and Schwarte[5] reported natural outbreaks of *Listerellosis* in swine and the isolation of *Listerella monocytogenes* from the central nervous system of naturally affected pigs showing clinical symptoms of a central nervous system disorder including incoordination, stilted gait, trembling, and paralysis of posterior extremities.

Summary. Isolation of *Listerella monocytogenes* from the liver of a pig unaccompanied by encephalitic symptoms is described. The presence of *Listerella monocytogenes* in the liver raises the question of its pathologic significance in non-encephalitic syndromes swine.

5 Biester, H. E., and Schwarte, L. H., *Am. J. Vet. Med. Assn.*, 1940, **92**, 339.

14952

Biological Activity of Synthetic d, l-Desthiobiotin.

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Optically active desthiobiotin was first obtained by du Vigneaud, Folkers and coworkers[1,2] as a degradation product of natural biotin. The Cornell group made the interesting observations that desthiobiotin is equally as effective as biotin in stimulating the growth of *Saccharomyces cerevisiae*,[2] but has an antibiotin effect for *Lactobacillus casei*. They further provided evidence[3] to show that the growth effect on the yeast is probably due to the conversion of desthiobiotin to biotin by the yeast cell. Lilly and Leonian[4] and Stokes and Gunness[5] have demonstrated the ability of desthiobiotin to replace biotin in the nutrition of various microorganisms.

Biological data on the activity of desthiobiotin for rats have, however, been lacking. In a recent report on the biological activity of synthetic biotin and several intermediates, Emerson[6] remarked that "desthiobiotin shows a low order of activity in biotin-depleted rats. However, the response was irregular and the slight activity of this compound may have been due to the presence of traces of biotin, since the compound was prepared from biotin." Since synthetic d,l-desthiobiotin[7] known to be free of biotin, has been available to us for some time, we have examined its activity for microorganisms and biotin-deficient rats in some detail.*

Experimental. The various samples of d,l-desthiobiotin tested melted at 163-164°C (corr.). The substance was dissolved for the assays in one of two ways: (1) When dilute solutions were required, as for the microbiological experiments or for some of the bioassays, it was dissolved in water, in which the solubility is about 0.1%, giving a solution of pH 3.5; (2) when more concentrated solutions were needed, as in bioassay dosage at higher levels, the compound was dissolved in somewhat less than the stoichiometric amount of sodium hydroxide solution to give a pH of 6-7.

Microbiological assays. The effect of d,l-desthiobiotin on the growth of *Saccharomyces cerevisiae* No. 139 was examined with 2 different media, that of Snell, Eakin and

1 du Vigneaud, V., Melville, D. B., Folkers, K., Wolf, D. E., Mozingo, R., Keresztesy, J. C., and Harris, S. A., *J. Biol. Chem.*, 1942, **146**, 475.

2 Melville, D. B., Dittmer, K., Brown, G. W., and du Vigneaud, V., *Science*, 1943, **98**, 497.

3 Dittmer, K., Melville, D. B., and du Vigneaud, V., *Science*, 1944, **99**, 203.

4 Lilly, V. G., and Leonian, L. H., *Science*, 1944, **99**, 205.

5 Stokes, J. L., and Gunness, M., *J. Biol. Chem.*, 1945, **157**, 121.

6 Emerson, G. A., *J. Biol. Chem.*, 1945, **157**, 127.

7 Duschinsky, R., and Dolan, L. A., to be published.

* The samples of d,l-desthiobiotin were made available to us by Dr. R. Duschinsky.

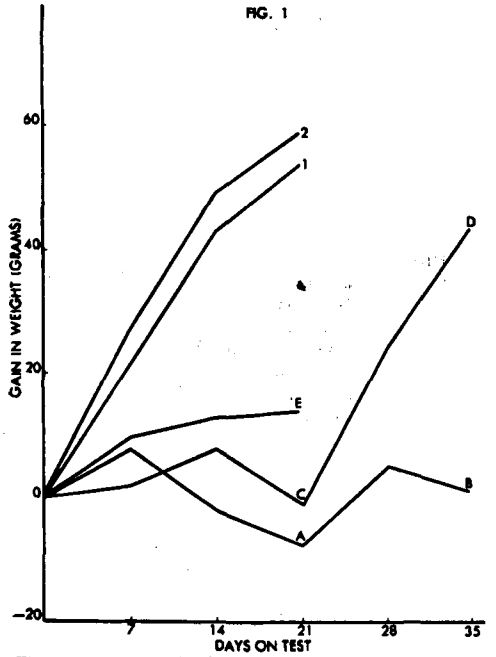
8 Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

