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Ultrastructure of the Adrenal Medulla of Normal and Insulin-Treated Hamsters

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Summary. Fine structural characteristics of the chromaffin cells both in normal and insulin-administered hamster adrenal gland were studied.

Exocytosis occurs in 5 per cent of nonstimulated cells especially on the apical cell surfaces. At the same time the occurrence of a great number of closely attached secretory granules was conspicuous on the lateral plasma membrane in the untreated hamster adrenal medulla.

Following insulin treatment (10 IU/100 g/body weight), characteristic was the development of large intercellular vacuoles between the lateral plasma membrane, in which electron-dense secretory material was frequently present. On the basis of this observation it is suggested that in the case of insulin-induced hormone secretion, exocytosis preferentially occurs on the lateral plasma membrane, and may play an important role in the discharge of secretory materials from the cells.

Key words: Adrenal medulla — Golden hamster — Insulin treatment — Induced exocytosis — Hormone discharge.

Introduction

The term exocytosis was introduced by De Duve in 1963 and since then a number of experiments have given evidence for the occurrence of this phenomenon in endocrine glands as well as in several other tissues (see Smith and Winkler, 1972; Benedeczky and Smith, 1972; Röhlich *et al.*, 1971; Masur *et al.*, 1972). Exocytosis has been extensively studied in the adrenal medulla by means of various methods; those of biochemistry (Banks, 1966; Helle, 1966; Blaschko, *et al.*, 1967; Schneider *et al.*, 1967) pharmacology, (Douglas and Poisner, 1966; Kirshner *et al.*, 1967; Viveros *et al.*, 1969) and morphology (Coupland, 1965; Diner, 1967; Benedeczky and Smith, 1972). Today it is generally accepted that the process is of basic significance in the secretion of catecholamines.

Exocytosis is easily detectable in the adrenomedullary chromaffin cells of the golden hamster. Nevertheless, some difficulties have emerged in connection with its detection in other species (Benedeczky and Smith, 1972) and there have also been discrepancies between the frequency of exocytosis and the stimulated secretion process (Smith *et al.*, 1973; Abrahams and Holtzman, 1973).

The electron microscopic results demonstrating exocytosis in the nonstimulated adrenal medulla of golden hamster have also been confirmed by the freeze-etching technique (Smith *et al.*, 1973). Moreover, these authors studied the adrenal medulla stimulated by drugs and described the increase of exocytosis.

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Since in the literature there were no conventional electron microscopic observations on stimulated hamster adrenal medulla available, the aim of this work was to study the fine structural changes of insulin-treated chromaffin cells, particularly in respect of the development of exocytotic profiles.

Materials and Methods

Adult golden hamsters (100–120 g) of both sexes were used: 10 animals were used as controls, and other groups of 10 animals were sacrificed at 2, 3, 4, and 6 hr after insulin treatment. The animals were not fed for 24 hr before injection of insulin, but water was allowed ad lib. The insulin was injected i.p. in a dose of 10 IU/100 g/body weight. To prevent convulsions, 1–2 ml 5% dextrose solution were given i.p. 90 min after administration of insulin. The animals were killed 2, 3, 4, and 6 hr following insulin treatment.

Two methods were used to fix the adrenal medullary tissue: immersion of the gland in fixative solution or perfusion in situ with fixative solution followed by immersion. For fixation by immersion, the animals were decapitated, and the adrenal glands removed immediately.

Before fixation by perfusion, the animals were anesthetized with ether. Perfusion fluid was introduced a needle into the left ventricle of the heart and was allowed to escape through a cut in the right auricle. The animals were perfused with saline (NaCl, 0.9% wt/vol) for 2 min to remove most of the blood, and then with fixative solution (2.5% glutaraldehyde and 4% formaldehyde) for 30 min. After perfusion, the glands were immersed overnight in the same solution.

The material fixed by perfusion and/or immersion was washed for 10 min in 0.1 M phosphate buffer, (pH 7.4) containing sucrose (7.5% wt/vol).

