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.

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The elaboration and release of the colloid of the thyroid.

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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 cells. With increasing age the thyroid again enters upon a stage of colloid accumulation. In animals 4.5 years of age, 56 per cent. of the thyroid mass is colloid. This relative increase is not due entirely to an absolute colloid increase, but also to an absolute decrease of the epithelium.

The colloid was studied on sections stained, according to Kraus' method, with polychrome methylene blue and acid fuchsin.

Colloid is elaborated long before follicles are formed, at a time when the cells still contain yolk and are devoid of granules, a structure frequently considered to be prerequisite to colloid elaboration. In thyroids of larvæ fed exclusively thymus gland, the cells remain permanently primitive, consisting almost entirely of nucleus, possess very small amounts of plasma and are devoid of granules. Yet these cells elaborate large quantities of colloid (55 per cent. of the total thyroid mass).

At first colloid is elaborated only by the formation of intracellular colloid globules of small size which stain red, the color of newly formed colloid. Owing to the increase in the number of cells containing intracellular globules, two or more globules may touch each other, become free, and form, by fusion, a primary follicle. Or the globule may grow within the cell, become free by retraction of the surrounding cell plasma and form a primary follicle. Primary follicles form, by fusion, the large secondary follicles. Colloid elaboration by intracellular globules, now in the individual cells of the follicle walls and in the cells of the reserve cell masses, continues, even after the formation of secondary follicles. These globules may grow within the cell to a large size, become an extracellular colloid mass in the follicular wall, by retraction of the cell plasma, and fuse with the main colloid mass; or they may be extruded by the inner cell end directly into the follicle recognizable for some time by the red coloration. The latter is a permanent mode of colloid elaboration.

Another mode of colloid elaboration is the elaboration of colloid from granules, the predominant mode after secondary follicles have formed. At first, fine granules of reddish color are scattered throughout the plasma. Gradually they increase in number and in size and tend to crowd near the inner surface of the cell, where a reddish substance accumulates between the granules, apparently the result of the fusion of the granules. It seems that this substance can diffuse directly through the cell membrane into the follicle, giving rise there to the marginal zone of red colloid around the yellowish brown, green, or blue center of the colloid mass.

Only one kind of stainable colloid is formed, the red colloid. It stains yellow, green, and finally blue, when it becomes old. It undergoes this change no matter if it ages in the follicle or within the cell. Blue colloid is not indicative of an actively releasing state of the thyroid, but of an accumulation of old colloid. Therefore, the colloid in the thyroid of old axolotls, which elaborate but do not release colloid, stains deep blue, almost black.

No explanation has been found as to how the colloid escapes from the follicle in the blood. It appears, however, that the vacuoles are somehow connected with the colloid release. There are two kinds of vacuoles in the colloid: closed vacuoles, and those which are in open communication with the inner cell ends. When the colloid first appears, it has no vacuoles. The colloid of all larvæ prevented from metamorphosing, by experimental procedures, and the colloid of larval axolotls are practically devoid of vacuoles. Apparently, the elaboration of stained colloid may take place in the absence of vacuoles. Yet, in the colloid of normal larvæ, the vacuoles increase steadily in number and size. Not only within the follicle, but also within the cell, the colloid develops vacuoles upon aging.

Communicating vacuoles are found only in thyroids releasing the colloid into the blood. They appear suddenly, when metamorphosis begins. At the same time, large vacuole-like spaces appear in the cells causing the swelling of the cells. It seems as if the content of the vacuoles is poured into the cells and escapes through the periphery into the blood. At any rate, communicating vacuoles are indicative of colloid release.

Feeding inorganic iodine to normal larvæ and axolotls, although it does not enforce the release of the colloid, produces ordinary vacuoles.

The elaboration of colloid is not necessarily followed by the release of the colloid. Feeding of inorganic iodine to old axolotl larvæ results in an increased rate of colloid elaboration, producing a wide marginal zone of red colloid; yet metamorphosis cannot be enforced by this procedure. Larvæ fed exclusively thymus, do elaborate colloid; yet they cannot metamorphose. Colloid elaboration and colloid release are, within certain limits, independent of each other.