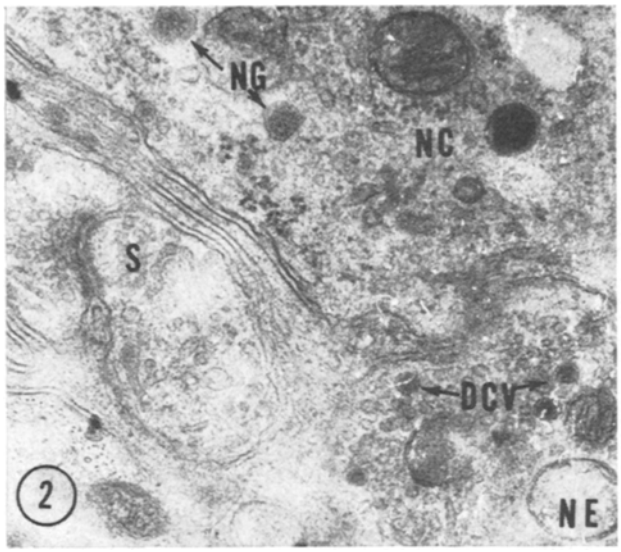

Fig. 2. Neuronal cytoplasm containing both granular vesicles and neurosecretory granules. Ca. × 25,000.

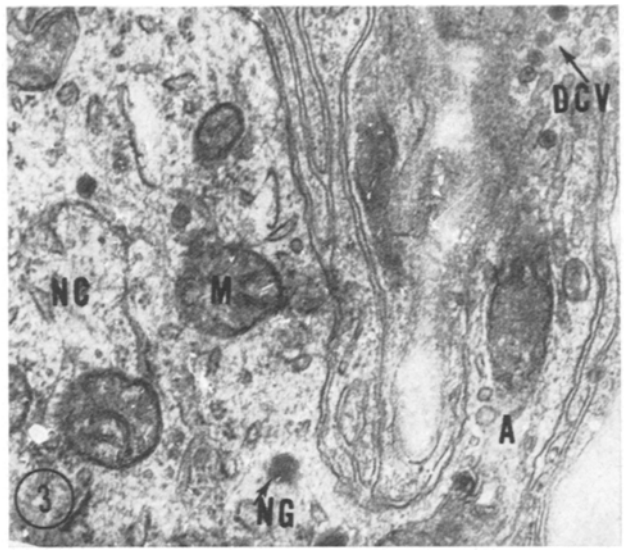

Fig. 3. Several granular vesicles within the nerve fibre (axon) departing from the respective perikaryon, and some neurosecretory granules in the cytoplasm. Ca. × 17,000.

adrenergic synapses, there were more numerous other synapses which displayed only agranular vesicles both on the pre- and postsynaptic side. Secondly, granular vesicles were also observed in the cytoplasm of some neurones. These granular vesicles were equal in size to those found in the nerve endings, but their concentration was smaller in the perikaryon (Figure 2). However, in some nerve fibres connected with the nerve cell body the granular vesicles showed much greater concentration than in the cytoplasm of the respective perikaryon (Figure 3).

While the granular vesicles in the nerve fibres, in the presynaptic terminals of some synapses and in the cytoplasm of some neurones varied in diameter in the to approximate range of 900–1200 Å, of which about 700 800 Å was occupied by the granule inside the vesicle, the agranular vesicles found in the synapses measured about 400–500 Å in diameter.

In the cytoplasm of all neurones, also larger granular vesicles were found. These vesicles had much larger diameter (about 2000 Å or more) than the synaptic granular vesicles. They were most often found in the vicinity of the Golgi apparatus, and they were diagnosed as neurosecretory granules surrounded by a membrane.

In the ciliary ganglion of the reserpinized rats, the number and localization of the granular vesicles had not been changed to any noteworthy extent. However, the density of the core seemed less clearly visible than in the controls. This suggests that the granules contained a catecholamine.

Zusammenfassung. Im Ganglion ciliare normaler ausgewachsener Ratten werden elektronenmikroskopisch granulierte oder «dense core» Vesikula von etwa 900 bis 1200 Å Durchmesser festgestellt (in Nervenfasern, im Zytoplasma von Nervenzellen und in Präsynapsen). Reserpin (Serpasil®, Ciba) verursachte keine nennenswerte Änderung in Zahl oder Lokalisation der Vesikula, hingegen eine Abnahme ihrer Kerndichte.

