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HASSALL'S CORPUSCLES—A SITE OF THYMOCYTE DEATH

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Summary.—Cortical thymocytes incorporated tritiated thymidine within ½–4 hours after injection and retained decreasing amounts of the isotope for 5–7 days. In contrast with the remainder of the medulla, Hassall's corpuscles selectively included tritiated nuclear material one day after injection. Corpuscular labelling reached a maximum by the 4th day, then became fragmented and disappeared by the 7th day.

It is deduced that some of the DNA generated in the cortex is transported to the medulla and disintegrates in Hassall's corpuscles. The biological significance of the apparently wasteful DNA synthesis remains unexplained.

THE GREAT majority of thymocytes produced in the thymus are thought to die *in situ* without leaving the gland (Metcalf, 1966). Yet the intrathymic fate of these cells is unknown. This problem has been studied by labelling cortical thymocytes with tritiated thymidine that was injected directly into the exposed guinea-pig thymus.

It has previously been suggested that Hassall's corpuscles provide a graveyard for dead thymocytes (Aubertin and Bordet, 1909; Blau, 1967*a*), a notion challenged by Mandel (1968*a*). Evidence is now presented that some DNA from thymocytes that have recently divided accumulates and disintegrates in Hassall's corpuscles.

MATERIALS AND METHODS

Albino Hartley guinea-pigs aged 6–8 weeks were anaesthetized with ether and the thymus exposed. Under visual control, 20 μCi of tritiated thymidine (Amersham TRK 120) contained in 0·1 ml of saline was injected by multiple punctures using a size 20 needle. The incisions were closed with Michel clips or with sutures. Animals were killed in groups of 2 at intervals of ½, 1, 2, 4 and 8 hours and 1, 2, 4, 5, 7 and 14 days after injection. The

EXPLANATION OF PLATE

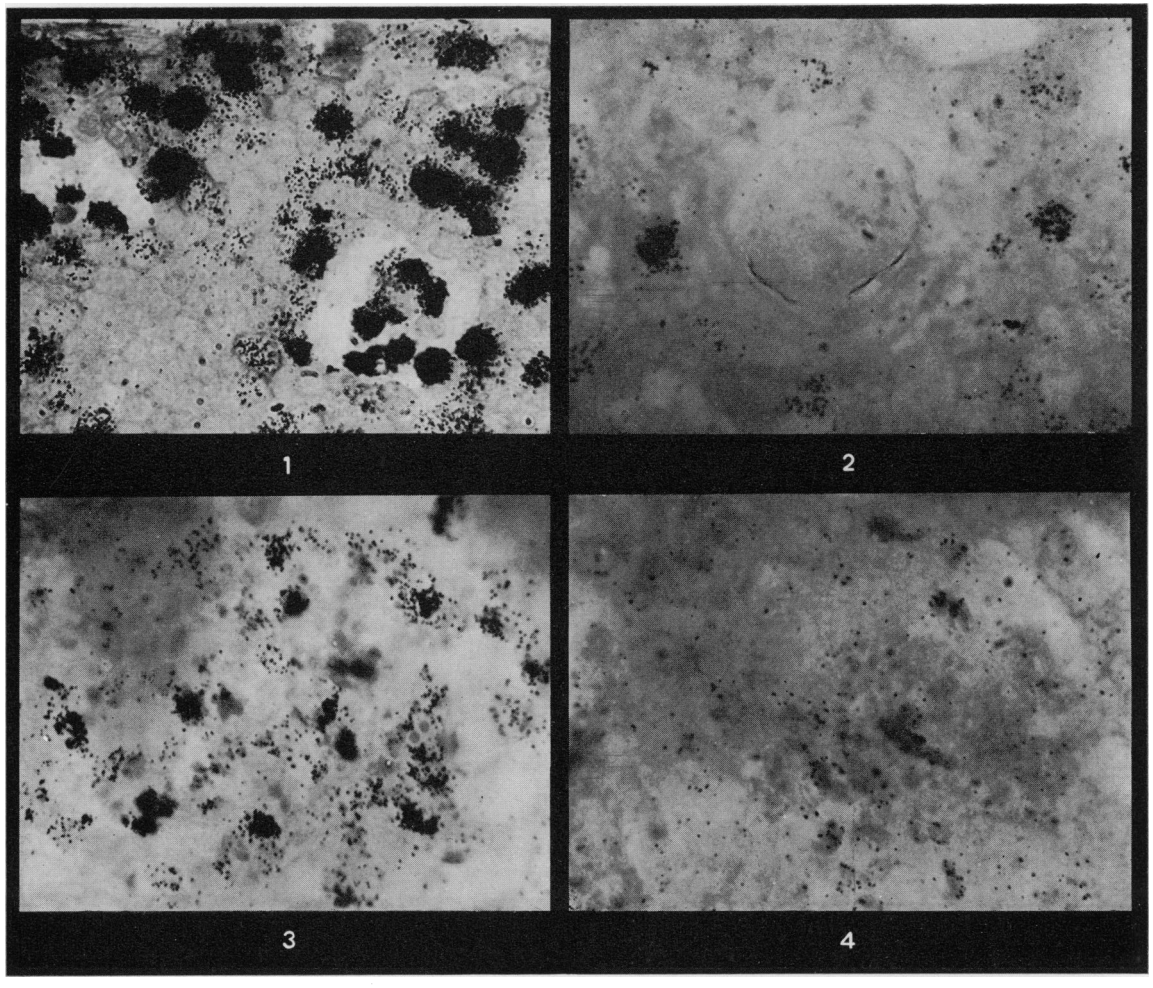
FIG. 1.—Eight hours after injecting tritiated thymidine into the exposed guinea-pig thymus, an unusually heavily labelled cortical "pockmark" shows the isotope overlying nuclear material. Heavily labelled thymocytes are also seen.

