Further studies are now being made on toxicity and the dosage suitable for human injection with the hope that an innocuous mixture can be effected combining high viscosity and radiopacity which is suitable for safe and practical angiography.

8080 P

Relation of the Phospholipins to the Reactivity of Antipneumococcus Sera.

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Hardy and Gardiner's method[1] for the removal of lipoids from sera is based upon the fact that extraction of protein solutions by alcohol or alcohol-ether at low temperatures does not cause denaturation. Using this method, Hartley[2] has shown that the removal of lipoids from certain antisera apparently abolishes their *in vitro* reactivity. Felton[3] has extracted antipneumococcus horse serum in a similar manner, and has demonstrated that the removal of lipoids does not diminish protective action.

These findings have been confirmed for Type I antipneumococcus horse serum. Extracted sera fail to agglutinate homologous type pneumococci and to give a precipitate with the specific capsular polysaccharide. *In vivo,* however, they show the presence of protective antibody in unaltered concentration.

Lipoid extraction was carried out in the following manner: The antiserum was introduced, with stirring, into 10 volumes of absolute alcohol at —10°C. After 6 hours' extraction the precipitate was collected by centrifugation at a temperature below —2°C., and again extracted with an amount of chilled absolute alcohol equal to that first used. After 12 hours the precipitate was collected in the same manner, and was then extracted with anhydrous ether for 10 hours at —10°C. and for a second time with anhydrous ether for 10 hours at room temperature. The precipitate was then collected and freed of ether by vacuum distillation, and finally was dissolved in an amount of saline equal to the original serum volume. The resulting solution does not differ in appearance from untreated serum.

[1] Hardy, W. B., and Gardiner, S., *J. Physiol.*, 1910, **40**, lxviii.

[2] Hartley, P., *Brit. J. Exp. Path.*, 1925, **6**, 180.

[3] Felton, L. D., and Kauffmann, G., *J. Immunol.*, 1933, **24**, 543.

Although failing entirely *in vitro,* the extracted antipneumococcus horse serum brought about agglutination *in vivo* as well as unextracted serum. As a consequence of this observation extracted immune horse serum was injected intraperitoneally into mice and the peritoneal fluid was withdrawn after 30 minutes. This recovered fluid possessed the capacity to agglutinate homologous type pneumococci. This result suggested that the modified antibody had in some manner been reactivated by a substance in the peritoneal fluid. In an attempt to duplicate this effect *in vitro* a series of separate purified lipoids was employed, and it was found that the addition of minute amounts of lecithin at pH 6.0 completely restored *in vitro* activity.

Antipneumococcus rabbit serum, on the other hand, retains all of its *in vitro* reactive properties after extraction by alcohol-ether in the cold. If, however, the process is altered to include 2 extractions with petroleum ether at room temperature after treatment with alcohol, the *in vitro* reactivity of immune rabbit serum is almost entirely abolished. Its protective action, however, remains unchanged. It was found that in this instance the *in vitro* reactivity of the extracted rabbit serum could be restored only by the addition of cephalin.

It is of some interest that although these sera can be specifically reactivated by certain lipoids they are not reactivated by the lipoid material originally extracted from them. In this connection it has been found that the addition of minute quantities of free cholesterol to the extracted sera, prior to the addition of either cephalin or lecithin, prevents the reactivation in both instances.

It has not yet been possible to determine whether or not these latter lipoids are essential constituents of the antibody molecule, although 2 facts may point in this direction. The first is the specific character of the reactivating process. The second is the change in the solubility of the immune fraction which is present in extracted horse serum, for this is no longer precipitated by ten times dilution with distilled water.

Antipneumococcus sera from several animal species have been studied from this point of view. Those from the guinea pig and the rat apparently belong to the rabbit or "cephalin" group, while those from the goat, the mouse, and man belong to the horse or "lecithin" group.

The results presented in this summary are a further demonstration of the wide differences in the properties of antipneumococcus sera derived from the rabbit and the horse.