NEUTRAL RED-INDUCED AUTOPHAGOCYTOSIS IN THE EXOCRINE PANCREATIC CELLS OF THE FROG (RANA ESCULENTA L.)

by

G. RÉZ and J. KOVÁCS

Department of General Zoology of the Eötvös Loránd University, Budapest

Received on 25th March, 1974

Introduction

Autophagosytosis has been widely investigated in recent years (for reviews see Ericsson 1969, Pfeifer 1971). It is readily induced by treatment with neutral red *in vivo*, especially in cell types characterized by a well-developed protein synthesizing apparatus. Thus, the dye was found to induce the formation of autophagic vacuoles in the cytoplasm of mouse exocrine pancreatic cells, too (Alousi, Stenger and Morgan 1968, Réz and Kovács 1967, 1973, Byrne 1964, Weiss 1955, Essner 1970), and the detailed morphological investigation of the phenomenon has been carried out (Réz and Kovács 1971).

In the present investigation our aim was to obtain comparative morphological data on neutral red-induced autophagocytosis in the amphibian pancreas.

Material and methods

The first part of the experiments was carried out on summer (August) animals. Twenty-five male frogs (*Rana esculenta* L.) were given single intraperitoneal injections of 2% neutral red (0.4 mg/g body weight) dissolved in 0.7% saline. They were decapitated 5, 8, 16, 24 and 48 hours after this treatment, 5 frogs at each time. