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ULTRASTRUCTURAL STUDY OF THE FORMATION OF SECRETORY GRANULES IN THE CHROMAFFIN CELLS OF THE ADRENAL GLAND

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Abstract

The author studied the granulogenesis of chromaffin cells in the adrenal gland of golden hamster at ultrastructural level. It was found that the Golgi apparatus of the glandular cells was in tight morphological relationship with the rough surfaced endoplasmic reticulum ,mitochondria and various types of vesicles. The development of the prosecretory granules was observed first of all in the terminal vesicles of the Golgi apparatus. A significant amount of degranulated tubules of the endoplasmic reticulum was also detected in the direct neighbourhood of the Golgi apparatus, where even the detachment of the smooth surfaced vesicles was observable on the terminal parts of the tubules. It was presumed that the smooth surfaced vesicles transport secretory proteins from the rough surfaced endoplasmic reticulum to the elements of the Golgi apparatus. Studying the granulogenetic role of the cell nucleus it was found that Actinomycin D and 5 Fluorouracil treatment significantly altered the chromaffin cells, especially the ultrastructure of the nucleus and the granulogenetic processes representing hormone resynthesis.

All these observations are in favour of the fact that the granulogenesis of the chromaffin cells stands under regulation even at cellular level; that is, this phase of the secretory process is nucleus-dependent.

Introduction

The biochemical analysis of isolated chromaffin granules has revealed that macromolecular material and substances of low molecular weight can be demonstrated in a rather significant amount in the granules (WINKLER, 1976). Amoung the macromolecular components proteins are present in the highest amount in the chromaffin granules, from which the chromogranin also contains amino sugars (SMITH and WINKLER, 1967) and sialic acid in 3% (BARTLETT and SMITH, 1974). Enzymes also occur among the protein components (dopamine β-hydroxylase, ATP-ase) and even the presence of cytochrome B 559 and certain flavoproteids could be demonstrated (FLATMARK et al. 1971). The high lizolecithin content of the chromaffin granules is striking (16.8% of the total amount of phospholipid), which may stand in connection with the exocytosis of the granules (WINKLER and SMITH, 1975).

The characteristic feature of the chromaffin granules is that the water soluble fraction contains a large number of material with low molecular weight, catecholamines and nucleotides (WINKLER and SMITH, 1975). In the knowledge of the mainer chemical components of the chromaffin argnules, arises the question; where and how many various chemical components develop and by which cell biological processes they "become packed" into the secretory granules? Certain events of the granulogenesis, like the incorporation of ^{3}H-labelled leucin into the chromogranin molecules, can be well followed by biochemical methods. Other partial processes are less clear for example the origin of the secretory granule membrane, the path and transport mechanism of the secretory proteins. The morphological relationship between the

Golgi apparatus and the endoplasmic reticulum in granulogenesis are not clear enough either, furthermore the cellular mechanism of the granulogenetic regulation and within this the direct morphogenetic regulatory role of the cell nucleus is almost completely unkpown.

Therefore, our studies aimed to describe the mode of formation of the chromaffin granules and to provide new data regarding the granulogenetic role of the cell nucleus on the basis of detailed ultrastructural analysis.

Materials and Methods

Golden hamsters *(Mesocricetus aureus)* used in our studies were obtained from the central animal house of the Oxford University. Before taking samples for electron microscopic studies the animals were narcotized with 3.5% chloral-hydrate, the chest was opened, cannula was led into the left ventricle of the heart, and the right auricle was incised with a single cut. Before perfusion fixation the circulatory system of the animals was rinsed with physiological salt solution, then after washing the blood perfusion fixation was carried out for 20—25 min. with a fixative mixture containing 2.5% glutaraldehyde (TAAB) and 4% formaline. Following perfusion the adrenal glands were taken out, cut into two pieces with a razor blade and further fixed in a refrigerator for 1 hr in the above fixative at 4 °C. Prefixation was followed by postfixation with osmium tetroxide in 2% OsO_4 lasting for 1—2 hrs, buffered according to Palade. Dehydration was carried out on ascending alcohol series and the samples were embedded in Durcupan ACM. The ultrathin sections were prepared with an LKB ultramicrotome. The sections were double contrasted, "stained" for 30 min. in saturated uranyl acetate diluted solution, and contrasted with lead citrate ofter being washed in destilled water.

Treatment of the experimental animals:

Before insulin treatment the animals were starved for 24 hrs with securing tap-water. A single i.p. treatment was carried out with insulin with the doses of 10 and 20 IU/100 g. 6 hrs following this, the animals received Actinomycin D (AD) and 5-Fluorouracil (5-FU) treatment. Doses of 250 ug/kg (AD) and 250 mg/kg (5-FU) were administered i. p.

Samples were taken for electron microscopic studies 3, 6, 24, 48, 72, 96, 120, 114 and 168 hrs after the insulin treatment. Following the insulin + AD and 5-FU treatment, respectively, the first samples were taken after 24 hrs, and the further time-points were the same. The electron microscopic pictures were prepared on a JEM 100 B type electron microscope.

Results

Morphological characteristics of granulogenesis in normal adrenal gland

Well developed Golgi area was observable in the direct neighbourhood of the cell nucleus in the chromaffin cells of the adrenal gland, as is general in glandular cells (Fig. 1).

The characteristic morphological feature of the Golgi area in the chromaffin cells was that many rough surfaced endoplasmic reticulum tubules and mitochondria could be found in loose substance, that is the membrane components forming the Golgi apparatus (saccules and vesicles) were in tight topographical connection with the afore mentioned cell organelles (Figs. 1, 2, 3).

The Golgi apparatus itself was mainly made up of saccular elements, 3—4 parallel saccules could be seen side by side (Figs. 1, 3). The Golgi vesicles occured in varying amount in the whole area of the Golgi, frequently in the direct neighbourhood of the saccules, but also further from them. Golgi vacuoles were only sporadically found in the chromaffin cells.

The size and morphological characteristics of the vesicles found in the Golgi area showed rather great variation. The smallest Golgi vesicles had diameters of

about 50 nm, the largest ones even reached 200 nm. The majority of the Golgi vesicles were electron lucent or moderately electron dense. Vesicles with spiny edges were also observable in significant number among the smooth surfaced Golgi vesicles (Figs. 2, 3, 4). The presence of electron dense material was frequently observed in the terminal vesicles of the Golgi saccules (Figs. 1, 2, 3, 4), but the prosecretory material was rarely present in the centrally developed vesicle of the saccule. The precursors of the secretory granules, the so-called prosecretory granules, were not only observable on the terminal part of the Golgi saccules, but also perisaccularly, in the whole Golgi area; in smaller-larger number (Figs. 1, 2, 3). The recognition and separation from the „mature" secretory granules was possible on the basis of their smaller size and the lower inner density.

