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I. CHROMAFFIN TISSUE

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The bulk of the catecholamines present in the chromaffin cell is stored in intracellular particles which we call the *chromaffin granules.* Our knowledge of these structural elements has been greatly advanced in recent years, by the use of two methods, centrifugation of tissue homogenates and electron microscopy.

Some time ago it was found that in homogenates of adrenal medulla the greater part of the catecholamines could be sedimented by centrifugation. This discovery was followed by work in which it was shown that the amine-carrying granules could be separated from the mitochondria by making use of the ability of the former to penetrate into sucrose solutions of greater density. Hagen and Barrnett (14), isolated chromaffin granules by using the sucrose density gradient described by Blaschko *et al.* (5) and obtained electron micrographs of them; they showed unequivocally that the elements rich in amines were identical with the granules that had been seen in electron micrographs of chromaffin tissue (see 8, 10, 12). Electron micrographs of particulate fractions obtained from a sucrose density gradient of bovine adrenal medulla have recently been published (1); in this work the chromaffin granules were separated not only from the mitochondria but also from the microsomes.

In recent months, the density gradient method has been used to separate the chromaffin granules from another type of structural element, apparently lysosomes, present in the large-granule fractions. It has been established that in homogenates of the bovine adrenal medulla and of human phaeochromocytoma there occur typical lysosomal enzymes; these lysosomal enzymes have a distribution which differs not only from that of the catecholamines but also from that of fumarase, a typical mitochondrial enzyme (7, see also 19). The enzymes tested are typical lysosomal enzymes; in the bovine medulla six enzymes have been studied: acid phosphatase, β-glucuronidase, cathepsin, arylsulphatase, ribonuclease and deoxyribonuclease. All these enzymes have a maximum of activity at a density intermediate between that of the mitochondria and the catecholamines. Particles that have the appearance of typical lysosomes have been seen in electron micrographs of rat adrenal medulla (11).

Much work has been carried out on the problems of how the chromaffin granules maintain their store of amines at rest, and how the amines are released when the chromaffin cell is stimulated. In isotonic sucrose at temperatures close to 0°C the amine content of the granules does not decline significantly, but when they are transferred to a hypotonic medium the amines are readily released into solution. The first of these observations indicates that the retention of amines within the granules is not dependent upon a continuous supply of energy, as is the retention of potassium within the red blood corpuscles. The second obser-

vation shows that the forces that maintain the concentration of amine within the granules are rather weak.

We owe to Hillarp the discovery of ATP in the adrenal medulla in unusually large amounts. The finding that the ATP is located in the chromaffin granules led to the idea that the positively charged catecholamines were bound to the negatively charged phosphate groups of the ATP, an idea that was strengthened when it was found that in the granules the molar ratio of catecholamines to ATP was close to 4, and therefore that the ratio of the sum of positive charges on the amines to the sum of negative charges on the nucleotides was close to unity. An ionic binding mechanism of storage of the catecholamines is consistent with the observation that radioactive phosphate is incorporated into the ATP of the chromaffin granules much more slowly than into the ATP of the mitochondria (20).

The possibility of an interaction between catecholamines and ATP to form a complex of the type: ATP (catecholamine)$_4$ finds some support in nuclear magnetic resonance studies (26, see also 25). It has also been suggested that a complex of this type in the chromaffin granules might make their contents approximately isotonic with the tissue fluids; if these constituents were free their combined concentrations within the granules would make their contents markedly hypertonic (14). However, the forces that hold the amines must be weak: when the granules are disrupted in hypotonic solutions, both the ATP and the amines are released. The same is true for much of the protein (4, 16). Possibly the ATP and the catecholamines interact with the protein to form a ternary complex and it is such a complex that is held in the granules.

Another possibility is that in the granules there exists an asymmetric diffusion barrier to nucleotides, so that the latter could enter the storage sites but could not diffuse outwards. They would thus provide a high concentration of nondiffusible anions, which would retain the positively charged amines in the storage site.

That the soluble protein may take a part in amine storage, has led to a renewed interest in this material (6, 21, 23). It has been established that there are at least two proteins in the soluble fraction. The major protein has been purified and appears homogeneous by electrophoresis and ultracentrifugation. The protein is acid, and amino-acid analysis has established the presence of a relatively large number of glutamyl residues. Smith and Winkler (22) have recently confirmed the presence of a small amount of cyst(e)ine (see 18). They have also obtained evidence of the presence of sugars (including hexosamines), indicating that this is a glycoprotein (22).

It seems unlikely that the storage of the catecholamines is associated with a turnover of ATP. On the other hand, observations by Kirshner (17) and by Carlsson *et al.* (9) have shown that the uptake of catecholamines by the granules at 20 to 30°C is stimulated by Mg^{++} plus ATP. It was for this reason that attempts have recently been made to settle the question as to the presence of an ATPase in highly purified preparations of chromaffin granules. In this work an ATPase activity was found in the granules which could not have been explained by contamination with either mitochondria or microsomes (1). The granule

ATPase was activated by magnesium ions, and it was suggested that this enzyme is involved in the magnesium-activated uptake of catecholamines. In contrast to the lysosomal enzymes, which are readily soluble, the ATPase is recovered in the insoluble stroma of the chromaffin granules (1, 15).

When catecholamines are secreted, the ATP content of the adrenal medulla declines. Stjärne (24) has found that perfusates from adrenal glands stimulated by acetylcholine contained purines; nucleotides could not be detected. Banks (2) was also unable to demonstrate nucleotides in such perfusates. Recent work carried out in Sheffield has indicated that the principal purines liberated during catecholamine secretion are inosine and hypoxanthine, accompanied by traces of adenosine. The molar ratio of catecholamines to purines found in the perfusates is about 10, indicating that approximately one-third of the adenine nucleotides lost can be accounted for (3).

The chromaffin cell is known to contain a very active microsomal ATPase (1, 13). Recently it has been found (3) that there is also an alkaline phosphatase present in the microsomal fraction; this enzyme is able to dephosphorylate adenosine-5′-monophosphate at a rate of 7.5 μmoles per g wet weight per hr at pH 9.5 (at pH 7.5 the rate is 5.2). It has been found that the microsomal fraction is capable of removing all the phosphate from ATP.

Evidence has also been obtained for the presence of an adenosine deaminase in the adrenal medulla (3); the specific activity of this enzyme is 4.3 μmoles per g wet weight at pH 7.4. There was no evidence of the existence of an enzyme able to deaminate adenosine-5′-monophosphate.

Thus, the recent observations on the enzymes present in chromaffin tissue allow us to conclude that the cell contains all the catalysts required for the breakdown of ATP, adenosine and inosine.

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