Autoradiographic Evidence for Iodine Binding Blood Cells in the Oozoid of *Amaroucium constellatum*, a Compound Ascidian

There have been a number of studies which indicate that organically bound iodine is present in ascidians[1-3]. The first reports that examined this topic emphasized the iodine-binding properties of the endostyle, because of the morphological evidence which indicated that this organ was homologous with the vertebrate thyroid. However, it soon became clear that much more iodine was bound by the blood and the tunic than by the endostyle of these animals[3]. A part of this organically bound iodine is found in thyroxine and its precursor, monoiodotyrosine and diiodotyrosine[3]. Thyroxine has been detected in extracts of the blood[3], the endostyle[4] and the tunic[5].

Two experimental studies have recently appeared which have partially defined one of the physiological roles of thyroxine in these animals. In *Perophora orientalis*, thyroxine and triiodothyronine increase the rate of vascular budding, while the goitrogen thiourea inhibits the elongation of the stolon and promotes the process of asexual reproduction[6]. Thyroxine and triiodothyronine also accelerate the rate of growth of the oozoid of *Amaroucium constellatum*, in particular the growth of the post-abdomen[7]; thyroxine has been identified using radiochromatographic techniques in extracts of the oozoid of *A. constellatum*[7].

In conjunction with the work on the effect of thyroxine on the growth of *A. constellatum* oozoids, a study was done on the uptake of iodine-125 using autoradiographic procedures. In this study silver grains were observed over certain cellular zones of the endostyle, some cells in the tunic and a certain kind of blood cell in the vascular system; the blood cells incorporated the largest amounts of iodine. This report will concern itself with the intracellular location of the bound iodine in these blood cells.

Tadpole larvae were obtained by placing sexually mature colonies, which had been kept in the dark for 12 h, in the light. The tadpole larvae were picked up with a pipette as they were liberated from these colonies and placed in small petri dishes in which they were allowed to metamorphose into oozoids. The 24 h-old oozoids were incubated in sea water which contained 1 μCi per ml of Na ^{125}I and penicillin G sodium salt (20 units/ml) and streptomycin sulfate (50 μg/ml) for 24 h at 19 °C. After washing briefly with sea water, they were fixed in 2% osmium sea water for 1 h at 0 °C. After washing with sea water, they were dehydrated with ethanol and embedded in Epon 812 resin.

For light microscopic autoradiography, 0.5 μm sections were obtained. Autoradiograms were prepared by the dipping method with Eastman-Kodak NTB-3 nuclear emulsion. After exposure for 10 days, they were developed with Eastman-Kodak D-76 for 4 min at 20 °C. They were then stained with methylene blue after the method of HENDRICKSON et al.[8]. For electron microscopic autoradiography, silver and gold sections were obtained. These thin sections were put on copper 150 mesh grids and stained with 2% uranyl acetate. These grids were then coated with Eastman-Kodak NTE nuclear emulsion by a slight modification of the method of SALPETER et al.[9]. The grids were kept for 1 month in the dark at 5 °C and then developed (Eastman-Kodak Dektol for 1 min at 24 °C for Figure 2a; Eastman-Kodak Microdol-X for 1 min at 19 °C for Figure 2b). They were then stained with Reynolds lead citrate method[10] for 30 min.

As shown in Figures 1a and b, certain kinds of blood cells bind ^{125}I. These iodine-binding cells represent only a small proportion of the blood cells which make up the vascular system; they are present throughout the adbomen

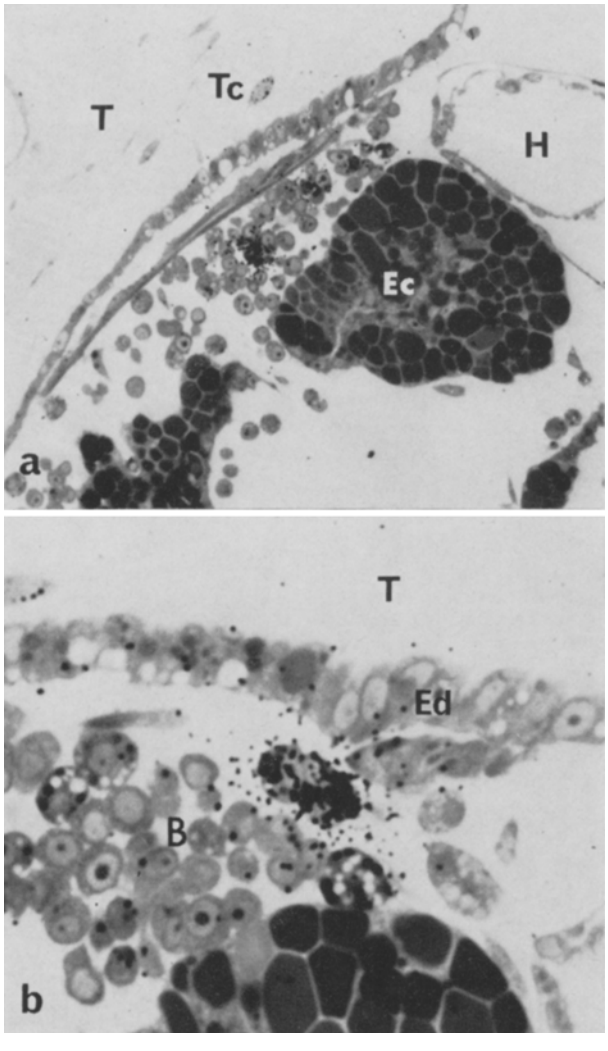

Fig. 1a) Light microscopic autoradiograph showing silver grains located over some of the blood cells in the abdomen of an oozoid of *A. constellatum*. ×960. b) Enlargement of one of the iodine-binding cells. ×2,400. B, blood cells; Ec, epicardium; Ed, epidermis; H, heart; T, tunic; Tc, cell in tunic.