Tight morphological relationship was detectable not only between the Golgi saccules and the prosecretory granules, but between the rough surfaced endoplasmic reticulum tubules and the newly formed granules as well. Thus, for example, the ribosomes were still well observable on the end of the membrane of the rough surfaced endoplasmic reticulum cistern which extended into the Golgi area (Fig. 3). However, the long tubule forming the extension of the cistern was already agranular and showed tight adjustment to the neighbouring secretory granules.

The development (Figs. 2, 3) and attachment of a few vesicles could also frequently be observed on the terminal part of the degranulated Er tubules, therefore it is probable that one part of the vesicles found in the Golgi area is of rEr origin.

Occasionally, the presence of so-called spiny sections was detectable on the central or terminal membrane of certain Golgi saccules. On the basis of the picture it cannot be decided whether these „spiny-edged" membrane sections were becoming attached or incorporated, nevertheless, their presence was noteworthy (Fig. 4), since the Golgi membranes are traditionally regarded to be smooth surfaced membranes. The rootlet of the centriole (Fig. 1), microtubules and multivesicular bodies were also observable in the Golgi area (Fig. 2).

The ultrastructure of the chromaffin cells of the adrenal gland following 5-Fluorouracil and Actinomycin D treatment

Following 5-Fluorouracil (5-FU) treatment so-called spotted nucleoli appeared in the majority of the nuclei of the chromaffin cells (Fig. 7). Due to their high electron density the „spots" were rather striking and were present in an even distribution in the substance of the nucleus. Both granular and filamentous components were detectable in the „spots" (Fig. 7). The 5-FU treatment rarely resulted in the development of ring-shaped nucleoli, too (Fig. 6).

Actinomycin D (AD) treatment produced the characteristic segregation of the nucleoli (Fig. 5). In the segregated nucleoli an electron dense granular and a fibrillar zone could be separated, and besides these the development of a lighter amorphous area was also detectable (Fig. 5). The cytoplasm was firstly characteristic of having lower amount of secretory granules, but the presence of many granules having irregular shape and low electron density was also a characteristic feature (Fig. 9).

The appearance of so-called quadrilamellar membranes in the chromaffin cells was also a new phenomenon (Fig. 8). They often developed in the direct neighbourhood of the cell nucleus, the inner membranes were agranular, and several ribosomes were observable on the outer ones. The Golgi apparatus was relatively small in the chromaffin cells treated with 5-FU (Fig. 9).

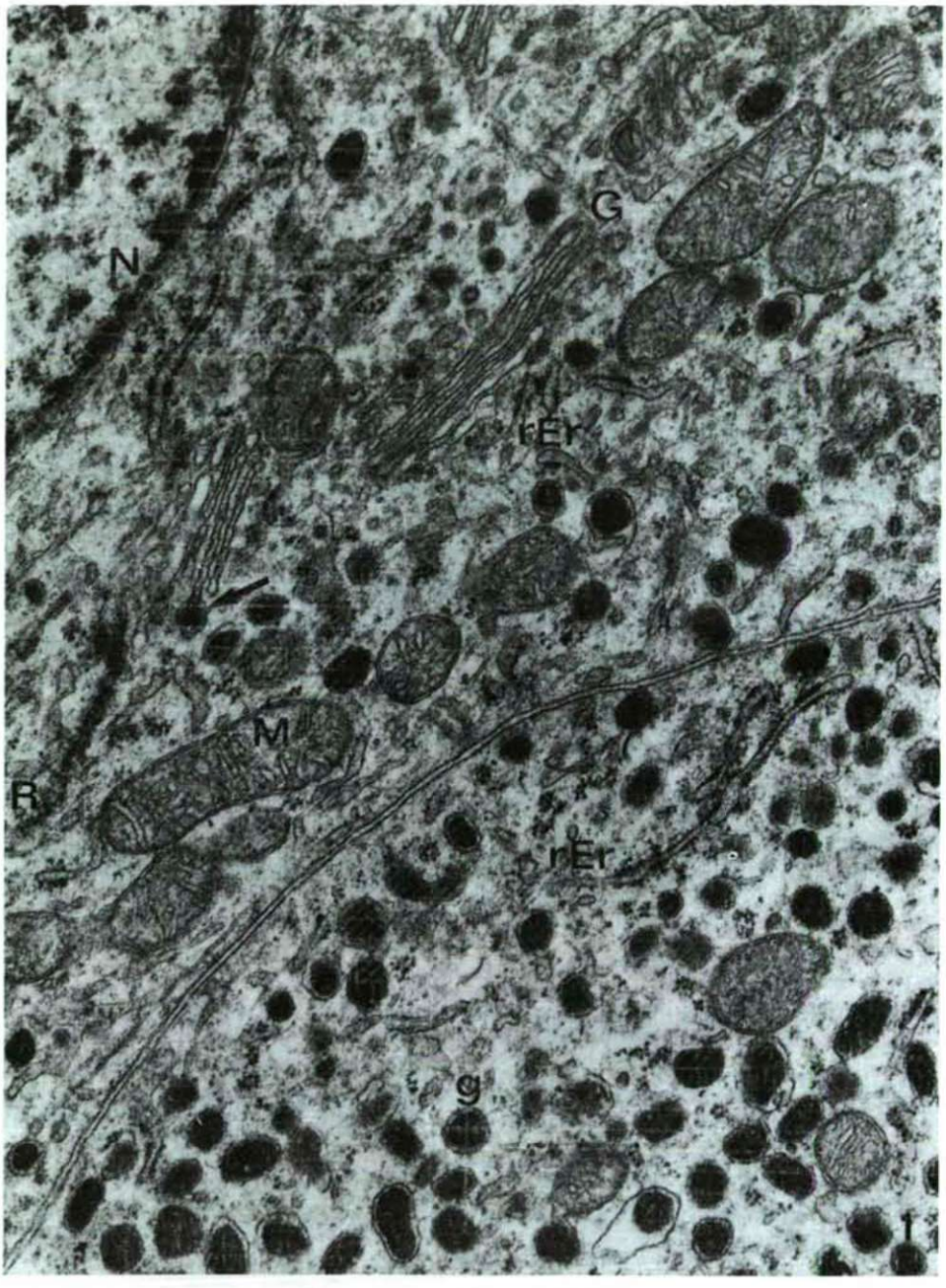

Fig. 1. Chromaffin cells of adrenal gland in untreated golden hamster. Extented Golgi area (G) can be seen beside the cell nucleus (N). The development of prosecretory granule is observable in the terminal cistern of some Golgi saccules (→). Several rough surfaced endoplasmic reticulum tubules (rEr) and mitochondria (M) can be seen in the Golgi area. R = rootlet, g = mature secretory granule. X 25 000.

