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THE FORMATION OF THYMUS CYSTS AND HASSALL'S CORPUSCLES IN MODEL EXPERIMENTS

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In previous experiments [4, 5, 6, 7, 11, 12] we have shown that certain processes under stress, especially when they are accompanied by tissue proliferation, may cause the thymus to enter different histological reactions which manifest themselves with multiplication of Hassall's corpuscles, appearance of cysts, accumulation in both of a PAS positive substance, and mast cell reactions. Concurrent with all these phenomena is a partial evacuation of the thymus. The experimental results seemed to reveal both the Hassall's corpuscles and the columnar epithelium-lined cysts as non-preformed structures of the thymus which arise in it owing to certain functions under stress and may, moreover, disappear after the stress has ceased.

It seemed therefore interesting to study the nature of Hassall's corpuscles and cysts, in order to throw some light on certain histophysiological problems in connection with the thymus.

The views concerning Hassall's corpuscles are different; some authors regard them as degenerative formations [1, 2], others as remnants of the preformed epithelial thymic primordia, others again as the result of epithelial keratinization [8, 9, 10]. The latter view (Norris, cited in 10) implies that the epithelial thymus may be of ectodermal rather than entodermal origin. Whether or not the process of keratinization is a genuine one is undecided. In our own experiments the temporary appearance of Hassall's corpuscles and the subsequent disappearance of their greater part seems to disprove the degenerative character.

In a study reviewing many aspects of the problem [10], Tesseraux makes repeated references to cysts, mainly as concomitants of pathological states. According to our experience, however, cysts may occur within physiological limits in several different states, for example under the effect of whole body X-ray irradiation or under Cortisone treatment, whereby a secretory action of the epithelial lining of the cyst is clearly observable. The secretion itself consists of a PAS-positive substance of neutral mucopolysaccharide character.

It may be irrelevant whether the appearance of cysts and Hassall's corpuscles in connection with thymic function is regarded as a pathological or a

normal process; what matters is to account for the elements taking part in it. Our present series of model experiments has been carried out to explain the problem of origin of these structures.

Material and method

The experiments were made by means of the transplantation method. The trachea and oesophagus was removed from embryonal or newborn rats under sterile conditions, minced, and placed into physiological salt solution. If the tissue hadn't been obtained from an embryo, but a newborn, we used it with 50,000 units of penicillin to the solution, saturating it with chloramphenicol, to prevent infection. A total of 25 young animals were used, as donors.

The prepared tissue pieces were implanted into the spleens of adult rats, close to the surface underneath the capsule. The transplantations were performed with oesophageal or tracheal tissue or with a combination of the two. The grafting was homologous, to animals selected at random, or syngenesiological, to the donors' own mothers.

The recipients were 52 adult rats of the Wistar strain. The results were controlled between the 2nd and the 25th day following operation. The spleen was removed, the transplantation area fixed in Carnoys fluid and embedded in methyl-benzoate-celloidin-paraffin. Sections were cut in 8 different levels and dyed according to the Tri PAS method.

Results

1. *Oesophagus*

Two days after homologous transplantation, the epithelium of the implanted organ was intact, but the connective tissue of the adventitia together with the submucosa and the tunica muscularis became loose an the cells were migrating. The epithelial lumina were surrounded by loose connective tissue with hardly distinguishable muscle cells in it. More distinct, owing probably to a reaction of the host organ, was the demarcation of the loose substance on the surface against the spleen. The spleen showed numerous macrophages containing blood pigment in the areas surrounding the graft. Slight PAS positivity, with a slight tendency for keratinization, was observable on the luminar side of the epithelial surface. An orangeophilic substance abounding in granules filled the whole of the lumen. There were more or less circular rings of epithelium round the greater part of the lumen (Fig. 2).

Two days later the epithelial connective tissue was slightly condensed around the lumina but remained loose elsewhere (Fig. 3). This was mainly the case when the transplantate had been engrafted near to the spleen surface instead of its depth. The surface of the spread-out epithelium showed the presence of a partly orangeophilic partly PAS-positive substance. The luminar surface itself was covered by vigorously developing orangeophilic layers.

Six to seven days after transplantation, the loose connective tissue became less and condensed around the epithelial cyst. On the interior luminar surface above the Malpighi layer a PAS-positive secretion was visible; at the same time a few cells in the deeper layer also displayed PAS-positivity. The keratinization process took a more pronounced character (Fig. 5).

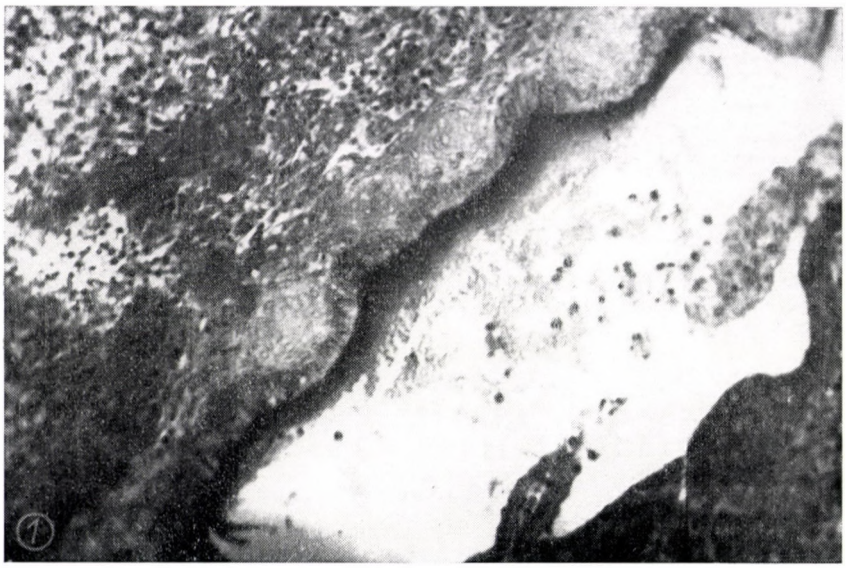

Fig. 1. Homologous tracheal graft after two days. Incipient secretion of PAS-positive substance. Tri PAS (×100)

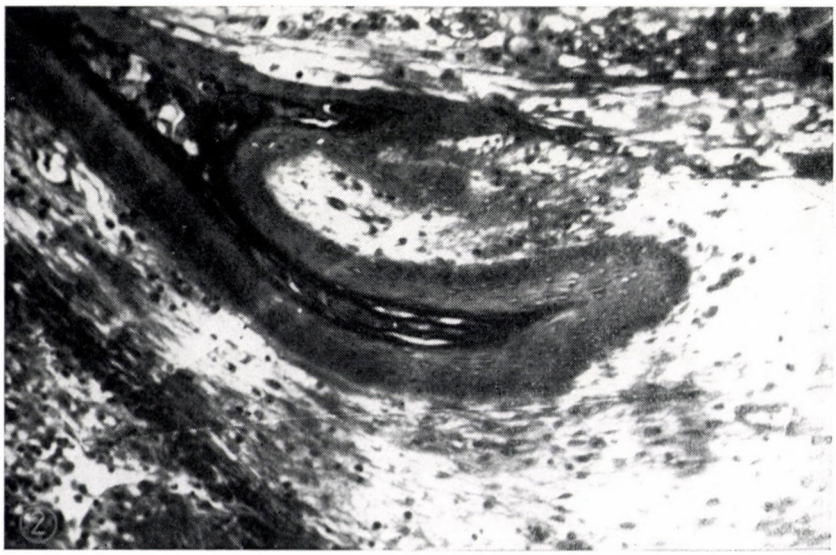

Fig. 2. Homologous oesophageal graft after two days. Epithelial islet, with keratinization on luminar surface. Tri PAS (×100)

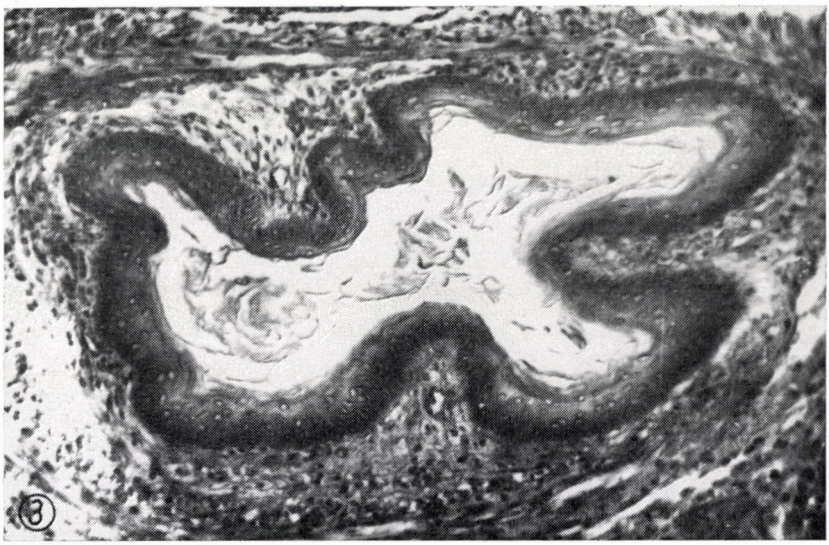

Fig. 3. Homologous oesophageal graft after four days. Progredient keratinization. Tri PAS (×100)

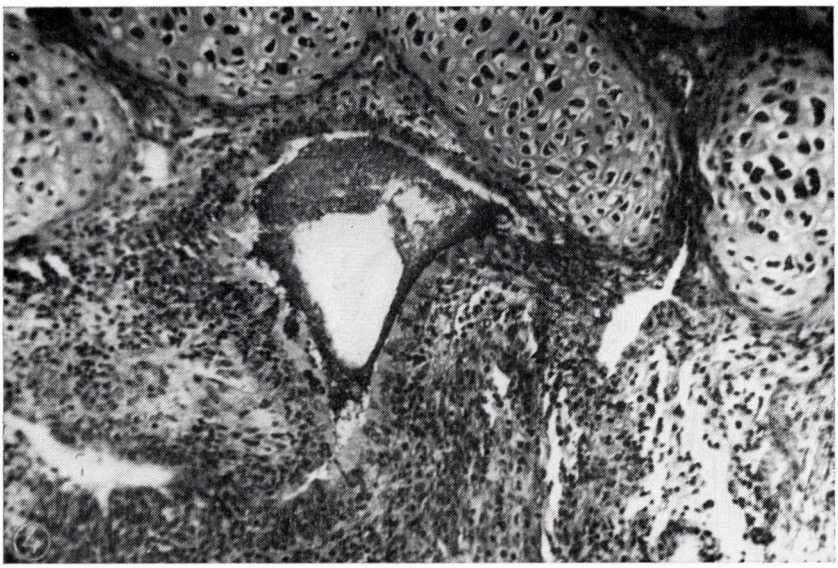

Fig. 4. Homologous tracheal graft after six days. Strongly PAS-positive cartilage cells. PAS-positive secretion in the small cyst lumen. Tri PAS (×100)

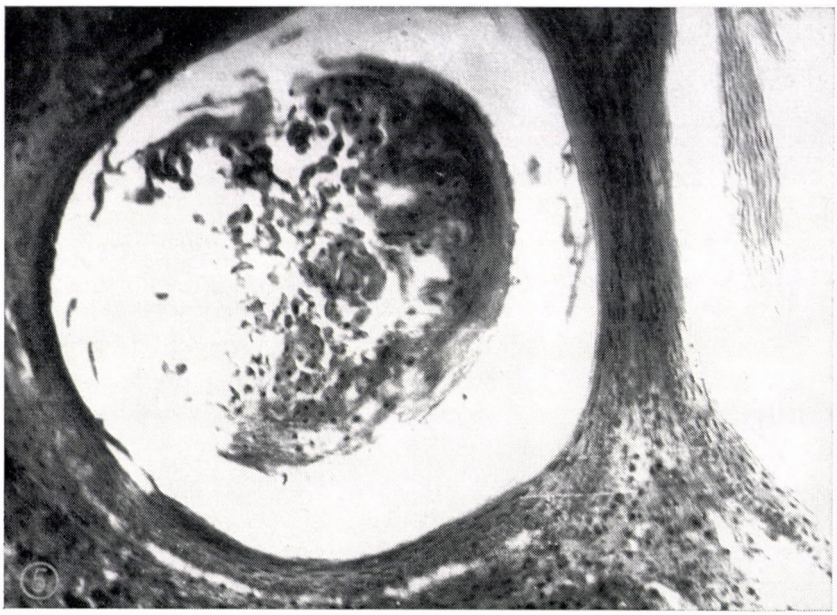

Fig. 5. Syngenesiologic oesophageal graft after seven days. Substance inside lumen partly orangeophilic partly PAS-positive. Secretion-like PAS-positive mass on epithelial surface. Tri PAS (×100)

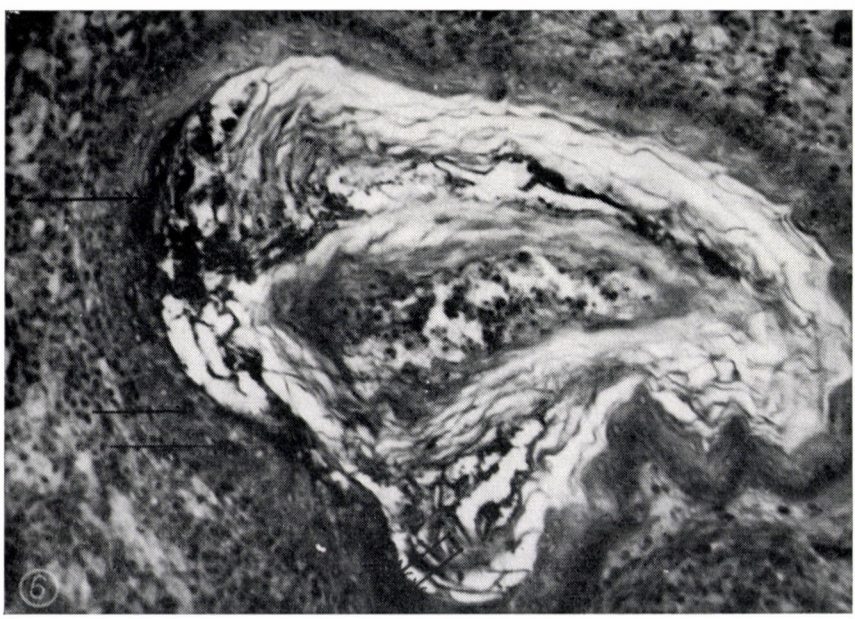

Fig. 6. Homologous oesophageal graft after eleven days. Most of the lumen-filling substance and a proportion of the lamellae are PAS-positive. In the Malpighi layer many PAS-positive cells (plain arrow) and a PAS-positive mass on the epithelial surface (double arrow). Tri PAS (×100)

After 11 days nearly the whole of the upper Malpighi layer was transformed into PAS-positive cells. Part of the superficial cells were secretory, whereas the rest were detached in the form of keratinized PAS-positive lamellae (Fig. 6). Elsewhere the structure was unimpaired; there were no degenerative symptoms.

