necrosis and interstitial leukocytic infiltration to extensive and widespread areas of necrosis and inflammation. In those animals that recovered from their paralysis and appeared to be approaching normalcy, the microscopic myocardial lesions varied, often showing marked calcification in the zones of necrosis, fibroblastic replacement of muscle and early scar tissue formation. These older lesions were always accompanied, however, by varying degrees of leukocytic reaction.

This filter-passing agent has been found to be sterile on repeated culture. It will withstand heating to 56 degrees C for 20 minutes, losing some of its potency but not its specificity and it is completely destroyed by heating to 70 degrees C for 20 minutes.

Extracts of organs of companion chimpanzees dying of other conditions have uniformly failed to produce any lesions in mice. The agent has been passed through Berkefeld and Seitz filters and has been transferred to seven-day chick embryos and again passed through Berkefeld filters without losing its potency or specificity.

On rare occasions small foci of round cells have been found in the kidneys and the pulmonary lesions range from hyperemia to patchy foci of edema and even occasionally broncho-pneumonia. Splenic hyperplasia has also been observed rather frequently. The other viscera have shown no lesions that could be attributed to the agent.

The agent has been found to be potent and specific when introduced intravenously, intraperitoneally, subcutaneously, intracranially and by intranasal instillations. Moreover, it is found to be present in the nasal washings of inoculated animals. Quite recently it has been employed to produce myocarditis in guinea pigs and rabbits and we are now studying these myocardial changes with the electrocardiograph. This work is still in progress.

The cardiac lesions of this ape disease and sporadic human acute interstitial myocarditis of unknown etiology are strikingly similar.

So far as we have been able to learn, this myocarditis producing agent has not been described previously.

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VITAMIN-SYNTHESIZING DEFICIENCIES IN YEASTS SUPPLIED BY HYBRIDIZATION[1]

VITAMIN-SYNTHESIZING deficiencies of *S. cerevisiae* can be supplied by hybridizing it with species able to synthesize the vitamins for which it is deficient. Most strains of *S. cerevisiae* are deficient in the ability to synthesize biotin and vary from good to poor in the ability to synthesize pantothenic acid. This species appears to be heterozygous for the genes controlling the ability to synthesize pantothenic acid. Our culture of *S. carlsbergensis* is capable of synthesizing both pantothenic acid and biotin in large quantities, but is unable to synthesize pyridoxine, which *S. cerevisiae* synthesizes well. Haplophase cultures[2] derived from *S. carlsbergensis* were biotin +, pantothenic acid + and pyridoxine –. They were mated with haplophase cultures of *S. cerevisiae*, which were biotin –, pantothenic acid – and pyridoxine +. The hybrid synthesizes all three vitamins in large quantities.

The technique was that developed by Burkholder[3] in his survey of vitamin-synthesizing ability of various yeast species. The standard medium containing glucose and asparagin and various minerals was supplemented with the six B vitamins (exclusive of B_2). In the medium containing the six vitamins, growth was nearly complete at the end of three days. The cultures were grown in $6 \times \frac{3}{4}$" Kimble test-tubes and the growth was determined by measuring the turbidity with a photoelectric colorimeter. Other nutrient media were made up corresponding to the complete medium described above except that single B vitamins were lacking. Since these B vitamins are essential to cell metabolism, it is assumed that a culture able to produce good growth in a nutrient lacking a given vitamin is able to synthesize this vitamin and that the converse is also true.

TABLE 1

	No vitamins	All vitamins	-pyridoxine	-pantothenic acid	-thiamin	-biotin	-niacin	-inositol
S. cerevisiae diploid	0	315	340	215	306	11	324	300
S. carlsbergensis diploid	1	350	24	280	355	280	340	315
S. cerevisiae haploid	0	309	285	13	290	12	306	230
S. carlsbergensis haploid	3	330	23	204	323	124	322	290
Hybrid	7	303	313	241	314	134	304	282

The amount of inoculum was tested and shown not to carry enough vitamin to obscure the results.

The haplophase segregant of *S. cerevisiae* is pantothenic acid –, while the diploid from which it was derived was pantothenic acid +. This indicates that

[1] This work was supported by a grant from Anheuser-Busch, Inc. St. Louis.

[2] Carl C. Lindegren, *Ann. Mo. Bot. Garden*, 32: 107–123, 1945.

[3] Paul R. Burkholder, I. McVeigh and D. Moyer, *Jour. Bact.*, 48: 385–391, 1944.

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*.

[1] A. Schatz, E. Bugie and S. A. Waksman, *Proc. Soc. Exp. Biol. and Med.*, 55: 66, 1944.

[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.