Experimental Studies on the Histology of the Mammalian Thymus.

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With Plates 6–12.

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INTRODUCTION.

It has been known for some time that a variety of experimental methods can be employed to bring about destructive changes in the mammalian thymus, accompanied by a conspicuous decrease in its size. In most cases if the treatment is not too severe (22) and the animal young, the organ subsequently regenerates. Injection of serum (8), weak acid (9), or aniline dyes (10), alcohol (18), paraffin (11), or adrenalin (12), fracture of a limb (13), and treatment with X-rays (1, 4, 22, 23) will all cause partial degeneration of the thymus which may be followed by regeneration. The time which elapses before the organ is
completely restored varies with the severity of the treatment, but histological investigation indicates that the process is the same in all cases. Autoplastic transplantation of the thymus into the muscle of the abdominal wall \((14, 16, 17, 21)\) is followed by a similar degeneration and regeneration, and pieces of thymus cultivated in plasma show the histological changes that occur in the whole organ during experimental involution.

A section through the normal mammalian thymus is composed of a number of dark cortical areas surrounding paler medullary regions, the two being distinct but not separated by any membrane (fig. 12, Pl. 7). The cortex consists almost entirely of small cells with little cytoplasm lying close together, while the medulla contains similar cells scattered among larger 'reticular' cells with pale nuclei. Blood-vessels ramify throughout the thymus, but are more abundant in the medulla; it is not uncommon to find agglomerations of erythrocytes lying free among the thymus cells \((19)\).1 Conspicuous in the human thymus and in that of the cat, but small in the mouse and certain other mammals, are the nests of more or less concentrically arranged cells known as Hassall's corpuscles, which occur in the medulla (fig. 2, Pl. 6). Strands of connective tissue penetrate the thymus from the surrounding sheath, and may divide it up more or less completely into a number of lobules, each with a distinct cortex and medulla.2

The modifications which have been described in the thymus after experimental treatment may be summarized as follows: the small thymus cells in the cortex (lymphocytes of some

1 A similar extravasation of erythrocytes has been observed by Dustin \((5)\) in the thymus of the axolotl and reptiles, and by the present writer in the Teleost thymus.

2 A comparison of sections through the thymus of a number of mammals indicates a connexion between the amount of connective tissue in the normal organ and the size of the corpuscles of Hassall. Thus in the thymus of man, the cat and the marsupial Trichosurus, the corpuscles are large, and the thymus subdivided by numerous strands of connective tissue; in the rabbit, rat, and mouse, on the other hand, connective tissue is relatively scarce in the thymus and the corpuscles insignificant in size.
writers) undergo pycnosis in large numbers, and are rapidly ingested by the larger elements (reticular cells). After about three days the thymus is considerably reduced in size, and most of the nuclear debris have been resorbed; the corpuscles of Hassall are conspicuously enlarged, and in some species ciliated cysts have developed. The outer layer of the thymus consists of cells with large nuclei described as epithelial or reticular. At this stage the medulla contains a number of persistent small thymus cells, and is darker staining than the cortical region—an inversion of the relation in the normal thymus. The blood-vascular and connective-tissue elements have become more prominent, and many polymorphs can be seen in the vessels. Later, the vascular and connective-tissue elements regress, and a rapid proliferation of small thymus cells takes place; after a few days characteristic cortical and medullary regions can once more be distinguished. The corpuscles of Hassall degenerate during this period, and are inconspicuous in the newly reconstituted organ. Histological regeneration may be completed in six days.

The discrepancies in the accounts of the writers cited above are largely due to fundamental differences of opinion concerning the histogenesis of the thymus, the nature of the small thymus cells and of the larger elements in the medulla. The controversy extends to the thymus of all vertebrates, and a full account can be found in recent papers by Hammar, Dustin, and Winiwarter (15, 7, 24). It has long been established that the original thymus rudiment is budded off from the epithelium of the pharynx. Hammar and others maintain that the small thymus cells are true lymphocytes, derived from mesodermal immigrants, which have penetrated the thymus during early development and multiplied there; the larger cells in the organ, including the corpuscles of Hassall, are the product of the original epithelium. Amongst recent authors Dustin states, on the contrary, that the small thymus cells are directly descended from the cells of the original rudiment, while the other elements are mesodermal and have passed into the thymus along with the blood-vessels. According to Hammar the thymus consists of lymphocytes lying in an epithelial reticulum; according to Dustin there is
no such persistent reticulum, since the original epithelium has been replaced by the small thymus cells to which it gave rise.

The changes in the thymus which degenerates after experimental treatment and subsequently reforms have been studied in detail, partly to throw light on the function of the organ and partly to elucidate its histology. The following summary of the conclusions of different workers with regard to the second problem shows that agreement has not yet been reached on essential details.

Rudberg (23) investigated the effect of X-rays on the dog's thymus; he states that lymphocytes—for as such he regards the thymus cells—degenerate, and are ingested by the reticular cells; later these too degenerate. Regeneration is brought about through mitosis of reticular cells or persistent thymus cells, or through immigration of lymphocytes along the connective tissue. The illustrations are inconclusive.

Aubertin and Bordet (1) studied the action of X-rays on the thymus of new-born cats and rabbits; they observed that the lymphocytes underwent pycnosis and were replaced by large epithelioid cells, continuous with the cells forming the corpuscles of Hassall; later these epithelioid cells form fibrous tissue. The authors incline to the belief that these are connective-tissue cells. They describe the hypertrophy of Hassall's corpuscles which contain nuclear debris.