After 20 days the epithelial structure in the homologous transplantate was slightly loosened, the cells showed a moderate degree of destruction but

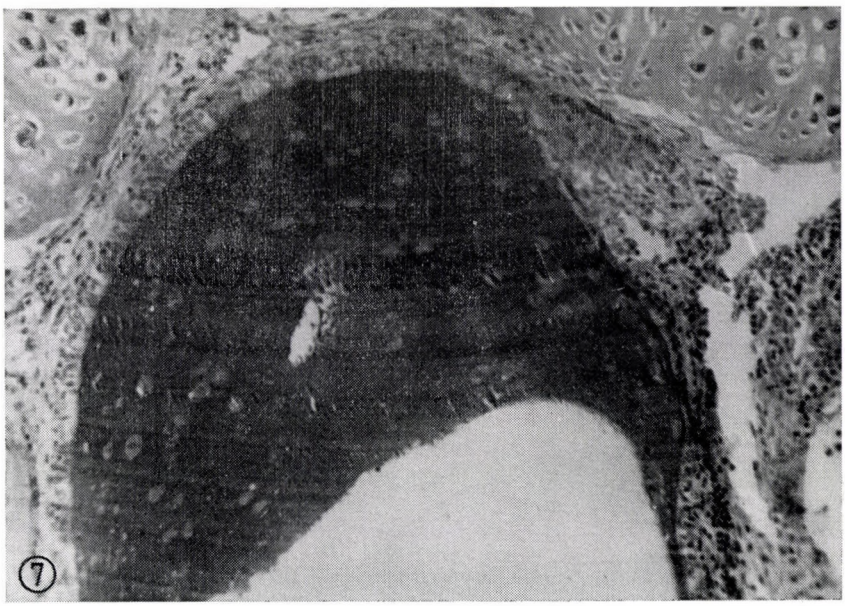

Fig. 7. Homologous tracheal graft after 25 days. Much secretion in the cyst, with some detached cells. Tri PAS (×100)

the presence of PAS-positive lamellae, even of epithelial cells, was still noted. In some cases there was no destruction, and PAS-positivity became increasingly pronounced.

2. *Trachea*

Two days following transplantation the picture was similar to that of the oesophageal grafts after the same period. The adventitial substance was loose, the cells were migrating, the epithelial cysts were sorrounded by loose connective tissue. The epithelium intensively discharged a PAS-positive substance; the secretion filled the cyst whose connection with the cells was clearly visible (Fig. 1). Secretory activity was similar to that of goblet cells.

In 6- to 8-day preparations the amount of connective tissue diminished and most of it had condensed around the epithelium where the cartilage was well observable. An intensely PAS-positive substance had accumulated in the cytoplasm of cartilage cells; the transition from marginal cartilage to connective tissue was gradual. The epithelium displayed intensive secretion; in the lumen there was a considerable amount of PAS-positive substance showing a close relationship with the cells (Fig. 4).

On the 20th day a more or less pronounced destruction of the epithelial substance was observed in homologous transplantates but PAS-positivity persisted to a certain degree. In other cases the structure was intact, discharging much PAS-positive secretion (Fig. 7).

3. *Combined oesophageal and tracheal graft*

The result was similar to that in the case of either oesophagus or trachea alone. In the three experimental groups there was no difference during the early period of observation between syngenesiological and homologous grafts but later the homologous showed a moderate degree of destruction. However, since not all preparations of one and the same age displayed destruction, we saw no reason for limiting the validity of our conclusions.

Discussion

As already mentioned, the experiments were intended to provide an explanation for the development of Hassall's corpuscles and thymus cysts. In our choice of the trachea and the oesophagus as experimental models we have been led by the consideration that, since these organs, like the thymus itself, develop from the entodermal primordia of the foregut, their epithelium probably had the same potential as that of the thymus. The results have answered expectations and we succeeded in establishing some facts concerning the origin of Hassall's corpuscles and thymus cysts.

