embryo survival, was obtained following inoculation of the lower dilutions. However, when the same fluid was titrated by inoculation into the yolk sac no evidence whatever of interference was found. It is clear, therefore, that inoculation into the yolk sac was a suitable method for the measurement of infectivity but not for the demonstration of interference. In another experiment allantoic fluid from dead embryos was partly inactivated by exposure for 20 minutes at 56°C and titrated in eggs by both the yolk sac and the allantoic sac routes. As anticipated, mortality following inoculation into the allantoic sac varied from 0 to 30% in all groups. However, when the same fluid was titrated by the yolk sac route, the LD_{50} was $10^{-2.8}$, and embryos inoculated with undiluted fluid died more rapidly (A.D.D. = 3.2) than those receiving fluid diluted 10^{-1} (A.D.D. = 5.3). Thus it was not possible to demonstrate interference following inoculation of partly inactivated allantoic fluids known to contain interfering material. It is suggested that inoculation into the yolk sac may prove to be of value in the detection of residual active virus in inactivated preparations.

Discussion. It has been shown in the preceding sections that interfering material was liberated or produced following death of the embryo infected by the allantoic sac route with influenza B virus and that such material was not destroyed by exposure to a temperature of 56°C for 5 hours. It would seem reasonable to assume for the moment that the interfering material present in allantoic fluid from dead embryos differs quantitatively rather than qualitatively from like material known to be present in fluid from living embryos(1-3), although the possibility of existence of an unrelated inhibitory material should not be ignored. The fact that virus was found to elute from the allantoic membrane in dead but not living embryos following inoculation into the allantoic cavity(14) may account, in part, for the increase in the amount of interfering material present in unheated allantoic fluid following death of the embryo. It is of considerable interest that the presence of excessive active virus in untreated allantoic fluid from dead embryos was capable of overcoming the effectiveness of the interfering material present in the same fluid (Table II). Indeed, it is not clear whether inactivation of infective allantoic fluid actually creates interfering material or simply reveals the presence of such material by the removal of excessive infective virus, or both.

Summary. Experiments are described illustrating (a) the use of death of the embryo as a criterion for the measurement of infectivity and interference, (b) an increase in the amount of interfering material present in allantoic fluid following death of the embryo and (c) the absence of demonstrable autointerference following inoculation into the yolk sac.

14. Stone, J. D., *Austral. J. Exp. Biol. and Med. Sci.*, 1948, v26, 49.

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Cholesterol and Fatty Acid Synthesis in Biotin Deficient Rats. (18243)

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Elevation of the serum cholesterol level in man(1) and in the rabbit(2) has been observed in biotin deficiency. To determine whether or not this rise represents an increased production of cholesterol, the rate of

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1. Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N., and Isbell, H., *Science*, 1942, v95, 176.
2. Curran, G. L., unpublished observations.

cholesterol synthesis in biotin deficient rats was measured by means of deuterium oxide. Because livers from biotin deficient rats have a decreased rate of pyruvate oxidation(3) and because biotin is necessary for the reversible carboxylation of pyruvic acid to oxalacetic acid(4,5), it was of interest to determine in addition whether the rate of fatty acid synthesis in the rat is influenced by the interference of biotin deficiency with normal pyruvate metabolism.

Experimental. Animal feeding experiment. Five male weanling rats of the Wistar strain weighing about 30 g each were placed in separate metabolism cages. Three of the rats received 15 g a day each of the egg white diet recommended by Emerson(6). The two control rats received similar portions of a diet identical except for the egg white, which was autoclaved to destroy its avidin content. After three weeks the three rats on the unheated egg white diet showed evidence of biotin deficiency. At this time all the rats were injected with sufficient 99.8% D_2O to bring the heavy water content of their body fluids to 1.5%. For the next 11 days the rats received drinking water containing 2.5% D_2O. One of the biotin deficient rats died 2 days after injection of the heavy water; the remaining 2 rats in this group showed the alopecia, dermatitis, spectacle eye and neuromuscular imbalance characteristic of biotin deficiency by the end of the experimental period. After the body fluids had been enriched with heavy water for 11 days, the rats were sacrificed.

Isolation procedures. A sample of body water was distilled from each rat carcass and then the fatty acids and the cholesterol, as the digitonide, were isolated from each carcass and liver(7).

Isotope analyses. Deuterium analyses were carried out by a micromethod(8).

3. Pilgrim, F. J., Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, v145, 237.

4. Shive, W., and Rogers, L. L., *J. Biol. Chem.*, 1947, v169, 453.

5. Lichstein, H. C., and Umbreit, W. W., *J. Biol. Chem.*, 1947, v170, 329.

6. Emerson, G., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 359.

TABLE I. Cholesterol Synthesis in Biotin Deficient and Control Rats.

