Since these results disagreed with those previously reported,[1] a third group of 15 deficient animals was given 1000 units of D† subcutaneously and the prothrombin time was determined 3-5 days and 1 week later (Table I, Group 4). In this group, the mean prothrombin values before and after treatment show a questionably significant difference. If the extreme variates are omitted in calculating the mean in column 6, the differences become insignificant. Individually, 7 of the 15 were definitely improved, 4 were more deficient after receiving the vitamin than before, 3 were essentially unchanged, and 1 died before a second determination was made.

Comment. These results fail to show that vitamin D has any definitely beneficial effect on the prothrombin deficiency produced by dietary means in the rat. Although certain individual cases showed improvement, the average results of the group show no striking effect. This does not agree with the interpretation of results previously reported.[1] The reason for this difference is not immediately apparent; however, the range of variation in the deficient rats is so great that it is difficult to evaluate any procedure unless a large series is used. This may explain the difference. The present results agree with those of Smith and coworkers[2] and of Greaves.[3] However, they fail to explain the beneficial effects of viosterol in clinical jaundice.

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Use of Avidin in Studies on Biotin Requirement of Microorganisms.*

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The recent discovery of anti-vitamins suggests a new principle in nutritional pathology, with far-reaching applications in the study of bacterial metabolism. Avidin,[1, 2] the first anti-vitamin to be iso-

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† Meade-Johnson Special Concentrate.

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1 Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1940, **136**, 801.

2 Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1941, **140**, 535.

lated,[3] belongs to a group[4] of nutritionally important substances which may be as potentially significant as the vitamins themselves, in that they act as governors for the biological processes which are catalyzed by the vitamins. The anti-vitamin, avidin (anti-biotin[5]), is a protein constituent of unheated egg white, which possesses the property of combining readily and specifically with biotin to form a stable complex—a complex in which the biotin is rendered unavailable for utilization by yeast cells,[2] by animals[6, 7] and, as we propose to demonstrate, by certain bacteria.

This report constitutes a study of the effect of avidin on various bacterial species. We anticipated a correlation between the biotin requirement of an organism and its subsequent growth inhibition by avidin. If such a correlation were found to exist, it would obviously follow that the biotin requirement of a given organism might be predicted by its reaction to avidin. It is necessary to point out that our tests were only qualitative and, due to circumstances beyond our control, fairly crude. Two properties peculiar to avidin make its sterilization difficult: (1) it is heat labile; *i. e.*, solutions are inactivated rapidly by steaming or autoclaving, and (2) it is, for the most part, retained by bacterial filters.† It was necessary, therefore, to employ fresh sterile egg white for many experiments, although a number have been carried out with purified avidin preparations yielding identical results.

The general method of procedure was as follows: Serial dilutions of avidin concentrate or egg white were added to duplicate sets of tubes of synthetic or infusion medium containing known amounts of biotin. The avidin in one set of dilutions was inactivated by autoclaving for 15 minutes while the other set was not heated. Both series of tubes were then inoculated with the test organism. The effect of avidin on the test organism, as indicated by the degree of growth inhibition produced, was determined by a comparison of growth in the heated and unheated avidin dilutions. The addition of biotin to those dilutions of avidin which brought about complete inhibition of growth resulted in growth of the organism upon reincubation.

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3 Pennington, D., Snell, E. E., and Eakin, R. E., *J. Am. Chem. Soc.*, 1942, **64**, 469.

4 Woolley, D. W., *J. Biol. Chem.*, 1941, **141**, 997.

5 Woolley, D. W., and Longsworth, L. G., *J. Biol. Chem.*, 1942, **142**, 285.

6 Gyorgy, P., Rose, C. S., Eakin, R. E., Snell, E. E., and Williams, R. J., *Science*, 1941, **93**, 477.

7 Gyorgy, P., and Rose, C. S., *Science*, 1941, **94**, 261.

† Unpublished data.

It was our practice to add sufficient avidin to any given culture to neutralize the biotin present and still insure an excess of avidin. It is important to emphasize that this investigation was limited to a study of the effect of avidin in such concentrations. That higher concentrations of avidin would yield entirely different results is, of course, a possibility. Serial dilutions of avidin were carried out in such a manner that complete inhibition, partial inhibition and complete growth of a susceptible organism were obtained.

Early in the investigation, it occurred to us that certain effects which we observed might be due to lysozyme present in egg white or avidin concentrate. That this was not the case was shown by a number of experiments in which it was found (1) that purified, highly potent lysozyme preparations had no effect on organisms which were markedly susceptible to avidin, *viz.*, the saccharomyces, the clostridia, and the lactobacilli, (2) that our avidin preparations had no effect on the usual lysozyme-assay with *Micrococcus leisodykticus.* In general, we found lysozyme and avidin to have separate, definite and apparently unrelated biological activities. In all experiments in which egg white or avidin inhibited growth of an organism, our final criterion of the specificity of such inhibition was its reversal by crystalline biotin.

