TABLE II.
Inactivation of Transaminase and of Vitamin B_6 by Ultraviolet Light.

Irradiation (min.)	Transaminase activity %	Vitamin B_6 compounds		
		Pyridoxal γ	Pyridoxamine γ	Pyridoxine γ
0	37.2	40.0	40.0	40.0
5	10.3	8.0	18.5	14.3
10	4.3	2.1	9.8	8.9
15	1.9	1.2	5.9	4.3
30	<1	0.6	2.9	0.8

that transaminase is rather resistant to hydrogen peroxide. The stability of the members of the vitamin B_6 group has been reported earlier.[7] The inactivated enzyme was not reactivated by addition of pyridoxine, pyridoxal, pyridoxamine, or boiled tissue extract.

Irradiation by X-rays. The conditions were as follow:† 200 K.V.; 16 milliamp; 50 cm distance; filter, 2.5 mm. Al inherent in tube, none added. Since no appreciable inactivation of transaminase (Table III) was observed, no vitamin B_6 determinations were carried out.

TABLE III.
X-Ray Irradiation of Transaminase.

Irradiation r units	Transaminase activity %
0	39.7
500	36.2
1,500	38.4
4,000	35.8
7,000	39.1
10,000	34.5

Summary. Glutamic-aspartic transaminase is inactivated by sunlight and by ultraviolet light. Destruction of enzymatic activity by these agents parallels the destruction of vitamin B_6, a derivative of which is present as the prosthetic group of the enzyme.[1-4] The irradiated enzyme is not reactivated by addition of pyridoxine, pyridoxal, pyridoxamine or boiled tissue extracts. Irradiation with X-rays leaves the enzyme intact.

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Biological Activity and Metabolism of d,l-O-Heterobiotin in the Chick.*

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O-Heterobiotin, an oxygen analog of biotin, has biotin-like activity for the chick[1] and for rats fed egg white.[1,2] Hofmann *et al.*[1] found the d,l analog 10% as active as d-biotin in curing egg-white toxicity in rats and approximately 6 and 20% as active in promoting growth in chicks when fed at different levels on a biotin deficient ration. Rubin *et al.*[2] found d,l-O-heterobiotin 5% as active as d-biotin in counteracting egg-white toxicity in the rat.

Since levels of 40 γ of d,l-O-heterobiotin

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TABLE I.
Activity and Storage of d,l-O-Heterobiotin* in the Chick.

Group No.	Supplement per 100 g biotin deficient basal	No. of survivors at 4 wks.	Avg. wt. at 4 wks.	No. of chicks with dermatitis at 4 wks.	d,l-O-Heterobiotin content of	
					Liver	Leg muscle
			g		γ/g	γ/g
1	None	11†	185	11	0.0	0.0
2	2.5 γ d-biotin	6	185	3		
3	5 γ "	3	205	0		
4	10 γ "	5	220	0		
5	20 γ "	11†	240	0	0.0	0.0
6	5 γ d,l-O-heterobiotin	6	180	4		
7	10 γ "	6	175	2		
8	20 γ "	6	195	0	0.3	0.0
9	200 γ "	6	205	0		
10	500 γ "	4	215	0	0.5	0.0
11	1000 γ "	6	210	0	5.3	0.2

* d,l-O-Heterobiotin is compared with d-biotin on a weight basis throughout this work. If only the d-form of the analog is active the values should be increased correspondingly.

† 12 chicks per group.

per 100 g of a biotin-deficient ration have given only partial responses in chicks[1] it was interesting to determine if higher levels could eventually give complete protection. At the same time it was possible to study the distribution of the analog in chick tissues when graded levels of the compound were included in the diet.

Day-old White Leghorn cockerels were maintained in cages with raised screen bottoms and heated by carbon filament lamps. The chicks were fed a purified ration having the following composition: dextrin 61 g, casein 18 g, gelatin 10 g, Salts V[3] 6 g, soy bean oil 5 g, l(—) cystine 0.3 g, i-inositol 0.1 g, choline chloride 0.15 g, thiamine hydrochloride 0.3 mg, pyridoxine hydrochloride 0.4 mg, riboflavin 0.6 mg, Ca pantothenate 2 mg, nicotinic acid 5 mg, 2-methyl-1,4-naphthoquinone 0.05 mg, α-tocopherol 0.3 mg, p-aminobenzoic acid 5 mg, and crystalline folic acid 0.05 mg. Water and feed were given *ad libitum*, and each chick received weekly one drop of a vitamin A and D concentrate containing 1200 U.S.P. units of vitamin A and 120 A.O.A.C. units of crystalline vitamin D_3 per drop. After 3 days on the basal ration chicks within a 10 g weight range were divided into uniform groups of 6 and given supplements of d-biotin or d,l-O-heterobiotin in the basal ration. The feeding trials were terminated when the chicks were 4 weeks old and samples of liver and leg muscle were taken from 4 chicks in each group for O-heterobiotin analysis. O-Heterobiotin was determined by the differential microbiological assay of Luckey *et al.*[4]

