MORPHOLOGICAL EXAMINATION OF AUTOPHAGIC VACUOLES FORMED IN MOUSE PANCREATIC EXOCRINE CELLS UNDER THE EFFECT OF NEUTRAL RED

by

G. RÉZ and J. KOVÁCS

Department of General Zoology of the Eötvös Loránd University, Budapest

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Introduction

Neutral red has been used for studying the various functions of cells for a long time (Storb 1965). Most processes which can be tested by vital staining with basic dyes are related to the functions of lysosomes. (De Duve – Wattiaux 1966.) Recently De Duve (1969) and Allison – Young (1969) published a survey of the results achieved in studying the vital staining of lysosomes. As verified by light microscopic examinations, lysosomes are capable of accumulating basic vital dyes (Allison – Young 1969, Bastos et al. 1965, Koenig 1963a, b, 1965, 1969, Kovács – Hafiek 1964, Ogawa et al. 1961, Robbins et al. 1964, Zelenin 1966). The swelling of the lysosomes and the release of their acid hydrolase content can be observed parallelly (Koenig 1963 b). In animal cells, neutral red usually accumulates in granules, (Möllendorf 1918) which also contain basophilic substances (RNA).These are the so-called Chlopin's Krinom granules discovered by Chlopin (Chlopin 1927, De Duve 1969, Kárpáti 1966, Kedrowski 1941, Koenig 1963 b, 1965, Kovács – Hafiek 1964, 1966, Kovács – Péczely 1966, Kovács 1969, Réz – Kovács 1967, Schmidt 1958).

The ultrastructure of the neutral red granules has been studied by numerous authors (Alousi et al. 1967, Byrne 1964 a, Kárpáti 1966, Koenig 1969, Kovács – Péczely 1966, Kovács 1969, Madarász – Kovács 1968, Morgan et al. 1966, Réz – Kovács 1967, Schmidt 1962, Tanaka 1962, Weiss 1955). Krinom granules containing a highly dense substance proved to be the foci of cytoplasmic degradation. According to Schmidt's results (1958, 1962) and also born out by a number of other data, the basophilic material of the Krinom granules originates in the cisternae of the rough surfaced endoplasmic reticulum. (Alousi et al. 1967, Kovács – Hafiek 1964, Kovács – Péczely 1966, Ko-

vács 1969, Madarász – Kovács 1968, Réz – Kovács 1967). In liver cells (Madarász – Kovács 1968), besides the autophagocytosis of the endoplasmic reticulum, an intensification in the production of dense bodies can also be observed. In the intestinal epithelial cells of parrots Krinom granules are formed by the fusion of multivesicular bodies and lysosomes (Kárpáti 1966). Cadmium ions and hyperosmotic sucrose solutions which induce lysosome formation and focal cytoplasmic degradation are also able to stimulate Krinom formation (Kovács 1968, 1969, Réz – Kovács 1967, 1969).

In pancreas cells neutral red also accumulates in the form of granules (Byrne 1964 b, Chlopin 1927, Morgan 1953, 1968, Morgan et al. 1966, Réz – Kovács 1967). Weiss (1955) was the first to study the neutral red granules of the pancreas and kidney cells by electron microscopy. A few papers dealing with the above question have been published since, with the conclusion that the dye induces autophagy (Alousi et al. 1967, Morgan et al. 1966, Réz – –Kovács 1967). However, no detailed examination of the process has been carried out yet.

In the present paper the types of degradation taking place in autophagic vacuoles will be discussed.

Material and Method

12 female white mice of 25–30 g body weight, each were used. 10 mice were given 0,3 mg neutral red (Chroma) per gram body weight in the form of a 1,5% aquous solution. Two animals served as control. Two hours after the treatment the animals were decapitated. Small pieces of pancreas were fixed in 1% osmium tetroxide dissolved in 0,125 M phosphate buffer and embedded in araldit. The ultrathin sections were contrasted with lead citrate and uranil-acetate solution and examined in an UEMV–100 type electron microscope.

