20.00 h ($p < 0.001$) and a reattainment of morning levels by 02.00 h. The depletion at 20.00 h could be expected since the period of greatest activity is known to begin at approximately 18.00 h (onset of darkness[5]). Muscular exercise is a known powerful stimulus for GH secretion in man[4].

Fasted rats: The effect of the 24-hour fast appears to influence the GH-activity in a manner opposite to that in fed rats. The highest content of GH-activity in the pituitaries occurred in the late morning (08.00 h) and dropped to its lowest level by 14.00 h ($p < 0.001$).

Diurnal fluctuations of plasma GH have been reported for children[6] and adults[7]. In children, GH levels are low during the day and high during the night, the latter being attributed to the long fact since the previous meal. It has been reported by CLARK[8] that high prolactin levels occur in rats at 16.00 h and low values were found at 22.00 h.

The data suggest circadian fluctuations in the pituitary content of tibia-test-assayable material, presumably GH. The fact that the removal of the exogenous stimulus of food intake did not eliminate significant variations in pituitary GH content suggests that the latter might be subject to the function of the 'Biological Clock'[9].

Zusammenfassung. Mit Tibiatest auf Wachstumshormonaktivität geprüftes Hypophysenmaterial zeigte trotz Fastenperioden zirkadische Gehaltsschwankungen. Die Wahrscheinlichkeit besteht, dass Wachstumshormone und eventuelle andere hypophysäre Hormone in ihrer Aktivität der «Biologischen Uhr» unterworfen sind.

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Injury and the Axial Organ of Echinoids

When foreign cells or organisms are injected into the perivisceral cavity of certain echinoids, the axial organ responds in a distinctive way[1]. Similar responses occur when coelomic fluid is removed, allowed to clot and re-introduced into the perivisceral coelom of the individual from which it was obtained. This suggested that the organ is responding to conditions likely to be associated with serious injury[1]. The observations to be reported substantiate this suggestion.

Material and methods. Injuries which penetrate the test lead to loss of coelomic fluid and coelomocytes both by escape and by clot formation. It is pertinent to discover whether the axial organ responds to such losses. Healthy individuals of *Arbacia punctulata* were deprived of coelomocytes in 2 ways. First, by withdrawing coelomic fluid into a sterilized syringe inserted through the peristome; second, by repeatedly removing the clot of coelomic fluid which sealed an opening 3–5 mm in diameter made in the test. The effects produced on the axial organ were compared and related to the condition of the organ in an individual having a naturally acquired lesion of similar kind.

Results and discussion. When *Arbacia punctulata* is robbed of about 1.0 ml of coelomic fluid the fluid lost is replaced within 24 h and the axial organ shows well-defined changes. Cells, cell debris and secretion begin to leave the lacunae and move into the contractile vessel, so that the channels in the trabeculae which connect the 2, become swollen with such material (Figures 1 and 2). Some of the cells resemble lymphocytes and fusiform cells[2], others peritoneal cells and those which produce mucus. Cell divison is active in both the migrating cells and those of the peritoneum which covers the axial organ and lines its central cavity. In such areas the peritoneum may lose its integrity, allowing cells and secretion to escape from the underlying lacunae and to pass, together with the loosened and proliferated peritoneal cells, into the lumen of the organ or into the perivisceral coelom. The nature of this secretion and that which passes into the contractile vessel in *Arbacia punctulata* has not yet been determined, but in other species much of it is acid mucopolysaccharide. It could be significant in wound healing[3].

These effects are evident $3^1/_2$ h after a single withdrawal but urchins can survive up to 9, provided that the withdrawals of fluid are separated by intervals of no less than 24 h. Under such conditions the coelomic fluid declines in clotting power, presumably owing to loss of coelomocytes and the lacunae show signs of depletion (Figure 3). Similar effects were clearly observed in all of 15 experiments in only 3 of which were there signs of debility or general tissue disintegration. One such case had suffered 6 withdrawals, the other two 9. The effects in instances such as the last mentioned produced by severe stressing can be accepted only with reserve, but they are useful in so far as they indicate that the depleted lacunae appear to be partially replenished with cells and secretion from the area where the mesentery and its contained 'haemal' vessel are attached to the axial organ. This hints at a possible function for the enigmatical 'haemal' system.

Parallel experiments in which the peristome was penetrated by the syringe, but no fluid withdrawn, produced comparably clear effects on the axial organ in only 2 instances out of 7. This suggests that loss of coelomocytes rather than unavoidable tissue damage is the major cause.

Clear and consistent effects were reproduced in 4 urchins from which healing clots were removed at intervals of 1–6 days. After 4 or 5 such operations clotting and healing were impaired or ceased and the axial organ showed changes precisely similar to those which followed withdrawal of coelomic fluid.

