is used alone in female mice presents the interesting possibility of a specific relationship between 8-azaguanine and flavotin. Studies are in progress to clarify the suspected relationship. It is conceivable, for example, that flavotin prevents the degradation of 8-azaguanine into a molecule lacking carcinostatic activity. Folic acid was found to potentiate 8-azaguanine's carcinostatic activity against the 755 tumor in this manner(6,15).

The positive results obtained with the riboflavin analog, flavotin, together with the demonstration that flavotin acts as a riboflavin antagonist in this tumor-host system, furnish additional support for the hypothesis presented at the beginning of this report.

Summary. Flavotin, a riboflavin analog, had no effect on the growth of a transplantable mammary carcinoma. However, the combination of flavotin and 8-azaguanine was carcinostatic, but only in female mice. The sex difference in response is not understood. Reversal experiments utilizing riboflavin-5-phosphate reveal the mechanism of increased carcinostatic activity afforded by the drug combination to be due to interference with riboflavin metabolism.

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1. Williams, R. J., *A.A.A.S. Research Conference on Cancer,* Science Press Printing Co., Lancaster, Pa., P. 259, 1945.

2. Greenstein, J. P., *Biochemistry of Cancer,* New York, Academic Press, Inc., 1947.

3. Ballantyne, R. M., and McHenry, E. W., *Cancer Research,* 1949, v9, 689.

4. Olson, R. E., *Cancer Research,* 1951, vII, 571.

5. Higgins, H., Miller, J. A., Price, J. M., and Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v75 462.

6. Shapiro, D. M., and Gellhorn, A., *Cancer Research,* 1941, vII, 35.

7. Skipper, H. E., *Cancer,* 1949, v2, 475.

8. Goldin, A., Greenspan, E. M., and Schoenbach, E. B., *Cancer,* 1952, v5, 153.

9. Stoerk, H. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 798.

10. Gellhorn, A., Engelman, M., Shapiro, D. M., Graff, S., and Gillespie, H., *Cancer Research,* 1950, v10, 170.

11. Taylor, A., and Carmichael, N., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 544.

12. Goldin, A., Greenspan, E. M., Goldberg, B., and Schoenbach, E. B., *Cancer,* 1950, v3, 849.

13. Heilman, F. R., and Kendall, E. C., *Endocrin.,* 1944, v34, 416.

14. Okey, R., Penchary, R., and Lepkovsky, S., *Am. J. Phys.,* 1950, v161, 1.

15. Kream, J., unpublished data.

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Surface Activities of Biotin and Biocytin. (19834)

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Evidence has been presented by numerous workers that biotin functions in a variety of systems concerned with a) decarboxylation of, or carbon dioxide fixation in, acetoacetate, aspartate, malate, oxalacetate or succinate, b) aspartic acid, serine, and threonine deamination, c) succinic acid dehydrogenation, and d) the synthesis of adenine, arginine, aspartic acid, bone carbonate, citric acid, citrulline, guanine, and unsaturated fatty acids. One explanation for the apparent multiplicity of functions of biotin is that the compound is involved in only one primary system whose failure in biotin deficiency manifests itself by a variety of secondary metabolic derangements. Williams and Williams have shown (1) that biotin is highly "surface active" as inferred from its remarkable effect in eliminating the diffusion current maximum encountered during the polarographic reduction of Cu^{++} at the dropping mercury electrode. If biotin is highly "surface active" and does indeed function in this way, for example, as the prosthetic group of a larger surface active molecule, or as a factor regulating cell permeability, then it is apparent that biotin-

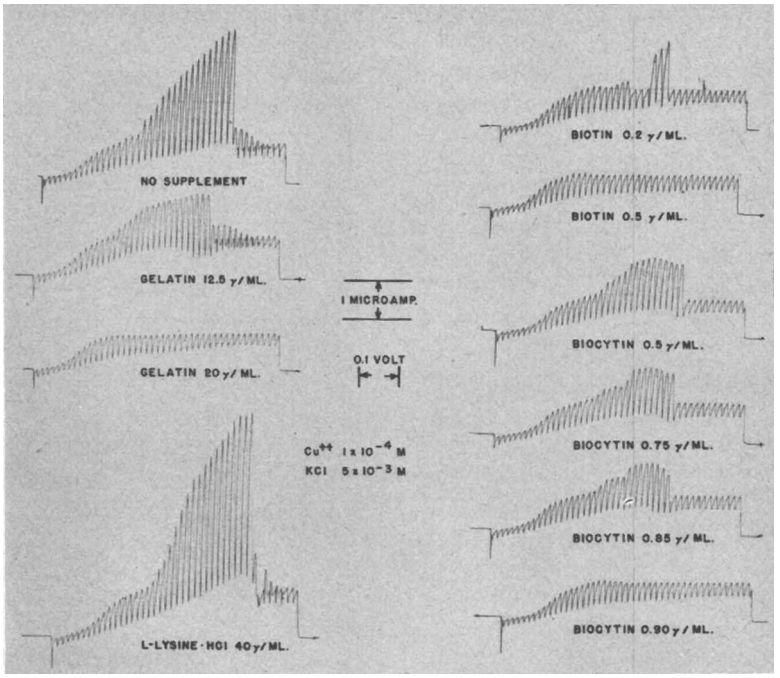

FIG. 1. The "surface activities" of biotin, biocytin, lysine, and gelatin as determined by concentrations required to eliminate the diffusion current maximum encountered during polarographic reduction of Cu^{++} at the dropping mercury electrode.

deficient cells would be expected to show a variety of impaired functions.