After washing, the aldehyde-fixed tissue was cut into cubed blocks immersed in osmium tetroxide (2% wt/vol), and dissolved in Palade's (1952) veronal buffer for 2 hours at 4° C. The blocks were then dehydrated in alcohol (30%, 70%, 95%, 100%) and embedded in Durcupan ACM. Ultrathin sections were cut with an LKB ultrotome and stained with a saturated aqueous solution of uranyl acetate for 30 min followed by Reynold's (1963) lead citrate for 2 min. Sections were examined in a JEM 7A electron microscope.

Results

1. Ultrastructure of Normal Adrenal Medulla of Golden Hamster

A great proportion of the chromaffin cells is located around the sinusoids (Fig. 1). In the lumen of the sinusoid, platelets are often visible. The cytoplasm of the endothelial cells is flattened and the basal lamina fused with the basement membrane of the chromaffin cells. Distribution of secretory granules in the cells is not uniform. The granules are generally concentrated at the apical poles of the cells; thus their number is low around the nucleus and in the area of the Golgi apparatus. Most of the granules are spherical and their membranes easy to detect around the highly electron-dense center. There are a great number of "closely attached" secretory granules along the plasma membranes, on the apical poles, and on the lateral sides of the cells. In a great proportion of chromaffin cells exocytosis in not observable (Fig. 1). Among 600 nonstimulated control cells only 29 cells (approx. 5% of the examined cells) showed exocytotic profiles on the cell membranes. In these cells where exocytosis occurs (Fig. 2), there are often four to five exocytotic profiles on a relatively short section of the apical plasma membrane. A few exocytotic profiles occur also on the lateral plasma membrane of the cell.

2. The Ultrastructure of the Adrenal Medulla Following Administration of Insulin

Three hours after insulin treatment, ultrastructural changes related to stimulated secretion were visible in a great proportion of medullary cells. Exocytosis

