

The extract is made faintly alkaline to litmus, then 0.3 per cent hydrochloric acid is added until a degree of acidity is produced such that the precipitate assumes a flocculent form, and settles rapidly, leaving a clear supernatant fluid.

Filtrate I is made up to a volume of 100 to 150 cc., adjusted to a pH of 5.5 to 5.8, and, sufficient anhydrous acetone added to bring to a concentration of 60 to 70 per cent in acetone. After standing 12 to 15 hours in the ice chest, precipitate II is separated from filtrate II.

Filtrate II is evaporated to dryness *in vacuo* at 40 to 45° C. The residue is dissolved in two and one half per cent trichloroacetic acid, corresponding in volume to filtrate II. A finely divided precipitate slowly develops, and after standing 12 to 15 hours in the ice chest, is separated by centrifugalization, or if still in suspension, by filtration. It is then dissolved in strong alcohol and precipitated with anhydrous ether.

Eight to 20 milligrams of such a preparation have been found to produce an increase of 4 to 5 milligrams, in the plasma calcium of dogs ranging in weight from 12 to 19 kilograms, within 12 to 15 hours following subcutaneous injection.

The active principle has been extracted by liquid ammonia from the residue obtained by the evaporation of Filtrate II. It has also been extracted by ethyl lactate and by ethyl butyrate. It is not adsorbed by kaolin at pH values of from 1 to 5, although at higher values it appears to be adsorbed, but has not been released.

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The Histogenesis of the Thymus as shown by Tissue Cultures, Transplantation and Regeneration.

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The histogenesis of the thymus is still unsettled. The majority of investigators, on the basis of the work of Hammar and Maximow,¹ consider the thymus to be a lymphoepithelial organ in which the original entodermal epithelium of the primordium transforms into a reticulum and is infiltrated with common lymphocytes. Some workers have recently resuscitated the old idea of Stöhr according

to which the small thymus cells merely resemble the lymphocytes in appearance and actually represent peculiarly modified epithelial cells.² Besides, the epithelial nature of the reticulum seems to be doubtful, mainly because in vitally stained animals, as some authors claim, the reticular cells store vital dyes in the same way as histiocytes.

The method of tissue cultures was applied by us to the study of the thymus problem. Pieces of the thymus were taken from newborn, half-grown and full-grown rabbits and cultivated in plain and carminized plasma with the addition of bone-marrow or embryonic extract.

In the deeper parts of the explant necrosis occurs; the peripheral layers of the lobules and sometimes patches of the medulla remain alive. During the first 24 hours the cultures show a massive emigration of the small cells which behave exactly like lymphocytes. The interstitial connective tissue also serves as a source of migrating lymphocytes, histiocytes and fibroblasts. During the second and third days, as a result of autolysis of the dead lymphocytes and emigration of the living ones, the volume of the lobules decreases, and a broad, pale epithelial layer develops on the periphery through the condensation of the reticular cells. Islands of epithelium eventually arise also in the debris of the deeper layers through contraction and collection of epithelial cells. All epithelial cells are connected by processes. Between them a varying number of dead and live lymphocytes remain to the latest stages. At the same time everywhere in the epithelium mitoses appear. During these changes in the epithelium the small vessels, which are very indistinct in the normal thymus, become more prominent. The epithelium recedes from them and they are now seen surrounded by broader or thinner layers of connective tissue cells of embryonic character, by singly scattered phagocytic carmine-storing histiocytes or macrophages, and by myelocytes. The true epithelium does not store the carmine. Thus in the cultures of early stages the most important relation between the epithelial reticulum and the vessels, with the accompanying connective tissue elements, becomes clearly manifested. The cells storing vital dyes in the normal thymus do not belong to the epithelial reticulum, but to the connective tissue of the adventitia of the blood vessels. After six, eight days of life *in vitro* proliferating epithelium forms strands and islets, sometimes showing intercellular and intracellular secretory vacuoles. The outlines of the epithelial cells are always sharp and no trace of their transformation into lymphocytes or connective tissue cells could be found. The

bodies of Hassall may remain unchanged or they disappear through degeneration and phagocytosis by histiocytes. Whether new ones are formed or not *in vitro* can not be stated decisively.