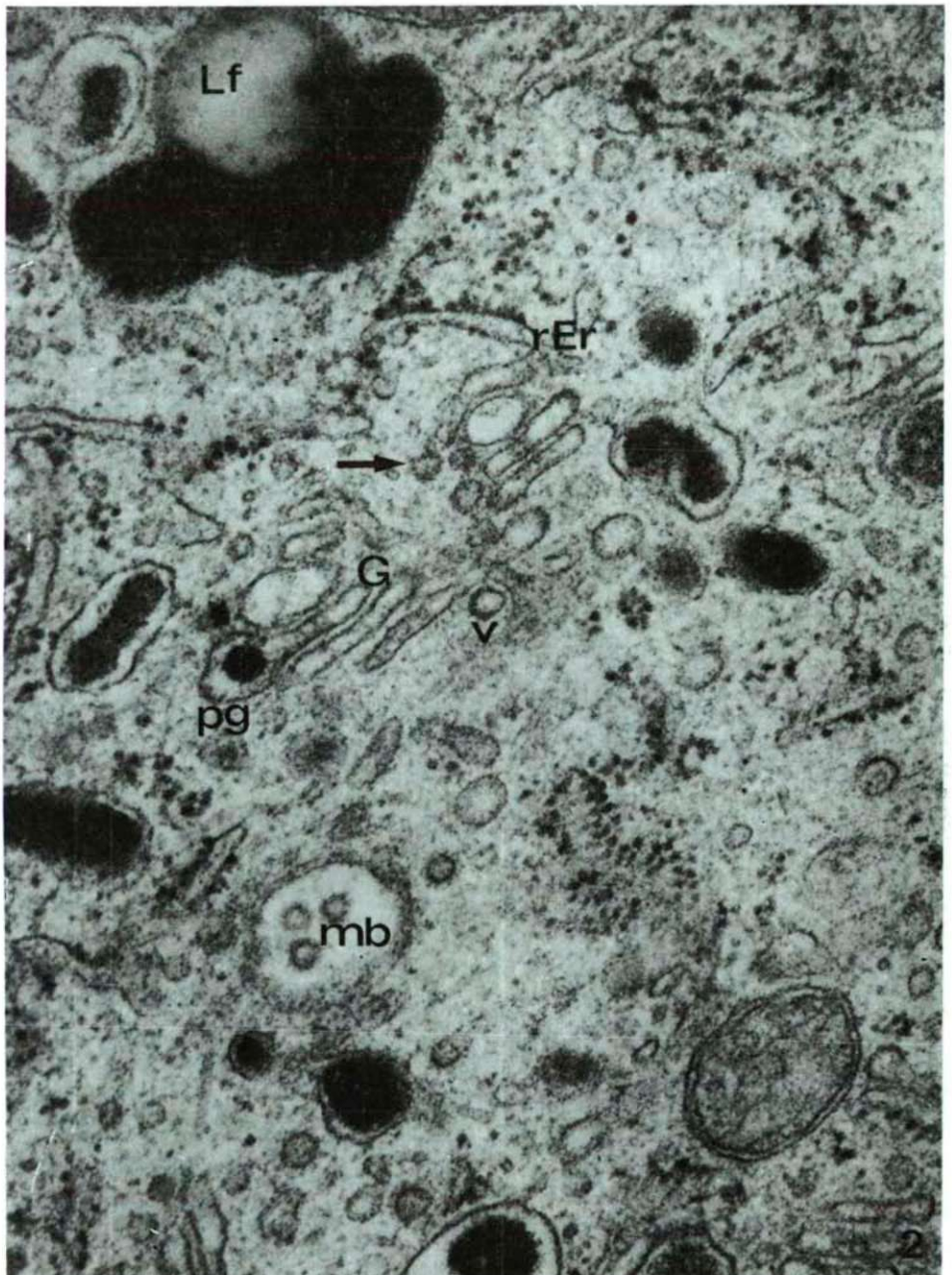

Fig. 2. Detail of Golgi apparatus (G). Several Golgi vesicules (v) are occupied beside the saccules. Degranulated tubules of rough surfaced endoplasmic reticulum (rEr) infolding into the Golgi area and a newly formed prosecretory granule (pg) are striking. The detachment of smooth surfaced vesicules from certain degranulated Er tubules is observable (→) Lf = lipofuscin granule, mb = multivesicular body. X 35 000.