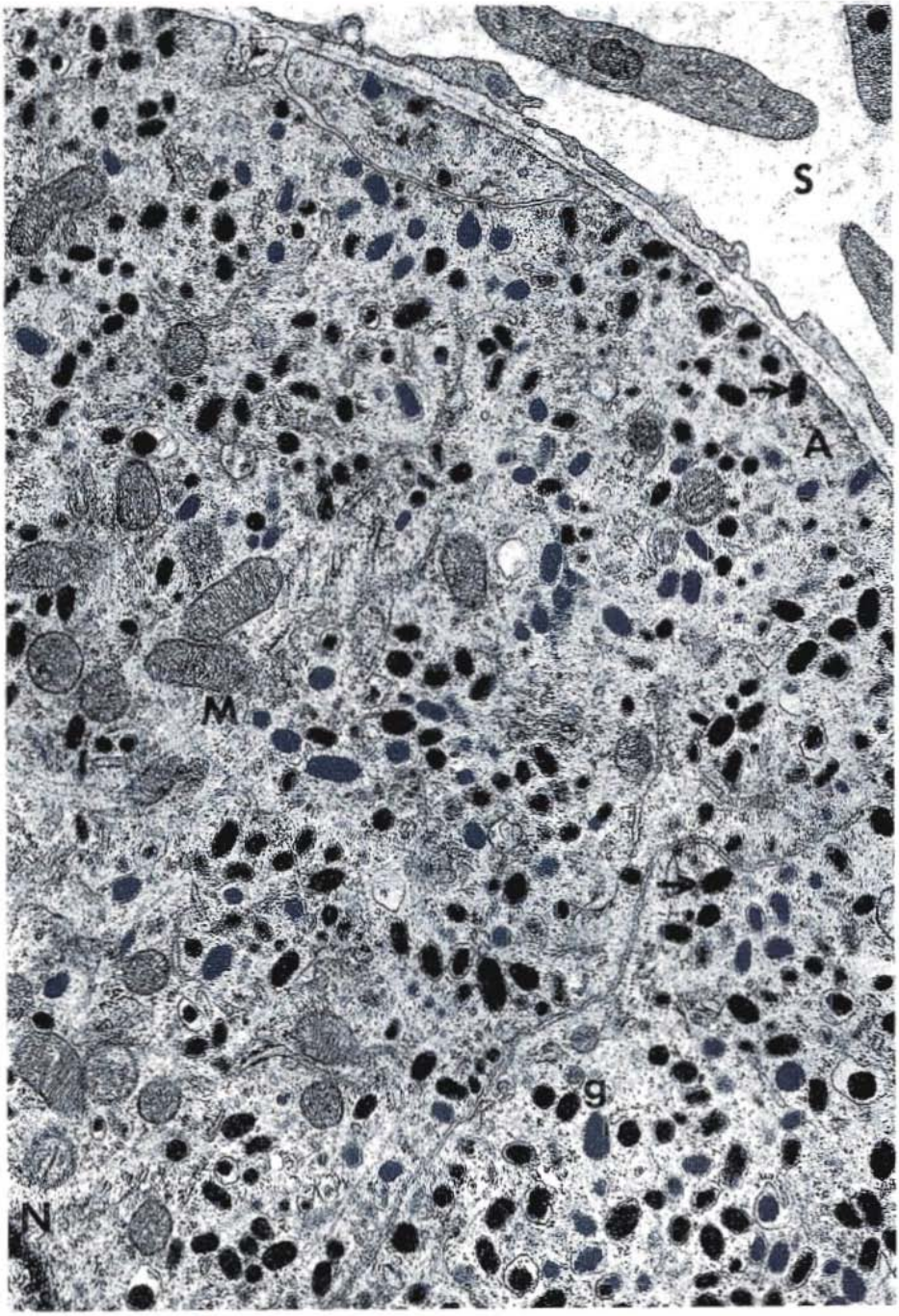

Fig. 1. Adrenomedullary cells of normal hamster, located around the sinusoid *(S)*. Secretory granules *(g)* are generally concentrated at apical poles of cells *(A)*. There are a great number of "closely attached" secretory granules along the apical and lateral plasma membranes (arrow). Exocytosis is not detectable on these cells. Note nucleus *(N)* and mitochondria *(M)*. × 18000