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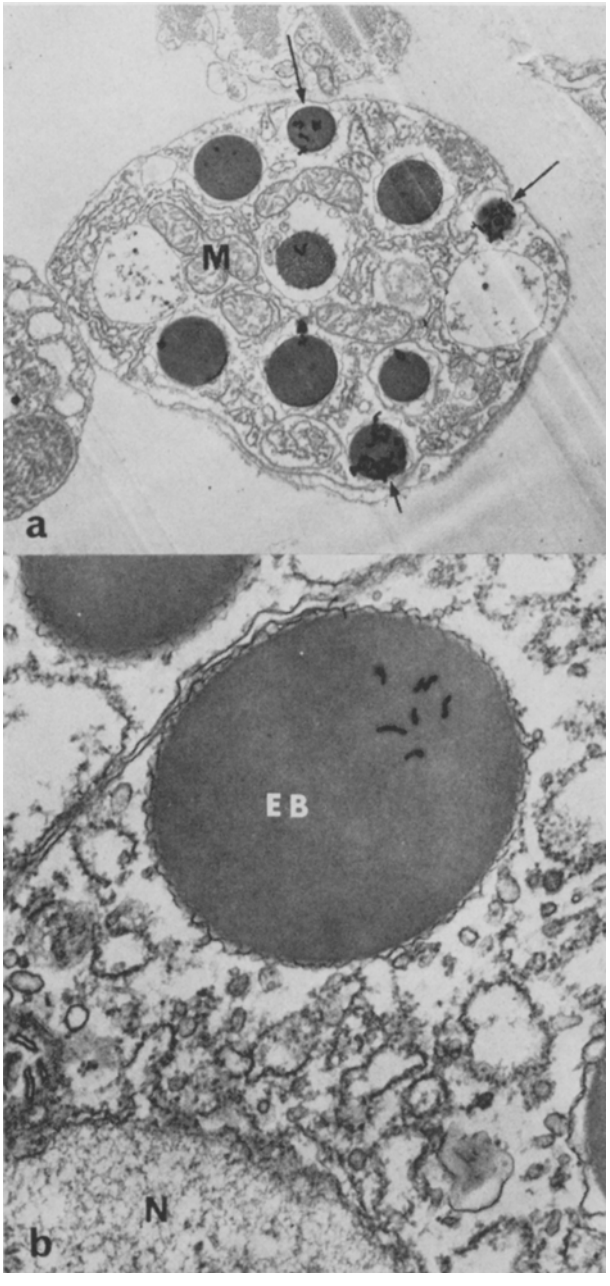

Fig. 2. a) Electron microscopic autoradiograph showing an iodine-binding blood cell which contains several electrondense bodies in its cytoplasm. Label (see arrows) is always associated exclusively with these electron-dense bodies. ×6,000. b) Enlargement of one of the electron-dense bodies which contains radioactive iodine. ×50,000. EB, electron-dense body; M, mitochondria; N, nucleus.

and postabdomen. These cells contain several membrane-bound electron-dense bodies in which the ^{125}I is found (Figures 2a and b).

In vertebrates, similar membrane-bound electron dense bodies are found in the cells that make up the follicular epithelium of the thyroid gland[11–13]. The number and size of these electron dense bodies is related to the functional demands that are placed on the thyroid gland. Autoradiographic studies show that iodine is bound by these electron dense bodies[13].

Membrane-bound electron dense bodies have also been observed in the cells that make up zone 7 of the ascidian endostyle[14,15]. In specimens that have been exposed to ^{125}I for a short period of time, silver grains have been observed over the apical surface and the multivesicular bodies of the zone 7 cells. Incubation for a longer period of time causes the transfer of the silver grains to the membrane-bound electron dense bodies in the same cells[14,15]. These are the cells of the endostyle where thyroxine biosynthesis is thought to occur.

The structural similarity of the membrane-bound electron dense bodies in the thyroid epithelium of vertebrates, the zone 7 endostyle cells and the iodine binding blood cells of ascidians, suggests that iodine-binding blood cells might elaborate thyroid hormones.

Zusammenfassung. Gewisse Blutkörperchen in den Oozoiden von *Amaroucium constellatum* nehmen ^{125}I in grosser Menge auf. Das Cytoplasma dieser Zellen besitzt membraneingewickelte elektronendichte Körperchen, wo sich die Silberkörner ausschliesslich befinden. Die Möglichkeit der Biosynthese des Schilddrüsenhormons in solchen Blutkörperchen wird diskutiert.

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Effect of Dibutyryl Cyclic AMP on Glucagon and Insulin Storage and Secretion in Organ Culture of Rat Islets

It is generally accepted that substances which enhance the intracellular level of cyclic AMP increase insulin release from endocrine pancreas[1–3]. It has also been suggested that changes of the cyclic-AMP value may be involved in glucagon secretion[4–8]. We have only a few results with respect to the cultured endocrine pancreas[2,7], especially to cultured islets, which are characterized by a continuous diminution of release and storage of hormone with the duration of cultivation[9–11].

The aim of this paper is to investigate the effect of N^6-2′-*O*-dibutyrylcyclic adenosine-3′,5′-monophosphate (DB-CAMP) on the content and secretion of hormones of cultivated rat islets. Collagenase isolated islets of adult Wistar rats (starved overnight with a body weight of