Results

After neutral red treatment, the following changes were found in the cytoplasm of the acinar cells:

1. A great number of highly vacuolated, large dense bodies had appeared among the cisternae of the endoplasmic reticulum (Fig. 1, 4 and 9). These contained electrolucent vacuoles, as well as electrodense granules and small rod-shaped particles of various sizes (Fig. 4). Dense bodies with single limiting membrane could often be seen near the Golgi apparatus, but also in the vicinity of the basal plasma membrane. No transitory form was found to indicate that the vacuolated dense bodies could have been produced by the degradation of some other cell component.

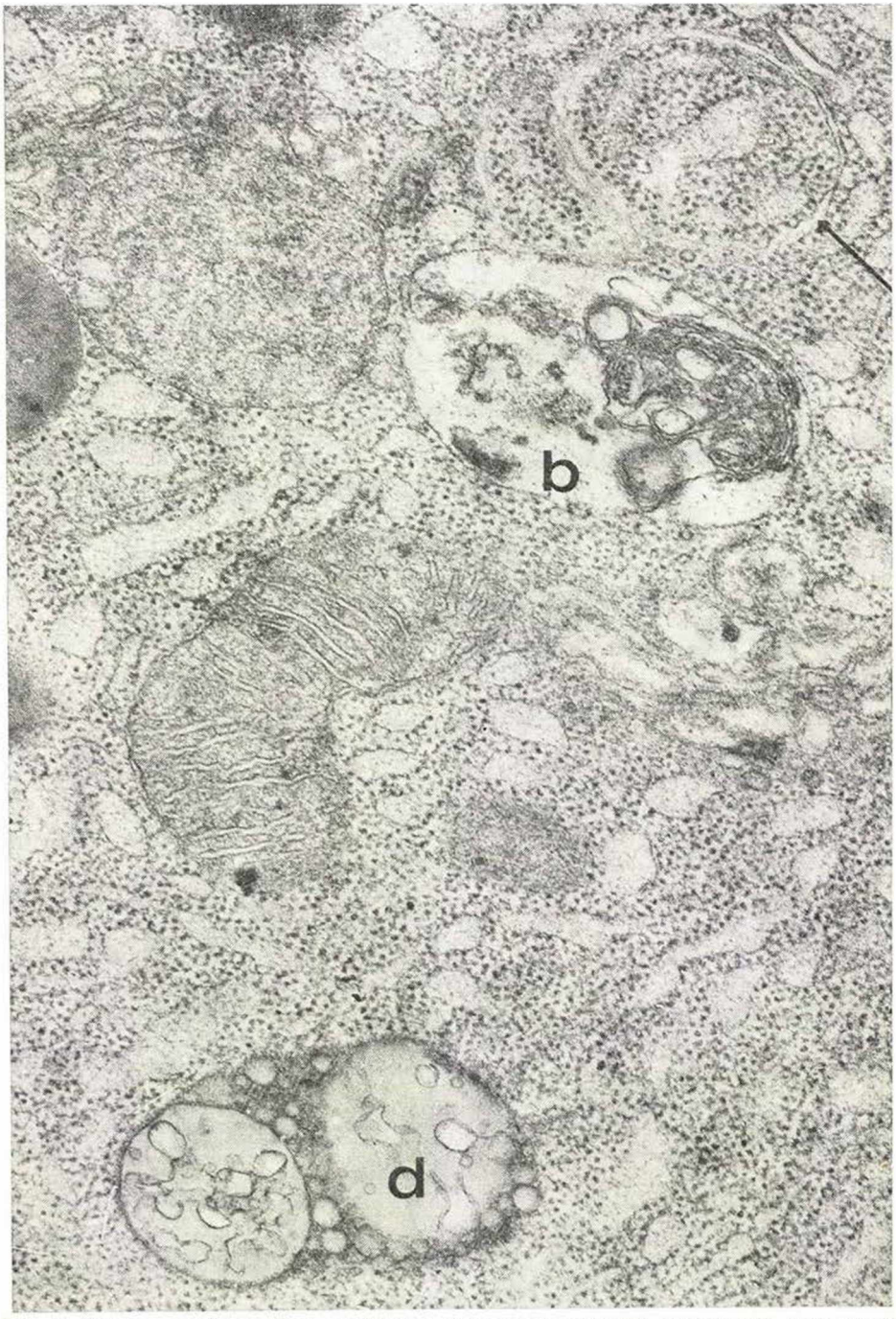

Fig. 1. Golgi area of an acinar cell 2 hours after treatment with neutral red. Autophagie vacuole (arrow) in an early stage and b-type autophagic vacuole (b) in a later stage of degradation; vacuolated dense body (d) (x35 600)