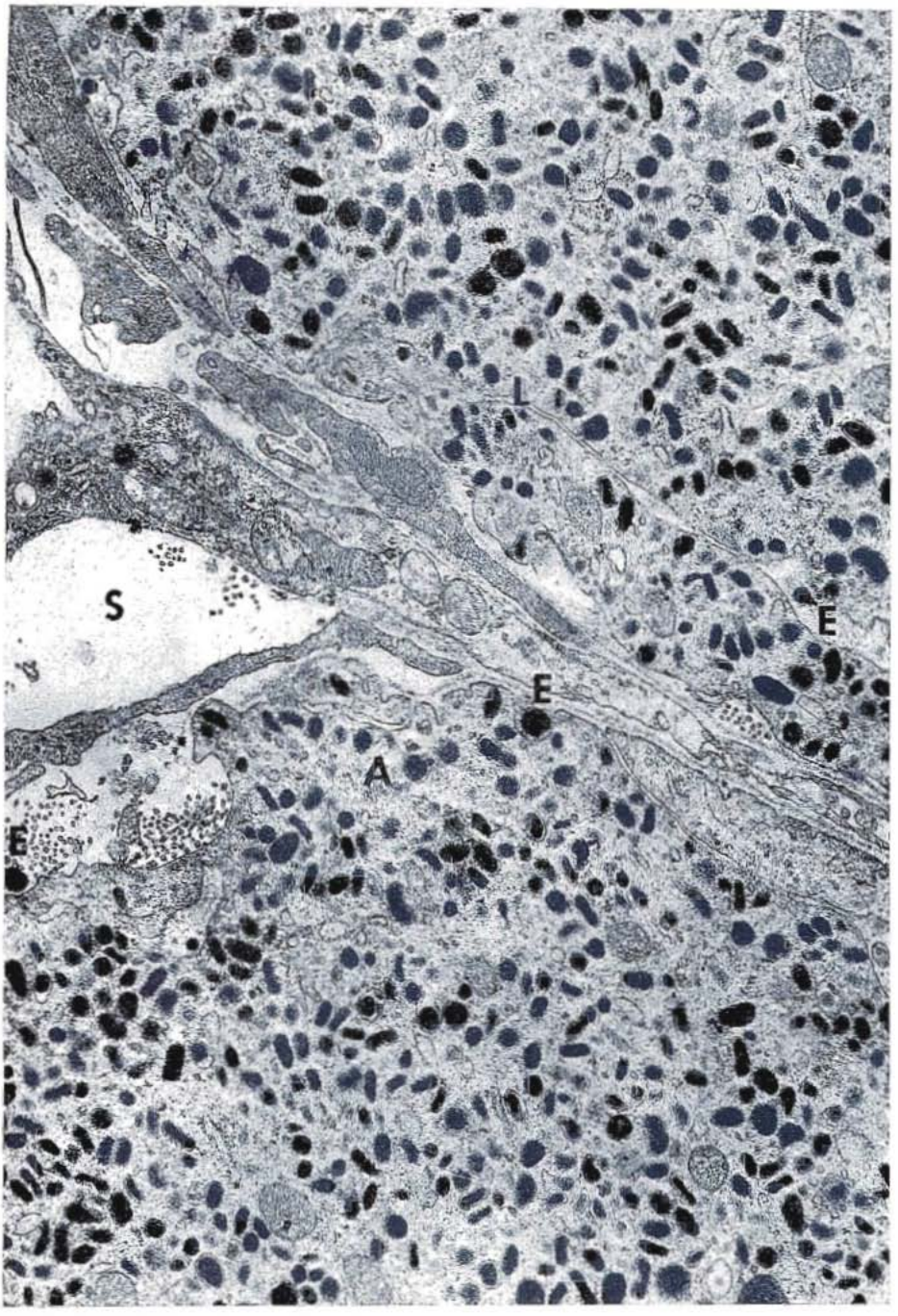

Fig. 2. Chromaffin cells in the nonstimulated control adrenal along a sinusoid *(S)*. Exocytosis *(E)* clearly and easily detectable, both on the apical *(A)* and lateral cell membranes *(L)*. × 18000

was frequent in these stimulated chromaffin cells, particularly on the lateral plasma membrane (Fig. 3). The omega shaped invaginations of the exocytotic profiles were often extremely dilated, given rise to vacuoles. However, the electron-dense material of the extruded granules was usually detectable in the lumen of the vacuoles. At the same time it was surprising that a conspicuous increase of exocytosis could not be observed at the apical poles of stimulated chromaffin cells (Fig. 4). In some instances exocytosis was found also on the lateral surface of cells containing norepinephrine.

Six hours after insulin treatment the vacuoles formed after exocytosis were still numerous and their lumina were constantly dilated, but the electron-dense material of secretory granules was no longer observable (Fig. 5). The number of secretory granules decreased in the cytoplasm whereas the amount of the rough-surfaced endoplasmic reticulum increased.