The occurrence of this hypertrophy was confirmed by Regaud and Crémieu (4, 22) in their more detailed study of the effect of X-rays on the thymus of the cat. They note variations in the small cells ('lymphocytes') of the normal thymus, and suggest that those in the medulla, which are less sensitive to X-rays, differ from those in the cortex. The regeneration of the small cells is not described in detail. Modifications in the blood-vascular and connective tissue following irradiation are described; a network of fibrous tissue develops in the interlobular clefts and spreads into the organ; the margin of the reduced thymus undergoes sclerosis. Polymorphs increase in number shortly after irradiation, but disintegrate in the corpuscles of Hassall when the organ is regenerating. Regaud and Crémieu
are uncertain of the origin of the reticular cells, but believe them to be epithelial; they appear to have a centripetal tendency and to form hypertrophied corpuscles of which the innermost cells are degenerating. The authors comment on the instability of these structures, and the rapidly reversible changes which they undergo in the irradiated thymus.

Dustin (8, 9, 10) injected various substances into mice, and studied the effects produced on the thymus. The immediate result of such treatment is the appearance of numerous pycnoses of small thymus cells. An accompanying metaplasia of the vascular and connective tissue takes place, and, from this, corpuscles of Hassall and ciliated cysts develop. Later the persisting small thymus cells undergo mitosis.

Goldner (12) found that the injection of adrenalin would bring about degeneration of the thymus. He concludes from his preparations that the reticular cells and corpuscles of Hassall are all epithelial structures. A subsequent study (13) of similar changes in the thymus of a guinea-pig, after the fracture of its limb, led this writer to extend his conclusion to the small thymus cells. These too may be changed into epithelial cells, or even into corpuscles of Hassall; they are not lymphocytes.

Jaffe, Plavska, and Gottesmann (14, 16, 17) have recently made a detailed study of the degeneration followed by regeneration which takes place in a thymus when the whole lobe or pieces are transplanted into the muscles of the abdominal wall. They assume the existence of a thymus reticulum, and describe all large cells seen in the thymus as epithelial or reticular, the terms being used interchangeably. Little reference is made to the vascular and connective-tissue elements. These writers conclude definitely that the young thymus cells in the regenerated organ together with the corpuscles of Hassall, ciliated 'ducts' and similar elements are all the products of the reticular cells. In this they agree with Goldner and disagree with Hammar and others, since they hold that their evidence shows that the thymus cell is not a lymphocyte.

It is clear from the accounts of the writers cited above that the corpuscles of Hassall increase in size while the thymus cells
undergo pycnosis, and degenerate as the thymus cells are being reformed. To assume that these diverse elements are all modifications of the original epithelial tissue raises considerable difficulties, and to substantiate such a theory requires more conclusive evidence than has yet been brought forward.

The most recent paper on the experimental histology of the thymus appears to be that of Popoff (21); he studied the changes in small pieces of rabbit thymus grafted into the abdominal wall according to Jaffe's technique, and cultivated in plasma. Popoff refers to the controversy on the nature of the thymus elements, and, unlike Jaffe, concludes in favour of the thymus as a lympho-epithelial organ; the histological changes in the transplant are essentially those found by previous workers, except that no reference is made to the behaviour of the corpuscles of Hassall. The activity of the connective tissue surrounding the graft is described; it penetrates the thymus and resorbs the pycnotic masses. Regeneration is initiated by mitoses in the peripheral layer formed of epithelial cells but, contrary to the statement of Jaffe, it is not these cells that give rise to the small thymus cells; the latter are produced by the connective tissue where it comes in contact with the epithelium—a process described by the author as agreeing with Hammar's lympho-epithelial theory. No illustrations are given.

It is well established (22, 16, 12, 19) that the corpuscles of Hassall are by no means constant structures, as was once thought, but highly variable cell aggregations. Often they appear concentric, since cells with large pale nuclei group themselves round the periphery, while at the centre degeneration and liquefaction is taking place. Transitions between corpuscles of Hassall, cysts and normal 'reticular' cells have been traced (19). The chief argument from these experiments in favour of the existence of an epithelial reticulum in the thymus (enclosing an infiltration of lymphocytes) is the appearance in the cortex, after the small thymus cells have degenerated, of large epithelioid cells. It is assumed that these are descended from the cells of the original thymus rudiment, and identical with the large cells of the thymus medulla which form corpuscles of
Hassall. It is a matter of dispute whether or not, in the regenerating organ, these epithelioid cells give rise to the small thymus cells; immigration of the latter may be excluded, but their development from embryonic connective-tissue cells accompanying the blood-vessels has been described (21); they do not appear to arise from the small thymus cells which have persisted in the medulla, but are regenerated in the cortex.

It was thought that a further investigation of the histological changes in an X-rayed thymus might clear up certain points which remain doubtful, especially the nature and behaviour of the thymus reticulum. If the reticular cells of the medulla which form corpuscles of Hassall are epithelial, as Hammar maintains, it should be possible to distinguish them from connective-tissue elements by their appearance or behaviour in the X-rayed thymus, and in tissue cultures. In this connexion the origin of the large cells forming a border round the reduced thymus is important. The manner in which cortex and medulla are reconstituted, when the thymus is regenerating, may throw light on the process whereby the normal organ is differentiated from the original epithelial rudiment.