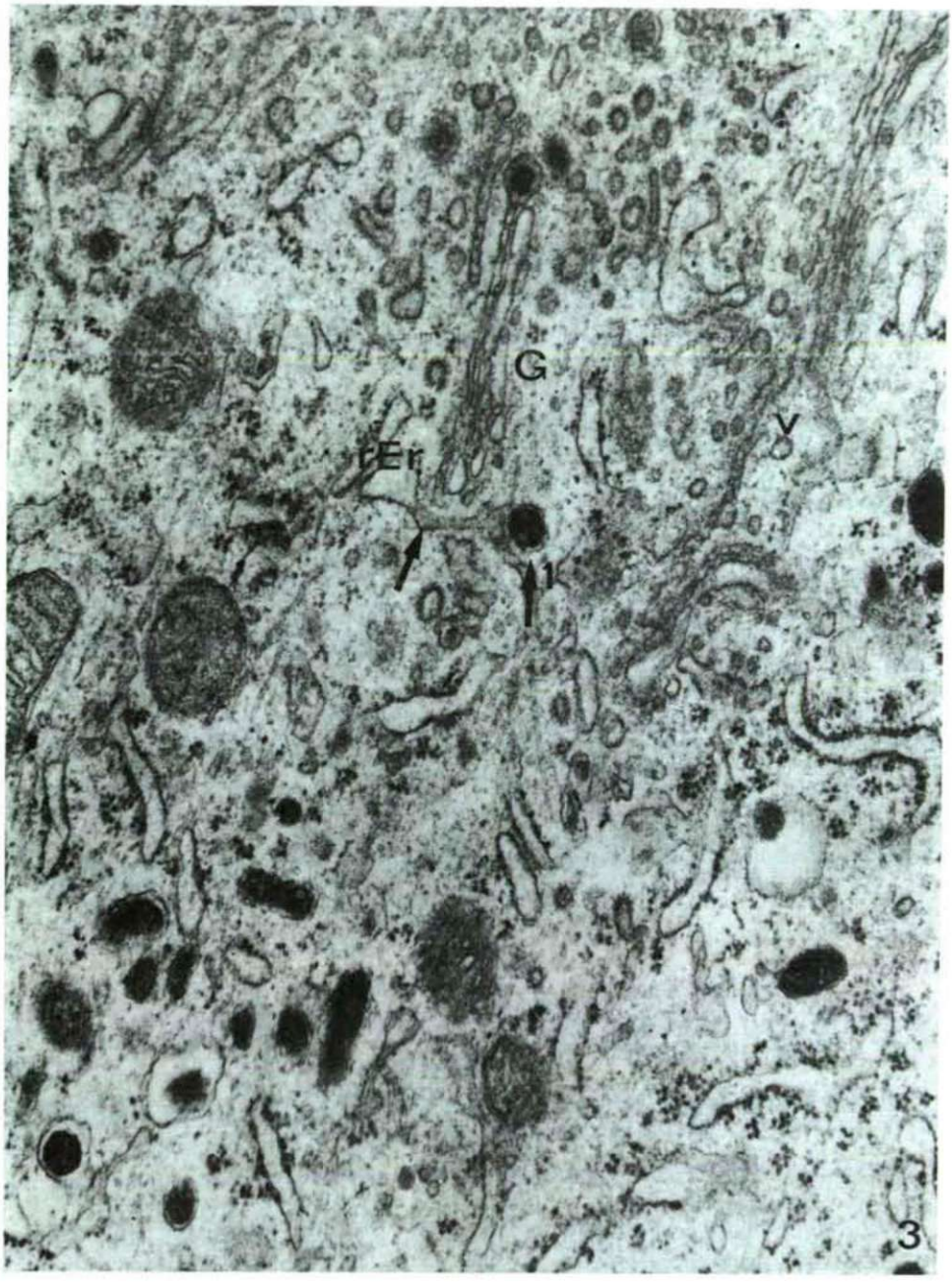

Fig. 3. Detail of chromaffin cell from untreated golden hamster adrenal gland. In the Golgi area (G) dilated rough surfaced endoplasmic reticulum tubules (rEr) are detectable in significant amount. The membrane surface of the tubules is partially degranulated (→). On some parts the tight morphological connection of the prosecretory granules and the degranulated endoplasmic reticulum is detectable (→ 1), v = Golgi vesicule. X 20 000.

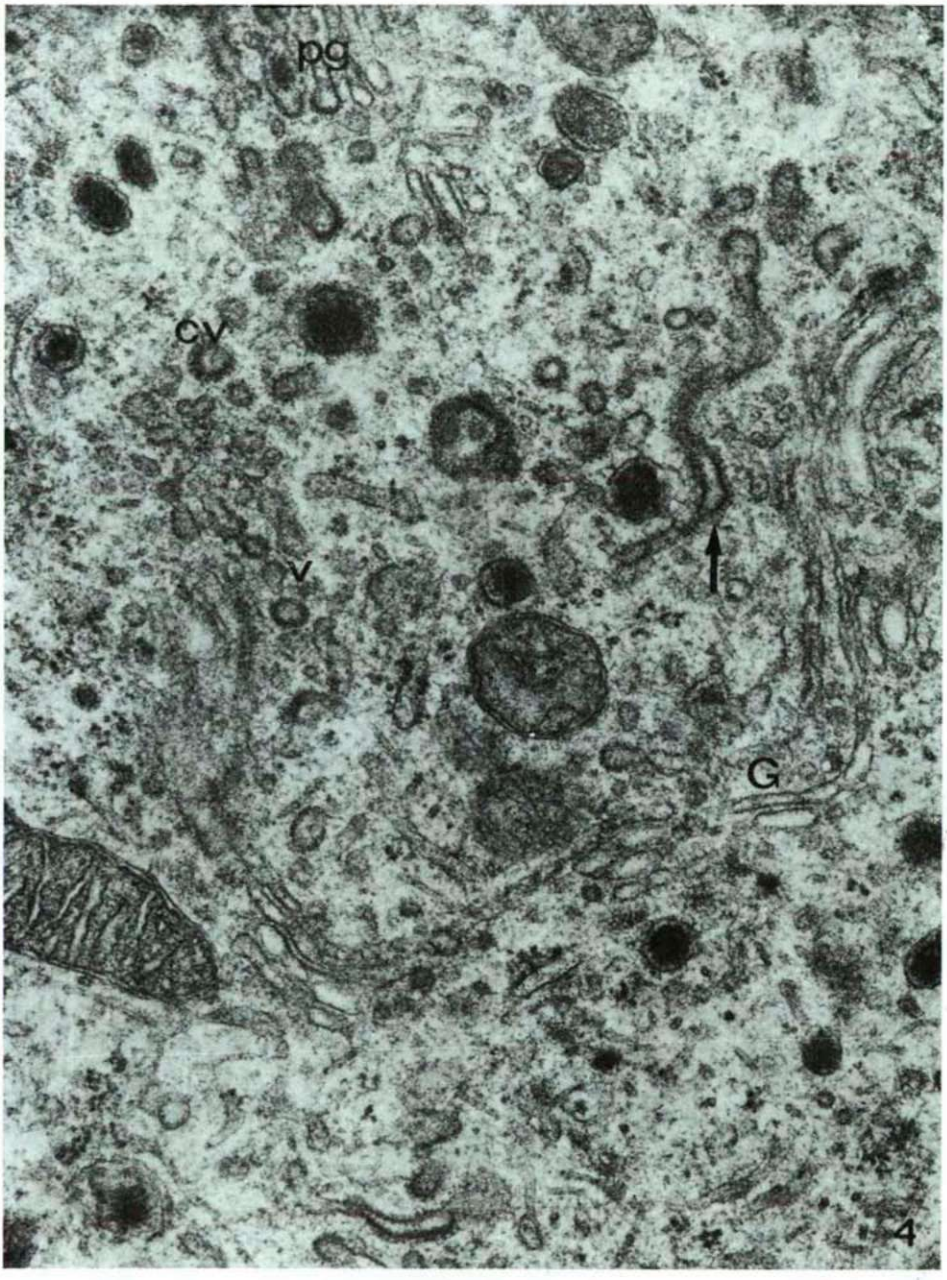

Fig. 4. High magnification of Golgi apparatus (G). The membrane of the Golgi saccule is covered by partial spiny coat (→). pg = prosecretory granule cv = coated vesicule, v = Golgi vesicule. X 62 000.