All microphotographs are taken from autoradiographs that were exposed for 4 weeks, stained with methylene blue and taken through an oil immersion lens with a final magnification of 800 (reduced to 530).

FIG. 2.—Twenty-four hours after injection labelled thymocytes and epithelial cells appear at the edge of Hassall's corpuscles.

FIG. 3.—Four days after injection some corpuscles contain large amounts of radioactive label in thymocytes and epithelial cells but mostly in nuclear debris.

FIG. 4.—By the 5th day corpuscular labelling diminishes and disperses parallel with decreased radioactivity in cortical thymocytes. The label disappears from Hassall's corpuscles between the 5th and 7th days after injection.



Blau.

tissues were processed and sectioned at 5–7 μm. Autoradiographs on Kodak AR10 stripping film were exposed for 1, 2, 4, 6 and 8 weeks, developed and stained with methylene blue. Haematoxylin and eosin sections were also examined.

RESULTS

Labelling in cortex

An unexpected finding was that thymocytes in the outer half of the cortex only were labelled ½–1 hour after injection. But by 4 hours the whole cortical thickness contained densely labelled cells as previously illustrated (Blau, 1972). During the subsequent days, progressively more thymocytes contained the isotope but the number of grains per cell diminished. Between the 5th and the 7th days most of the radioactivity disappeared from the cortex so that by the end of the 1st week only a small number of grains remained over cortical thymocytes.

Labelled nuclear debris was occasionally seen in cortical " pockmarks " as early as 4 hours after injection and persisted at the same low level for the next 5 days. A particularly good example is illustrated in Fig. 1.

Labelling in medulla

Occasional epithelial cells and thymocytes in the medulla contained a small amount of the isotope during the 1st week. Labelling of these cells gradually increased up to the 14th day, when the medulla contained more radioactivity than the cortex.