**Material and Technique.**

The material used was taken from male mice 4–6 weeks old at the time of irradiation, when normal involution of the thymus has not yet begun. Owing to the small size of the thymus in mice compared to other mammals, it is possible to fix and section each lobe with the surrounding tissue in its entirety. This procedure renders the interpretation of sections less liable to error, and reveals any immigration into the irradiated tissue. The irradiation was carried out in the Department of Anatomy, University College, by Mr. F. Melville, under the direction of Dr. A. S. Parkes. The dosage was found to give approximately \( \frac{4}{5} \) B. tint with Levi’s pastilles. Details:

- Kilovolts, 50.
- Milliamps, 5.
- Wave-length, 0.285 A.
- Spark gap (point-point), 6.3 cm.
Tube, Coolidge universal, medium.
Distance from anti-cathode, 18 in.
Filter, none.
Time of exposure, 80 minutes.

The treatment appeared to have no deleterious effect upon the mice, which were killed at varying intervals after it in order that the changes in the thymus might be followed. The latter was dissected out with its adjacent tissue and fixed in Bouin's fluid; one or both lobes was then embedded and sectioned, usually completely. Sections were cut at 5µ and 10µ, and the following stains were employed among others:

- Iron haematoxylin and Van Gieson.
- Eosin, iron haematoxylin, and light green (Prenant).
- Ehrlich's haematoxylin and Pasini.
- Mallory's connective-tissue stain.

The Normal Thymus in a 4–6 Weeks Mouse.

In a 4–6 weeks mouse no involution of the thymus has taken place. As in all mammals the bulk of the organ consists of the small close-lying thymus cells which have little cytoplasm (fig. 12, Pl. 7); the nuclei of these stain deeply, and vary in diameter from 3.5–6µ (fig. 1, Pl. 6). A few of these cells in the cortex are dividing mitotically, and a few are pycnotic. The medulla is small; corpuscles of Hassall and cysts can be seen in continuity with the pale reticular cells. The corpuscles of Hassall are not large as in the cat, but have a diameter varying from 20 to 40µ. They consist of more or less concentrically arranged groups of cells, of which the middle ones are frequently degenerating (fig. 2, Pl. 6); some of them contain the remains of polymorphs. The cysts are lined by a more or less irregular layer of reticular cells, which are sometimes ciliated (fig. 3, Pl. 6); the larger ones may have a diameter of 60µ and be 90µ long. Their contents include cell debris, leucocytes, and thymus cells. Blood-vessels are most abundant in the medullary areas. Erythrocytes and polymorphs can be found lying free among the thymus cells. The thymus of the mouse (unlike that
of the cat, fig. 28, Pl. 11) is not divided up into separate lobules by connective-tissue septa; only thin irregular strands from the enclosing fibrous sheath penetrate the organ.

Histological Changes in the X-rayed Thymus.

2 Hours after Irradiation.

No reduction in size has taken place in the thymus, but sections show that pycnosis of the small thymus cells has already begun. Groups containing six to twelve pycnotic nuclei are evenly distributed in the cortex, but rare in the medulla. Mitoses are uncommon, and the corpuscles of Hassall normal.

6 Hours after Irradiation.

This thymus is rather smaller than the last, and well-marked histological changes have occurred. The larger blood-vessels have increased considerably in size, and are crammed with red blood-corpuscles; fresh vascular and fibrous tissue is pushing in at the periphery of the organ. The thymus cells in the medulla are almost all intact, but the cortical ones are degenerating in large numbers. It is important to note that mitoses are rare; there are none among the reticular cells of the medulla with pale nuclei. Active amitotic proliferation, however, is taking place in the cortical strands of fibrous connective tissue (figs. 4, 5, 13, Pls. 6 and 7), and a similar activity can be observed in the connective tissue and smooth muscle in the walls of the blood-vessels.\(^1\) Amitosis takes place frequently by simple median cleavage.

The following types of cells can be found in the inpushing vascular and connective tissue: endothelial cells, red blood-corpuscles and polymorphs belonging to the capillaries, and fibroblasts, some with rounded, pale nuclei containing one or more nucleoli, others whose nuclei are irregular or spindle shaped. The first type of fibroblast is indistinguishable from the 'epithelioid' reticular cell of the medulla. Both actively ingest the nuclei of the degenerating thymus cells; the small thymus

\(^1\) See Discussion.
cells left intact do not appear to be phagocytic (cf. Popoff, 21). As in the normal thymus, corpuscles of Hassall are comparatively rare, and cysts are few and small.

10 1/2 Hours after Irradiation.

At this stage the cortex shows a reduction in size, and is filled with degenerating thymus nuclei (fig. 14, Pl. 7); these are being ingested by connective-tissue cells and polymorphs. The complete disappearance of the outer part of the cortex, and the occurrence of whole areas containing nothing but pycnotic debris shows conclusively that there is no continuous persistent reticulum. Some of the thymus cells in the medulla have degenerated, but the greater number are intact (fig. 16, Pl. 8).