Williams[8] and that of Hertz.[9] With both media, half-maximum growth was obtained with about 0.03 millimicrograms of biotin per cc of medium; the Hertz medium supported better growth. *d,l*-Desthiobitin showed an average biotin activity of 53% by weight in the medium of Snell *et al.* and 50% in the Hertz medium. These values are in essential agreement with the values reported by Wood and du Vigneaud[10] for synthetic d,1-desthiobiotin and by Harris *et al.*[11] for d,1-desthiobiotin obtained from d,1-biotin.

The "molar inhibition ratio" of d,1-desthiobiotin was determined in the manner described by Dittmer and du Vigneaud[12] with the *L. casei* method of Shull, Hutchings and Peterson,[13] modified as described by Shull and Peterson.[14] The average value of the "molar inhibition ratio" found in 3-day runs was 17,000; Dittmer and du Vigneaud[12] reported 9,100 for d-desthiobiotin. Since our value for d,1-desthiobiotin is approximately twice the value for d-desthiobiotin, the inference is that 1-desthiobiotin does not act as an antibiotin. This conclusion cannot be drawn with certainty, however, because of the somewhat empirical nature of the test for inhibition. The final decision must await simultaneous runs with the pure optical isomers.

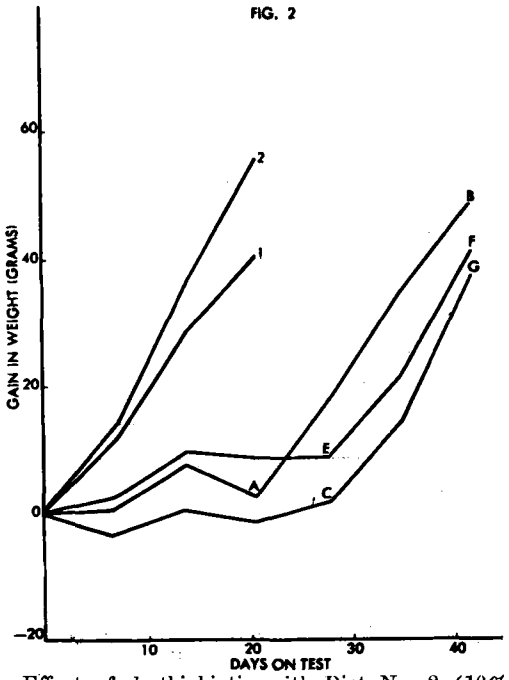
The "molar inhibition ratio" is usually determined with a 3-day incubation period in accord with the usual assay practices. When

FIG. 1



Effect of Desthiobiotin with Diet No. 1 (30% egg white).
Curve 1 : 0.5 μg of biotin
2 : 1 μg of biotin
O-A: Negative controls; A-B: 1 mg desthiobiotin; O-C: 1 mg desthiobiotin; C-D: 10 mg desthiobiotin; O-E: 0.5 mg desthiobiotin.
All given by intraperitoneal injection.

FIG. 2



Effect of desthiobiotin with Diet No. 2 (10% egg white).
Curve 1 : 0.5 μg of biotin
2 : 1 μg of biotin
O-A: Negative controls; A-B: 1 mg desthiobiotin; O-C: 4 μg desthiobiotin; C-G: 1 mg desthiobiotin; O-E: 8 μg desthiobiotin; E-F: 1 mg desthiobiotin.
All administration was by intraperitoneal injection except C-G where administration was oral.

[9] Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 15.

[10] Wood, J. L., and du Vigneaud, V., *J. Am. Chem. Soc.*, 1945, **67**, 210.