Discussion

Although a number of authors (Diner 1967; Grynszpan-Winograd, 1971; Benedeczky and Smith, 1972) provided morphologic examples of exocytosis in the hamster adrenal medulla, the stimulated gland—as far as we know—has not been examined so far by conventional electron microscopy. The studies of Yates (1964) were concerned with the effect of insulin administration on the Syrian hamster but at that time exocytosis had not yet been described in endocrine tissues and the author suggested that the release of catecholamines was not dependent upon the complete disintegration of secretory granules. In view of recent data on exocytosis the question arises whether or not there is a morphologically detectable correlation between stimulated secretion and exocytosis in the hamster adrenal medulla? Before answering the above question it is considered necessary to analyze briefly the fine structural features of the nonstimulated adrenal medulla. In this regard the following observations were made:

1. Frequency of exocytosis was low in the nonstimulated (normal) adrenal medullary cells (approx. 5%).

2. Numerous exocytosis may occur at the same time on the surface of a given chromaffin cell.

3. A great number of secretory granules are in close morphologic contact with the plasma membranes.

The incidental occurrence of exocytosis in the nonstimulated hamster adrenal medulla is not surprising. According to physiological data the so-called "resting secretion" of the adrenal medulla is very small (0.1 μg/min body weight kg: Euler, 1956; Holtz *et al.*, 1952; Malmajec, 1952). It is also possible that exocytosis, as a single extrusion process, is able to perform this function satisfactorily. The fact, however, that at the same time, a large number of secretory granules is attached to the plasma membranes suggests that hormone discharge can take place also in other ways.

According to Smith and Winkler (1972) there may be "a high frequency of random collisions between the vesicles and the inner side of the plasma membrane as a result of Brownian motion."

