the society's journal, *Civil Engineering*. Carlton B. Jansen, member of the society, engineer of the Dravo Corporation in Pittsburgh, received the award for a most interesting paper on "Submerged Shipways with Steel Sheeting Walls," describing a recent installation of great engineering interest in Wilmington, Del.

Another prize under the auspices of a society division rather than the society as a whole is the Karl Emil Hilgard Prize, in memory of a celebrated Swiss engineer who lived for many years in America. The hydraulics division of the society determined that this prize should go to Professor Harold A. Thomas, member of the society, of the Carnegie Institute in Pittsburgh, and Emil P. Schuleen, associate member, of the U. S. Engineer Office, in the same city. Their paper was entitled "Cavitation in Outlet Conduits for High Dams." Cavitation is the mechanical deterioration, in this instance of concrete, due to high water pressures and velocities. Two types of apparatus for studying cavitation are described, with analysis of the results, and a development of the hydraulic theory involved.

The last award for engineering studies was made to George J. Schroepfer, member of the society, chief engineer of the Minneapolis-St. Paul Sanitary District, for a paper entitled "Experiences in Operating a Chemical-Mechanical Sewage Treatment Plant." This paper received the Rudolph Hering Medal at the hands of the sanitary engineering division of the society. This medal commemorates a famous American engineer. The paper describes the problems that arose in the first two years of operation of a new plant, the expedients developed to overcome the difficulties, and further changes to effect economy or simplification. The results have a direct appeal to sanitary engineers faced with similar practical problems.

Four celebrated American engineers were awarded honorary memberships, highest recognition among American civil engineers. Best known of these men was Thomas H. MacDonald, for many years head of the government roads program, now the Public Roads Administration in Washington. Another well-known engineer is Francis T. Crowe, who has been construction superintendent on Boulder, Shasta and other huge western dam projects. Gerard H. Matthes is well known among American civil engineers for his outstanding work in the fields of river hydraulics, surveying and geology. Still another new honorary member is Edward H. Connor, long a leader in the field of large bridge construction and difficult deep foundations.

In their presentation for these distinguished honors, the new honorary members were accorded the following citations:

EDWARD HANSON CONNOR: Long a leader in the contracting field, attacking difficult bridge and foundation problems; whose character and integrity have earned success in a most hazardous engineering business.

FRANCIS TRENHOLM CROWE: Construction engineer specializing in tremendous dams; whose masterworks have brought protection to flood-stricken valleys, vital power to great industrial centers and life-giving water to a thirsty land.

THOMAS HARRIS MACDONALD: Pioneer in American transportation engineering, devoting a lifetime to distinguished public service; through whose clear vision and administrative powers the world's greatest highway development is being consummated.

GERARD HENDRIK MATTHES: Happy combination of Dutch and American training; master of many engineering fields, now lending great talents to solving the hydraulics of the Mississippi River; cultured gentleman who honors a great profession.

At the same meeting the following newly elected officers were installed: *President,* Malcolm Pirnie, New York City; *Vice-Presidents,* Richard E. Dougherty, New York City, and Franklin Thomas, Pasadena, Calif.; *Directors,* S. C. Hollister, Ithaca, N. Y.; Gail A. Hathaway, Washington, D. C.; R. W. Gamble, Milwaukee, Wis.; Wilbur M. Wilson, Urbana, Ill.; Frank C. Tolles, Cleveland, Ohio; William D. Shannon, Seattle, Wash., and Royce J. Tipton, Denver, Colo.

SYDNEY WILMOT,
Manager of Publications

SPECIAL ARTICLES

THE POSSIBLE SYNTHESIS OF BIOTIN FROM DESTHIOBIOTIN BY YEAST AND THE ANTI-BIOTIN EFFECT OF DESTHIOBIOTIN FOR L. CASEI[1]

RECENTLY the yeast-growth-promoting activity of desthiobiotin has been described, together with an improved method for its preparation from biotin by hydrogenolysis with Raney nickel.[2] Desthiobiotin was found to be equally as effective as biotin in stimulating the growth of *Saccharomyces cerevisiae,* but could not replace biotin as a growth stimulant for *Lactobacillus casei.* These differences in the growth-stimulating properties of biotin and desthiobiotin for

[1] The authors wish to express their appreciation to Mrs. Glenn Ellis and Miss Carol Tompkins for carrying out the bioassays.

[2] V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *Jour. Biol. Chem.,* 146: 475, 1942. D. B. Melville, K. Dittmer, G. B. Brown and V. du Vigneaud, SCIENCE, 98: 497, 1943.

yeast and *L. casei* suggested their utilization as a differential biological assay method for the determination of biotin and desthiobiotin in systems containing either or both compounds.

The surprisingly high yeast-growth activity of desthiobiotin raised the question of whether desthiobiotin was a yeast-growth factor *per se* or showed activity because of a conversion to biotin by the yeast cell. With a differential assay method available it became possible to investigate this problem, and the results of some experiments in this direction are described herein.