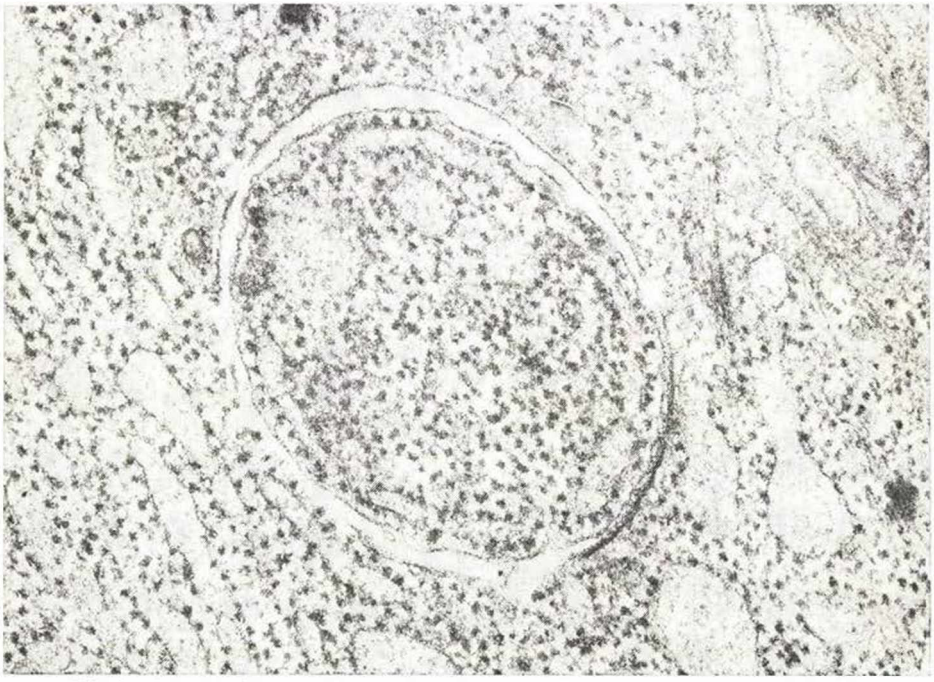

Fig. 2. Autophagic vacuole of early stage near the Golgi apparatus (x 41 500)

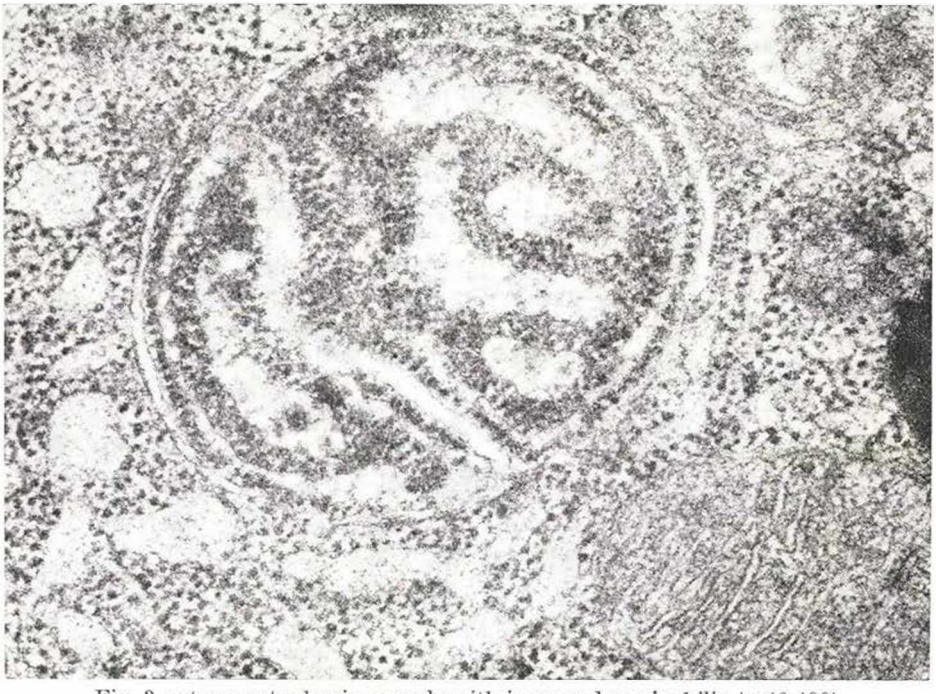

Fig. 3. a-type autophagic vacuole with increased osmiophilia (x 43 400)

