

This protocol shows that 0.2 cc. of M/2200 copper chloride protects against 1,000 but not against 10,000 fatal doses of pneumococcus. The doses used in the antibody experiments (0.01 and 0.06 cc.) are 1,000,000 and 6,000,000 fatal doses respectively.

The material and mice (of which over 2,000 were used in these studies) were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

103 (2626)

Studies on the purification of antibodies. IV. The removal of extraneous material from antipneumococcus extracts at an approximately neutral reaction.

By R. OTTENBERG and F. A. STENBUCK.

[From the Pathological Laboratory, Mount Sinai Hospital, New York City.]

Experiments with precipitates and supernatant fluids, obtained by bringing antibody-containing extracts of sensitized bacteria to a known range of hydrogen ion concentrations, revealed another method of purifying such antibody solutions of extraneous material, which method is different from that described in the preceding paper. It was found that at hydrogen ion concentrations between pH 7.0 and 7.6, that is, well to the alkaline side of the point of precipitation of antibody itself (about pH 4.0), pneumococcus protective antibody remained in solution, but a large amount of indifferent material, containing considerable nitrogen, was removed from solution. It was possible to remove a fraction of this indifferent material before adding copper chloride; and then, after addition of copper chloride, to remove a further fraction, as illustrated in the following protocol.

Two and a half liters pneumococcus antibody solution were brought to pH 7.0 by the addition of hydrochloric acid. A precipitate formed. This was allowed to stand in the ice box over night, and centrifuged. The supernatant was designated "M-20 C." Trial precipitations of 10 cc. portions of this supernatant by addition of copper chloride indicated complete recovery of the

antibody in the supernatant fluid, after removal of the precipitate formed at pH 7.2, with the copper chloride in final concentration of M/2100. Two liters of "M-20 C." were then treated with 100 cc. of M/100 copper chloride and brought to pH 7.2. A pale green precipitate formed. It was allowed to stand over night in the ice box and then centrifuged. The original material, "isoelectric supernatant" (*i. e.*, supernatant after removal of the first precipitate) and "copper supernatant" were tested on mice. Each mouse received 0.5 cc. of a 1/200 dilution of 18-24 hour pneumococcus Type 1 culture, (*i. e.*, 0.0025 cc. of culture) and 0.5 cc. of the protective material diluted as indicated.

Comparison of original antibody solution and "isoelectric supernatant".

Material	Dilution of Material		Nitrogen per 100 cc.	
	0	1:10		
Original antibody extract	S	48	12.1 mg.	
	S	64		
Supernatant of pH 7.0 isoelectric precipitation	S	48	8.2 mg.	
	S	S		
Virulence control dose of culture	0.000,000,01	0.000,000,005	0.000,000,0025	0.000,000,00125
	48	48	48	S

This shows that the antibody was all recovered in the supernatant.

Comparison of "Isoelectric Supernatant" with Supernatant after Further Precipitation with Copper Chloride.

Material	Dilution of Material		Nitrogen per 100 cc.	
	0	1:10		
Supernatant of isoelectric ppn. at pH 7.0.	72	24	8.2 mg.	
	S	S		
Supernatant of above after copper precipitation.	S	92	3.0 mg.	
	S	S		
Virulence control dose of culture	0.000,000,01	0.000,000,005	0.000,000,0025	0.000,000,00125
	24	48	41	S

This shows that the antibody is still in the supernatant after copper precipitation at 7.2, although this supernatant contains only one-fourth of the nitrogenous matter of the original material.

The removal of indifferent material is easy to bring about, and occurs with much greater regularity than the precipitation of the antibody itself at a more acid point as described in the preceding paper. It would therefore seem to offer a more practical method for partial purification of the antibody.

The material and mice used in these studies were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

104 (2627)

Observations on the extra-cardiac circulation.*

By C. S. DANZER.

[*From the Physiological Laboratory and the Medical Clinic of the Johns Hopkins University, Baltimore, Md.*]

Experiment (I)

If the bulbus arteriosus of the frog is ligated, the heart gradually dilates. In about ten minutes the heart has more than doubled its size. (Fig. 1.) The heart muscle appears more cyanotic. If at the same time the peripheral circulation in the frog's web is observed microscopically, it is seen that the blood keeps moving in the capillaries for 5-10 minutes after aortic ligation. While corpuscular flow is slower, it simulates the normal as far as direction is concerned, going from the arteries to capillaries, then to the veins.

Thus the enlargement of the heart after aortic ligation is associated with the movement of blood from the large arteries to the capillaries, and from these back to the heart. It appears as though co-ordinated contractions of the vessels themselves were capable of circulating the blood along its usual course. This vascular mechanism comes into play when the cardiac output is prevented by ligation of the aorta.

If now the vessels of a second frog are injured quite another reaction takes place following the ligation of the aorta. In order to produce a profound vascular injury, the spinal cord of the animal was destroyed by pithing. This, as is known, has a tre-

* This work was completed in 1919.