Cysts have become numerous; the diameter of the pluricellular ones varies from 17 to 170 μ. The majority occur in close connexion with vascular tissue, often on the borders of cortical and medullary areas. They are lined by an irregular layer of reticular cells, of which some or all may terminate in a brush of cilia (figs. 6, 7, 15, Pls. 6 and 7). Often the cavities are not round but branching, two or more cysts being connected by diverticula, or by strands of ciliated cells (Fig. 19, Pl. 8). It can be clearly established that the reticular cells lining the cysts are derived from the perithelial connective tissue of the blood-vessels; not only are the staining reactions similar, but actual continuity can be traced (fig. 6, Pl. 6). In many cases cavities have been formed in the thymus through the mass degeneration of the small cells, and have been subsequently lined by the perithelial cells of adjacent blood-vessels. Cysts can be found in process of development which lack a complete border of cells (fig. 6, Pl. 6). Many of the cells lining the cysts are cleaving amitotically; there are no mitoses among these cells, which are frequently arranged in pairs round the border of the cavities, indicating recent cell division (fig. 7, Pl. 6). Among the reticular

1 These cysts resemble those found, not uncommonly, in the normal thymus, but irradiation has led to an increase in their number and size. Cf. figs. 3, 6, 7, Pl. 6.
elements of the medulla in general, mitoses are as rare as in the normal thymus.

Sometimes the degeneration of a blood-vessel brings about cyst formation; such cysts, bordered by the perithelial cells (which develop conspicuous brushes of cilia), contain degenerating erythrocytes and polymorphs in addition to collagenous debris (fig. 18, Pl. 8).

Cysts of another type may arise through the coalescence of two or more unicellular cysts, which are not uncommon. Others again may be formed by the degeneration of the central mass of epithelioid cells in a corpuscle (fig. 17, Pl. 8). Although the cysts may be formed in various ways they all alike are the product of connective-tissue cells;¹ usually they contain leucocytes and degenerating masses (fig. 15, Pl. 7). It is interesting to note the close similarity between these cysts in the irradiated thymus of the mouse and those described and illustrated by Dustin (5, 6) in the thymus of the axolotl and the frog.

Corpuscles of Hassall are more numerous than in the normal thymus; many have erythrocytes or polymorphs in their degenerating centres, indicating that they have arisen round the remains of a blood-vessel (fig. 8, Pl. 6; cf. Jordan, 20). The reticular cells of the corpuscles are indistinguishable from normal connective-tissue cells, such as can be seen invading the organ from the sheath, and also forming the adventitia of the blood-vessels (cf. fig. 24, Pl. 10, from another thymus).

18 Hours after Irradiation.

In this specimen, but not in any other, the two thymus lobes have partially fused; the whole organ is greatly reduced, and

¹ Drew ("Experimental Metaplasia", J. Exp. Zool., 10) describes the formation of columnar ciliated epithelium from fibroblasts in Pecten, as the result of implanting pieces of ovary into the adductor muscle. The implant degenerates leaving a closed cyst lined by amitotically dividing fibroblasts; after 21–32 days those revert to an embryonic type, and afterwards become converted into columnar ciliated epithelium, which may persist for at least four months. The histological changes can be clearly followed, and they afford a good illustration of the potentialities of connective-tissue cells.
corresponds roughly in size to the medullary region of the normal thymus (fig. 20, Pl. 9). The large masses of nuclear debris filling the cortex in the last stage have almost completely disappeared. The edge of the section is occupied by branching connective-tissue cells, among which lie pycnotic and intact thymus nuclei (fig. 9, Pl. 6). The latter are easily distinguishable from those of the connective-tissue elements. Thymus cells are more common in the medulla, which occupies the greater part of the section, than at the edge, and pycnoses are rare among them. The destruction of thymus cells in the cortex and their persistence in the medulla has led to the apparent 'inversion' of the two regions noted by other writers. The corpuscles of Hassall and cysts are similar to those seen in the preceding stage.

28 Hours after Irradiation.

As in the last thymus, the normal cortex has disappeared, but rather more small thymus cells have escaped injury. Fresh fibrous connective tissue, accompanied by capillaries, is penetrating the organ at all points from the surrounding sheath, and ingesting the few remaining pycnotic nuclei. Large blood-vessels and numerous corpuscles of Hassall can be seen, but cysts are rare, probably because resorption of the debris is nearly complete. Occasional mitoses are occurring which are too large to be those of thymus cells; the latter are not dividing.

41-67 Hours after Irradiation.

During this period the thymus does not undergo any conspicuous change. Pycnotic nuclei are rare. Connective-tissue elements continue to invade the organ; they form an irregular lighter-staining border and are pushing farther in among the persisting thymus cells; some of these connective-tissue cells at the edge are dividing by mitosis.

3 Days 4 Hours after Irradiation.

This thymus shows no increase in size; the peripheral region remains lighter-staining than the medulla, but the distinction between the two is not so marked as before, owing to the centri-
petal growth of the connective tissue. Corpuscles of Hassall can be found near the edge of the thymus apparently derived from the connective tissue which penetrated the organ after irradiation. The first indications of regeneration can be found at this stage; the nuclei of the thymus cells scattered among the invading connective tissue at the edge have increased in size till the majority resemble the largest found in the normal thymus (fig. 10, Pl. 6; cf. figs. 1 and 9, Pl. 6). These nuclei do not stain as deeply with haematoxylin as those in the medulla, but can still be distinguished by their dark chromatin masses, from the adjacent pale nuclei belonging to the connective tissue. Mitoses corresponding in size to these nuclei are fairly common round the edge; similar mitoses, and also smaller ones, can be seen in the medulla, where no general enlargement of the thymus nuclei has taken place.

3 Days 19 Hours after Irradiation.

Thymus cells of the large type previously described have increased in number at the edge, and they continue to divide. Connective tissue still forms a considerable proportion of the organ, but the increase in the thymus cells tends to carry it inwards to the medulla. Corpuscles of Hassall are numerous, many of them occurring as before in the peripheral zone of the thymus; they are not confined to the medulla as in the normal organ.