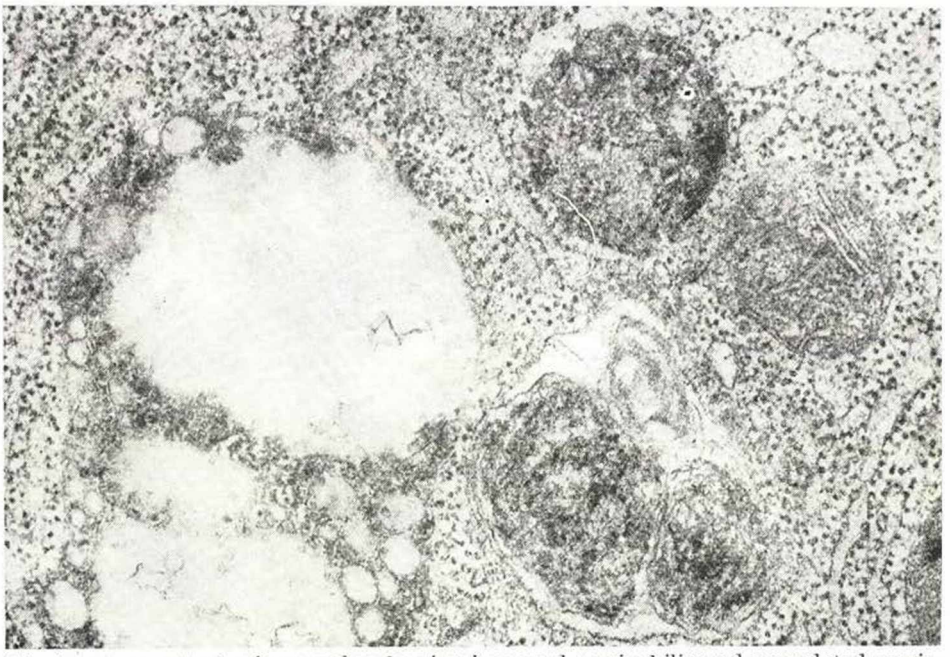

Fig. 4. a-type autophagic vacuoles showing increased osmiophilia and vacuolated osmiophilic body seen side by side (x 32 700)

