these bottom precipitates, as they will be found to be present in the controls as well.

It may be of interest in this connection to give the titration results of an extract antigen prepared as outlined in a previous paper of these Proceedings,[1] and the same extract containing 400 and 800 mgm. of cholesterin per 100 c.c., respectively.

This titration would indicate that cholesterin plays an important rôle in necessitating proportionally larger amounts of salt solution to bring about opalescent antigen-salt solution mixtures. It might be added also that in a general way, cholesterin proportionally increases the sensitiveness of the antigen after mixing with syphilitic serum. The problems involved in the cholesterinization of antigen are reserved for further studies.

241 (2201)

Employment of different antigens in Kahn precipitation test.

By R. L. KAHN and W. W. DUEMLING.

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

Employing the antigen titration outlined in the previous paper, the question arose to what extent the preparation of antigen may be varied without affecting the final results. The following antigens were accordingly prepared, titrated with salt solution and smallest amounts of the latter used which produced opalescent antigen-salt solution mixtures. The tests were carried out with positive and negative sera according to procedures I and II.

Antigen 1. This was prepared as described in a previous paper of these *Proceedings.*[1] Dried beef heart was freed from ether extractives and subsequently extracted in 95 per cent. alcohol for 9 days in the ice box and overnight at incubator temperature. Color approximated potassium bichromate color standard.

Antigen 2. After extracting the dried heart with ether in the usual manner, boiling alcohol was poured on the dried material, shaken and extraction continued for 1 day in the incubator. Color approximated potassium bichromate standard.

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[1] Kahn, R. L., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 325.

Antigen 3. Same as antigen 2, except that the alcohol extraction was carried out in the water bath at 37.5° C. for 2 days with frequent shaking. Same color index as above.

Antigen 4. Same as Antigen 1, to which was added a second alcoholic extract carried out for 2 days at incubator temperature having approximately the same color range.

Antigen 5. Ether extractives were removed in the usual manner and dried heart muscle extracted with alcohol for three hours in reflex condenser.

Antigen 6. Ether extraction carried out for 1 hour in reflex condenser, followed, after filtration and washing with fresh ether and drying, by alcohol extraction also by means of a reflex condenser for 1 hour.

Antigen 7. Dried heart muscle was extracted in 95 per cent. alcohol for three days in the incubator. The alcohol was then filtered off, evaporated to dryness and taken up in a small amount of ether. About ten times the amount of acetone was then added, permitted to stay over night, the acetone decanted and the precipitate redissolved in absolute alcohol. (Noguchi.)

Antigen 8. Ether extractives were removed in the usual manner, and dried beef heart was extracted with alcohol for 3 days in the incubator. After filtration, the alcohol was evaporated to dryness, taken up in a small amount of ether and precipitated with an excess of acetone. The precipitate was finally taken up in absolute alcohol.

Antigen 9. Ether extract of *Antigen* 8 was evaporated to small volume, precipitated with excess of acetone and precipitate suspended in alcohol and placed in incubator overnight, filtered next morning.

Antigen 10. Equal quantities of antigens 8 and 9.

Antigen 11. Alcoholic solution of acetone insoluble product from ether extract of beef heart obtained after 1 hour extraction in reflex condenser.

Antigen 12. Equal quantities of Antigens 11 and 6.

Antigen 13. Ether extraction of dried beef heart was prepared and saved. An alcoholic extract was then obtained, evaporated to dryness and taken up in a small amount of ether. This was mixed with original ether extract and acetone insoluble antigen in [illegible]nol prepared from it. This was finally mixed with [illegible]nd alcoholic extract of the same dried muscle. (W[illegible].)

Antigen 14. Alcoholic solution of acetone insoluble product obtained from an ether extract of beef heart mixed with an alcoholic extract of the same beef heart obtained as outlined in Antigen 3.

To each of the above extracts were added 400 mgm. of cholesterin per 100 c.c. before testing with the various sera.

The results of preliminary experiments indicate that most of these antigens compare favorably with one another. In a general way the acetone insoluble antigens are somewhat weaker than the others. Necessarily, a large number of tests will have to be carried out before establishing the degree of sensitiveness and particularly the specificity of these various antigens.

It is of interest to note that "Antigen 6" which can be prepared in about three hours, appears to give unusually sensitive as well as specific reactions. This as well as the other antigens outlined are still being investigated and other antigens are under preparation.

242 (2202)

The elaboration and release of the colloid of the thyroid.

By EDWARD UHLENHUTH.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

The development of the thyroid of the salamander *Ambystoma opacum* was studied on serial sections of thyroids in various stages before and after metamorphosis.

The proportion of colloid and epithelium was found by weighing separately wax models of the colloid and epithelium. During the larval period the colloid increases more rapidly than does the epithelium. From 13 per cent., shortly after hatching, it increases to 45 per cent. of the total thyroid mass just before metamorphosis. The larval period is not a period of colloid release, but of colloid elaboration and storage. At the beginning of metamorphosis, the colloid percentage drops suddenly below 30 per cent. This drop is due partly to the sudden and excessive release and disappearance of the colloid from the follicles, and partly to an excessive increase of the epithelial mass, owing to the swelling of the individual