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.

Received September 3, 1952. P.S.E.B.M., 1952, v81.

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

J. R. A. SCHMIDT AND A. F. RASMUSSEN, Jr.

From the Department of Medical Microbiology, University of Wisconsin Medical School, Madison.

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

* Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

one hour before to 18 hours after inoculation of the virus; however, very little inhibition resulted when 24 hours elapsed between the inoculation of the virus and cobalt. The decrease in hemagglutinating titer of allantoic fluids was accompanied by a corresponding drop in infectivity. When eggs were treated with 0.5 mg of cobaltous chloride at the time of inoculation with virus, ID_{50} values of treated fluids 24 hours later were an average of three logs lower than those of the control fluids.

In order to determine the efficacy of the drug on larger and smaller inocula (100-500 ID_{50} having been used in previous experiments) the inoculum of virus was varied from 10 to 500000 ID_{50}. The results of this experiment indicated that the material was less effective when 1000 ID_{50} or more of virus were used. The agglutinating titers of these fluids showed only a 2-fold difference from those of the control eggs, as compared with the 5- to 10-fold difference evidenced in eggs receiving smaller inocula.

Treatment of infected eggs with 3 doses of 0.2 mg of cobaltous chloride at 0, 12 and 24 hours after inoculation resulted in a more prolonged inhibition than that observed with a single large dose. After 36 hours of incubation, there was still a 5-fold difference between the titers of the treated and control fluids, whereas the use of a single large dose resulted in only a 2-fold depression in hemagglutinating titer at this interval.

In order to determine whether cobalt salts had a direct inactivating effect on the virus, mixtures of the virus and cobaltous chloride were made, incubated *in vitro* for 2 to 8 hours, and tested for infectivity in embryonated eggs. Results of these experiments indicated that cobaltous ion, in concentrations approximating those existing in the chorioallantoic fluids of treated eggs, had very little virucidal effect *in vitro*.

Since histidine and cobalt combine (chelate) to form a cobalto-histidine complex(5) the action of histidine on the multiplication of influenza virus in eggs treated with cobalt salts was investigated. From the data presented in Table I, it is evident that the inhibitory effect on virus proliferation was reversed by the introduction of histidine into the system, the degree of reversal being proportional to the amount of histidine employed. Complete reversal was attained with a ratio of 2 mols of histidine per mol of cobaltous ion, the ratio existing in the cobalto-histidine complex. Cysteine, which chelates with cobaltous ion in a ratio of 3 to 1, respectively, was likewise active in overcoming the cobalt-induced inhibition when present in the ratio indicated. Sodium thioglycolate also forms a complex with cobalt but was much less effective in preventing the virustatic effect of cobalt, even in concentrations of 22 mols per mol of cobaltous ion.

TABLE I. Effect of Various Compounds on Multiplication of Virus in Cobalt-Treated Eggs.

Amino acids	mg	Yield of hemagglutinin (% of control) 24 hr
Alpha-alanine	4	19.6
Beta-alanine	4	12.3
Arginine	4	6.2
Aspartic acid	4	12.3
Cysteine	4	50
	8	96
Glutamic acid	4	6.2
Histidine	.5	28.5
	1	50
	2	57
	4	98.2
Phenylalanine	4	3.1
Tryptophan	4	12.3
Miscellaneous		
Sodium thioglycolate	5	38.5
	10	98
Imidazole	.5	13
Histamine	.5	12.3
Adenine	4	14.1
Thymine	4	16
Uracil	4	9.4

Inoculum: 100-500 ID_{50} of Influenza A (PR8) virus. Compounds listed were administered simultaneously with 0.5 mg $CoCl_2$ at time of virus inoculation.

In view of the observation by Broquist and Snell(6) that purine bases are converted to histidine in the embryonated egg, adenine, uracil and thymine were tested for their ability to counteract the cobalt effect. All proved to be inactive. The major components of the histidine molecule, imidazole and alpha-alanine, when tested individually and in combination, were also found to be devoid of activity.

Discussion. Knight has shown that highly purified suspensions of influenza virus contain

histidine(7). From this it could be argued that cobalt inhibits virus growth by depriving the virus of histidine, an essential nutrient. However, cysteine which has not been shown to be a constituent of influenza virus and sodium thioglycolate which should be inert nutritionally also counteract the virustatic action of cobalt. Both the imidazole group of histidine and the sulfhydryl (–SH) group of cysteine or thioglycollic acid with which cobaltous ions chelate are also important chemically active groups in larger protein and nucleic acid molecules. Recent work by Burney and Golub(8) has suggested that –SH groups are present in the elementary bodies of psittacosis. These authors have demonstrated that p-chloromercuribenzoic acid completely inactivates psittacosis virus and that glutathione or cysteine reactivates the virus. In analogous experiments Klein *et al.*(9) have restored influenza virus activity with BAL and thioglycolate following inactivation by mercuric chloride. The influenza virus-cobalt-cysteine and virus-cobalt-thioglycolate relationships described above may involve –SH groups as well as imidazole groups in compounds necessary for viral growth. The compounds might be constituents of the virus particle or cellular enzymes of the host. With a sufficient excess of either the –SH bearing compounds, cysteine and thioglycolate or of histidine with its imidazole group, cobaltous ions would be removed from complexes previously formed through both types of linkage; *i.e.* chelation between cobaltous ions and imidazole groups or between cobaltous ions and sulfhydryl groups.

Summary. The growth of the virus of influenza in embryonated eggs is inhibited by cobaltous ion. This inhibition is overcome by the subsequent addition of histidine, cysteine and sodium thioglycolate.

1. Burk, D., Schade, A. L., Hesselbach, M. L., and Fischer, C., *Fed. Proc.*, 1946, v5, 126.
2. Schmidt, J. R. A., and Rasmussen, A. F., Jr., *Bact. Proc.*, 1950, 70.
3. Salk, J. E., *J. Immunol.*, 1944, v49, 87.
4. Rasmussen, A. F., Jr., Stokes, J. C., and Smadel, J. E., *Am. J. Hyg.*, 1948, v47, 142.
5. Burk, D., Hearon, J., Caroline, L., and Schade, A. L., *Biol. Chem.*, 1947, v165, 723.
6. Broquist, H. P., and Snell, E. E., *Fed. Proc.*, 1949, v8, 188.
7. Knight, C. A., *J. Exp. Med.*, 1947, v86, 125.
8. Burney, T. E., and Golub, O. J., *J. Immunol*, 1948, v60, 213.
9. Klein, M., Brewer, J. H., Perez, F., and Day, B. J., *J. Immunol.*, 1948, v59, 135.

Received September 4, 1952. P.S.E.B.M., 1952, v81.

Indwelling Pulmonary Arterial and Venous Catheters in the Dog.* (19836)

R. C. STROUD,† K. R. STETSON, AND H. RAHN.

From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

In order to obtain pressure measurements or blood samples from the pulmonary vessels of unanesthetized animals over a long period of time, investigators have usually resorted to externalizing the blood vessels to the chest wall by various types of cannulas. This requires major surgery. The following method describes a relatively simple procedure for placing and securing polyethylene tubes in the pulmonary artery or vein without resort to major surgery. They are introduced through the external jugular vein and common carotid artery, respectively. Catheters in the pulmonary artery have been retained in dogs up to two months. Such animals seem to keep in perfect health.

Placing the catheter into the pulmonary artery. Polyethelene tubes having an inside diameter of 1.27 mm (Clay-Adams cat. No.

* This study was supported by the Air Research and Development Command, Wright Field.

† Present address: Dept. of Physiology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.