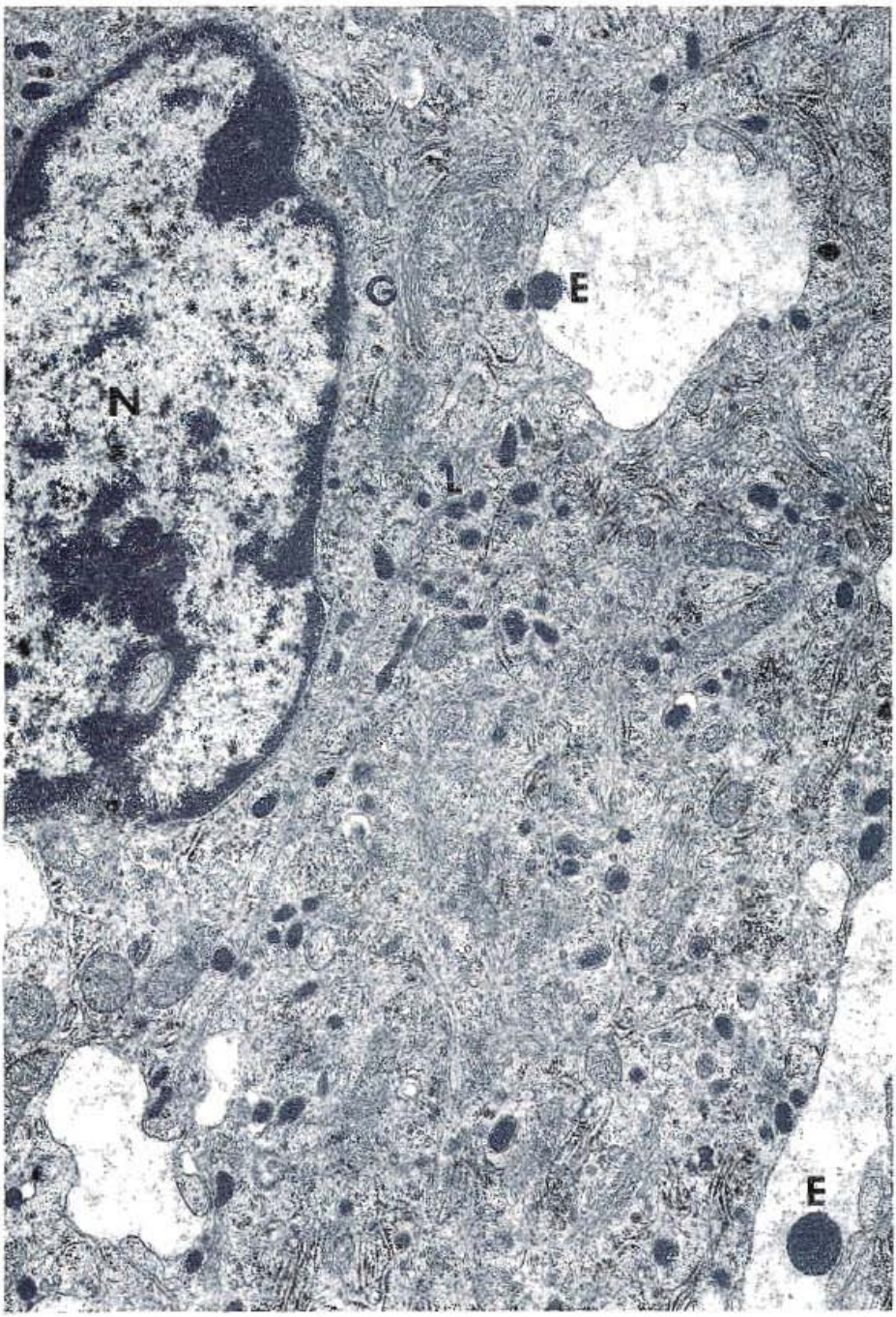

Fig. 3. Exocytosis *(E)* was frequent in a great proportion of chromaffin cells, particularly on the lateral plasma membrane *(L)* after 3 hours of insulin (10 IU/100 g) treatment. Note nucleus *(N)* and well-developed Golgi apparatus *(G)*. × 18000