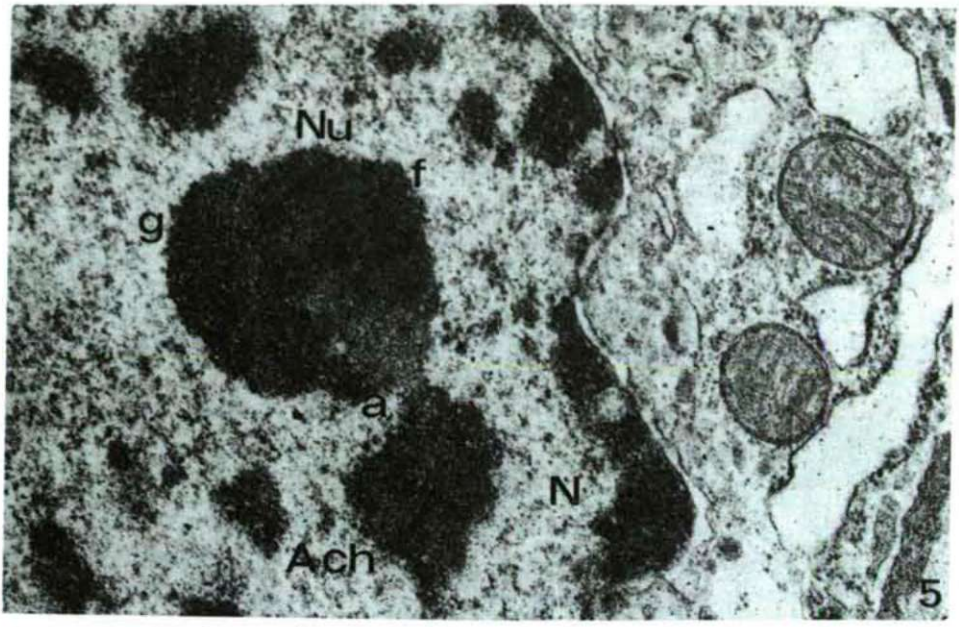

Fig. 5. Nucleus (N) of chromaffin cell 3 hrs following AD treatment. The substance of the nucleolus (Nu) is segregated to granular (g), fibrillar (f) and amorphous (a) components. Ach = associated chromatin. X 35 000.

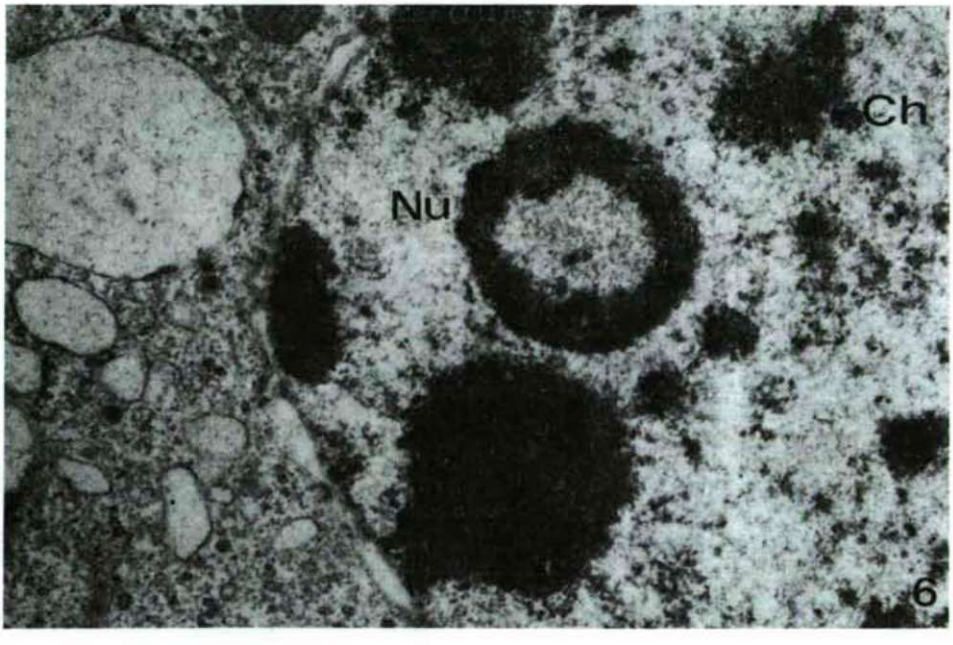

Fig. 6. Picture of "ringshaped" nucleolus (Nu), 3 hrs following 5-FU treatment. The phenomenon of segregation is partially observable. Ch = chromatin.