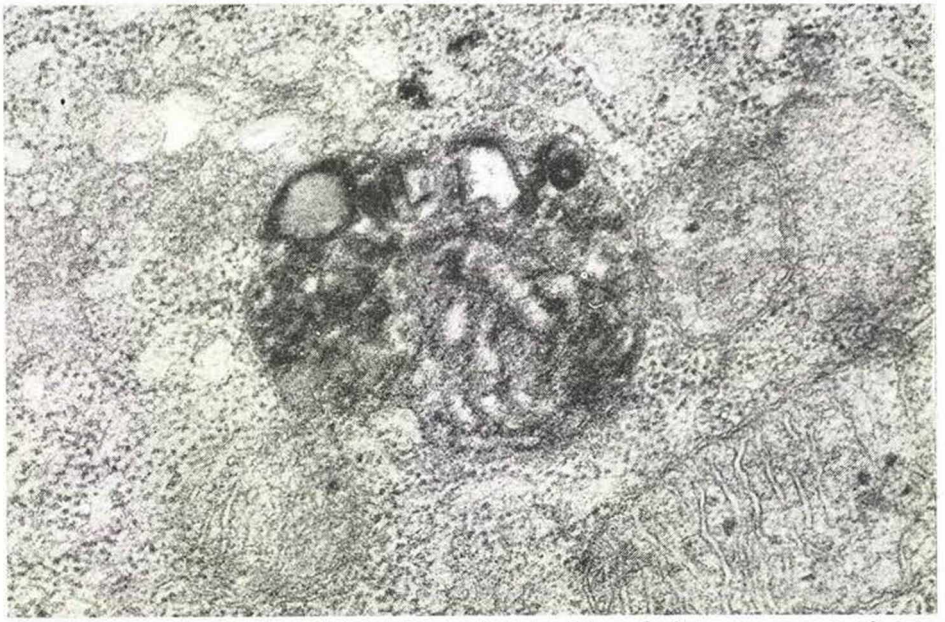

Fig. 5. a-type autophagic vacuole in advanced stage of degradation, seen near the Golgi apparatus (x 37 400)

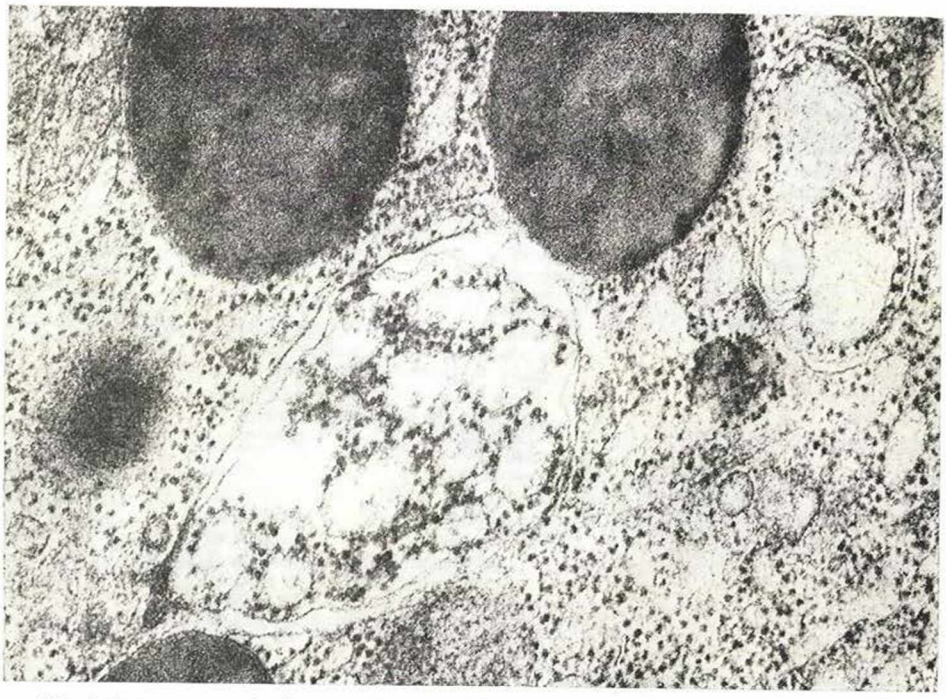

Fig. 6. b-type autophagic vacuoles containing cisternae of vacuolated endoplasmic reticulum near the Golgi area (x 46 800)