As to the former, the structure developing under the influence of the implanted piece of oesophagus, even though it cannot be identified as a genuine Hassall's corpuscle, bears a few characteristic marks of it. The upper epithelial layers constitute concentrical lamellae which scale off and enter the lumen, sometimes filling it entirely. Outwardly the lamellae show such a close resemblance to a keratinous tissue that, unless one remembers have transplanted an oesophagus, one can easily take them for a multi-layered horny squamous epithelium. Obviously, when the oesophagus is placed in an alien environment, its otherwise non-keratinizing layers of entodermal epithelium tend to undergo keratinization or some similar process. Therefore the morphological picture

alone, namely the presence of concentrical lamellae in Hassall's corpuscles, does not furnish an adequate ground for the conclusions that the thymus is an organ of ectodermal origin.

As we have seen, very soon after the transplantation the concentrical system of lamellae displays a slight PAS-positivity which so increases in the course

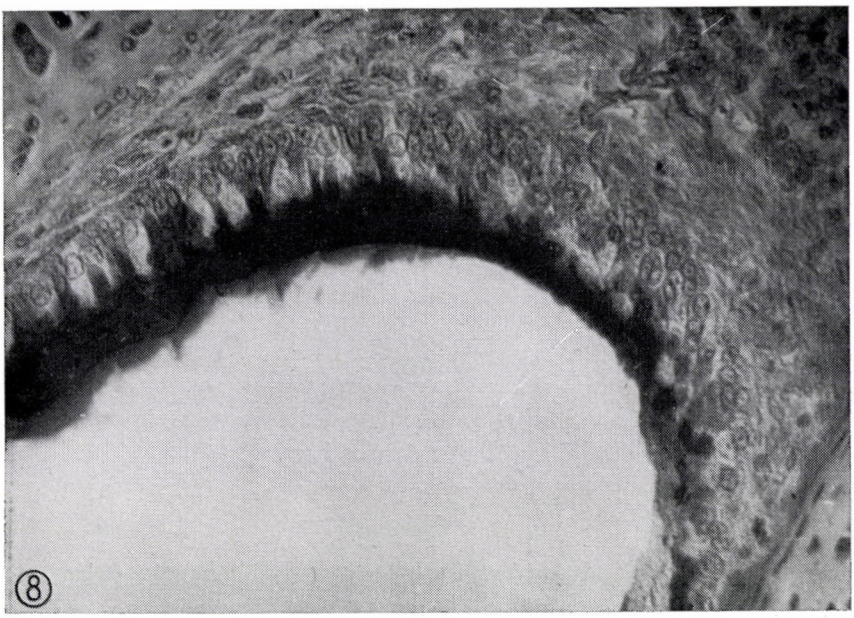

Fig. 8. Homologous tracheal graft after eight days. Copious discharge of PAS-positive secretion by columnar epithelium. Tri PAS (×100)

of time that the lamellae sometimes become undistinguishable, being replaced by the PAS-positive substance. Much the same process is observable in Hassall's corpuscles. An increasing quantity of PAS-positive cells makes its appearance in the oesophageal epithelium, mainly in the stratum spinosum.

The tracheal graft proved to be an apparently suitable model to demonstrate the process of cyst formation. The secretion of a PAS-positive substance by the epithelial cells was observable from the outset. The phenomenon in itself is not very remarkable since the epithelial cells of the trachea under normal conditions secrete a mucinous PAS-positive substance, yet it merits some attention that under our experimental conditions the secretory action of the tracheal epithelium showed a striking resemblance to the processes occurring in thymus cysts. The similarity appears clearly when we compare the picture on an 8-day graft (Fig. 8) with the one (Fig. 9), we had taken in a previous experiment, showing a thymus cyst in an X-ray treated rat where

there is a close functional analogy in the cell mechanism to produce a secretion and to fill the cyst with it. The intensive mucin formation in the trachea not under experimental but under the prevailing normal conditions escapes observation probably because the secretion is discharged continuously through the outlet ducts.