The average weight at 4 weeks, incidence of dermatitis, and O-heterobiotin content of the tissues are summarized in Table I. A comparison of the relative effectiveness of d,l-O-heterobiotin and d-biotin in curing dermatitis indicates that the d,l analog is about ⅓ as active as d-biotin. However, in a comparison based on growth the activities varied inversely with the amount of analog fed. 20 γ of the d,l analog had an activity of approximately 20%. With higher levels the activity fell off rapidly and values from

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1 Hofmann, K., McCoy, R. H., Felton, J. R., Axelrod, A. E., and Pilgrim, F. J., *Arch. Biochem.*, 1945, **7**, 393.

2 Rubin, S. H., Flower, D., Rosen, F., and Drexter, L., *Arch. Biochem.*, 1945, **8**, 79.

3 Briggs, G. M., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1943, **148**, 163.

4 Luckey, T. D., Moore, P. R., and Elvehjem, C. A., in press.

0.5 to 3% were obtained. It is apparent that a level of d,l-O-heterobiotin as high as 1000 γ per 100 g of ration does not give a complete growth response in chicks on our biotin deficient ration and it appears that higher levels would not have afforded complete protection since the 1000 γ level actually gave no better response than the 500 γ level. This failure of O-heterobiotin to completely replace d-biotin for growth when it is highly active in the prevention of dermatitis indicates that the analog is capable of fulfilling only part of the function of d-biotin in the chick. On the other hand, the plateau in growth observed above the 500 γ level (compare group 11 to 10) suggests an inhibitory effect of the d,l analog on growth. In this connection, Luckey *et al.*[4] have observed an inhibitory effect of low levels of d,l-O-heterobiotin on the growth of *Streptococcus fecalis*. Further experimental data are required to clarify this point.

The analyses for d,l-O-heterobiotin indicate that this compound does not occur naturally in chicks receiving our biotin deficient diet alone or when supplemented with d-biotin. No appreciable accumulation of the d,l analog occurred in the livers of the chicks receiving O-heterobiotin until 1000 γ were included in the diet. It is interesting to note that this represents the same level at which a growth plateau was established. Similarly, no O-heterobiotin could be detected in muscle tissue until the 1000 γ level of the analog was reached.

Summary. d,l-O-Heterobiotin has biotin-like activity for the chick. Levels of 20 γ per 100 g of purified biotin-deficient basal ration give complete protection against dermatitis and some growth response. The growth activity of the oxygen analog varies inversely with the level fed and growth comparable to that on an adequate biotin diet was not obtained. d,l-O-Heterobiotin can be detected in the liver and muscle tissue of chicks fed high amounts of this compound.

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The Inactivation of Streptomycin and its Practical Applications.*†

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Because of the rapidly accumulating information on the production, purification and practical utilization of streptomycin,[1] a knowledge of the effects of experimental conditions upon its activity is increasingly important. It is known, for example, that a weakly alkaline environment, namely pH 8.0 to 8.2, is favorable to the action of streptomycin and that acidic conditions are injurious.[2] Likewise, its activity is diminished by the presence of glucose and of phosphates. Cysteine and several ketone reagents also inactivate streptomycin.[3] Since factors of this sort can be of importance in choosing media for the testing of the potency of streptomycin, in developing tests for the sterility of streptomycin preparations, and in other practical applications, it was believed desirable to reinvestigate and extend some of the earlier observations. The presence of reducing agents, such as cysteine and glucose, among the substances that inactivate streptomycin, as well as the observation that anaerobic bacteria are somewhat resistant to its activity, has suggested that special attention be paid to these conditions, in an effort to learn whether their effects are of a fundamental

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[1] Waksman, S. A., and Schatz, A., *J. Am. Pharm. Assn.*, 1945, **34**, 273.

[2] Waksman, S. A., Bugie, E., and Schatz, A., *Proc. Staff Meet. Mayo Clinic*, 1944, **19**, 537.

[3] Denkewalter, R., Cook, M., and Tishler, M., *Science*, 1945, **101**, 12.