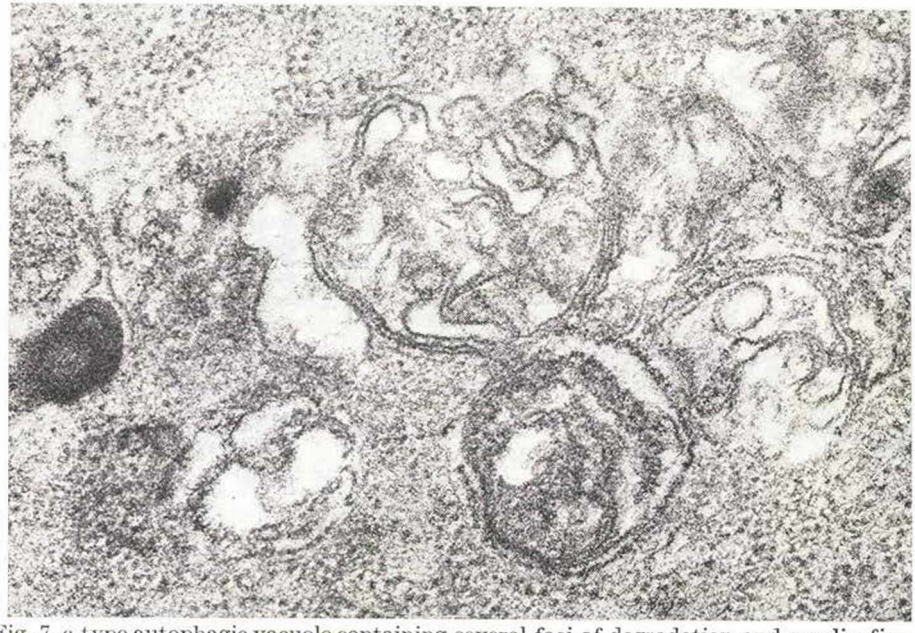

Fig. 7. c-type autophagic vacuole containing several foci of degradation and myelin figures. No bordering membrane can be discerned. (x 35 600)

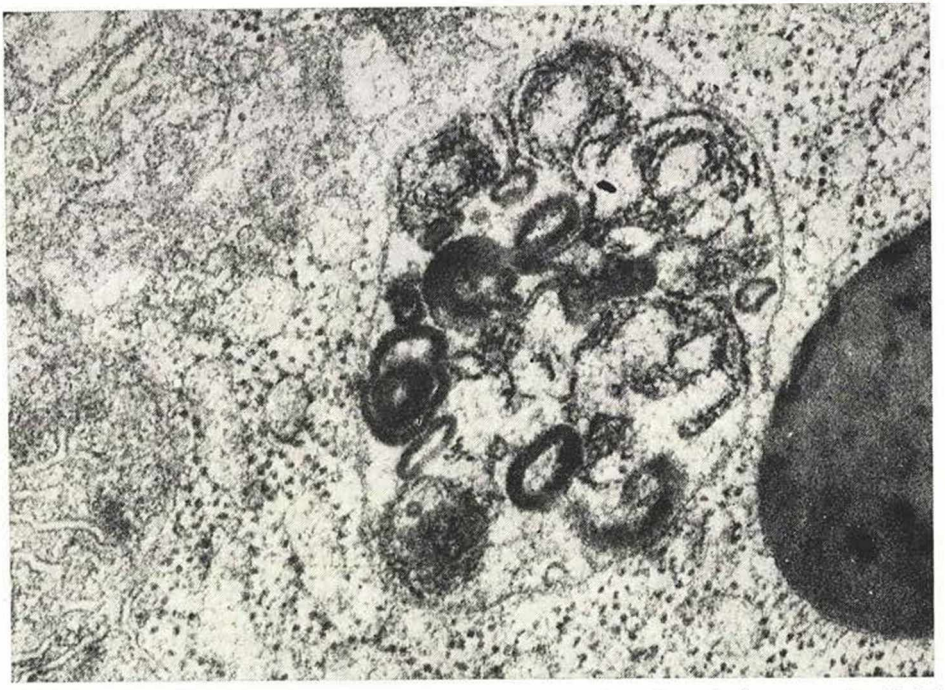

Fig. 8. c-type autophagic vacuole containing several foci of degradation near the Golgi apparatus (x 43 900)

2. In the cytoplasm, a great number of autophagic vacuoles containing rough surfaced endoplasmic reticulum cisternae were observed. These were surrounded either by a pair of membranes or a single membrane. Often the members of the membrane pair were fused in places (Fig. 2, 6). Figs. 1 and 2 show an autophagic vacuole in its early phase. The rough endoplasmic cisternae surrounded by a pair of membranes show no trace of degradation. Autophagic bodies in which the degradation of the sequestered substance could be observed were of three types:

a) In Figs. 3, 4, and 5 autophagic vacuoles containing well-discernible cisternae of unchanged form and arrangement could be seen. However, the osmiophilia of the contents of the vacuoles was greatly increased, as compared to that of the surrounding cytoplasm. The figures are arranged in the presumed sequence of the progress of the above process. At the end of the process (Fig. 5) the structure of the endoplasmic reticulum was scarcely discernible and the myelin figures also appeared. The body seen in Fig. 4 is presumably the product of the fusion of two autophagic vacuoles.