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Intracellular Localization of Acid Phosphatase and Aryl Sulfatase in Rat Metrial Gland Cells

The granulated metrial gland cells of the pregnant rat uterus are complex structures, the function of which is at present unclear. The cells are rich in glycogen[1] and their granules are acidophilic and metachromatic[2]. Furthermore they give a diastase-fast reaction with the PAS-technique[3]. From these tinctorial properties as well as the incorporation of ^{35}S by the cells, it has been concluded that the cytoplasmic granules contain a lysine-rich protein component[3] as well as a sulfated mucopolysaccharide[1,4] which, however, does not appear to be heparin[5]. It has also been postulated that the hormone relaxin may be produced by these cells[3,6]. This hormone has recently been demonstrated in the cells by immunofluorescence techniques[7].

More recently, light microscopic and histochemical studies on the granulated cells of the metrial gland of the pregnant rat have shown these cells to have a high content of hydrolytic enzymes[8,9]. On these grounds it has been suggested that the metrial gland cell granules may be lysosomal in nature[9]. It was therefore considered of interest to investigate the nature of the metrial cell granules – at the electron microscopic level – with respect to the lysosomal enzymes acid phosphatase and aryl sulfatase.

For the experiments 10 pregnant Sprague-Dawley rats were used. The animals were sacrificed at late stages of gestation (15–20 days). After ether anesthesia and exsanguination small pieces of the sub-placental uterine

wall were fixed for 2 h at 4 °C in 4% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.3). The specimens were rinsed overnight at 4 °C in 0.1 *M* cacodylate buffer containing 0.2 *M* sucrose. Nonfrozen sections (40–50 μ) of the fixed tissue specimens were cut on a Sorwall tissue sectioner according to the technique of SMITH and FARQUHAR[10]. For acid phosphatase the sections were incubated at 37 °C for 60 min in a GOMORI[11] medium as modified by BARKA and ANDERSON[12] with β-glycerophosphate (Grade I, Sigma Chemical Co., St. Louis, Missouri) as substrate. For aryl sulfatases a GOLDFISCHER[13] medium (pH 5.5) as modified by SELJELID and HELMINEN[14] containing *p*-nitrocatechol sulfate (2 hydroxy-5-nitrophenyl sulfate, Sigma) as substrate was used. These sections were incubated at 37 °C for 30 min. For both enzymes lead nitrate was employed as capturing ion. After incubation the sections were rinsed in the buffers used in the incubation medium and were post-fixed for 1 h in 1% osmium tetroxide in 0.1 *M* cacodylate buffer containing 0.2 *M* sucrose (pH 7.3) at 4 °C. As controls, specimens were incubated in media without substrate or lead. Some non-frozen sections were treated with 1% $(NH_4)_2S$ in distilled water before postfixation and embedding. After postfixation the sections were dehydrated in ethanol and embedded in Epon 812 according to the method of LUFT[15].

Sectioning was carried out on an LKB ultrotome using glass knives. Thick sections (1 μ) were placed on glass slides, lightly stained with toluidine blue and examined in the light microscope. Thin sections were collected on copper grids and examined in a Philips EM 300 electron microscope either unstained or stained with uranyl acetate, lead citrate or a combination of both.

Aryl sulfatase. Thick epon sections of incubated specimens treated with $(NH_4)_2S$ and investigated in the light microscope showed a uniform and distinct positive reaction for aryl sulfatases in the granules of the metrial gland cells (Figure 1). A positive reaction was also observed in the cytoplasm of some surrounding fibroblasts as well as in certain endothelial cells. A study of unstained thin sections with the electron microscope revealed heavy deposits of lead sulfate in the large granules of the specific cells (Figure 3). Some granules with lead deposits showed a tendency to chip out of the sections leaving holes and tears in the latter. Those sections treated with $(NH_4)_2S$ often showed a diffuse background precipitate of lead sulfide. Staining with lead citrate enhanced contrast in the specimen and was not deleterious to the lead deposits. Staining with uranyl acetate on the other hand, led to a marked decrease in amount of the reaction product.