4 Days 18 Hours after Irradiation.

In this thymus regeneration has proceeded far enough for a partial differentiation into a true cortex and medulla to have taken place (fig. 22, Pl. 9). This can be seen in both transverse and horizontal sections. It has been brought about by the centripetal movement of the connective tissue and vascular elements, and by the proliferation of persistent thymus cells, particularly those of the cortex. The nuclei of the thymus cells

1 Jaffe (17, fig. 1, Pl. 17) illustrates the formation of a corpuscle of Hassall in the peripheral region of a piece of the thymus of a guinea-pig (after autoplastic grafting) in an area extensively invaded by connective tissue.
vary in size, but average larger than those in the normal organ. Mitoses are numerous in the cortex, but uncommon in the medulla. Differentiation of the cortex is farther advanced. Large cysts, some ciliated, are conspicuous at this stage; they appear to be derived from regressing vascular tissue; corpuscles of Hassall occur in the reconstituted medulla, but can no longer be found in the cortex.

5 Days 18 Hours after Irradiation.

This thymus, though only a day older than the last, is histologically reconstructed (fig. 23, Pl. 9); it has increased in size very considerably, and the cortical and medullary regions are well marked. No large blood-vessels can be seen at this stage. Mitoses are common in the cortex, where the thymus nuclei are still large. The connective-tissue and blood-vascular elements which invaded the organ immediately after irradiation are now regressing in the medullary regions. Ciliated cysts and corpuscles of Hassall are being formed from degenerating vascular tissue (figs. 25 and 26, Pl. 10); they are larger and more numerous than in the normal thymus medulla. Thin strands of fresh connective tissue can be seen pushing into the regenerated cortex (fig. 25, Pl. 10), but this invasion is far scantier than the last, and less conspicuous owing to the continued proliferation of thymus cells.

11½ Days after Irradiation.

This thymus resembles the normal organ more nearly than the last, since both corpuscles of Hassall and cysts are rare and there is little fibrous or vascular tissue invading the cortex (fig. 27, Pl. 10). The regression of the connective and blood-vascular tissue prominent in the X-rayed thymus has been rapid; the corpuscles of Hassall and cysts conspicuous in the newly regenerating thymus (figs. 23 and 25, Pls. 9 and 10) represented late stages of the process. The degenerated tissue has been replaced in the medulla by the fresh connective and blood-vascular elements which were pushing into the cortex in the last stage, these having been carried centripetally inwards like their predecessors. Mitoses are very numerous; the thymus nuclei
vary in size, but approximate to those in the normal organ. Scattered pycnoses are not uncommon.

**Discussion.**

The above account of degenerative changes in the irradiated thymus resembles that of Regaud and Crémieu (22), but although these writers describe the hypertrophy both of corpuscles of Hassall and also of connective-tissue and blood-vascular elements, they continue to regard the reticular cells forming the corpuscles as epithelial, though offering no positive evidence in support of this theory.\(^1\) Jolly, in his 'Traité technique d'Hématologie' (published 1923), adopts a similar view; he classes the thymus as a lymph-organ and explicitly rejects the possibility of a vascular or connective-tissue origin for the corpuscles of Hassall; he describes and illustrates cysts arising through the confluence of several corpuscles, and regards these formations also as products of reticular epithelial cells, and centres of their involution.

Winiwarter, in a critical article on the histology of the thymus (24), concludes for various reasons that the corpuscles of Hassall are of epithelial origin, though he agrees with Dustin that the thymus cells are not lymphocytes.

Jordan and Horsley (20), however, in a recently published note on the thymus of man and the rabbit, state that 'concentric corpuscles arise chiefly from hypertrophied endothelial cells of precapillary arterioles and the immediately investing reticulum cells'. These authors have found structures closely comparable to Hassall's corpuscles in atrophic subcutaneous lymph-nodes in the rabbit.

As described above the present material offers conclusive evidence of the development of both corpuscles of Hassall and cysts from the blood-vessels and their investing connective tissue (fig. 8, Pl. 6; figs. 18, 19, Pl. 8; fig. 21, Pl. 9; figs. 24, 26, Pl. 10). It is probable that the periodic degeneration of blood-

\(^1\) The present writer has not seen a copy of the more detailed account of this work published by Crémieu (4); portions of it have been quoted and criticized by Dustin (6).
vessels in the normal thymus to form corpuscles of Hassall is the cause of the numbers of free erythrocytes found not infrequently among the thymus cells.

Irradiation has the effect of increasing considerably the numbers of cysts and concentric corpuscles, but the process by which these develop appears to be perfectly normal. The cavity of a cyst may be that of a blood-vessel which has broken down, or a space filled with degenerating thymus cells; in either case it becomes lined by perithelial connective tissue, the cells of which are not damaged by the X-rays. Probably the majority of the smaller blood-vessels in the thymus undergo change, but their loss is made good by the fresh blood-vascular tissue which simultaneously invades the organ from the sheath. The cysts are transitory structures, and have mostly disappeared by the time resorption of the thymus cell debris is complete. A new series of cysts and large corpuscles of Hassall arises later (4-6 days after irradiation, fig. 23, Pl. 9; fig. 25, Pl. 10), when the cortex of thymus cells has been reconstituted, coincidently with the regression of the additional vascular tissue which penetrated the organ during its involution. This new series becomes resorbed in its turn, and six days later corpuscles of Hassall and cysts are no more numerous than in the normal thymus.