TABLE 1

YEAST AND L. CASEI ASSAY VALUES OBTAINED WITH BIOTIN AND DESTHIOBIOTIN, SEPARATELY AND IN COMBINATION, AND AFTER AUTOCLAVING IN ACID SOLUTIONS

Growth stimulant added	Total amount of stimulant added	Yeast assay biotin and desthiobiotin values	L. casei assay biotin values
		γ	γ
Biotin	1.00 γ	1.01	1.02
Desthiobiotin	1.00 γ	1.00	0
Biotin 0.5 γ + Desthiobiotin 0.5 γ	1.00 γ	0.96	0.52
Biotin, autoclaved*	1.00 γ	0.98	0.96
Desthiobiotin, autoclaved*	1.00 γ	0.37	0
Moist yeast, autoclaved*	1.0 gm	0.72	0.67
Desthiobiotin + Moist yeast, autoclaved*	6.33 γ + 1.0 gm	2.12	0.67

* 1 hour at 120° C. with 2 *N* H_2SO_4.

The growth effects of pure biotin and desthiobiotin, alone and in combination, were first determined on cultures of *S. cerevisiae*.[3] A mixture of biotin and desthiobiotin showed an additive effect on the growth of this yeast, as shown in Table 1.

Biotin and desthiobiotin, singly and in combination, were tested for their growth effects on *L. casei* ε[4]. At concentrations of desthiobiotin lower than 0.47×10^{-7} molar, the growth produced by mixtures of the two compounds was identical with that produced by the biotin present alone, as shown in Table 1. This permitted the use of this method for differentiating between biotin and desthiobiotin in mixtures of the two.

However, it was observed that at concentrations of desthiobiotin higher than 0.47×10^{-7} molar, desthiobiotin possessed a definite anti-biotin effect toward *L. casei*. The growth of this organism due to 0.82×10^{-10} molar biotin was decreased to one half its value by the addition of 2.3×10^{-6} molar desthiobiotin prior to incubation. This inhibition of growth by desthiobiotin was completely reversed by increasing the biotin concentration to 4.1×10^{-10} molar. The use of the yeast-*L. casei* differential method of assay for desthiobiotin in biological materials is not complicated by the anti-biotin effect of desthiobiotin if the concentrations of desthiobiotin are not greatly in excess of 0.47×10^{-7} molar. In the following experiments sufficiently low concentrations of desthiobiotin are used to prevent the anti-biotin effect from playing an appreciable role in the assays.

Since preliminary acid hydrolysis was used to liberate any bound biotin in the yeast cultures, the effect of acid hydrolysis on the activity of desthiobiotin and of desthiobiotin and yeast mixtures was investigated. These results, also included in Table 1, show that while treatment of biotin with 2 N H_2SO_4 at 120° for 1 hour has no effect on its growth-promoting activity, the same treatment of desthiobiotin destroys between 60 and 80 per cent. of its yeast-growth activity. It is evident from these results that in fractions exhibiting growth activity for *L. casei*, and not affected by acid hydrolysis, this activity could not be due to desthiobiotin, but could be due to biotin.

In experiments designed to determine the possible formation of biotin from desthiobiotin used as a growth stimulant for yeast, 40 cc of biotin-free medium were inoculated with 2.4 mg of moist yeast from 24-hour culture of *S. cerevisiae* (Strain 139) and added to various amounts of desthiobiotin in 125 cc pyrex Erlenmeyer flasks. These cultures were incubated at 30° C. for either 16 or 46 hours. At the end of the incubation period, the cells were separated from the medium by centrifugation. The medium was autoclaved without acid for 15 min-

TABLE 2

DATA OF SEVERAL TYPICAL DESTHIOBIOTIN CONVERSION EXPERIMENTS

Compound added	Amount added	Inoculum per 40 cc.	Incubation period	Yeast assay (biotin plus desthiobiotin)			L. casei assay (biotin)			Amount of desthiobiotin converted
				Cells	Medium	Percentage of amount added	Cells	Medium	Percentage of amount added	
	γ	*mg.*	*hrs.*	γ	γ	*Per cent.*	γ	γ	*Per cent.*	*Per cent.*
Desthiobiotin	0.1212	2.40	16	0.1130	0.0025	95	0.1130	< 0.0008	93	100
Desthiobiotin	1.212	2.40	16	0.1315	1.016	95	0.1540	< 0.0008	13	13
Desthiobiotin	1.212	2.40	46	0.185	0.889	90	0.216	< 0.0025	18	18
Biotin	0.1217	2.40	16	0.1212	< 0.0004	99	0.100	< 0.0008	83	..