Lactobacillus arabinosus 17-5,[8] *Streptococcus lactis* R,[9] *Staphylococcus aureus* X-3,[10] *Clostridium butylicum,*[11] and *Clostridium acetobutylicum*[12] have been shown to require biotin for growth. As will be observed in Table I, growth of the above organisms was, in every case, completely inhibited by avidin, which inhibition could, in turn, be reversed by the addition of biotin. On the other hand, the growth of such organisms as *Eberthella typhi, Proteus vulgaris, Escherichia coli, Aerobacter aerogenes,* and *Serratia marcescens* which grow in simple media and synthesize biotin[13] was unaffected by avidin. Since avidin inhibits the growth of the above organisms which require biotin, and has no effect upon those which do not require biotin, this logical correlation suggests the use of avidin as an agent in the determination of the biotin requirement of microörganisms.

On the basis of the evidence presented we feel justified in predicting that those organisms given in Table I which are inhibited by

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8 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

9 Snell, E. E., and Mitchell, H. K., *Proc. Nat. Acad. Sc.*, 1941, **27**, 1.

10 Porter, J. R., and Pelczar, M. J., *J. Bact.*, 1941, **41**, 173.

11 Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

12 Oxford, A. E., Lampen, J. O., and Peterson, W. H., *Biochem. J.*, 1940, **34**, 1588.

13 Landy, M., and Dicken, D. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 449.

TABLE I.
Effect of Avidin on the Growth of Bacteria.

Organism	Medium	Incubation °C	Hrs	Degree of Inhibition	Biotin Reversal of Inhibition
Lactobacillus casei ε	Synthetic[14]	37	72	Complete	+
Lactobacillus arabinosus (17-5)	" 8	30	48	"	+
Lactobacillus acidophilus	" 14	37	72	"	+
Streptococcus lactis (R)	" 9	30	72	"	+
Staphylococcus aureus (X-3)	Heart Infusion Broth	37	48	"	+
Staphylococcus aureus (Y)	" " "	"	"	"	+
Diplococcus pneumoniæ (Mellon II)	" " "	"	"	"	+
Corynebacterium diphtheriæ (Park 8)	" " "	"	"	None	
Clostridium welchii (SR-12)	Thioglycollate	37	72	Complete	+
Clostridium welchii (Spray)	"	"	"	"	+
Clostridium botulinum (201-B)	"	"	"	"	+
Clostridium histolyticum (Spray)	"	"	"	"	+
Clostridium chauvei	"	"	"	"	+
Clostridium sporogenes	"	"	"	"	+
Clostridium butylicum (2-D)	Synthetic[11]	"	"	"	+
Clostridium acetobutylicum (A-211)	" 15	"	"	"	+
Klebsiella pneumoniæ	Heart Infusion Broth	37	48	None	
Eberthella typhi	" " "	"	"	"	
Salmonella enteritidis	" " "	"	"	"	
Serratia marcescens	" " "	"	"	"	
Bacillus subtilis	" " "	"	"	"	
Bacillus brevis	" " "	"	"	Complete	+
Bacillus anthracis	" " "	"	"	None	
Escherichia coli	" " "	"	"	"	
Alkaligenes fœcalis	" " "	"	"	"	
Aerobacter aerogenes	" " "	"	"	"	
Proteus vulgaris	" " "	"	"	"	
Proteus morganii	" " "	"	"	"	
Shigella paradysenteriæ	" " "	"	"	"	
Vibrio comma	" " "	"	"	"	

avidin (inhibition reversed by biotin) require biotin as a growth-essential. In partial confirmation of this prediction we have since demonstrated that *Lactobacillus casei* ε can be cultured in a medium of essentially-known composition only when biotin is supplied.[14]

Summary. A study was made of the effect of avidin on the growth of a number of bacterial species. A correlation was found between the biotin requirement of an organism and its growth-inhibition by avidin. In general, those organisms which have been found to require biotin as a growth-essential were inhibited by avidin, while those organisms which were found to synthesize biotin were unaffected. On the basis of this growth-inhibition by avidin, it is predicted that *Cl. chauvei, Cl. botulinum, Cl. histolyticum, Cl. sporogenes, D. pneumoniae, L. casei* ε*, L. acidophilus,* and *B. brevis,* whose biotin requirements have not yet been established require biotin as a growth-essential.

[14] Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.*, 1942, in press.

[15] Lampen, J. O., and Peterson, W. H., *J. Am. Chem. Soc.*, 1941, **63**, 2283.