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.

TABLE 1

THE GROWTH OF LACTOBACILLUS CASEI IN THE PRESENCE OF VARYING AMOUNTS OF BIOTIN, DESTHIOBIOTIN, AND COMBINATIONS OF THE TWO, AFTER 72 HOURS AT 37° C.

Micrograms biotin per liter	Photometer readings	Micrograms desthiobiotin per liter	Photometer readings	0.025 micrograms biotin per liter, varying amounts desthiobiotin	Photometer readings	2,500 micrograms desthiobiotin per liter and varying amounts of biotin	Photometer readings
0.0	4.5	0.0	4.5	0.0	19.0	0.0	5.0
0.015625	15.0	0.015625	11.0	7.181	22.0	0.0125	3.0
0.03125	22.0	0.03125	6.0	15.625	20.0	0.025	3.0
0.0625	34.0	0.0625	7.5	31.25	19.0	0.05	6.0
0.125	53.0	0.125	7.5	62.5	18.0	0.1	8.0
0.25	64.0	0.25	7.5	125.0	14.0	0.2	22.0
				250.0	11.0	0.4	56.0
				500.0	13.0	0.8	60.0
				1000.0	9.0	1.6	62.0
				2000.0	5.0		
2,500.0	70.0	2,500.0	4.0	4000.0	4.0		

desthiobiotin was added to the biotin can not be ascribed to the effect of the high concentration of this substance in the medium because the organism made an excellent growth in the presence of 2,500 micrograms of biotin. An examination of the last two columns of Table 1 furnishes more conclusive evidence of the anti-biotin effect of desthiobiotin. While smaller amounts of biotin failed to overcome the blocking effect of desthiobiotin within the time limit of the experiment, larger quantities readily neutralized the anti-biotin effect of this substance. Even after an incubation of 24 hours, 0.4 microgram of biotin per liter effectively overcame the effect of 2,500 micrograms of desthiobiotin.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID METHOD FOR MAKING PERMANENT MOUNTS OF MOSQUITO LARVAE

MOSQUITO larvae as well as other soft-bodied insects are frequently mounted on glass slides for taxonomic study and permanent safekeeping. There are several common mounting methods, plus numerous variations now being used by mosquito taxonomists. Rather serious disadvantages, however, are encountered in the use of three of the most commonly used techniques.

Canada balsam is an old and excellent mounting medium, but the generally accepted technique of dehydration in alcohols and clearing in xylol frequently results in a collapsed, brittle specimen with many lost and broken hairs. In addition the use of this technique requires a great deal of time, since the clearing process is usually quite lengthy.

Euparal is another resin frequently used, but it is now almost impossible to obtain and if obtainable is quite expensive. To secure best results with the use of this material, specimens should be placed in ethylene glycol mono-ethyl ether (Cellosolve solvent) before being mounted in euparal.

Many workers advocate mounting in an aqueous medium such as Berlese's chloral gum solution or one of its modifications. By the use of this substance specimens may be mounted directly from water after being killed. This is an excellent temporary medium, but it should not be used for permanent mounts because it evaporates badly, hardens very slowly, and even if the cover slip is ringed it will frequently evaporate and ruin the slide. This medium also tends to discolor after a period of a few years.

A technique has been worked out at this laboratory which eliminates many of the above disadvantages and results in a permanent preparation. The procedure is as follows: larvae are killed in hot water and then placed in 70 to 75 per cent. ethyl alcohol for 10 to 15 minutes. This time may be shortened somewhat if the venter is pierced in several places with a minuten nadeln or similar fine pointed needle. The specimen is next placed in 95 per cent. alcohol for 3 to 5 minutes and from there is dropped into absolute alcohol for about 5 seconds. It is then placed in creosote U.S.P. until the specimen has cleared sufficiently. In the case of a very delicate specimen the creosote should be diluted with equal parts of absolute alcohol before being placed in undiluted creosote. The time the specimen should remain in creosote will vary but is generally only a matter of a few minutes. Several larvae may be placed in the creosote at one time and the clearest ones removed first. The specimen is finally placed on a clean glass slide, excess creosote removed, but care should be used not to touch the larva. It is then covered with Canada balsam, oriented, and the cover slip applied. If the tip of the abdomen is severed while in the balsam, the slide should be held for several days to permit the balsam to harden. The cover slip can then be applied. This prevents the severed portion from drifting and makes a more presentable mount.

A wide-mouth medicine dropper or a small curved spatula should be used to transfer the specimens and care should be used to handle them as little as possible.

This procedure is quite rapid, the ingredients are readily available, the specimens do not collapse or harden and lastly the preparation is a permanent one.

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