Rat No.	Atom % excess deuterium in body water, day 8*	Atom % excess deuterium in carcass cholesterol	Atom % excess deuterium in liver cholesterol	Deut. content in chol. of carcasses relative to that in body water†	Deut. content in chol. of livers relative to that in body water†
Control No. 1	.668	.213	.321	32	48
Control No. 2	.650	.183	.294	28	45
Biotin deficient No. 3	.721	.135	.264	19	37
Biotin deficient No. 4	.589	.168	.248	29	42

* Rat No. 4 died on 7th day of D_2O administration and deuterium values are for that day.

† $\frac{\text{Atom \% deuterium in cholesterol}}{\text{Atom\% deuterium in body water}} \times 100.$

TABLE II. Fatty Acid Synthesis in Biotin Deficient and Control Rats.

Rat No.	Atom % excess deuterium in body water, day 8*	Atom % excess deuterium in carcass fatty acids	Atom % excess deuterium in liver fatty acids	Deut. content in fatty acids of carcasses relative to that in body water†	Deut. content in fatty acids of livers relative to that in body water†
Control No. 1	.668	.060	.102	9	15
Control No. 2	.650	.070	.102	11	15
Biotin deficient No. 3	.721	.084	.155	12	22
Biotin deficient No. 4	.589	.041	.105	7	17

* Rat No. 4 died on 7th day of D_2O administration and deuterium values are for that day.

† $\frac{\text{Atom \% deuterium in fatty acids}}{\text{Atom \% deuterium in body water}} \times 100.$

Discussion. It will be seen from the last column in Table I that there is no significant difference in the rate of cholesterol synthesis in the biotin deficient rat as compared to the

TABLE III. Body Weights and Weights of Carcass and Liver Fatty Acids from Biotin Deficient and Control Rats.

Rat No.	Body wt at termination of D_2O adm. in g	Wt gain or loss as % of body wt at start of D_2O administration	Wt of carcass fatty acids in g	Wt of liver fatty acids in g	Combined wt of liver and carcass fatty acids relative to body wt at termination of experimental period in %*
Control No. 1	150	+43%	3.76	.13	2.5
Control No. 2	165	+50%	5.41	.13	3.4
Biotin deficient No. 3	83	−10%	.71	.05	.9
Biotin deficient No. 4	95	−11%	1.84	.07	2.0

* $\frac{\text{Wt of liver fatty acids + wt of carcass fatty acids}}{\text{Body wt at end of experimental period}} \times 100.$

normal. Therefore the elevation of the serum cholesterol level in biotin deficiency cannot be explained on the basis of an increased rate of cholesterol production. The most likely explanation remaining is suggested by the data of Okey(9), who found that avidin prevents the accumulation of cholesterol in the liver by making biotin unavailable. Thus in biotin deficiency, even with a normal rate of cholesterol synthesis, the newly formed sterol cannot be deposited in the liver but rather enters the blood stream with a resultant hypercholesterolemia.

The deuterium contents of the fatty acids (Table II) show that the rate of fatty acid synthesis in biotin deficient rats is slightly increased. It is apparent that biotin deficiency, despite interference with the reversible carboxylation of pyruvate to oxalacetate, does not present a major obstacle to fatty acid synthesis in the intact rat. The last column of Table III gives the ratio of the combined weight of liver and carcass fatty acids relative to the body weight. It will be seen by comparing this column with the last column in Table II that the two biotin deficient rats with the smallest ratio of fatty acids to body weight have the highest rates of fatty acid synthesis. This finding is in agreement with the observations of Bernhard and Steinhauser (10) who found that the rate of fatty acid synthesis in fasting rats increased as the ratio of total weight of body fatty acids to body weight decreased. The increased rate of fatty acid synthesis found in biotin deficient rats is therefore a non-specific reaction to inanition rather than a characteristic of biotin deficiency.

Summary. The rates of cholesterol and fatty acid synthesis in normal and biotin deficient rats were determined by means of heavy water administration. In biotin deficient rats the rate of cholesterol synthesis was found to be normal and the rate of fatty acid synthesis slightly increased. It was shown that the increased rate of fatty acid synthesis is related to the inanition accompanying biotin deficiency and not to the deficiency itself.

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7. Rittenberg, D., and Schoenheimer, R., *J. Biol. Chem.*, 1937, v121, 235.

8. Rittenberg, D., unpublished.

9. Okey, R., *J. Biol. Chem.*, 1946, v165, 383.

10. Bernhard, K., and Steinhauser, H., *Helv. chim. acta*, 1943, v27, 207.

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