the diploid culture was heterozygous for the gene-pair controlling pantothenic acid synthesis.

Table 1 shows the turbidity readings of the original cultures of *S. cerevisiae* and *S. carlsbergensis*, their haplophase segregants and the hybrid. Analysis of the hybrid by dissection of ascospores indicated that it was heterozygous for the ability to synthesize pyridoxine, pantothenic acid and biotin.

S. globosus[4] is capable of synthesizing pantothenic acid, but is incapable of synthesizing thiamin. A hybrid was made with a haplophase from a homozygous pantothenic-deficient culture of *S. cerevisiae* (different from the one used above) and a haplophase culture of *S. globosus*. Several hybrids were produced by this mating, and one of them sporulated well, but only a few of the ascospores were viable. One of the hybrid-haplophases was backcrossed to the original pantothenic-deficient *S. cerevisiae*. The resulting diploid synthesized both pantothenic acid and thiamin efficiently. Since neither parent could synthesize both vitamins, the hybrid had obviously obtained its ability to synthesize pantothenic acid from *S. globosus* and thiamin from *S. cerevisiae*. The hybrid was a poor synthesizer of biotin, but this was according to expectation, since neither parent possessed the ability. The data appear in Table 2.

TABLE 2

	-All	all	-Py	-Pa	-T	-B	-N	-I
S. cerevisiae	8	362	355	50	313	19	355	300
S. globosus	9	270	254	241	26	20	258	250
Hybrid	5	290	293	258	290	15	294	300

These experiments prove that a vitamin-synthesizing deficiency in a yeast can be supplied by hybridization and that the heterozygote usually synthesizes the vitamin nearly as well as the homozygote.

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STREPTOMYCES ANTIBIOTICS. I. CRYSTALLINE SALTS OF STREPTOMYCIN AND STREPTOTHRICIN

METHODS have been found for securing certain crystalline salts of streptomycin and streptothricin.

These antibiotics are water-soluble, nitrogenous, thermostable, basic organic substances produced by species of *Streptomyces* in suitable culture media. Streptomycin[1] was obtained first in 1944 as a crude concentrate which was prepared from cultures of *Streptomyces griseus*. It is strongly bacteriostatic against gram-positive organisms, including *Bacillus mycoides* and *Bacillus cereus*, and against gram-negative organisms, including *Pseudomonas fluorescens*, *Pseudomonas aeroginosa* and *Serratia marcescens*, while its toxicity for animals is sufficiently low that it has therapeutic interest for diseases such as tularemia,[2] typhoid fever,[3] tuberculosis[4] and brucellosis.[5] Streptothricin[6] was obtained first in 1942 as a crude concentrate which was prepared from cultures of *Streptomyces lavendulae*. It is also active against gram-negative organisms, including the three mentioned above, but is relatively much less active[1] against such gram-positive organisms as *Bacillus mycoides* and *Bacillus cereus*. Streptothricin also appears to have possible therapeutic applicability.[7]

The concentrates of streptomycin and streptothricin which were used for these biological studies[1, 6] were prepared by adsorption of the active substance from the culture medium by means of Norite-A, elution of the active substance from the adsorbate with dilute acid, neutralization of the eluate and concentration *in vacuo* to a residue. A concentrate of streptothricin was made also by eluting the adsorbate with acidified alcohol, neutralizing the eluate and adding ether to the eluate.[8]

We have studied methods for the purification and isolation of these active substances and have found that when rather highly purified concentrates of streptomycin were treated with methyl orange (the sodium salt of helianthine) a crystalline salt formed which served for purification of the active principle. This helianthate can be converted into the hydrochloride, sulfate or any other suitable salt for chemical or therapeutic purposes. Concentrates of streptomycin hydrochloride were treated with methyl orange and by metathetical reaction yielded the insoluble crystalline streptomycin helianthate. Since this salt is relatively insoluble in water, it separates satisfactorily from an aqueous methanol solution and was recrystallized from the same solvent. Solvent of crys-

[4] We are indebted to Dr. Wickerham of the NRRL, Peoria, for the culture of *S. globosus*.

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[2] F. R. Heilman, *Staff Meetings of Mayo Clinic*, 19: 553, 1944.

[3] H. J. Robinson, D. G. Smith and O. E. Graessle, *Proc. Soc. Exp. Biol. and Med.*, 57: 226, 1944; H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Asn.*, 128: 175, 1945.

[4] W. H. Feldman and H. C. Hinshaw, *Staff Meetings of the Mayo Clinic*, 19: 593, 1944; A. Schatz and S. A. Waksman, *ibid.*, 57: 244, 1944.

[5] D. Jones, H. J. Metzger, A. Schatz and S. A. Waksman, SCIENCE, 100: 103, 1944.

[6] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exp. Biol. and Med.*, 49: 207, 1942.