b) In Fig. 6 another type of autophagic vacuoles is shown, with a pair of bordering membranes fused in certain places and containing cisternae of endoplasmic reticulum showing marked degradation without

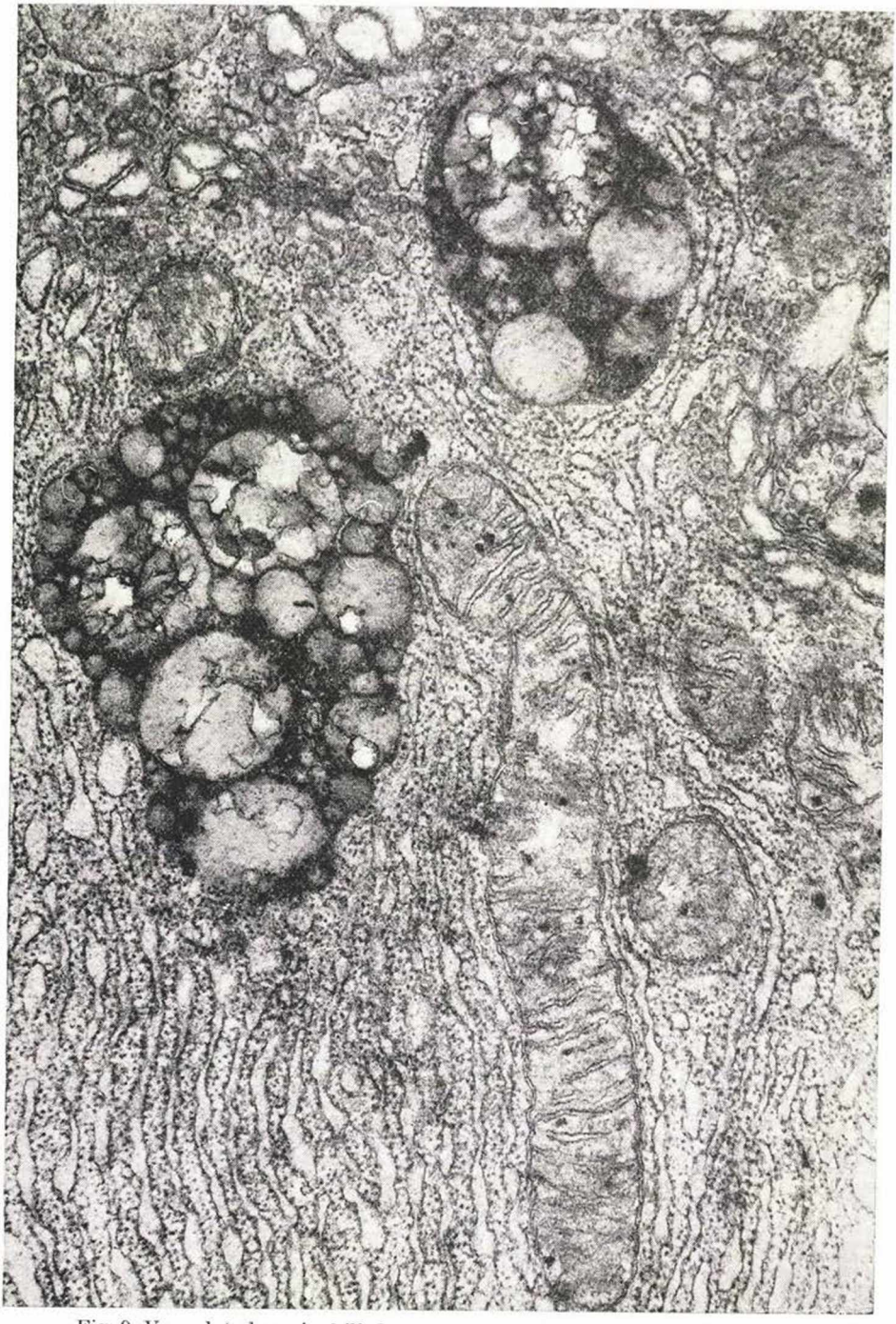

Fig. 9. Vacuolated osmiophilic bodies near the Golgi apparatus. (x 27 800).