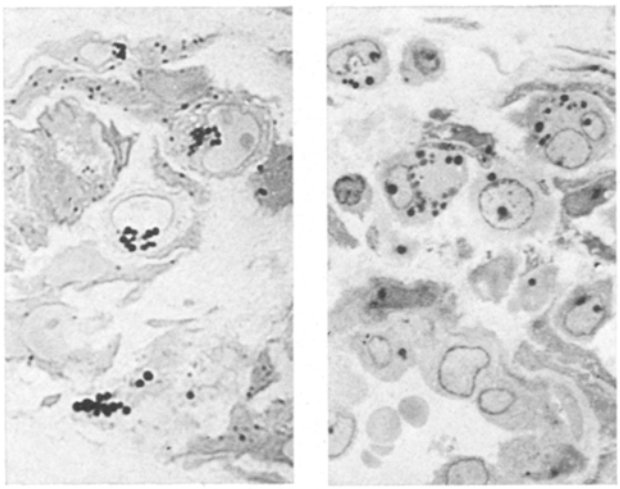

Fig. 1. Thick epon section of non-frozen specimen incubated for aryl sulfatase and posttreated with $(NH_4)_2S$. Heavy deposits of lead sulfide are seen in cytoplasmic granules of metrial gland cells. Light micrograph. × 920.

Fig. 2. Thick epon section of specimen incubated for acid phosphatase. Section lightly stained with toluidine blue. A positive reaction is seen in granules of typical metrial gland cells located in the vicinity of a blood vessel. Light micrograph. × 880.

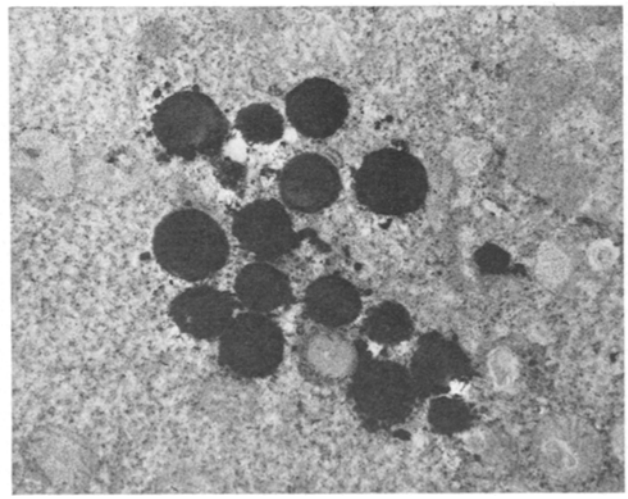

Fig. 3. Electron micrograph of a granulated metrial gland cell incubated for aryl sulfatase. The reaction product (lead sulfide) is localized in the cytoplasmatic granules. Chopped specimen posttreated with $(NH_4)_2S$, thin section unstained. × 13,300.

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Acid phosphatase. The positive reaction seen in the light microscope after incubation in a β-glycerophosphate-containing medium and post-treatment with $(NH_4)_2S$ (Figure 2) was never as intense as that observed with the sulfatase medium after $(NH_4)_2S$. It was also found in the electron microscope that the deposits of lead phosphate were not as dense as those of lead sulfate but appeared as needle-like precipitates. The reaction product appeared

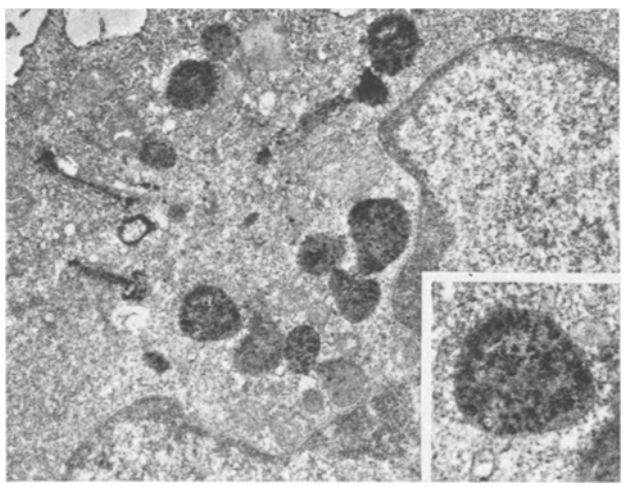

Fig. 4. Electron micrograph showing acid phosphatase activity in granules and Golgi-apparatus of a metrial gland cell. Chopped specimen treated with $(NH_4)_2S$ and thin section stained with uranyl acetate and lead citrate. × 11,600. Inset × 26,600.