The re-establishment of the medulla can be clearly traced in this material (figs. 22, 23, Pl. 9; fig. 25, Pl. 10); during the intense proliferation of thymus cells in the cortex, the connective-tissue and blood-vascular elements which invaded it after irradiation move centripetally, becoming aggregated in small areas near the middle of the organ. No fresh proliferation of epithelial elements can be distinguished during medulla formation, as would be expected from the lympho-epithelial theory of the thymus. The reconstitution of the medulla in the regenerating organ by the ingrowth of mesodermal cells suggests strongly that these areas are so formed in the embryonic thymus, and that they should not be regarded as condensations of the original epithelium.

Proliferation of the connective tissue and walls of the blood-vessels accompanied by degeneration and scarcity of the thymus
cells, and the contrasting condition, have been previously described and discussed by Dustin, as seasonal variations in the frog, and also under experimental conditions in both the frog and the mouse (6, 8, and 9).\footnote{Dustin (8 and 9): these papers on the thymus of the mouse are short and do not include illustrations.}

Although the derivation of both corpuscles and cysts from the blood-vascular tissue can be clearly traced, it is impossible to write with complete certainty of the exact part played in these transitions by endothelium, connective tissue, and smooth muscle. Dustin (5) has described cysts in the thymus of the axolotl which are similar in almost every detail to those in the thymus of the mouse; their formation and the nature of the cells from which they arise is discussed at some length, and the conclusion is reached that these cells, which may divide amitotically, all belong to the connective-tissue group. Dustin regards the cells adjacent to the blood-vessels in the frog's thymus as of the same type; these, too, undergo irregular cleavage after autoplastic grafting (6).

Carleton and Florey (3) in a recent paper discuss the difficulty of distinguishing smooth muscle from connective tissue in the mammalian lacteal; they state that nuclear lines and cleavages (present in cells which have ceased to divide mitotically and suggestive at first sight of amitosis)\footnote{It is recognized that the question of amitosis is controversial, and that it is almost impossible to obtain conclusive evidence of its occurrence. In the mouse thymus after irradiation the increase in the connective-tissue elements, within a few hours, is so conspicuous, that a consideration of the appearance of the nuclei and the absence of mitotic figures (especially among the perithelial cells lining the newly formed cysts), leads almost inevitably to the inference that cell division is taking place amitotically.} are characteristic of smooth-muscle nuclei and serve when present to distinguish them from those of connective tissue.

In the thymus both kinds of tissue would normally be present in the walls of the blood-vessels, and might be expected to proliferate under the influence of the same stimulus; it is noticeable that mitoses are absent among the cells which form the cysts at a time when they are increasing rapidly in number. Lines of
cleavage and other indications of amitotic divisions can be clearly seen in the nuclei of these cells (figs. 6 and 7, Pl. 6; cf. fig. 3, Pl. 6), which appear to be all of one type though some develop cilia and others do not. This capacity to develop cilia renders it unlikely that they are specialized contractile cells, though some of the nuclei in process of cleavage resemble the smooth-muscle nuclei depicted by Carleton.

On the whole the evidence from the mouse supports Dustin's conclusions that the cells forming the cysts belong to the connective-tissue group, although it is possible that there are smooth-muscle cells among them which have reacted similarly to the irradiation. The endothelial cells appear to degenerate when the cyst is formed actually in the cavity of a blood-vessel (figs. 18, 19, Pl. 8; fig. 26, Pl. 10), and their nuclei can sometimes be found in the lumen.

Jaffe (17) mentions rapid amitotic division of 'reticulum epithelial' cells four days after autoplastic grafting in the thymus of the guinea-pig. In another paper (16) he notes proliferation of the 'reticular' cells, which are at first spindle-shaped but become epithelioid—an indication of their connective-tissue nature.

The absence of a continuous epithelial reticulum, such as is postulated by advocates of the lympho-epithelial theory, can be clearly demonstrated in sections through a thymus fixed ten hours after irradiation, when the mass of cortical thymus cells are degenerating; there are so few large cells in the cortex similar to those in the medulla (which are untouched by the X-rays), that they cannot possibly be said to constitute a reticulum (fig. 16, Pl. 8). The cells forming a border round the reduced thymus, which have been described by other observers as epithelial or epithelioid and have been erroneously regarded as a condensation of pre-existing reticular cells, are fibroblasts. They can be seen shortly after irradiation pushing in from the surrounding sheath and resorbing the pycnotic thymus nuclei (fig. 4, Pl. 6; fig. 18, Pl. 7; fig. 21, Pl. 9). Jaffe and others (14, 16, 17, 21) appear to have confused these connective-tissue cells and the enlarged 'epithelioid' thymus cells which by repeated
division reconstitute the cortex in the regenerated thymus; just before their period of mitotic activity the nuclei of these cells become enlarged till they are comparable to those of the connective-tissue cells among which they lie (fig. 10, Pl. 6). Similar nuclei having a diameter larger than the average can be found among the small thymus cells of the normal organ. If these latter are the products of the original epithelium of the pharynx, their regeneration from larger 'epithelioid' cells may be considered a repetition of the embryonic process.

This experimental study of the histology of the thymus fails completely to support the lympho-epithelial theory of its structure, commonly expounded in the text-books. No epithelial elements can be distinguished in the thymus, other than the larger thymus cells. Both corpuscles of Hassall and ciliated cysts are derived from the blood-vessels and accompanying connective-tissue. It will be seen below that these conclusions are reinforced by a study of sections through tissue cultures of cat thymus.

