with regulation of blood potassium, may be considered debatable. However, it is possible that an influence upon regulation of potassium in the blood can be exercised by epinephrine secretion from the medulla, loss of which can be compensated for by the function of other mechanisms. It is conceivable that such a compensatory mechanism, or mechanisms, could be so affected by the profound metabolic disturbance which results from loss of cortical function that the ability of the organism to maintain a normal level of blood potassium is lost. We believe that the foregoing evidence, in support of the possibility that regulation of blood potassium may be accomplished by functional activity of the adrenal medulla, is at least as credible as that which attributes such a function to the cortex.

Summary. 1. The physiological role of the adrenal glands in relation to blood potassium, hitherto attributed to function of the cortex or one of its steroid components, can be ascribed to a function of epinephrine secretion from the medulla.

2. The level of potassium in the blood serum is diminished by intravenous administration of adrenalin in such amounts and rate of injection as correspond with the physiological rate of epinephrine secretion from the adrenal glands.

3. This effect is obtained in normal dogs and in animals with reduced or suppressed epinephrine secretion. The elevation of blood potassium, which occurs in adrenalectomized dogs, can be corrected by constant intravenous injection of physiological dosage of adrenalin.

4. Higher dosage may effect a rise in serum potassium. A moderate increase in dosage usually induces a preliminary rise followed by a more lasting reduction in the level of potassium. This can also result from too rapid injection of smaller doses.

5. Significant changes in sodium were not constant. The supposed dependence upon the adrenal cortex for maintenance of a physiological equilibrium between sodium and potassium is not supported by the results of our experiments.

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Effect of Cholesterol on Antigenicity of Streptolysin O.* (17613)

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The hemolysins contained in culture filtrates of hemolytic streptococci have provided abundant research material since their discovery by Marmorek(1). Despite initial controversy, the existence of 2 distinct varieties of hemolysin is now well established, Todd(2), Barnard and Todd(3), Herbert and Todd(4), Herbert and Todd(5). The oxygen labile streptolysin O has received much attention in the literature, and among other interesting properties is the neutralization of its hemolytic effects by suspensions of cholesterol in high dilution, Hewitt and Todd(6). Cohen and Schwachman(7) noted that pneu-

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1. Marmorek, A., *Ann. Inst. Pasteur*, 1895, v9, 593.

2. Todd, E. W., *J. Path. Bact.*, 1938, v47, 423.

3. Barnard, W. G., and Todd, E. W., *J. Path. Bact.*, 1940, v51, 423.

4. Herbert, D., and Todd, E. W., *Biochem. J.*, 1941, v35, 1124.

5. Herbert, D., and Todd, E. W., *Brit. J. Exp. Path.*, 1944, v25, 242.

6. Hewitt, L. F., and Todd, E. W., *J. Path. Bact.*, 1939, v49, 45.

mococcus hemolysin, whose properties are similar to those of streptolysin O, was also neutralized by cholesterol. More recently Fisher(8) has noted that cholesterol and other sterols inhibit the hemagglutinins of *Hemophilus pertussis*. Saponin and digitonin, both of which are actively hemolytic, are inactivated by suspensions of cholesterol and plasma, Ponder(9). Hewitt and Todd(6) also reported that the intraperitoneal injection of sufficient cholesterol protected mice against subsequent lethal intravenous doses of streptolysin O. Although their observations were confined to a few mouse protection tests, this finding suggested that there could be *in vivo* neutralization of streptolysin O by cholesterol. They recorded no data on the effect of cholesterol on the behavior of streptolysin O as an antigen. The experiments reported here were conducted to determine the effect of cholesterol on the antigenicity of streptolysin O.

Materials and methods. Streptolysin O. Actively hemolytic culture filtrates were obtained from strain Richards grown overnight in Todd-Hewitt broth(10). Purification of the filtrate was taken to stage two of the method described by Herbert and Todd(4) and then lyophilized. The material was standardized by titrating against a standard serum following the method given by Todd(2) in Appendix 1. The material used in the majority of the experiments contained one unit of streptolysin O in 0.8 mg.

Cholesterol suspensions. Suspensions were prepared by dissolving U.S.P. cholesterol in a small quantity of 95% ethyl alcohol, adding an equal volume of 5% sodium oleate and adding dropwise to hot saline with constant shaking. By this means sufficiently smooth suspensions were obtained. Dilutions of these cholesterol suspensions in the region of 10^{-6} to 10^{-7} effectively neutralized one unit of streptolysin O. Hemolysis which might be due to sodium oleate was diluted out at this concentration.

