the lack of biotin stimulates the multiplication of the parasites *per se,* whether it interferes with a defensive mechanism in the host, or whether both factors may be involved.

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Effect of Nerve Compression on Wallerian Degeneration *in Vitro.**

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Wallerian degeneration is an essential preliminary to successful nerve regeneration in that it prepares the distal stump for reinvasion by regenerating fibers. Physiological insight into its mechanism, however, lags greatly behind our descriptive acquaintance with its morphological features. The following experiments represent part of a broader study of the factors determining the character and rate of Wallerian degeneration.

It was known that when a nerve locally constricted by a ligature,[1] clamp,[2,3] or arterial cuff,[4] undergoes Wallerian degeneration, those parts of the axis cylinders lying within the compressed zone may fail to break down. This effect could be ascribed (1) to injury of the fibers, or (2) to paralysis of the mechanism of degeneration either (a) by the local ischæmia, or (b) by direct pressure action on the nerve fiber. Alternative a and b could be decided by reproducing the compression effect *in vitro, i.e.,* with the whole nerve removed from blood supply; alternative 1 and 2 by the demonstration that fibers arrested in their degeneration by compression may degenerate after decompression.

The capacity of nerve fibers to undergo typical Wallerian degeneration in a properly composed medium *in vitro* has been known.[5,6] Our own observations confirm, with certain qualifications, the earlier reports: Degeneration occurs promptly in Ringer's, Tyrode's solution, blood serum, but less regularly or not at all in NaCl solution or blood plasma. Our present experiments were carried out with fragments (*cca.* 1 cm) of rat nerves (sciatic, brachial, intercostal; 100-200 g donors), explanted into Ringer's solution in Petri dishes at 37° C, with or without compression, fixed after varying intervals sectioned longitudinally, and silver-impregnated according to Bodian.[7] Criteria of degenera-

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1 Cajal, S. Ramôn y, *Degeneration and Regeneration of the Nervous System,* Oxford University Press, London, 1928, Vol. I, p. 292.

2 Stroebe, H., *Beitr. z. path. Anat.,* 1893, **13**, 160.

3 Denny-Brown, D., and Brenner, C., *Arch. Neurol. and Psych.,* 1944, in press.

4 Weiss, Paul, and Davis, Hallowell, *J. Neurophysiol.,* 1943, **6**, 269.

5 Ingebrigtsen, Ragnvald, *J. Exp. Med.,* 1916, **23**, 251.

6 Nageotte, Jean, *L'Organisation de la Matière,* Paris, Félix Alcan, 1922, p. 274.

7 Bodian, David, *Anat. Rec.,* 1937, **69**, 153.