It proved possible to relate these findings to one instance taken from the normal environment. Naturally occurring lesions of the test proved rare in *Arbacia*:

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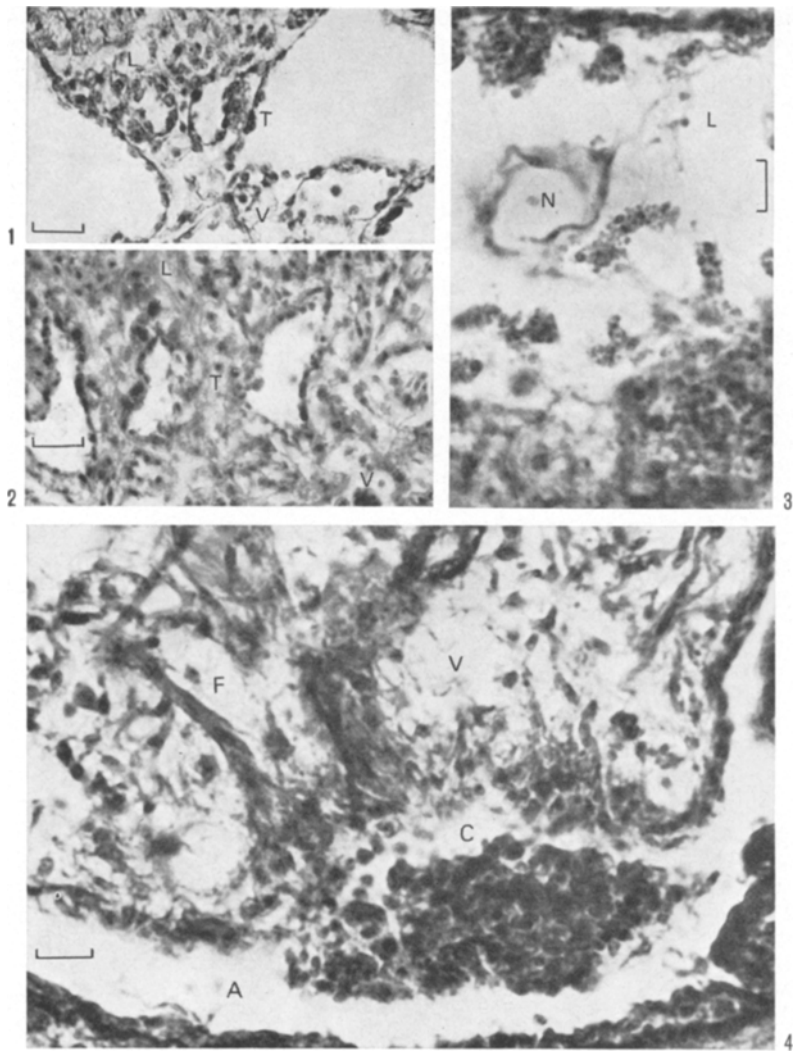

Fig. 1. Portion of the axial organ of an untreated individual of *Arbacia punctulata* showing the normal condition of the trabeculae (T), the channels in which connect the lacunae (L) and the contractile vessel (V). Note the sparse contents of the channels and the distinct peritoneal layer investing the trabeculae. Fixed: Bouin; stained: Mallory; scale: 13 μm.

Fig. 2. A comparable region of the axial organ of an individual from which approximately 1.0 ml of coelomic fluid had been withdrawn at intervals of 48 h. Note the channels within the trabeculae (T), swollen with cells and secretion discharged from the lacunae (L) and passing into the contractile vessel (V). The peritoneum around the trabeculae shows signs of proliferation. Fixed: Duboscq-Brasil; stained: Heidenhain's iron hematoxylin, eosin and Alcian Blue; scale: 12 μm.

Fig. 3. Depletion of the lacunae (L) following 9 withdrawals of coelomic fluid in 16 days. Note that the canaliculus (N), normally surrounded by dense lacunar contents, now appears almost isolated. Fixed: Duboscq-Brasil; stained: Mallory; scale: 10 μm.

Fig. 4. Portion of the axial organ of an urchin which had a lesion penetrating the body wall so as to expose the perivisceral coelom. Note the clump of cells and cell debris (C) in the lumen (A). This substance resembles lacunar contents and appears to have been discharged from the contractile vessel (V) by the funnel (F). Fixed: Duboscq-Brasil; stained: Mallory; scale: 10 μm.

among 200 urchins examined at Woods Hole in July and August 1968, 6 showed lesions of the test. Of these only one showed a lesion that had penetrated to the perivisceral coelom and was sufficiently recent to be imperfectly sealed. In this instance the axial organ showed extensive proliferation of cells from the peritoneal lining into the lumen and the discharge of cells, secretion and cell debris from the lacunae into the contractile vessel.

The ultimate fate of the lacunar substance discharged has yet to be determined. Some of that passed into the contractile vessel appears to leave it by pores or small funnels, which have not previously been described in its walls, to enter the lumen of the axial organ (Figure 4). From here, together with lacunar substance and peritoneal cells which have passed into the lumen directly (see above), the discharge could pass into the perivisceral coelom or into the stone canal by way of the communications known to exist between them[4].

Some potentialities of peritoneal cells in the regenerative processes of *Arbacia* have been clearly revealed[5]. An abundance of these and other cells, together with mucus, mobilized by the contractile vessel and discharged into the perivisceral coelom could be very significant in aiding emergency repairs to the test, especially when accompanied by a mechanism for dealing with infection and with effete or disorganized cells[1,3].

The changes observed therefore fit with the previous contention[1] that at least some of the activities of this enigmatical organ are defensive responses induced by injury. To a limited extent only the idea is in keeping with the recurrent notion[6,7], that the organ is lymphoid, but when knowledge is limited, such analogies can be dangerous oversimplifications[4,8].

Résumé. Chez *Arbacia punctulata*, quand on enlève le liquide périviscéral, ou quand on endommage le test plusieurs fois, la glande brune réagit. Les coelomocytes, les cellules péritonéales et les «mucocytes», sécrétés, sont mis en liberté. Ces éléments entrent dans le vaisseau contractile et dans la cavité périviscérale.

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