[7] H. J. Metzger, S. A. Waksman and L. H. Pugh, *Proc. Soc. Exp. Biol. and Med.*, 51: 251, 1942; H. J. Robinson, O. E. Graessle and D. G. Smith, SCIENCE, 99: 540, 1944; H. J. Robinson and D. G. Smith, *Jour. Pharm. and Exp. Therap.*, 81: 390, 1944.

[8] S. A. Waksman, *Jour. Bact.*, 46: 299, 1943.

tallization was present, but after heating at 100° *in vacuo* the anhydrous form was obtained. This anhydrous product darkened at 205° and melted with decomposition at 220–226° (micro-block).

Assays were carried out by Dr. H. B. Woodruff and Mr. D. Hendlin, of the Microbiological Department. Employing *B. subtilis* as a test organism in a cup assay method which they developed, they found an activity of about 350 units/mg for streptomycin helianthate. The various other salts described in this paper were assayed in the same manner.

The results of some microanalytical determinations on the dried streptomycin helianthate follow: Found: C, 50.38, 50.29; H, 5.86, 5.56; N, 14.48, 14.64; S, 5.76.

Streptomycin helianthate was converted to the hydrochloride by treating the salt with a mixture of methyl alcohol and hydrochloric acid. The liberated helianthine was removed and the hydrochloride was precipitated with ether from the filtrate. The hydrochloride was obtained as a white powder. When dried at 25° *in vacuo* over phosphorus pentoxide, streptomycin hydrochloride showed a specific rotation $(\alpha)_D = -84°$ (C, 0.5 per cent. in water), and an activity of about 800 units/mg. The results of microanalytical determinations on a sample dried at 100° *in vacuo* follow: Found: C, 36.60, 36.42; H, 6.04, 6.20; N, 13.42; Cl, 14.80; ash, none. Tests for the presence of sulfur and phosphorus gave negative results. The ultraviolet adsorption spectra of streptomycin in phosphate buffer at pH 7, in glycine buffer at pH 2 and in borate buffer at pH 9 showed only end absorption below about 2,300 Å.

The above analytical data alone are not sufficient for establishing firmly the true empirical formulae of streptomycin, its hydrochloride and its helianthate. These results need to be interpreted in conjunction with molecular weight determinations and other information. Furthermore, the possible molecular weight of about 700 for streptomycin hydrochloride adds to the difficulties of exact determination of empirical formulae. After more complete data are obtained, the results will be presented in detail.

The crystalline salt of streptomycin and *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonic acid was prepared from streptomycin hydrochloride and Orange II. This salt showed an activity of about 300 units/mg. The preparations of streptomycin sulfate, which have been obtained crystalline, have shown about 520 units/mg.

Streptothricin helianthate was prepared using the same method. The crystals melted with decomposition at 220–225° (micro-block) and had an activity of about 400 units/mg.

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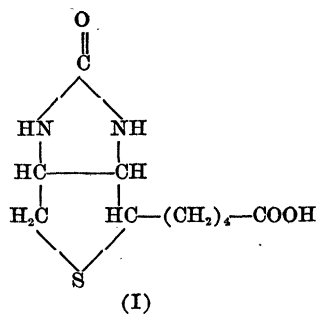
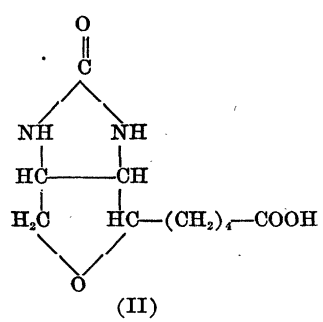
THE MICROBIOLOGICAL ACTIVITY OF AN OXYGEN ANALOG OF BIOTIN

THE synthesis of a *dl*-hexahydro-2-oxo-l-furo [3, 4] imidazole-4-valeric acid, an oxygen analog of biotin, has been recently described.[1] The structural relationship between biotin (I) and this new compound (II) is indicated by the following formulae.



(I)



(II)

The growth-stimulating activity of the oxygen analog under conditions in which biotin is the limiting factor has now been determined for three microorganisms: *Lactobacillus arabinosus*,[2] *Lactobacillus casei*[3] and *Saccharomyces cerevisiae*.[4] The methods were, with slight modifications, those described in the literature cited.

On a weight basis, the *dl* oxygen analog is one half as active as natural *d* biotin when assayed with *L. arabinosus*. Its activity for *L. casei* is slightly lower, approximately 40 per cent. that of *d* biotin. With

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[2] L. D. Wright and H. R. Skeggs, *Proc. Soc. Exp. Biol. and Med.*, 56: 95, 1944.
[3] M. Landy and D. M. Dicken, *Jour. Lab. and Clin. Med.*, 27: 1086, 1942.
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