It is the purpose of this paper to present data a) confirming the activity of biotin in the system of Williams and Williams, and b) showing that biocytin, a "combined form" of biotin, recently identified as ε-N-biotinyl-L-lysine(2), also shows activity equal to that expected from the biotin content of the molecule.

Procedure. The data obtained were accumulated with a Leeds and Northrup Electrochemograph, Type E. The procedures followed in measuring the surface activities of the compounds studied were essentially those described by Williams and Williams and consisted of a determination of the minimal concentration of each compound required to just eliminate the diffusion current maximum encountered during the polarographic reduction of Cu^{++} at the dropping mercury electrode. After several trial runs, an aqueous solution of copper sulfate containing a copper ion concentration of 1.002×10^{-4} M was selected. As supporting electrolyte, to conduct the electrons through the solution, a concentration of 5×10^{-3} M KCl was found to be satisfactory. The range of potentials used in obtaining the polarograms was from 0 to –0.6 volt relative to the standard saturated calomel reference electrode. By trial and error, concentrations of biotin, biocytin, and gelatin were established, which, when each was dissolved in the KCl-$CuSO_4$ solution just described, would eliminate the potential maximum.

Results and discussion. The concentrations of the various compounds studied that were just sufficient to eliminate the diffusion current maximum under the conditions described (see Fig. 1) may be summarized as follows:

Biotin	.5 γ/ml
Biocytin	.9 γ/ml
Gelatin	20 γ/ml

These results indicate that biotin is the compound with the greatest activity studied. Biocytin is about 60% as active as biotin. This value agrees quite well with the biotin content of biocytin. L-Lysine is inactive in the present test.

An evaluation of the results summarized

above and an indication of the extent to which the effect observed is specific for biotin cannot be answered at this time because of paucity of similar data on a variety of biological compounds. The effects obtained would appear, however, to be of sufficient significance to warrant continued examination of the hypothesis that biotin may function through some "surface" mechanism.

Summary. The "surface activity" of biotin as measured by its effect in eliminating the diffusion current maximum encountered during the polarographic reduction of Cu^{++} at the dropping mercury electrode is confirmed. Biocytin (ϵ-N-biotinyl-L-lysine) is as active on an equimolar basis.

1. Williams, V. R., and Williams, H. B., *J. Biol. Chem.*, 1949, v177, 745.

2. Wright, L. D., Cresson, E. L., Skeggs, H. R., Peck, R. L., Wolf, D. E., Wood, T. R., Valiant, J., and Folkers, K., *Science*, 1951, v114, 635.

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Inhibitory Effect of Cobaltous Ions on Multiplication of Influenza Virus.* (19835)

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Interest in the action of cobalt on viruses was aroused by Burke's demonstration of the inhibition of growth and respiration of bacteria and animal tissues by cobaltous ions(1). It was subsequently found that cobalt salts inhibit the multiplication of influenza virus in embryonated eggs(2). Therapeutic trials conducted in our laboratory have yielded disappointing results, *i.e.* maximally tolerated doses of cobalt chloride administered orally and intranasally were of no value in the treatment of experimental influenzal infection in mice. Despite this fact, a study of the inhibitory action of cobalt was continued with the thought that some indirect information on the requirements for viral growth might be obtained. This report presents the results of further experiments dealing with the inhibitory action of cobalt on the viruses of influenza in embryonated eggs and the mechanisms of growth inhibition.

Methods. Three strains of influenza virus were used: namely, influenza A, PR8 and FM1, and influenza B, Lee. Groups of six to twelve 11-day-old chick embryos were inoculated with 0.1 ml of suitably diluted stock virus usually containing 100-500 ID_{50} by the allantoic route and returned to the 35 degree incubator. Inoculated embryos for each experimental series were removed from the incubator at the desired intervals and 0.4 ml aliquots of allantoic fluid were withdrawn from each of the eggs. The fluids were placed at 4°C as they were collected and maintained at that temperature until all eggs in a group were tapped, at which time they were assayed for their content of virus by hemagglutination tests employing the pattern endpoint (3) and, in some instances, by infectivity titration *in ovo*(4).

Results. When eggs were treated with 0.5 mg of cobaltous chloride or acetate at the time of virus inoculation, considerable inhibition of growth of PR8, FM1 and Lee viruses resulted. Results with the three strains of virus were essentially similar; in the experiments described the PR8 strain of influenza A was employed. The inhibition was most pronounced during the phase of rapid virus multiplication and persisted for a period of 32 hours after virus inoculation. Thus, in one series of 98 eggs treated with cobaltous chloride, only 11 failed to show at least a fourfold reduction in hemagglutination titer. Cobalt exerted a similar degree of inhibition when introduced at intervals varying from

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