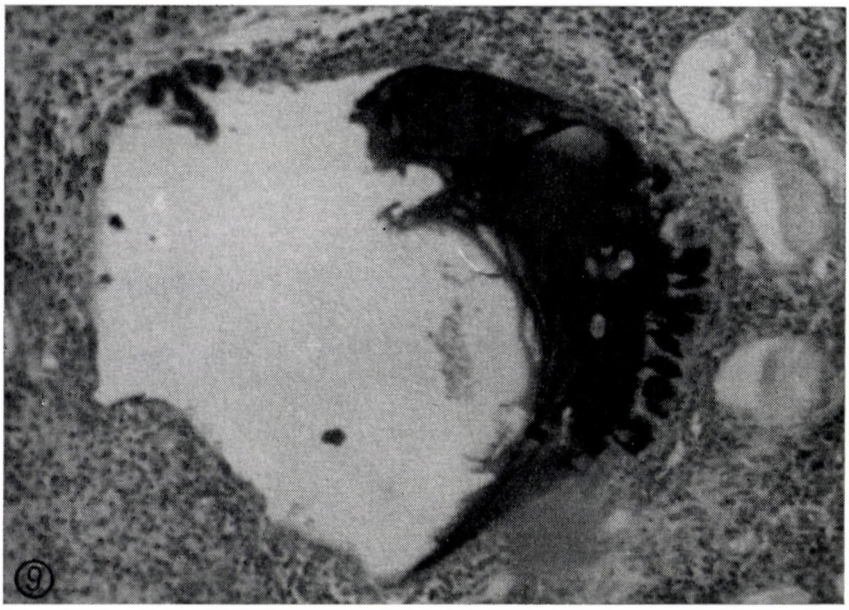

Fig. 9. Thymus of an X-ray treated rat. Epithelial cells showing signs of secretion. PAS-positive substance in the lumen. PAS (×60)

Conclusions

The experiments seem to indicate that both Hassall's corpuscles and the thymus cysts may be regarded as non-preformed structures which develop in close connection with the organ itself as a result of its function. The epithelial buds, growing out from the entoderm of the 3rd and 4th pharyngeal pouch, obviously carry such potentials with them as are leading to the development of both the multi-layered squamous and the columnar epithelium. The idea also suggests itself that the epithelial buds as long as they grow, comprise a cavity in which the cells are secreting and later, when they have established themselves and the lymphatic tissue has developed — irrespective of whether from the epithelium itself or from the surrounding connective tissue — the connection with the starting point breaks off and the secretion changes from the initial exocrine one to an endocrine secretion. The product, however, is not a specific

hormone, like those discharged by the endocrine glands, but — since the epithelium has preserved its original nature — a mucopolysaccharide (Fig. 10).

The epithelium spreads in the course of development over the lymphatic tissue; although in some places it takes the appearance of preformed groups (Hassall's corpuscles, minor cysts), it resumes under the action of stress its original form (multiplication of Hassall's corpuscles, development of large

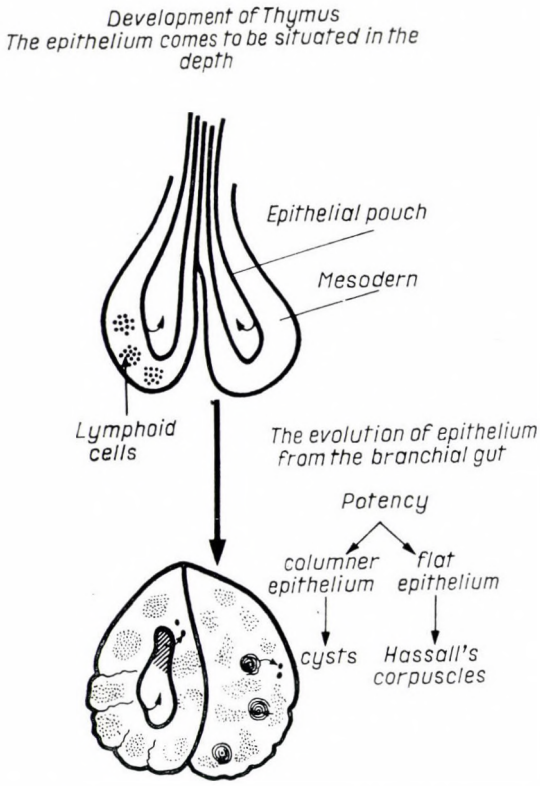

Fig. 10. Schematic course of thymus cyst and Hassall's corpuscle formation

cysts) and performs the corresponding function (production of mucopolysaccharides).

