First, much smaller amounts of the hypoprothrombinemic agent were used; and second, much larger doses of synthetic vitamin K were administered. That these were the significant differences was shown further by repeating the experiment in Case 3 using the same dosage of synthetic vitamin K and a larger dose (300 mg) of Dicumarol. Prolongation of the prothrombin time was not prevented at these respective levels.

It appears that the Dicumarol-induced hypoprothrombinemia involves a mechanism separate from that of a prothrombin deficiency consequent to a nutritional (vitamin K) lack. This is suggested by the fact that the doses ordinarily adequate for therapeutic effect do not detectably alter the prolonged prothrombin time caused by the anticoagulant although the extent of prothrombin time prolongation might be comparable.

If a quantitative relationship can be established between the activity of Dicumarol and of vitamin K it might be utilized as a means of biologically standardizing preparations of compounds exhibiting vitamin K properties. Work is now in progress to determine this.

Our findings are in accord with those of Link and his associates.[1,2]

Conclusion. In man, as has been demonstrated in animals, synthetic vitamin K administered in relatively large amounts has been found to counteract or neutralize the hypoprothrombinemia induced by a smallest single effectual dose of Dicumarol.

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Miss Frances Kaufman gave technical assistance.

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Modification of the Yeast-Growth Assay Method for Biotin.

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We wish to report a modification of the yeast-growth method of Snell *et al.*[1] for the assay of biotin in tissues and body fluids. This alteration was considered desirable since the existing method was found to be useful over a very limited range of biotin concentrations. Moreover, we encountered many instances of non-specific stimulation of yeast growth, particularly by human and canine urine samples. Our experience suggested that the medium previously employed was somewhat deficient for maximal growth of *S. cerevisiae.*

Mitchell and Williams[2] have demonstrated that certain amino-acids as well as a casein digest greatly enhance the growth of several strains of *S. cerevisiae* in a synthetic medium. These findings suggest that the addition of a biotin-free casein digest to the medium of Snell *et al.* may afford more nearly maximal growth and eliminate the occurrence of non-specific stimulation. We have accordingly developed a new medium containing an optimal amount of biotin-free casein hydrolysate.

Since our performance of the assay differs in many details from that of the earlier workers it is advisable to describe our procedure in some detail.

Method. The medium requires the following ingredients of highest purity:

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

[2] Mitchell, H. K., and Williams, R. J., *Biochem. J.*, 1940, **34**, 1532.

Sucrose (Reagent)	34	g
$(NH_4)_2 \cdot SO_4$	5	"
$KH_2 \cdot PO_4$	3.4	"
d-l aspartic acid	170	mg
$MgSO_4$	.45	g
$CaCl_2$	.45	"
Mineral mixture (*cf.* Snell[1])	.18	mg equivalent
Inositol	9	"
10% biotin-free casein hydrolysate	1	g equivalent (pH 3.0)
Thiamine	1	mg
Pyridoxine	1	"
Calcium pantothenate	.5	"

Add distilled water to make 1 liter. The pH of this preparation without adjustment is usually between 3.8 and 4.0.