**Observations on Tissue Cultures of the Thymus of the Cat.**

The changes occurring in pieces of thymus taken from advanced cat embryos and cultivated in plasma, were studied in order to throw light on the nature of the thymus reticulum and the corpuscles of Hassall.

Pieces of thymus, 1 mm. or less in diameter and about 0.5 mm. thick, were incubated for 1–13 days in maternal plasma, without subculturing or the addition of embryo extract. The method employed was that of tube cultures previously described by Carleton (2), but a few cover-slip preparations were also made for comparison with the others. At intervals of 24 hours or more the implants were fixed in Bouin's fluid, diluted with two parts of distilled water to one of the fixative; they were subsequently embedded in paraffin and sectioned at 6μ. Mitoses were occurring at the time of fixation in all cultures included in this description (fig. 31, Pl. 11). Control pieces of thymus were similarly fixed without incubation.
An examination of sections through the latter showed that the thymus was of the adult type and contained conspicuous corpuscles of Hassall, all lying in the medullary region of the lobules; the latter were small and separated by connective-tissue septa containing capillaries. In the medullary regions reticular cells were sparsely distributed, and the blood-vessels small and inconspicuous (fig. 28, Pl. 11).

After 24 hours incubation conspicuous changes have occurred in the tissue; many of the small thymus cells are degenerating, including the few which have emigrated into the surrounding plasma. Connective tissue has proliferated until it forms an almost complete fibrous sheath round the implant; mitoses are occurring among the cells with large nuclei, but the spindle-shaped cells in the walls of the blood-vessels appear to be cleaving amitotically, as in the X-rayed thymus (fig. 11, Pl. 6). Groups of reticular cells, indistinguishable from large connective-tissue fibroblasts, can be seen in the medulla surrounding corpuscles of Hassall; such cells are aggregated together unlike those in the normal medulla, with relatively few thymus cells lying together and those pycnotic (fig. 29, Pl. 11). The reticular cells of the medulla and the connective-tissue cells derived from the interlobular septa are both engaged in phagocytosis of the degenerating thymus cells. Corpuscles of Hassall only occur in the medullary region.

Sections of older cultures show a continuation of the process initiated 24 hours after implantation, multiplication of fibrous connective tissue and pycnosis of the small thymus cells. The connective tissue forms a complete sheath round the implant, and a few cells branch out into the surrounding plasma (fig. 32, Pl. 12). Three days after implantation corpuscles of Hassall can be seen at the edge of the tissue on either side a degenerating central region containing pycnotic thymus cells. Their position clearly indicates that they have been formed since the tissue was incubated; in the normal thymus the lobules are small and the corpuscles of Hassall always situated in the medulla. Cultivation in plasma has brought about a reversal of the normal dark- and light-staining cortical and medullary regions, owing
to the proliferation of connective tissue round the edge. The corpuscles of Hassall embedded in this connective tissue, and continuous with it, are similar in all respects to normal ones (fig. 33, Pl. 12). In a 5-day culture corpuscles of Hassall can be found in isolated bays projecting from the implant into the plasma. In the two oldest cultures (18 days) corpuscles of Hassall persist in connective tissue, but the small thymus cells have all degenerated.

The above account does not agree with that recently published by Popoff (21), who gives neither illustrations nor complete details of his technique. He distinguishes certain reticular cells as epithelial, since, unlike connective-tissue cells, they do not store carmine and vital dyes. My own tissue cultures were made before Popoff’s paper was published, and I have not repeated the latter’s experiment of adding carmine to the plasma. The sections through the cultures of the cat’s thymus show plainly that it is connective tissue that forms a border round the implant and proliferates in the medulla, and I am unable to accept Popoff’s statement that these cells are epithelial.¹

This ‘epithelium’ he describes as forming strands and islets, sometimes showing intercellular and intracellular vacuoles; such vacuoles are common in the connective tissue of my cultures.

Corpuscles of Hassall are normally small in the rabbit thymus, which was the material used by Popoff; their formation is more readily observable in the thymus of the cat.

The conclusions which can be drawn from a study of cultures of the cat’s thymus may be summarized as follows: the reticular cells of the medulla resemble the large fibroblasts of the interlobular connective tissue and behave similarly; the cells forming a border round the implant are also connective-tissue cells, and it is impossible to distinguish any epithelial

¹ It is possible that the non-phagocytic cells which Popoff believes to belong to an epithelial reticulum are large thymus cells, such as occur normally. The addition of the bone marrow or embryo extract to the culture may have caused enlargement of the peripheral thymus cells of the implant and a consequent increase in the epithelioid type; cf. the enlargement of thymus cells in the thymus regenerating after irradiation.
reticulum. New corpuscles of Hassall arise at the edge of the cultures, and their outer cells are continuous with those of the connective tissue which surrounds them; these corpuscles of Hassall may persist after all the thymus cells have degenerated.

I wish to express my thanks to Dr. H. M. Carleton, without whose assistance I should have been unable to make these tissue cultures. The work was done in the Department of Physiology at Oxford.

Observations on the Effect of X-rays on the Lymph Nodes of the Mouse.

The lymph nodes are much less affected by the action of X-rays than is the thymus. Six hours after irradiation those adjacent to the thymus contain a number of pycnotic nuclei, scattered fairly evenly throughout their tissue; these are being phagocyted by neighbouring cells. Seventeen hours after irradiation pycnotic nuclei are rare; owing to the destruction of a number of lymphocytes the tissue of the node appears less dense than usual, but there are no conspicuous changes. The reticular tissue of the capsule shows no sign of proliferation; mitoses are rare. Twenty-four hours later mitoses of the large type are fairly common, and the lymph node has regained its normal appearance.