increased osmiphilia. Degradation is indicated by the fact that the encapsuled cisternae have disintegrated into vesicles and their surface is partly free of ribosomes. The supposed further stage of the process is shown in Fig. 1 where the autophagic vacuole contains smooth membranes embedded in "light" matrix. Thus, as the end product of the process, an autophagic vacuole containing a substance of decreased density emerged.

c) Another type of autophagic vacuoles is presented in Figs. 7 and 8. Highly increased osmophilia is not characteristic of this type either. In the initial stage (Fig. 7) foci showing various stages of degradation can usually be found near each other. The ribosomes of the cisternae in the different stage of degradation can scarcely be discerned (Fig. 7). Mainly it is only the paired arrangement of the membranes which permits us to draw the conclusion that the above formations (Fig. 7) are the remnants of the endoplasmic reticulum. In the process of autophagic transformation the substance is formed into a filamentous material with granules of loose structure (Fig. 8). The great number of myelin figures is also characteristic of this stage (Fig. 8). In the initial stage, a welldiscernible membrane cannot always be observed around the substances showing signs of degradation (Fig. 7). However, the autophagic vacuole seen in Fig. 8 is surrounded by a clearly discernible membrane.

Discussion

In the acinar cells of the pancreas, neutral red induces strong autophagy, i. e. it causes the focal degradation of the cytoplasm as described by Hruban et al. (1962, 1963 Swift – Hruban 1964). In accordance with the data on autophagy as recently reviewed by Ericsson (1969), we have found that in the majority of cases one pair of membranes fused in some places borders the autophagic vacoules in their early stages of formation. In agreement with Ericsson (1969) we are of the opinion that in certain cases, the bordering membrane of the autophagic vacuoles originates in the rough surfaced endoplasmic reticulum. With the epithelial cells of the seminal vesicle (Kovács – – Péczely 1966, Kovács 1968, 1969) and with autophagic vacuoles forming at the basal pole of the pancreatic cells, this supposition seems well founded, since no smooth surfaced reticulum or GERL is contained in these cells.

On the basis of the ultrastructure of the degrading subtance 3 morphological types of autophagic vacuoles can be distinguished. It is suggested that in accordance with the morphological picture, autophagic vacuoles differ in their rate and way of degradation. In the case of type a, the gradually increasing osmiophilia, with the structure being still intact, indicates a very slow process of degradation or rather only a slow process of denaturation within the bordering membrane. This might well be the case, since a great number of stages can be observed in the growth of osmiophilia. This phenomenon suggests that the inner condition

of the vacuole, i.e. the permeability of the bordering membrane can change only very slowly. According to our unpublished observations similar vacuoles produced by sucrose and cadmium treatment do not contains acid hydrolases, thus the latter appear only in a later stage of degradation by the mediation of primary or secondary lysosomes (E r i c s s o n 1969).

With b- and c-type autophagies the process is probably more rapid. At least this was the conclusion drawn by us from the fact that in these cases a lesser number of transitory forms could be observed. Probably the bordering membranes of the b-type autophagic vacuoles have a greater permeability than those of the a-type. The early vesiculation of the encapsuled cisternae indicates a rapid change in the inner conditions of the vacuoles. The rapid change in the structures, i.e. the early appearance of myelin figures suggests that these autophagic vacuoles (or their environment) contain more acid hydrolase than the variant with a slow rate of degradation.

No literary data are available as to the highly vacuolized dense bodies which form in a large number in the pancreatic cells under the effect of neutral red treatment. According to our unpublished results, these bodies also form upon treatment with cadmium ions and give a positive acid phosphatase reaction. Since these bodies can also be found on the basal pole of the cells, we do not consider them to be lysosomes swollen under the effect of basic dye (K o e n i g 1969). L u t z n e r and co-workers (1965) described pigment granules of very similar ultrastructure in the Chédiak-Higashi syndrome.

Summary

Neutral red produces massive autophagy in mouse pancreatic exocrine cells. According to their morphology, autophagic vacuoles can be grouped into 3 types. The authors ascribe the difference in type to the speed and intensity of degradation taking place in the vacuoles.

Besides of autophagy a great number of large-sized highly vacuolized dense bodies of uncertain origin were observed in the cells.

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