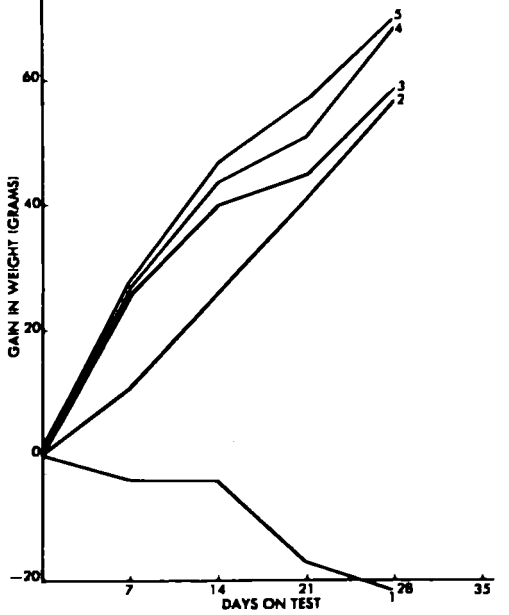
[11] Harris, S. A., Mozingo, R., Wolf, D. E., Wilson, A. N., Arth, G. E., and Folkers, K., *J. Am. Chem. Soc.*, 1944, **66**, 1800.

[12] Dittmer, K., and du Vigneaud, V., *Science*, 1944, **100**, 129.

[13] Shull, G. M., Hutchings, B. L., and Peterson, W. H., *J. Biol. Chem.*, 1942, **142**, 913.

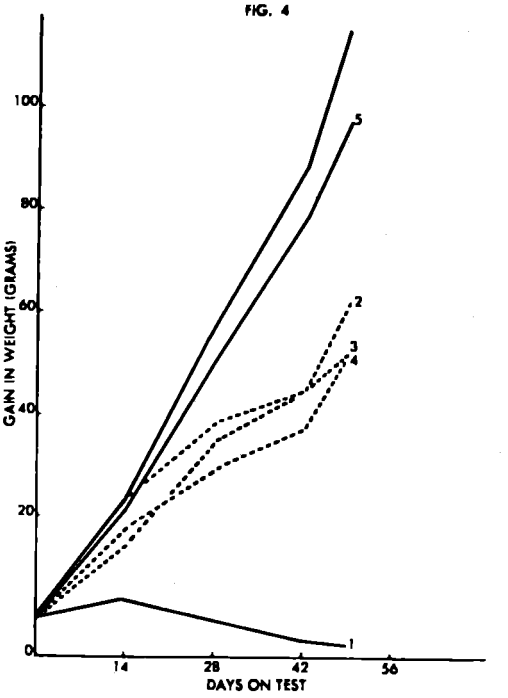
[14] Shull, G. M., and Peterson, W. H., *J. Biol. Chem.*, 1943, **151**, 201.

FIG. 3



Effect of desthiobiotin with Diet 1 (30% egg white).
Curve 1 : Negative controls
2 : 0.35 μg biotin
3 : 4 mg desthiobiotin
4 : 0.7 μg biotin
5 : 8 mg desthiobiotin.
All administered by intraperitoneal injection.

FIG. 4



Effect of desthiobiotin with Diet No. 3 (Succinylsulfathiazole).
Curve 1 : Negative controls
2 : 3000 units folic acid concentrate
3 : 0.4 μg of desthiobiotin + 3000 units of folic acid
4 : 0.8 μg of desthiobiotin + 3000 units folic acid
5 : 1 mg of desthiobiotin + 3000 units folic acid
6 : 0.2 μg of biotin + 3000 units folic acid.
All administered orally. The broken lines indicate groups which showed marked biotin deficiency symptoms at the end of 7 weeks.

the time of incubation was extended to 6 days, it was found that the value of the ratio increased somewhat, indicating a small diminution in antibiotin activity. The increases observed, however, fell within the limits of variation of random runs and hence were not necessarily significant.