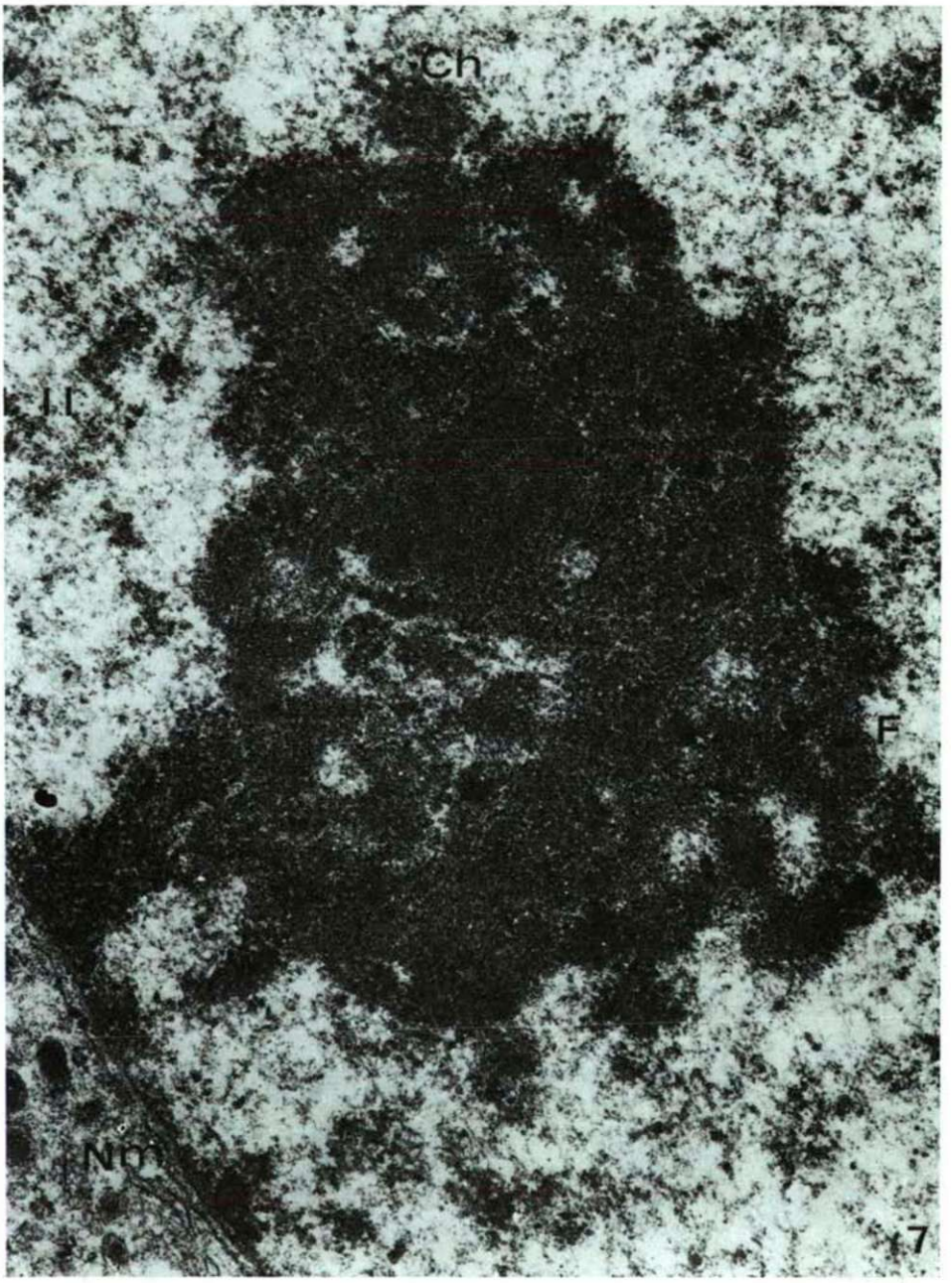

Fig. 7. "Spotted nucleolus" in the nucleus of chromaffin cell 72 hrs after treatment with 5-FU. Electron dense "spots" are striking in the nucleolonema having loose substance. F = fibrillar components, Nm = nuclear membrane, Ch = chromatin. X 75 000.

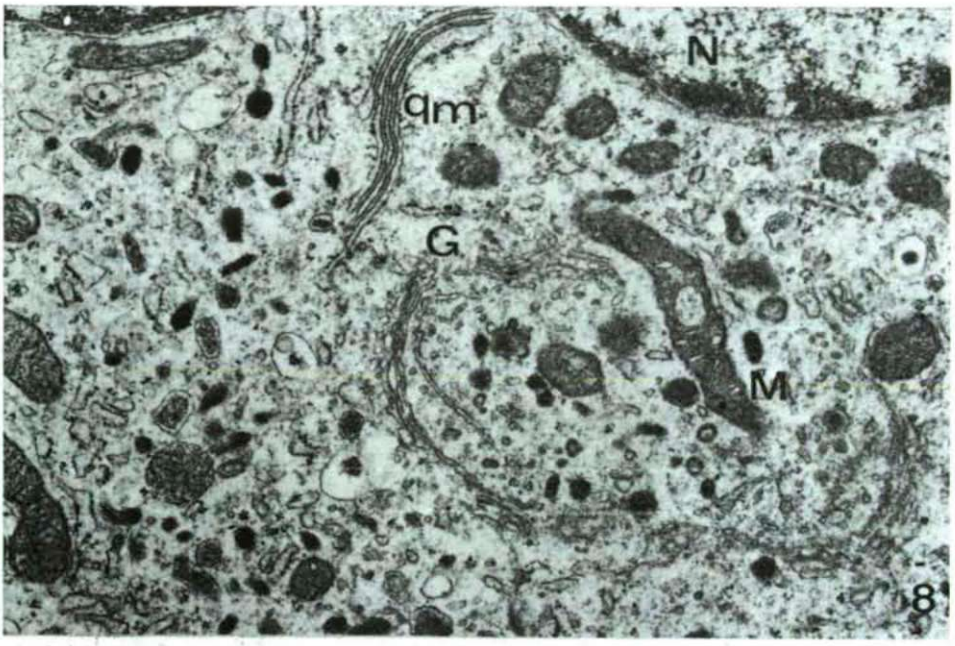

Fig. 8. Detail of chromaffin cell 48 hours following AD treatment. A quadrilamellar membrane (qm) can be observed in the neighbourhood of the cell nucleus (N), from the inner membrane surface of which the ribosomes are missing. The Golgi area (G) is relatively small. Certain mitochondria (M) are vacuolised. X 21 000.

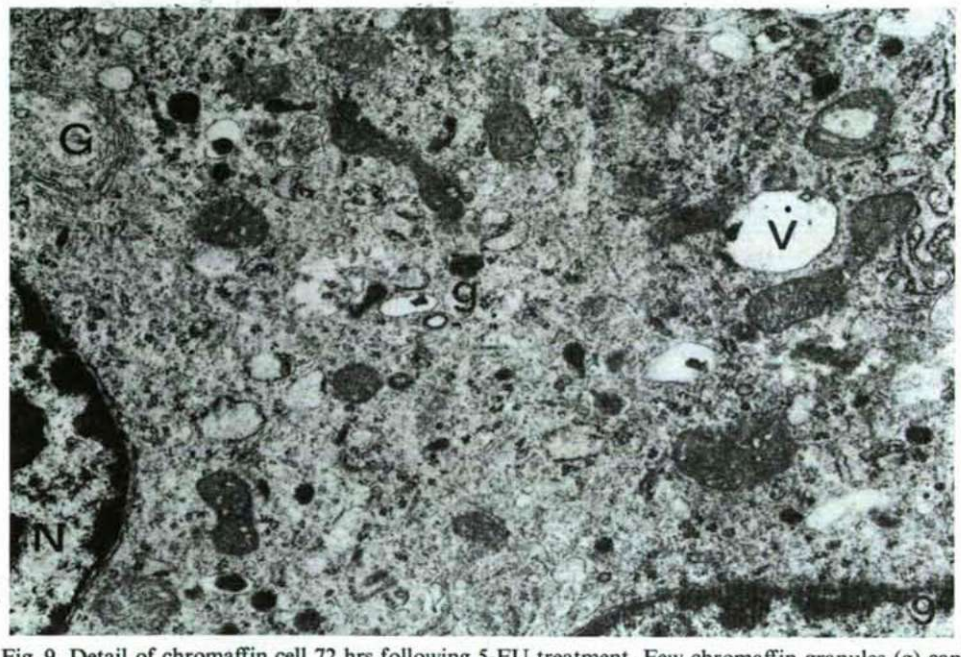

Fig. 9. Detail of chromaffin cell 72 hrs following 5-FU treatment. Few chromaffin granules (g) can be seen in the cytoplasm. The Golgi area (G) collapsed and does not contain prosecretory granule. Several empty vacuoles (V) are observable in the cytoplasm. N = nucleus, X 18 000.