not only in the granules but also in some cisternae of the Golgi complex (Figure 4). Furthermore, all granules were not consistently reactive, and there was also a certain variation between cells with respect to number of granules with a positive reaction. A positive reaction was also noted in lysosome-like bodies in fibroblasts and endothelial cells. Also with respect to the acid phosphatase reaction it was noted that staining with uranyl acetate removed considerable amounts of the reaction product from the sections.

Acid phosphatases and aryl sulfatases are both regarded as lysosomal enzymes. However, acid phosphatases have also been demonstrated in cellular structures not considered to be lysosomes, such as developing secretory granules in both endocrine and exocrine cells[16–18]. It has been suggested that this type of enzyme may be involved in secretory granule condensation processes[18] or that it may be included with secretory granules in an inactive form which may become active if the granules are destined for destruction rather than discharge[19]. The findings of acid phosphatase activity in structures belonging to the Golgi complex as well as in the granules of the metrial gland cells suggest the possible involvement of this organelle in granule production.

It is interesting to note that more metrial cell granules showed a positive reaction for aryl sulfatase than for acid phosphatase. Such a variance was reported among lysosomes in some tissues already by GOLDFISCHER[13] in his original report on the histochemical method. It is at present unknown if this difference is due to a real heterogeneity among the granule population or to technical diversities. The presence of aryl sulfatases in the granules is interesting from the point of view that the granules appear to contain a sulfated polysaccharide component of a low degree of esterification[2,3]. However, lacking evidence for the normal occurrence of aryl sulfates as sulfate donors any suggested connection between the sulfatase and the sulfated compound of granules would be merely speculative.

Although it is evident from the present as well as previous investigations[8,9,20] that the specific granules of the metrial gland cells contain lysosomal enzymes, it is not clear if the granules are to be classified as lysosomes, secretory granules or a type of storage granule.

In view of the fact that the metrial gland cells are not phagocytic[21,22], it is difficult to believe that the high content of hydrolytic enzymes can be solely required for intracellular metabolism[9]. It has also been shown that the metrial gland has a high collagenase activity[23]. BULMER[9] has correlated this finding with the possible location of relaxin in the cells and forwards the suggestion that the cells may be involved in the post-partum resolution of the placental site. It is a vexed question whether or not the metrial gland cells of the pregnant rat uterus produce relaxin. This has been suggested by VELARDO et al.[6] and WISLOCKI et al.[3] and more recently by DALLENBACH-HELLWEG et al.[7] who, in using immunofluorescence techniques, have traced this substance to the cells. However, BLOOM, PAUL and WIQVIST[24], using an in vitro technique, investigated a number of pregnant rats tissues for relaxin, including the uterine sites of the metrial gland. Only the pregnant rat's ovaries contained significant amounts of relaxin. These findings were confirmed by STEINETZ, BEACH and KROC[25]. In the light of these findings also DALLENBACH-HELLWEG et al.[7] consider their negative immunofluorescent results with respect to rat ovaries difficult to explain. BULMER[9] suggests that the relaxin demonstrated by DALLENBACH-HELLWEG et al.[7] may simply be a storage material derived from a primary source elsewhere rather than a hormone synthesized by the metrial gland cells.

Further studies are in progress to elucidate the function of the metrial gland cells and the nature of their granules[26].

Zusammenfassung. Die beiden lysosomalen Fermente (saure Phosphatase und Aryl Sulphatase) wurden mit elektrohistochemischer Technik in den zytoplasmatischen Zellgranula der Glandula myometralis gravider Ratten demonstriert. Die Befunde werden in Beziehung zur heutigen Kenntnis der funktionellen Granulanatur diskutiert.

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