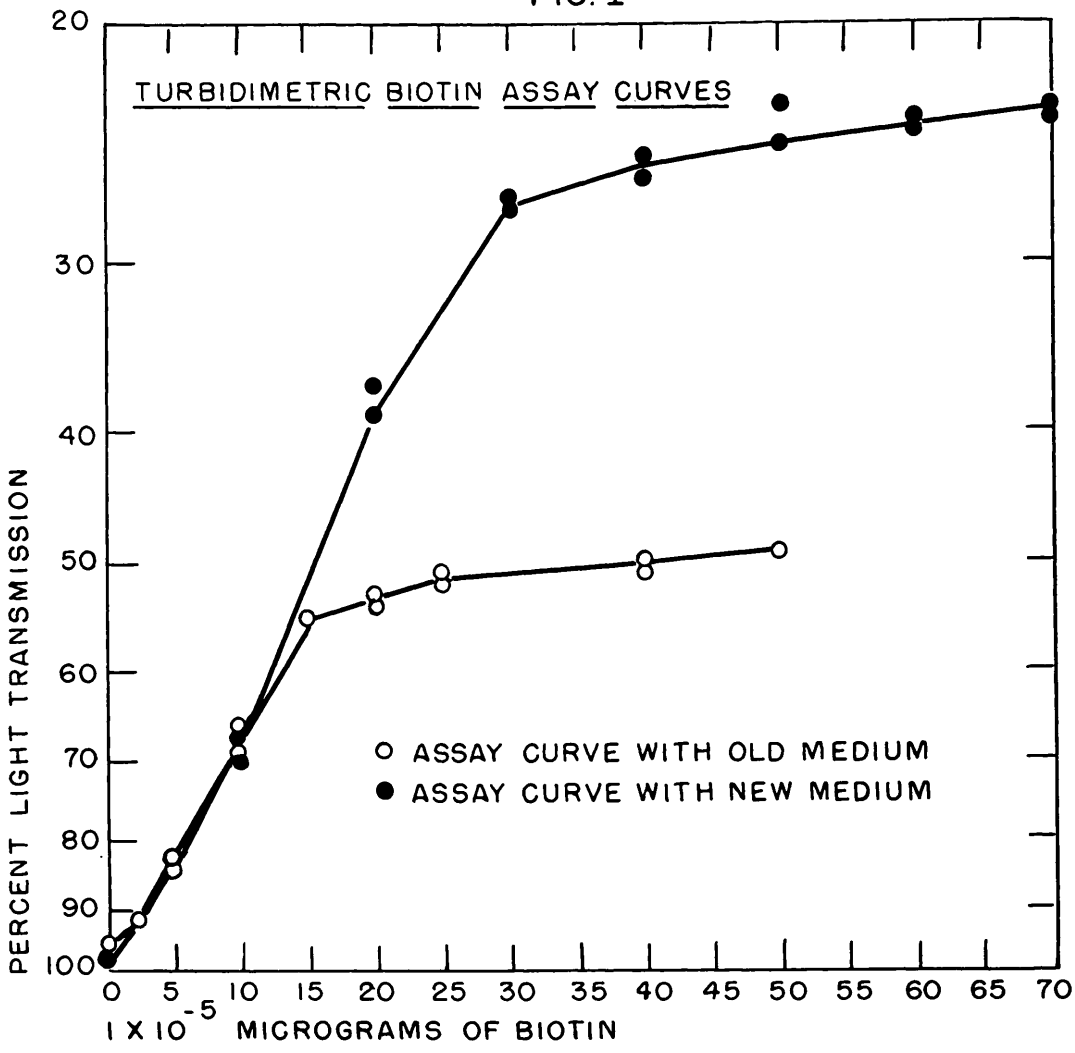
These materials are added successively. Gentle warming may be required to dissolve completely. The medium may be stored in the refrigerator for at least 3 weeks without deterioration.

The test is carried out in previously calibrated photometer tubes measuring 18 cm x 2 cm. In each culture tube 5 cc of the above medium are added to variable portions of the test material and the final volume in all tubes is adjusted to 10 cc with distilled H_2O.

A stock culture of *Sacharomyces cerevisiae* (No. 139)* is carried at room temperature

*Obtained through the courtesy of Doctor C. N. Frey, Fleischmann Yeast Co. Laboratories.

FIG. I



on Sabboraud's agar, transfers being made at least every 3 days. The test inoculum is prepared from a 24-hr culture grown at 37° in test medium to which has been added a maximally effective quantity of biotin. This culture is washed twice with normal saline and its final concentration adjusted turbidimetrically so that the .05 cc used for each test culture will contain approximately .05 mg of suspended yeast.

The test cultures are incubated without plugs for 18 to 22 hr at 37°C in a forced-draught incubator.

Assay values are determined turbidimetrically in relation to a standard curve obtained for each run in duplicate with crystalline biotin dilutions ranging in content from 1 x 10^{-4} to 5 x 10^{-4} μg per culture.

Samples may be prepared either by hot water or acid extraction, depending on the nature of the material to be tested. Only water-clear preparations may be employed. We have used this test almost exclusively for canine and human urine samples and have found direct dilution of the urine to yield satisfactory results.

Results and discussion. Fig. 1 presents a characteristic assay curve. For purpose of comparison, this figure also includes a characteristic curve obtained with the medium of Snell *et al.*[1] Some daily variation of absolute values is encountered, but the character of the curve is remarkably constant. The test is most practicable in the range from 1 x 10^{-4} through 3 x 10^{-4} μg. As the biotin concentration is increased sensitivity is rapidly lost. Hence the growth obtained with 5 x 10^{-4} μg of biotin is only slightly changed by further increasing the biotin concentration as much as 5-fold. Recovery tests range between 90 and 110% and successive dilutions of an unknown material check within 5%.

We have found that the following additional factors added singly or in combination to the test medium do not affect the biotin values obtained: Pimelic acid, nicotinic acid, riboflavin, adenine, guanine and uracil, choline and ethanolamine.

Considerable care must be taken to keep the age and preparation of the inoculum quite constant. We have found that variations in this regard will alter the results from day to day.

The advantages of the modified test are (1) an increased specificity due to the elimination of the non-specific stimulation which may result from the amino-acid content of test materials. (2) A wider working range which facilitates biotin determinations in completely unknown materials.†

Summary. The addition of a biotin-free casein hydrolysate to the test medium increases both the specificity and the range of biotin determinations by the yeast growth method.

† Assays obtained for several natural materials will be presented elsewhere.