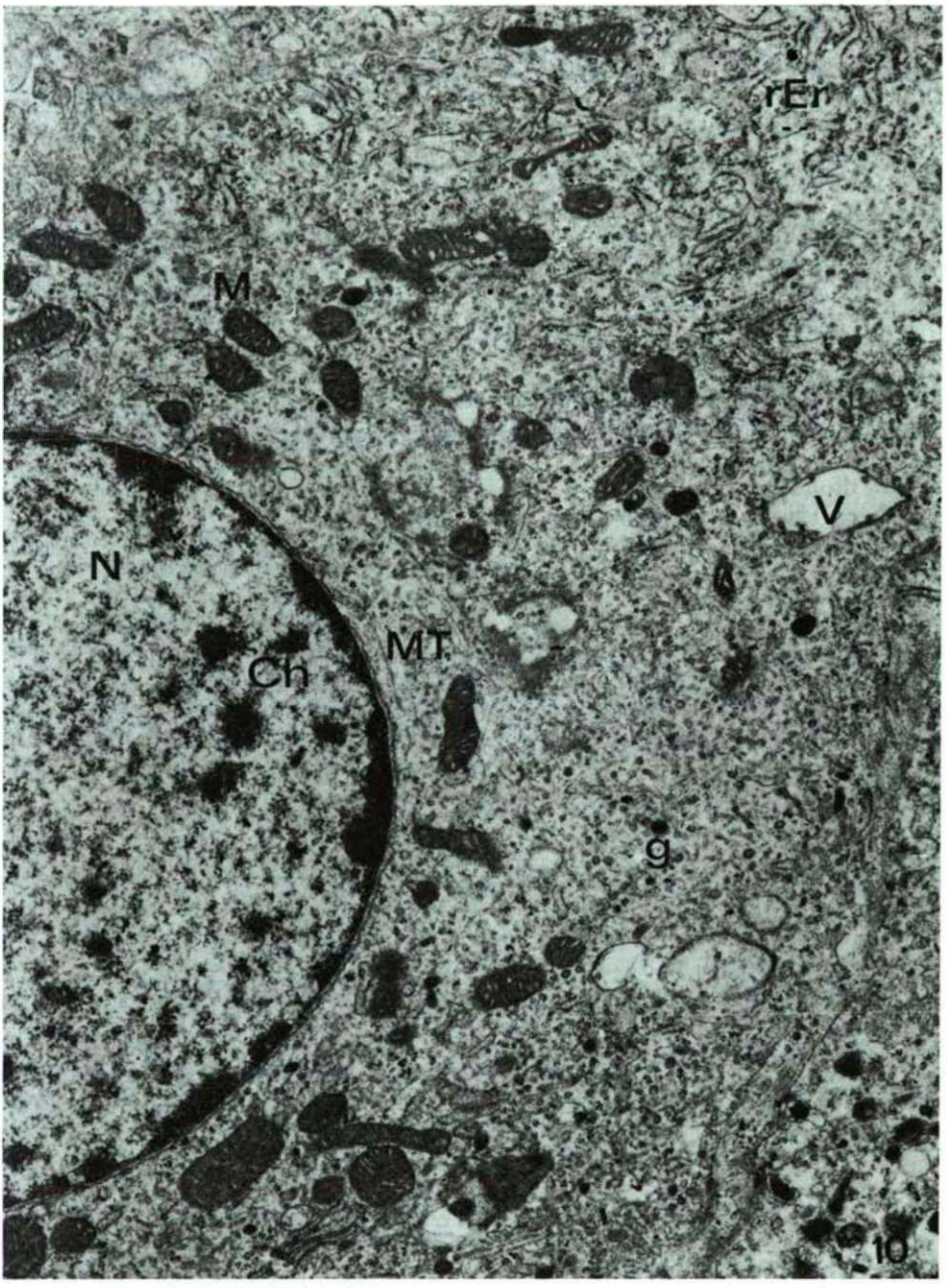

Fig. 10. Chromaffin cell 48 hrs after AD treatment. The structure of the cell nucleus (N) is well preserved, but it is rather poor in chromatin (Ch). In the cytoplasm many electron dense mitochondria (M), large number of rough endoplasmic reticulum tubules (rEr) and free ribosomes can be seen. Besides the cytoplasmic vacuoles (V) the small amount of secretory granules (g) is striking. MT = microtubule. X 15 000.

In certain mitochondria the appearance of vacuoles was also detectable following 5-FU treatment (Fig. 9). Ribosome aggregates, rough surfaced endoplasmic reticulum tubules were observable in large amount in the cells treated with 5-FU and AD (Figs. 9, 10).

Although the structural preservation of the chromaffin cells was rather good following 5-FU and AD treatment, and furthermore, abundant rEr and mitochondria were present in the cytoplasm — the development of new secretory granules was hardly detectable in the chromaffin cells; referring to the fact that the process of granulogenesis significantly altered due to the treatment with the above agents.

Discussion

Studies had been started rather long ago on the genesis of the secretory granules of the chromaffin cells in the adrenal gland (LEVER, 1955; WETZSTEIN, 1957; FUJITA et al. 1965; BENEDECZKY, 1966, 1969; RATZENHOFER and MÜLLER, 1967; ELFVIN 1967).

On the basis of these studies a conception developed that the granulogenetic process of the chromaffin cells of the adrenal gland is rather similar to that of described in other glandular cells (SIEKEWITZ and PALADE, 1958; FARQUHAR and WELLINGS, 1957; PALADE et al. 1962; SJÖSTRAND, 1962; CARO and PALADE, 1964; MUNGER, 1964; HERMAN et al. 1964; DASS and BAYLEY, 1965; REDMAN et al. 1966). Regarding the formation of the chromaffin granules HOLTZMAN and DOMINITZ (1968) set forth a new conception. During the course of their cytochemical studies they observed the presence of acid phosphatase in the secretory granules, therefore they proposed the Gerl-origin of the secretory granules. However, regarding the acid phosphatase content of the chromaffin granules BENEDECZKY and SMITH (1972) propounded that it may originate from the fusion of the coated vesicles and the prosecretory granules, during the course of which the coated vesicles, as primary lysosomes, contain and transport the enzyme in question to the secretory granules. Furthermore, BENEDECZKY and SMITH (1972) also proposed that the coated vesicles may transport other secretory base materials, too. Thus, for example, they may take part in the transport of the secretory proteins from the rEr towards the Golgi saccules; as also reported by JAMIESON and PALADE (1967), in the case of pancreas. The coated vesicles may transport proteins and parts of the membrane from the Golgi cisternae towards the prosecretory granules (BENEDECZKY and SMITH, 1972), and bypassing the Golgi apparatus, they may transport secretory proteins from the rough surfaced endoplasmic reticulum directly to the prosecretory granules.

