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Studies on the purification of antibodies.

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It has been known for years that when precipitates are produced in serum by salts of the heavy metals, immune bodies present are more or less completely precipitated. Whether the immune bodies are precipitated by the metal directly or whether merely adsorbed by the rather voluminous protein precipitate formed has never been determined. It seemed a possibility that the metal might actually enter into combination with the immune body, and that by repeated precipitation of such a combination, purification of the immune body might be brought about. In preliminary experiments with anti-sheep hemolysin and typhoid agglutinin, using the chlorides or sulfates of copper, nickel, zinc, mercury and lead as precipitants, it was found that a good deal of these antibodies could be dissolved out of the precipitates. On account of the large amount of protein present, however, it was found that any really effectual purification of the antibody was impossible.

It was determined therefore, to try the effect of metallic salts on antibody already partially freed of protein by the method of specific absorption followed by subsequent dissociation from appropriate cellular antigens. This we have done both with anti-sheep hemolysin and with typhoid agglutinin. But on account of the interference of the metal salts with the action of complement in hemolysin experiments, the work has been more successful with bacterial agglutinin.

With typhoid agglutinin the preliminary purification was brought about by first sensitizing large amounts of formol-killed typhoid bacilli as nearly as possible to saturation with agglutinin, then washing the sensitized bacteria free of serum in repeated changes of saline solution, and then dissociating the agglutinin from the bacteria either with water at 55° C for one half to one hour, or with N/200 NaOH at room temperature

for five minutes. The extracts contained on the average about 0.15 per cent solid matter of which about 0.03 per cent was ash. Their titre ranged from 1/300 to 1/1200. More than half of the solid matter present was undoubtedly derived from the bacteria themselves as was found when control extracts were made of bacteria which had not been sensitized.

When to such extracts metallic salts were added, (copper, nickel, zinc, platinum and mercuric chlorides) precipitates formed, but only when the solutions were brought to a certain hydrogen ion concentration zone. The limits of this zone can not yet be exactly defined; it is not the same for the different metals. The maximum precipitation was usually near pH 6.4. When the amount of metal added was not excessive (not over M/200 strength) the precipitate contained not only nearly all the agglutinin but nearly all the metal present. When these precipitates were redissolved in very weak (N/200) HCl, they were found to contain most of the agglutinin. In order to re-precipitate the agglutinin from this solution again it was only necessary to bring this solution again to the proper hydrogen ion concentration. It was not necessary to add further metallic salt. We have been able to reprecipitate and redissolve such metal-antibody combinations as many as five times in succession with only a slight loss of agglutinin each time.

The specificity of the agglutinin effect and the absence of agglutinination by either the metallic salts, or the acid, or both together, were carefully controlled.

In the case of copper, a second zone of precipitation of the agglutinin was found far to the alkaline side. The hydrogen ion concentration was beyond pH 9.6 and could not be precisely measured by the colorimetric system of Clark, which we employed. This alkaline copper precipitate brings down agglutinin almost quantitatively and is deep blue in color, whereas the precipitate obtained in the nearly neutral zone is pale blue. Like the neutral precipitation the alkaline precipitation can be brought about repeatedly with only slight loss of agglutinin. It seems probable that the precipitate brought down in the alkaline zone is composed partly of copper hydroxide because copper hydroxide can be precipitated in about the same zone by the addition of suitable amounts of alkali to copper chloride solution.

When the control extracts were made from bacteria which

had never been sensitized, and treated in the same way (with copper only), a similar looking precipitate, only smaller in amount, was formed in the "neutral" precipitation zone. Just as in the earlier experiments with serum, therefore, the question is again open as to whether the precipitation of agglutinin is here brought about merely by adsorption to precipitated bacterial protein, or whether a separate combination of antibody and metal exists. The almost quantitative recovery of the antibodies after repeated precipitation, however, offers promise that the latter may be the case. If so, since each precipitation seems to leave some of the extraneous bacterial substance behind, this method of repeated precipitation may afford the possibility of ultimate purification of the antibody. Further work is being done in this direction.

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On the nature of the action of vegetable extracts on the blood sugar of normal rabbits.

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The recent work of Winter and Smith[1], Best and Scott[2], Funk and Corbitt[3], and of Collip[4] establishes the point that there is present in yeast and in plants and vegetables a substance which, upon injection into normal rabbits, causes a fall in blood-sugar. In this connection, the experiments of Thalhimer and Perry,[5] and Fetzer[6] led to similar observations.

Best and Scott, working with extracts of potatoes and rice, obtained results like those following an injection of insulin. On the other hand, Funk and Corbitt (unpublished data), as well

1 Winter, L. B., and Smith, W. J., *J. Physiol.* 1922, lvii, 100.
2 Best, C. H., and Scott, D. A., *J. Metabol. Research*, 1923, iii, 177.
3 Funk, Casimir, and Corbitt, H. B., *Proc. Soc. Exp. Biol. and Med.* 1923, xx, 422.
4 Collip, J. B., *J. Biol. Chem.* 1923, lvi, 513; 1923, lvii, 65.
5 Thalhimer, W., and Perry, M. C., *J. Am. Med. Assoc.*, 1923, lxxx, 1614.
6 Fetzer, L. W., *J. Am. Med. Assoc.*, 1923, lxxxi, 772.