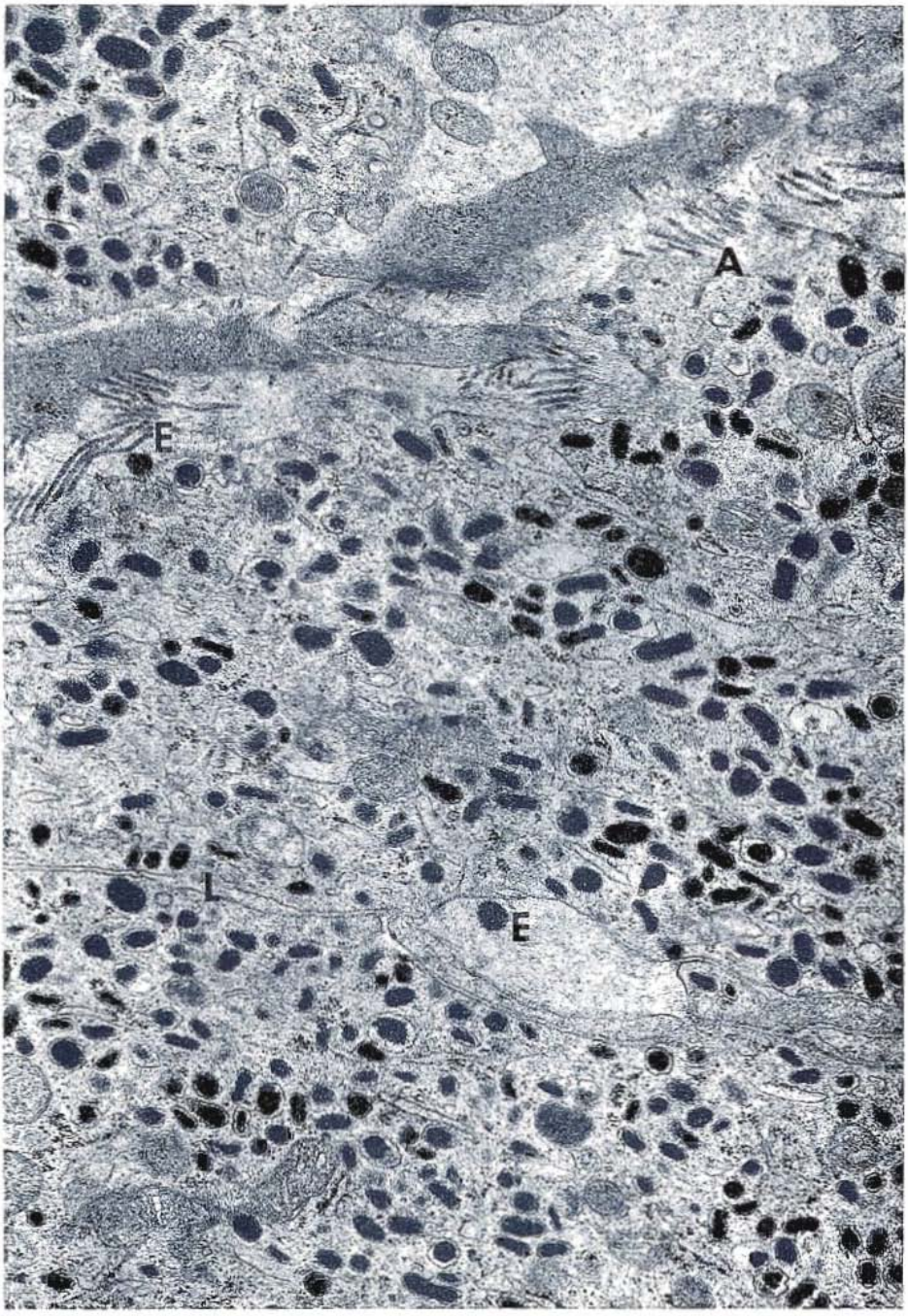

Fig. 4. Chromaffin cells, 3 hours after insulin (10 IU/100 g/body weight) administration. In a group of cells occasional exocytosis *(E)* was observed on apical *(A)* and lateral *(L)* plasma membrane. × 18000