Biological assays. Two procedures can be used for inducing biotin deficiency in the rat, *viz.*, by the avidin mechanism or by repression of intestinal synthesis of biotin by means of sulfonamide compounds. Both approaches have been used in the present experiments. Because of the dearth of information regarding the quantitative aspects of biotin assay with diets containing egg white, it seemed desirable to vary the content of egg white. Accordingly, diet No. 1 (Table I) is patterned after that employed by Dittmer, du Vigneaud, György and Rose[15] and contains 30% dried egg white as the source of avidin and as the sole source of protein; diet No. 2 is based upon that used by Nielsen and Elvehjem[16] and provides 10% egg white and 8% casein. For the sulfonamide experiments, 1% succinylsulfathiazole was included in a diet similar to that employed by Daft and Sebrell[17] in their experiments on folic acid. The diets are detailed in Table I.

25-day-old male rats (Sprague-Dawley), weighing 35-45 g, were housed in individual

[15] Dittmer, K., du Vigneaud, V., György, P., and Rose, C. S., *Arch. Biochem.*, 1944, **4**, 229.

[16] Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 405.

[17] Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1943, **58**, 1542.

TABLE I.
Composition of the Diets.
(g per 100 g of diet)

	115	216	317
Sucrose		73	
Glucose (Cerelose)			72
Corn starch	48		
Dried egg white	30	10	
Vitamin-free casein (Labco)		8	18
Salts (USP No. 1)	5	4	4
Wesson oil	14		3
Primex		5	
Cod liver oil	3		2
Succinylsulfathiazole			1

The following supplement was supplied to the rats receiving diets 1 and 2 three times weekly in 1 ml of distilled water: 47 μg of thiamine chloride, riboflavin and pyridoxine, 470 μg of calcium pantothenate, 1.17 mg of inositol and nicotinic acid, 580 μg of p-aminobenzoic acid, and 7 mg of choline chloride. In addition the rats on diet 2 received 0.2 ml of USP Cod Liver Oil weekly.

The rats on diet 3 received the following supplement: 233 μg of thiamine chloride and pyridoxine, 470 μg of riboflavin and calcium pantothenate, 1.17 mg of inositol, 580 μg of p-aminobenzoic acid, 187 μg vitamin K (Synkavite), 2.33 mg nicotinic acid, 23.3 mg choline chloride, 3 times weekly in 4 ml of water.

cages and fed these diets *ad libitum*. Each curve in the figures represents the average response of a group of 3 to 5 rats. All supplements are given in terms of daily dosages.

Activity of d,l-Desthiobiotin in Egg White Experiments. After an average period of 45 days on diet 1 (30% egg white) and 65 days on diet 2 (10% egg white), the rats showed marked symptoms of biotin deficiency, characterized by cessation of growth, spectacled eye, seborrheid dermatitis and alopecia; arched back and profuse salivation were also manifested by many of the rats. Supplementation with biotin or d,l-desthiobiotin was begun at the times stated above.

Preliminary experiments with both diets showed that d,l-desthiobiotin, administered either orally or intraperitoneally in daily doses up to 8 μg, had less than 5% of the activity of biotin. However, subsequent experiments with larger doses revealed that definite biotin activities of the order of magnitude of 0.1-0.01% could be elicited. Thus, rats that had shown no weight gain on diet 2 (10% egg white) when given 0, 4 or 8 μg per day of d,l-desthiobiotin (Fig. 2: O-A, O-C, O-E), responded with marked weight gains (Fig. 2: A-B, C-G, E-F) and amelioration of the deficiency symptoms when dosed with 1 mg of the compound (A-B and E-F intraperitoneally, C-G orally). When, however, 1 mg was administered intraperitoneally to rats on diet 1 (30% egg white), no such response resulted (Fig. 1: O-C, A-B); 10 mg was required to cause a marked weight response (Fig. 1: C-D) and cure of symptoms. The experiment with diet 1 was repeated (Fig. 3) with essentially the same result. It appears, therefore, that the small biotin activity of d,l-desthiobiotin was inversely proportional to the concentration of egg white in the diet.

This inverse effect suggests that desthiobiotin may have acted in these experiments by displacement of biotin from avidin-biotin complex. Dittmer and du Vigneaud[12] have shown that desthiobiotin combines with avidin and that two analogs of desthiobiotin, namely, 4-(imidazolidone-2)-caproic acid and 4-(imidazolidone-2)-valeric acid, can displace biotin from avidin-biotin as tested by yeast or *L. casei* cultures. The latter has not been demonstrated for desthiobiotin because of the experimental difficulties imposed by the behavior of desthiobiotin towards these microorganisms. The present animal experiments provide some evidence for this viewpoint.