[3] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[4] G. M. Shull, B. L. Hutchings and W. H. Peterson, *Jour. Biol. Chem.*, 142: 913, 1941.

utes, while the cells were autoclaved at 120° C. for 1 hour in 2 N H_2SO_4. The solutions from the autoclaved cells were neutralized, adjusted to volume and filtered to remove any precipitate. Both the autoclaved medium and the acid-hydrolysed cells were assayed for yeast-growth-promoting activity, which represents activity due to both biotin and desthiobiotin, and *L. casei* growth-stimulating activity, which is a measure only of biotin or some other biotin vitamer which has been synthesized by the yeast and which is capable of supporting the growth of *L. casei*.

The data of several typical desthiobiotin conversion experiments together with a biotin control are presented in Table 2. These results show that desthiobiotin disappears from the incubating yeast cultures and is replaced by an equivalent amount of a substance possessing growth-promoting powers for *L. casei*. The most logical assumption is that desthiobiotin is transformed to biotin by the growing yeast cell.

As can be seen in Table 2, the conversion of desthiobiotin is not complete with increasing amounts of desthiobiotin added, even with the longer incubation period. Apparently only sufficient amounts of desthiobiotin are converted to supply the needs of the growing cells. This is also borne out by our finding that resting yeast did not convert any measurable amount of desthiobiotin to biotin. Increased concentrations of other components of the growth medium did not affect the conversion. The use of such a biological synthesis of biotin, from the relatively easily synthesized desthiobiotin, on a preparatory scale might be feasible with micro-organisms which could convert larger amounts of desthiobiotin to biotin.

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THE ANTI-BIOTIN EFFECT OF DESTHIOBIOTIN[1]

ACCORDING to Melville, Dittmer, Brown and du Vigneaud[2] *Lactobacillus casei* does not grow when desthiobiotin replaces the biotin of the medium, whereas *Saccharomyces cerevisiae* strain 139 grows readily. Through the courtesy of Dr. R. T. Major, of Merck and Company, the writers secured a sample of desthiobiotin and by using 45 biotin-requiring organisms confirmed and extended the findings of Melville *et al.* The results show that the biological effect of desthiobiotin could be classified into the following four groups according to the responses of the individual organisms:

1. *Desthiobiotin replaced biotin* for 25 strains of *Saccharomyces cerevisiae,* for *Saccharomyces chaudati, S. macedoniensis, Endomycopsis fibuliger, Debaryomyces matruchoti v. subglobosus, Mycoderma valida, Mycotorula lactis, Schizosaccharomyces pombe, Torula lactosa, Zygosaccharomyces marxianus, Zygosaccharomyces lactis, Neurospora crassa, N. sitophila, Ceratostomella ips 438, C. Montium* and *Leuconostoc mesenteroides.*

2. *Desthiobiotin did not replace biotin* for *Ceratostomella pini* 416, *Sordaria fimicola, Lactobacillus casei, L. arabinosus* and *Rhizobium trifolii* 205.

3. *Desthiobiotin did not act as anti-biotin* in the presence of an exogenous supply of biotin for *Lactobacillus arabinosus* and *Rhizobium trifolii.* These were not inhibited by 1,000 micrograms of desthiobiotin and 0.025 microgram of biotin per liter; in fact, *L. arabinosus* showed nearly a threefold increase in growth over the controls, and a still greater growth when desthiobiotin was augmented to 4,000 micrograms per liter. This stimulation may be ascribed to one of the following two causes: either this organism is able to utilize a certain amount of desthiobiotin in the presence of biotin, or else the sample of desthiobiotin at our disposal carried biotin as impurity. However, it is doubtful if there was enough biotin to support so much growth, otherwise why did this organism fail to grow when no biotin was added to desthiobiotin?

4 *Desthiobiotin acted as anti-biotin* for *Sordaria fimicola, Ceratostomella pini* 416 and *Lactobacillus casei.* The first one of these three organisms may be considered a borderline case: it averaged 45 milligrams of dry mycelium per flask in the presence of 0.1 microgram of biotin per liter; when 250 micrograms of desthiobiotin was added to this amount of biotin, the yield went up to 83 milligrams, but when desthiobiotin was increased to 4,000 micrograms per liter and the amount of biotin remained the same, the yield dropped back to 44 milligrams per flask. *Ceratostomella pini* 416 showed a more clear-cut effect of anti-biotin action. It averaged 34 milligrams of dry mycelium per flask in the presence of 0.25 microgram of biotin per liter; upon the addition of 2 micrograms of desthiobiotin per liter, the yield increased to 50 milligrams per flask. But when desthiobiotin was increased to 1,000 micrograms per liter, the yield dropped to 12 milligrams per flask.

Table 1 gives the responses of *Lactobacillus casei* in detail.

Failure of *Lactobacillus casei* to grow whenever

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 326.

[2] D. B. Melville, K. Dittmer, G. B. Brown and V. du Vigneaud, SCIENCE, 98: 497, 1943.