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Dialysis of Complement Against 1.0 Normal Sodium Chloride.*

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Chow and Zia[1] reported that when guinea-pig serum is dialyzed against normal NaCl solution through a cellophane membrane at about 5°C its complementary activity rapidly decreases. They also stated that they were able to restore practically all of the original activity of the serum by combining the dialysate with the non-dialyzable fraction, and concluded that the loss of activity was due to the removal of a dialyzable component of the complement. Since these authors were unable to reactivate dialyzed serum by the addition of yeast-inactivated complement, or ammonia-inactivated complement, or by complement inactivated by oxidation with iodine, but were able to reactivate with dialysates of serums which had previously been inactivated by any of the above methods, they state that "these results, therefore, indicate that the dialyzable component of complement is different from the third or fourth or the oxidizable components of complement."

Because of the introduction of a new factor or factors in the constitution of complement it was thought of interest to study the reactivation of complement which had been inactivated by dialysis. However, upon repetition of the experiments of Chow and Zia, it was not possible to confirm their results in respect to the inactivation of complement by dialysis under the conditions reported by them.

It was observed that serum dialyzed in the cold (4.5°C) against 1.0 N NaCl, even over long periods of time, loses its hemolytic activity no more rapidly than serum which is stored without dialysis under the same conditions.

Experimental. Five cc of fresh guinea pig serum were measured into a 5/8-inch cellophane tube (No. 341), the bottom of which had been folded and tied off with silk thread in such a way as to prevent leaking. The top of the tube was sealed with a rubber stopper and tightened with a rubber band. The cellophane sac containing the

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1 Chow, B. F., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 695.

serum was then suspended in a large stoppered test tube containing 50 cc of cold 1.0 N NaCl solution. The sac was suspended in the saline at such a height that the level of the saline was about a centimeter above the level of the serum within the sac. A control sample of the same guinea-pig serum was measured into a similar cellophane sac and sealed in the same manner as above. This was suspended in an empty, large, stoppered test tube.

The large test tubes containing the sacs were placed in a cold room at 4.5°C. At the end of 24 hours, the sacs were opened and 0.05 cc of serum was removed from each sac. These samples were diluted 1:30 with 0.9% NaCl solution and the complement-activity of each sample was determined by the method of initial hemolysis of Ecker *et al.*[2] After removal of the samples for titration, the cellophane sacs were again sealed. Each serum undergoing dialysis was suspended in another 50 cc portion of cold 1.0 N NaCl solution. Dialysis was allowed to proceed at 4.5°C for another 24-hour period at the end of which time the above procedure was repeated. This was continued throughout the course of the experiment. The volume of each dialyzed serum and of the control serum was measured at intervals during the experiment. It was found that in no case did the volume increase by as much as 10% during the entire experiment. There was no change in the volume of the control.

The table shows the complement values obtained for three dialyses of 2 different serums, and also the titration values for the controls.

Conclusions. It is seen from the above table that guinea-pig serum slowly loses some of its complementary activity during dialysis against 1.0 N NaCl solution at a low temperature over a period of 2 weeks. This loss in activity is in no way comparable, however, with that reported by Chow and Zia who stated that in a

TABLE I.
Complement-activity of Serum During Dialysis in the Cold Against 1.0 N Sodium-chloride Solution.

Period of dialysis in hr	0	24	48	72	96	144	168	192	216	240	264
Dialyzed serum A	.03	.04	.03—	.04—	.04+	.04		.08		.08	
Control A	.03	.03	.03+	.04—	.04+	.04		.07+		.08	
Dialyzed serum B_1	.02	.03	.03	.04—	.04		.03		.04		.03
Dialyzed serum B_2	.02	.03	.03	.03	.04		.03		.04		.03
Control B	.02	.03	.03	.03	.04		.03		.04		.03

[2] Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

typical experiment a sample of serum lost nearly 90% of its original activity upon dialysis for 4 days under the same experimental conditions, while a control sample lost only 25% of its activity during storage for the same period. It will be noted from the results of the present experiments that both the total loss and the rate of loss of complement activity in the dialyzed serum is in each case substantially identical with that of the undialyzed control serum. It is upon this evidence that the conclusion is drawn that none of the components of complement are removed from the serum by dialysis against 1.0 N NaCl. The loss of complementary activity obtained by allowing serum to remain in the cold for long periods of time may be explained upon the basis of oxidation alone, or other factors, since neither complement nor any of its parts is dialyzable through a cellophane membrane against 1.0 N NaCl solution in the cold. In additional experiments, little loss of activity was obtained in dialyzing against cold 1.0 N NaCl solution complement which had been purified by a fractional precipitation method which will be reported in a subsequent paper. Dialysis in this case has been allowed to proceed for periods as long as 10 days.

Summary. Dialysis against 1.0 N NaCl at 4.5°C does not remove a dialyzable component from the guinea-pig complement. The gradual loss of activity noted may be accounted for on the basis of changes within the protein-molecule.

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Butter Fat in Dermatitis-Producing Diets.

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Butter fat has been revealed both as a curative agent[1] for rat dermatitis and also as a component (9%) of a dermatitis-producing diet.[2] The curative property was demonstrated by a daily supplement of 500 mg of fresh butter fat, a quantity approximately equal

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1 Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., *J. Nutr.*, 1939, **18**, 99.

2 György, P., *Biochem. J.*, 1935, **29**, 741.