All these presumptions prove that even in the begining of the 70s several basic processes were unclear in the mechanism of formation of the chromaffin granules. As cytomorphology and cytochemistry enriched our knowledge on the Golgi area with newer data, the picture regarding the genesis of the chromaffin granules became more and more complex. Firstly it became certain that the large number of proteins present in the chromaffin granules —; the synthesis of the chromogranin begins in the rough surfaced endoplasmic reticulum (WINKLER et al. 1972; BAUMGARTNER et al. 1974; GEISSLER et al. 1977). Concerning how the chromogranin reach up to the prosecretory granules, COUPLAND and KOBAYASHV (1976) provided new data by their autoradiographic studies. However, it is a principle problem in the work of COUPLAND and KOBAYASHY (1976) that they were unable to detect the accumulation of ^{3}H leucin in the rough surfaced endoplasmic reticulum before the incorporation into the Golgi apparatus. To surmount this problem authors declared that the chromaffin granules are poor in rEr. This statement cannot be strengthened on the basis of our earlier

studies (BENEDECZKY and SMITH, 1972) as well as our present results, and we unambiguously found that the chromaffin cells are rich in rEr elements.

During our observations we also emphasized that the rEr tubules are present exactly in the Golgi area — impacted among the saccules, being in tight topographical relationship with them; therefore we interpret the autoradiographic studies of COUPLAND and KOBAYASHY (1976) as follows: The incorporation of ^{3}H leucin observed at the early time-point (15 minutes) begins in the rEr tubules found in the Golgi area and only reaches as far as the Golgi saccules later (if doing so at all!). Now we have reached the critical question of granulogenesis; how the secretory proteins get from the rough surfaced endoplasmic reticulum to the Golgi saccules, where according to the classical conception the final encasement takes place.

According to JAMIESON and PALADE (1967) the secretory proteins are transported to the elements of the Golgi apparatus through the so-called transitional vesicles, becoming unattached from the rEr. Since we frequently observed the detachment of degranulated vesicles on one end of the rEr tubules found in the Golgi area, in agreement with the opinion of JAMIESON and PALADE (1967) we also think that the secretory proteins reach the saccules of the Golgi apparatus through these vesicles — where the uptake of the rest of the secretory materials also takes place (sugars, sulphate groups, etc.).

The uptake of the other components of low molecular weight of the chromaffin granules, like the nucleotides and hormones, is well clarified on the basis of biochemical and pharmacological studies (WINKLER, 1977; ABERER et al. 1978) and is not so followable by ultrastructural methods. The role of the cell nucleus is a point of further interest in the process of secretion. It is known on the base of literary data (LEEMAN, 1959a, b; ROELS, 1963; VIOLA MAGNI, 1968) that the cell nucleus shows various alterations in the secretory phases produced in different ways. The nature of these changes, however, is not always unambiguous, thus, not even the conclusions which could be drawn. In our studies we established such a secretory model where first the hormone content of the chromaffin cells was "depleted" by insulin loading (BENEDECZKY et al. 1965, BENEDECZKY, 1967), then following this 5-FU and AD treatment was carried out and the process of hormone resynthesis was studied in the function of the altered nucleic acid metabolism (BENEDECZKY et al. 1972). It was determined that both agents inhibited the process of hormone resynthesis strongly and durably (BENEDECZKY et al. 1972). It is known that AD it preferentially binds to chromatin in the early time-points following treatment, hampering in such a way the transcription of RNA by the inhibition of the RNA polymerase (HURVITZ et al. 1962; GOLDBERG and RABINOVITZ, 1962; REICH, 1964; RINGERTZ et al. 1969; RECHER et al. 1971). As a consequence of the inhibition of the process of transcription a long-standing disturbance takes place in the RNA metabolism (GIRARD et al. 1964; PENMAN, 1966; WEINBERG etal. 1967; SINGER and PENMAN, 1972; COPOER and BRAVERMAN, 1977). 5-FU, besides its direct DNA synthesis inhibitory effect (HEIDELBERGER, 1963), also influences the metabolism of RNA; it infiltrates into the RNA, occupying the place of uracil (KOPPER et al. 1972).

Both 5-FU and AD bring forth characteristic ultrastructural alterations in the cell nuclei (LAPIS and BENEDECZKY, 1966; STENRAM, 1969). These characteristic alterrations — spotted nucleolus, ring-shaped nucleolus, nucleolus segregation — also developed in the nucleus of the glandular cells; therefore we came to the conclusion that the applied agents significantly changed the nucleic acid metabolism of the glandular cells.

The consequences of the altered nucleic acid metabolism were also detectable in

the cytoplasm. We have proved by our biochemical measurements (BENEDECZKY et al. 1972) the inhibition of hormone resynthesis, and simultaneously, we observed the large degree of decrease in the number of the hormone-storing granules in the cytoplasm of the chromaffin cells. Many irregular shaped granules of low density were present among the granules found in the cytoplasm, which may also be brought into connection with the previous 5-FU and AD treatment, respectively. The appearance of quadrilamellar membranes, furthermore, the development of small "collapsed" Golgi apparatus may be evaluated as degenerative ultrastructural signs. However, besides the degenerative ultrastructural changes caused by the agents, regenerative signs were also detectable. The increase in the amount of free ribosomes, polyribosomes and the Er tubules (mainly smooth Er) in the cytoplasm of the chromaffin cells was especially striking. All these ultrastructural signs refer to the fact that through "feedback mechanism" the hormone release and depletion-developing on the effect of the insulin treatment-set forth the resynthetic activity of the medullary cells. It is known, for example, that the amount of dopamine β-hydroxylase and tyrosine hydroxylase increases significantly in the adrenal gland following intensive hormone depletion (VIVEROS et al. 1969; THOENEN, 1975). The synthesis of these enzymes proably stands in tight connection with the rough surfaced endoplasmic reticulum of the glandular cells, and so it is understandable that this process resulted in the increase in the amount of rough surfaced endoplasmic reticulum. Namely, the inhibitory effect of the 5-FU and AD treatment applied under the resynthesis did not prevail immediately and some partial processes of the hormone resynthesis could come into action from the RNA reserves synthetised before the inhibition, however, this reserve was not enough for the complete process of granulogenesis, therefore the glandular cells became blocked in an initiative „rEr increased" state.

These data are in favour of the fact that the process of granulogenesis takes place only in glandular cells with intact nucleic acid metabolism, that is, the hormone resynthesis and process of granulogenesis are nucleus-dependent cell physiological processes.

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