Standard Antistreptolysin O. The antistreptolysin used consisted of refined serum globulins from hyper-immunized horses, prepared by E. W. Todd, and employed by many investigators as an arbitrary standard for antistreptolysin titrations. The sample used contained 20,000 units per ml and 0.35% trikresol as preservative. All dilutions were made in 0.85% sodium chloride solution.

Experimental. Titrations were carried out by mixing 1.0 ml volumes of a descending series of tenfold dilutions of cholesterol with 0.5 ml volumes of streptolysin O containing one unit in 0.5 ml. Active streptolysin O solution was prepared in saline containing 0.2% sodium thioglycollate as a reducing agent. After allowing 15 minutes in the water bath at 37° for neutralization to take place, 0.5 ml of a 3% washed rabbit red cell suspension was added and incubation continued for one hour. From these experiments the dilution of cholesterol suspension required to neutralize one unit of streptolysin O was determined. Mixtures of streptolysin O and cholesterol were prepared, in which 10 times the required amount of cholesterol was added. These over-neutralized mixtures were incubated in the water bath at 37° for 15 minutes and then inoculated intravenously into 2 rabbits. At the same time a mixture was prepared containing the same amount of streptolysin O but 10 times the required amount of standard antistreptolysin O. This too was incubated in the water bath at 37° before intravenous inoculation into a second pair of rabbits. Finally, streptolysin O alone was given to another pair of rabbits after having been incubated in the same manner. All rabbits were males weighing approximately 2 kg. The volume of all the inocula was 4.0 ml. The cholesterol in the streptolysin O mixture gave excellent dispersion, probably due to the protein present.

The animals were bled prior to immunization, and one week following the last dose. Two courses of immunization injections were given to each of the 3 groups of animals. The first course consisted of 50 units of streptolysin O, 50 units of streptolysin O plus 0.5 mg of cholesterol, 50 units of streptolysin O plus 500 units of antistreptolysin O to the

7. Cohen, B., and Schwachman, H., *J. Bact.*, 1937, v33, 67.

8. Fisher, S., *Brit. J. Exp. Path.*, 1949, v30, 185.

9. Ponder, E., *J. Gen. Physiol.*, 1944, v28, 357.

10. Todd, E. W., and Hewitt, L. F., *J. Path. Bact.*, 1932, v35, 973.

TABLE I.
Antistreptolysin Titers in Immunized Rabbits.

	Streptolysin O alone		Streptolysin O + cholesterol		Streptolysin O + antistreptolysin O	
Rabbit	A_1	A_2	B_1	B_2	C_1	C_2
Before immunization	<1	<1	<1	<1	<1	<1
After first course	60	50	50	20	<1	<1
After second course	50	16	30	20	1	2

respective groups. After 4 days this dosage was doubled and after a further 4 days, 4 times the original dosage was given. After an 18 day rest period a second series of injections were given and bleedings taken as before. This consisted of 3 injections at 4 day intervals of the original doses. Antistreptolysin titres of all the sera were determined by the method outlined by Todd. All values are recorded with reference to the standard serum.

Results. Table I shows the antistreptolysin titres of the rabbits before and after each course of injections. It will be seen that the titres of antistreptolysin produced by the inoculation of streptolysin-cholesterol mixtures do not differ significantly from those produced by streptolysin alone. The inoculation of streptolysin-antistreptolysin mixtures produced no change in the antistreptolysin titre whatever after the first course of injections and very little after the second.

Discussion. The results indicate that the antigenicity of streptolysin O is not impaired when its hemolytic activity is inhibited by cholesterol. The explanation offered is that some easily dissociated streptolysin-cholesterol complex is formed which loses its ability to lyse red cells but still functions as an antigen. The chemical grouping by which cholesterol combines with streptolysin is a matter for further study although there is evidence which implicates certain regions on the cholesterol molecule(8). Hewitt and Todd point out that sulfhydryl groups added in the form of cysteine did not effect the neutralization of streptolysin O by cholesterol. We have found that oxidized, inactive streptolysin O is neutralized in the same way as the active reduced form. The addition of -SH containing reducing agents to mixtures of oxidized, inactive streptolysin O and cholesterol failed to restore any hemolytic activity except to tubes containing less than a neutralizing dose of cholesterol.

Summary. 1. The observation of Hewitt and Todd that cholesterol neutralizes the hemolytic activity of streptolysin O is confirmed.

2. The antigenicity of streptolysin O remains unchanged in cholesterol-neutralized mixtures.

3. Under similar conditions streptolysin O neutralized by its specific antibody elicits virtually no immune response to streptolysin O.

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Bioassay of Desoxycorticosterone-like Material in Urine.* (17614)

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Many explanations have been offered for the retention of sodium which is characteristic of the various edematous states. Injury,

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