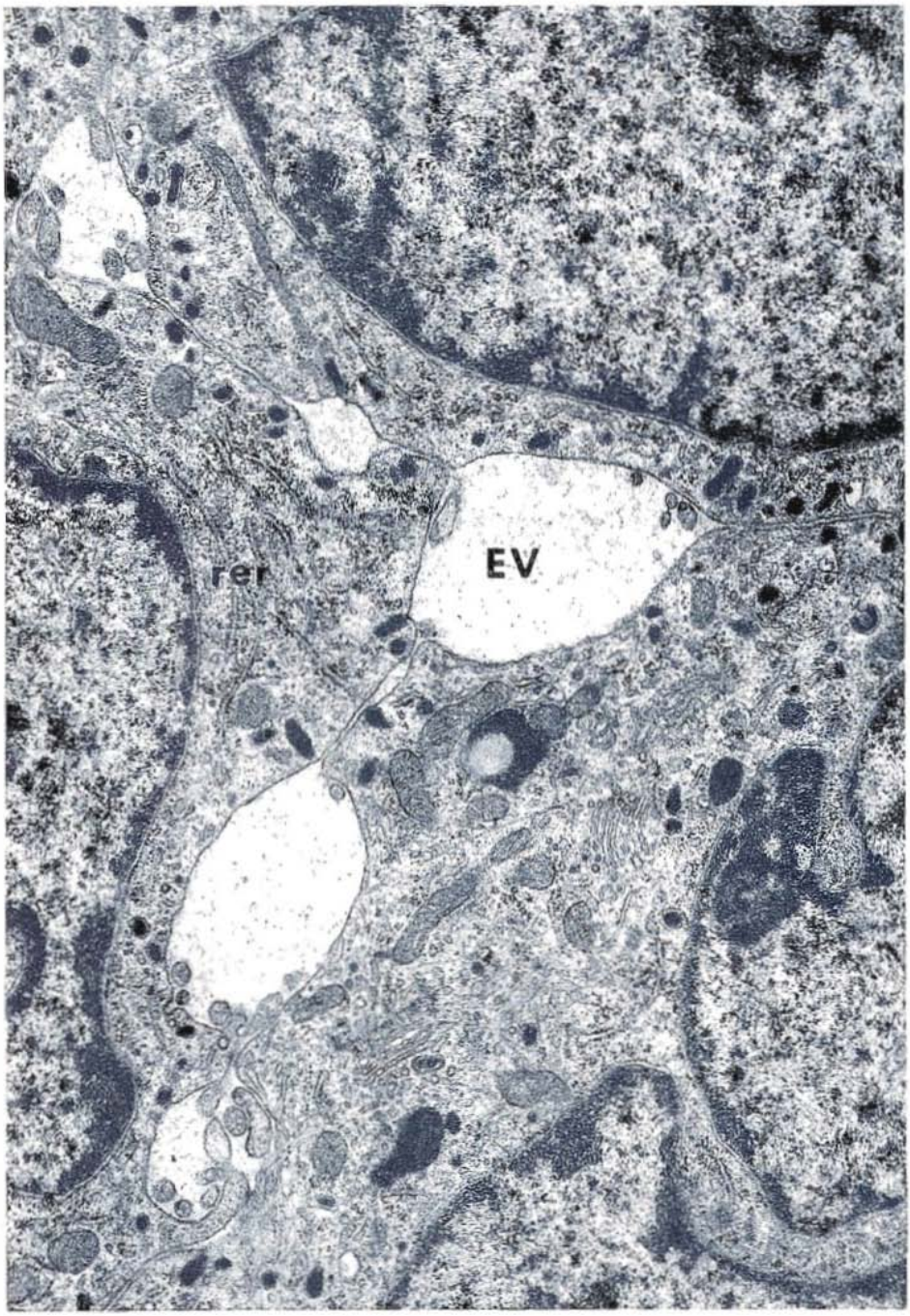

Fig. 5. Chromaffin cells, 6 hours after insulin treatment. Exocytotic vacuoles *(EV)* were still numerous but electron-dense material of secretory granules was no longer observable. Note abundance of rough-surfaced endoplasmic reticulum *(rer)*. × 18000

In consequence of the collision the membrane of the granules may fuse with the plasma membrane, and the fusion, gives an opportunity for the release of secretory material. If the fusion is short (1 ms or less) the release of the low molecular weight substances takes place preferentially and the secretory granules become relatively rich in protein (e,g., dopamine β hydroxylase: Smith and Winkler, 1972). All of this suggests that exocytosis is not the exclusive way of hormone release. In the nonstimulated hamster adrenal medulla therefore both exocytosis and the close morphologic contact of secretory granules to the cell membranes (tight junction) may represent a form of hormone secretion.

In order to study whether the ultrastructural organization of actively secreting cells changes in the stimulated gland, golden hamsters were subjected to insulin treatment. Since the catecholamine-mobilizing effect of insulin is well known, an increase in the frequency of exocytosis was expected to occur first of all on the apical surfaces of chromaffin cells. Contrary to this, however, an increased incidence of exocytosis was not observed on the apical cell membrane, but on the lateral one. It was conspicuous that at the site of exocytosis, large vacuoles also developed. On the basis of these the question arises as to why the exocytotic profiles preferentially develop on the lateral plasma membranes. An answer can be suggested if we assume that during an increased and long lasting secretion, (i.e., induced by insulin) the apical pole of chromaffin cells becomes unable to perform the discharge of secretory material. In consequence, it is obvious that the large surface of lateral plasma membranes furnishes an excellent opportunity for hormone liberation and so they become the main point for the events of exocytosis. On the basis of morphologic observations it is rather difficult to explain the significance of large exocytotic vacuoles on the lateral cell surface under intensive secretion in the hamster adrenal medulla.

Our preliminary morphometric measurements (Benedeczky *et al.*, 1973) showed that the concentration of secretory granules in the cytoplasm decreased. This indicates that the insulin-induced hormone mobilization also causes an absolute decrease in the number of secretion granules. In other words, their disappearance occurs by means of a complete disintegration process, that is, by exocytosis.

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