Activity of d,l-Desthiobiotin in Experiments with Succinylsulfathiazole. The results were similar to those obtained in the egg white experiments in that the apparent activity was of a low order of magnitude, approximately 0.02% of biotin. Thus, as shown in Fig. 4, 1 mg of d,l-desthiobiotin was required to prevent deficiency symptoms and effect a weight increase similar to that obtained with 0.2 μg of biotin.

These experiments were complicated by folic acid deficiency as demonstrated by depression of the leucocyte count. After 4 weeks on the basal diet, the rats had reached stationary weight, but did not show clean-cut symptoms of biotin deficiency. At that point, supplementation with the biotin compounds and a folic acid concentrate was begun. The latter was treated with 0.3% hydrogen peroxide for 24 hr at room temperature to render the biotin present unavailable to the rat.[18] Microbiological assay with *S. lactis*[19] showed that

the folic acid was not affected by the peroxide treatment. Daily supplementation with 3000 Snell-Peterson units[20] of folic acid *precipitated marked symptoms of biotin deficiency* in groups 2, 3 and 4 (Fig. 4). The negative controls, group 1, which received neither biotin nor folic acid, showed only slight symptoms of biotin deficiency (spectacled eye). Group 5 and 6, which received 1 mg of d,l-desthiobiotin and 0.2 μg of biotin respectively, showed no manifestations of biotin deficiency. This must therefore be considered as a prophylactic experiment. The apparent biotin activity due to d,l-desthiobiotin was of the order of magnitude of 0.02%.

The striking effect of the folic acid concentrate in accentuating the biotin deficiency symptoms may arise from the fact that folic acid induces better growth and survival and thus increases the biotin requirement of the rats. Similar observations have been recorded by Daft, Ashburn and Sebrell,[21] who produced a dermatitis in rats by supplementing a diet containing succinylsulfathiazole with a liver concentrate which provided folic acid, among other things; in "the 40 rats given neither biotin nor liver extract, ... any degree of biotin deficiency present in these animals was difficult to diagnose." There are indications in the recent report of Nielsen and Black[22] that the same is true for mice.

Summary. Synthetic d,l-desthiobiotin induces the following biological responses. (1) It is 50% as active as d-biotin in promoting the growth of *S. cerevisiae*. (2) It is approximately half as effective as d-desthiobiotin in inhibiting fermentation by *L. casei*. The inhibitory effect may diminish slightly over a period of 6 days. (3) On diets containing egg white or succinylsulfathiazole, the apparent vitamin activity in rats is of a low order of magnitude, 0.1 to 0.01% that of biotin.

18 Nielsen, E., Shull, G. M., and Peterson, W. H., *J. Nutrition*, 1942, **24**, 523.

19 Luckey, T. D., Briggs, G. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **152**, 157.

20 Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 173.

21 Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

22 Nielsen, E., and Black, A., *J. Nutrition*, 1944, **28**, 203.

The assistance of Miss Gertrude Engel with some of the microbiological assays is gratefully acknowledged.

14953

The *In vitro* Antibacterial Effects of Sulfanilamidoindazoles.

C. A. LAWRENCE AND G. R. GOETCHIUS. (Introduced by M. L. Tainter.)

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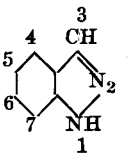
In a previous report from these laboratories, Kwartler and Lucas[1] described the preparation of several sulfanilamidoindazoles. This paper presents the results of the bacteriological investigations on 4 of the compounds, 3-, 5-, 6-, and 7-sulfanilamidoindazole. To our knowledge the only reference to the use of compounds of this nature in chemotherapy is the work of Coggeshall and Maier[2] who found that 5-sulfanilamidoindazole is ineffective in experimentally induced influenza and poliomyelitis infections in mice. The structural formula for indazoles, together with the system for numbering, is as follows:



1 Kwartler, C., and Lucas, P., *J. Am. Chem. Soc.*, 1943, **65**, 1804.

2 Coggeshall, L. T., and Maier, J., *J. Pharm. and Exp. Therap.*, 1942, **76**, 161.