Further inquiries into this phenomenon might perhaps explain the accumulation of numerous PAS-positive cells in the thymus around the cysts and Hassall's corpuscles. Since the lumen is lacking a direct outlet, the mucopolysaccharides escape by way of cell transport and this phenomenon is likely to be in close relation with the mast-cell activity of the thymus which we have observed and described earlier.

The thymus in the different animal species shows a predominance of Hassall's corpuscles or of cysts, accompanied by more pronounced reactions

of one kind or the other, it may be presumed that the potentials preserved by the epithelium are mainly those of the squamous or of the columnar type, respectively, with a possible effect on the thymus itself. This may account to some extent for the unequal and sometimes contrary effects elicited by thymectomy in different species.

The thymus seems to perform an endocrine function, but not in the sense of other endocrine glands in which the original epithelium, together with the discharged secretion, has acquired a certain specificity. The thymic epithelium also becomes specialized in the exocrine sense, with the difference that it has no outlet duct. The polysaccharide secretion escapes not only with the blood stream but by way of cell transport. It may thus be termed a *cytocrine* process. While the original cells turn into mast cells, they transform the discharged polysaccharides into heparin which cannot be regarded, according to our present knowledge, as a hormone although it has recently been shown to have a strong aldosterone inhibiting effect [3] which might have a part in the thymus-adrenal antagonism; it also plays some part, as Heilbrunn's and our own experiments seem to indicate, in cell division and cell proliferation. It might even represent the substance through which the thymus exerts its influence upon neuroendocrine regulation.

Summary

The formation of Hassall's corpuscles and thymus cysts has been studied in model experiments. Tissue pieces from the trachea and oesophagus of embryonal or newborn animals were transplanted into the spleen whereupon the epithelium of these organs showed the same morphological and functional changes as characterize Hassall's corpuscles and cysts. Conclusions have been drawn concerning the genesis of these structures and the histophysiology of the thymus.

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ОБРАЗОВАНИЕ КИСТ ЗОБНОЙ ЖЕЛЕЗЫ И ГАССАЛЕВСКИХ ТЕЛЕЦ В ОПЫТАХ НА МОДЕЛИ

ДЬ. ЧАБА и И. БЕРНАД

Авторы проводили опыты на модели с образованием Гассалевских телец и кист зобной железы. От эмбрионов или от новорожденных животных они пересаживали трахею и пищевод в селезенку; эпителий этих органов имеет функциональные и морфологические признаки, характерные для Гассалевских телец и для кист. Обсуждается способ возникновения этих образований и заключения, сделанные в связи с гистофизиологией зобной железы.

DIE ENTWICKLUNG VON THYMUSZYSTEN UND HASSALLSCHEN KÖRPERCHEN IM MODELLVERSUCH

GY. CSABA und IRÉN BERNÁD

Zur Entwicklung der Hassallschen Körperchen bzw. Thymuszysten wurden Modellversuche durchgeführt, indem man Trachea und Ösophagus von Embryos bzw. von neugeborenen Tieren in die Milz implantierte. Das Epithel dieser Organe zeigt ähnliche morphologische und funktionelle Erscheinungen, wie sie für die Hassallschen Körperchen und die Zysten charakteristisch sind. Der Mechanismus des Zustandekommens dieser Gebilde und die sich aus der Histophysiologie der Thymusdrüse ergebenden Schlußfolgerungen werden erörtert.

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