Labelling in Hassall's corpuscles

During the first 8 hours no label was evident in Hassall's corpuscles in spite of intense radioactivity in the cortex. However, at the end of the 1st day a few labelled cells appeared at the edge of corpuscles (Fig. 2). The number of labelled cells adjacent to, as well as inside, corpuscles then increased, reaching a maximum between the 2nd and 4th days after injection, when some corpuscles contained large amounts of radioactivity (Fig. 3). By the 5th day the label became scattered and less intense (Fig. 4), and disappeared from corpuscles 7 days after injection.

Inside the corpuscles some tritium was found overlying epithelial cells and thymocytes, but the majority was evident over nuclear debris, none being visible over hyaline material.

It needs to be stressed that radioactivity in corpuscles was confined to lobules in which the cortex was well labelled.

DISCUSSION

The selective inclusion of labelled nuclear material in Hassall's corpuscles in excess of the remainder of the medulla was noted by Murray and Woods (1964) but not studied in detail. The serial observations reported here support the concept that these thymic corpuscles act as a repository for dead or dying thymocytes and provide further confirmation that the corpuscles belong to the reticuloendothelial system (Blau, 1968). Histochemical studies also reveal high concentrations of nucleotidase and other degradative enzymes in the Hassall's corpuscles of man and chickens (Kouvalainen, 1964; Fennel and Pearse, 1961).

Whether thymocytes, as such, migrate from the cortex to the medulla or

nuclear fragments are carried there by macrophages is not certain. Some of the label inside the corpuscles was of the same intensity as that in cortical thymocytes of the particular lobule. More commonly, however, corpuscular labelling was lighter than in the cortex. Hence it seems likely that both thymocytes and nuclear debris migrate, or are transported, to the corpuscles from the cortex.

The rapid DNA labelling in cortical " pockmarks " has not previously been reported, yet bears comparison with the speed of incorporation of thymidine in germinal centres of the rat spleen. There, portions of, or whole, pyknotic nuclei became labelled within 30 minutes of injecting a pulse of tritiated thymidine, a maximum intensity being reached at the end of 24 hours (Fliedner, 1967).

The total amount of radioactivity in corpuscles and " pockmarks " cannot account for all dead thymocytes, assuming that the majority of these cells die *in situ*. Hence, a large proportion of dead cells must be removed in a manner not so far elucidated. Although the dose of tritium used in this experiment was high, there was no evidence of x-irradiation damage, *i.e.* no excess of dead thymocytes, no corticomedullary inversion and no increase in size or number of Hassall's corpuscles.

Analysis of the structure of Hassall's corpuscles should provide a lead to their origin, as yet an unresolved problem. Light and electron microscopy, as well as dynamic studies such as reported here, show that these corpuscles consist of macrophages, polymophonuclear leucocytes, epithelial cells, nuclear material derived from thymocytes and hyaline material with staining properties similar to keratin (Kostowiecki, 1963; Mandel, 1968*a*, *b*; Murray and Woods 1964; Smith and Parkhurst, 1949). The cellular origin of this keratin-like substance is still unclear but each of the remaining cells is motile and could thus account for the waxing and waning in corpuscular size, the variation in nuclear content and the presence of antigen and antibody (Blau, Jones and Kennedy, 1968; Blau, 1967*b*). No evidence seems forthcoming to support a vascular origin of Hassall's corpuscles (Jaroslow, 1967), although contact between blood vessels and the edge of corpuscles is demonstrable in serial sections (Kater and van Gorp, 1969).

The current emphasis is on release of thymocytes from the thymus, giving rise to circulating T lymphocytes. Yet Metcalf (1966) has repeatedly stressed the high incidence of intrathymic death of recently generated thymocytes. The observations recorded here indicate two sites of accretion of dead nuclear material inside the gland: Hassall's corpuscles and to a lesser extent, cortical " pockmarks ". The biological significance of this apparently wasteful DNA production remains unresolved although reminiscent of the overproduction of erythrocytes in bone marrow and lymphocytes in germinal centres.

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