In the oldest cultures (20 days) the epithelium undergoes progressive atrophy, becomes smaller and the vacuoles disappear. Gradually the connective tissue completely overgrows the epithelium.

The experiments with autotransplantations according to the method used by Jaffe were made on half-grown rabbits; the transplants were excised at 2, 4, 6, 8, and 10 day intervals, fixed in Zenker-Formol, embedded in celloidin, and stained with Hematoxylin-Azur II Eosin. The regeneration of the remains of the thymus was also studied.

During the first six days the transplants show degeneration of lymphocytes in the central area. The epithelium forms, on the periphery of the lobules, a capsule-like layer containing dead and live lymphocytes. The vessels, with the accompanying embryonic connective tissue cells, myelocytes and histiocytes, become more prominent. The surrounding local connective tissue shows the usual signs of aseptic inflammatory reaction, infiltration with special leucocytes, mobilization of histiocytes, emigration of lymphocytes and monocytes. It is impossible to discriminate between this local connective tissue and the interstitial tissue of the transplant. The connective tissue with its enlarged vessels penetrates into the transplanted tissue, resorbs the necrotic masses and breaks up the epithelium into islands of different size and shape.

After 7 days regeneration becomes very distinct. The epithelium presents itself in the form of thick growing lobated buds containing many mitoses. The vessels accompanied by embryonic connective tissue penetrate deep into the folds between the epithelial buds. Wherever the connective tissue comes in contact with the epithelium the cells of the former become mobilized as large, medium sized and small lymphocytes. The latter penetrate by means of ameboid movements between the epithelial cells and show many mitoses as do also the epithelial cells. In many places the lymphocytes give rise to special myelocytes; these are mostly of the darkly nucleated, micromyelocytic variety. After 8 days the lymphocytes outnumber the epithelial elements.

The regenerative processes in the transplants of the thymus duplicate the histogenetic processes of this organ in the embryo. The essential feature in both cases is the infiltration of epithelium with true lymphocytes. The epithelium gradually becomes transformed into a reticulum. At the same time the vessels continue to branch

between the epithelial masses and form a network of embryonic connective tissue, penetrating the epithelial network, and serving as a source of lymphocytes and histiocytes.

The regeneration of the stump of the remaining part of the thymus duplicates in all essential features the regenerative process in the transplants.

The lympho-epithelial nature of the thymus and the presence of a perivascular mesenchymal reticulum in this organ are decisively shown by these experiments with tissue cultures, autotransplantations and regeneration. No evidence of the development of the small thymus cells from the epithelium was found.

¹ Hammar, A., *Arch. mikroskop. Anat.*, 1909, lxxiii, 1.

² Maximow, A., *Arch. mikroskop. Anat.*, 1912, lxxx, 39.

³ Maximow, A., *Arch. mikroskop. Anat.*, 1911-1912, xxxix, 560.

⁴ Stöhr, Ph., *Anatom. Hefte*, 1906, xxxi, 409.

⁵ Schridde, H., *Cent. alg. Path.*, 1923, xxxiii, N. II, 284.

⁶ Dustin, A., *Arch. Biol.*, 1920, xxx, 601.

⁷ Winiwarter, H., *Bull. Histol. appliquee.*, 1924, N. I.

⁸ Jaffe, H., Gottesman, J., *J. Exp. Med.*, 1926, xliii, 403.

⁹ Jaffe, H., *J. Exp. Med.*, 1926, xlv, 523.

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An Immune Reaction in Tissue Culture.*

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The method of tissue culture has not been extensively used for immunological purposes. Carrel and Ingelbritsen¹ and Przygode² have shown that lymph node, bone marrow and spleen react in tissue culture to the presence of antigens by the formation of specific antibodies.

This report is the first on a series of experiments in which we have cultivated various tissues from normal and immunized animals and have watched the effect of the addition of antigens to the cultures.

In one set of experiments we cultured the lungs of 8 normal rabbits and those of 5 rabbits immunized against pigeon erythrocytes.

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