Although occasional groups of undegenerating reticular cells can be found both in the normal and in the irradiated lymph nodes of the mouse, corpuscles similar to those in the thymus do not arise, as they are said to do, in the rabbit (20). It is possible that their absence is connected with the meagre vascularization of the lymph node, as well as with its different structure and mode of development.

Summary.

1. A number of male mice 4–6 weeks old were subjected to irradiation and killed at intervals of 2 hours to 12 days after the treatment. A detailed study was made of the resulting histological changes in the thymus. Immediately after irradiation most of the small thymus cells comprising the cortex of the
organ degenerate. Simultaneously the existing connective-tissue elements in the thymus proliferate amitotically, and fresh connective tissue and blood-vessels invade the periphery of the organ from its sheath. Numerous ciliated cysts develop in the thymus, derived from the blood-vessels and their adjacent connective tissue; the majority of these disappear 28 hours after irradiation when resorption of the cell debris is complete.

Regeneration is initiated 3 days after irradiation by an enlargement of the nuclei of the thymus cells persisting at the edge of the organ; these, by repeated division, reconstitute the cortex. Fresh medullary areas are formed by the aggregation of the connective tissue and blood-vessels which are carried centripetally during regeneration; most of this tissue degenerates, giving rise to a fresh series of corpuscles of Hassall and cysts. These are transitory structures and have almost all disappeared from the medulla by the time histological regeneration of the thymus is complete, 12 days after irradiation.

2. Small pieces of thymus from an advanced cat embryo were cultivated in normal maternal plasma; the implants were fixed after various periods of incubation, embedded in paraffin and sectioned. The histological changes after incubation are similar to those in the irradiated thymus, before the latter begins to regenerate; they include pycnosis of the small thymus cells and proliferation of fibrous connective tissue. This forms a border round the implant in which fresh corpuscles of Hassall develop, their outer cells being continuous with the fibrous connective tissue.

3. The significance of these results with regard to the normal histology of the thymus is discussed, as are the conclusions of other workers who have studied similar changes in the thymus after experimental involution. Both these experimental studies of the histology of the thymus indicate the complete absence of an epithelial reticulum, such as is frequently postulated; the corpuscles of Hassall, and cysts generally associated with it, are derived from the blood vascular and accompanying connective tissue.

4. A note is added on the reaction of typical lymph nodes to
the irradiation; this is slight in comparison with that of the thymus.

I wish to express my thanks to Professor J. P. Hill, F.R.S., for his advice, and to Dr. A. S. Parkes for supplying me with the irradiated mice. With the exception of the tissue cultures, the work was carried out in the Department of Anatomy and Embryology, University College, London.

Dec. 1927.

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**ADDENDUM.**

Since this paper was completed, a further paper has been published by Popoff:

"The Histogenesis of the Thymus as shown by Tissue Cultures", 'Arch. f. exp. Zellforsch.', 4, 1927.

This is a detailed account of the work referred to above (21). Lack of space prevents a full criticism, but the following points may be noted, in addition to previous comments.

The author assumes that the lympho-epithelial theory of the nature of the thymus has been proved by the embryological studies of Maximow and Hammar; actually these have been severely criticized and not confirmed by later investigators such as Dustin. The latter has accumulated a formidable body of evidence against the lympho-epithelial theory, which cannot lightly be disregarded. Popoff, however, assumes the existence of an epithelial thymus reticulum, and endeavours to confirm Maximow's theory by a study of tissue cultures; previous investigators in this field have failed to reach agreement.
Maximow’s cover-slip technique was employed, and cultures of rabbit thymus were examined in vitro, and subsequently fixed, sectioned, and stained with haematoxylin and eosin-azur II. Popoff presents a detailed analysis of the constituent cells of the cultures, including those related to the blood-vessels, which are clearly mesodermal. Curiously enough, he makes no mention among these of the large connective-tissue cells, with pale oval vesicular nuclei, which are prominent in my own cultures; reference to Popoff’s description and figures, particularly figs. 1 and 4, indicates that these cells are described as epithelial, on the ground that they do not actively ingest carmine. Cells morphologically similar to these, with the characteristic staining reactions of connective tissue, occur in the normal thymus, both in the interlobular clefts and medullary regions. After 24 hours cultivation in plasma these cells are far more prominent (figs. 29 and 30), considerable proliferation, not condensation, having taken place. No difference can be distinguished between those forming isolated medullary islands, often round corpuscles of Hassall, and others belonging to the interlobular clefts, which are unmistakably connective-tissue cells.

Figs. 32 and 33 in this paper afford evidence that the prominent cells with round vesicular nuclei, which other writers have described as epithelioid, are large connective-tissue fibroblasts, which participate in the formation of the characteristic corpuscles of Hassall. It appears to the present writer that a study of tissue cultures of mammalian thymus not only lends no support to the lympho-epithelial theory of the thymus, but renders it no longer tenable.

EXPLANATION OF PLATES 6–12.

All illustrations are from haematoxylin-stained sections, unless it is otherwise stated. The drawings on Pl. 6 were all made to the same scale with the aid of a camera lucida. I am indebted to Mr. F. Pittock of the Department of Anatomy, University College, for the remaining illustrations, which are untouched microphotographs. Figs. 1–10 and 12–26 inclusive are taken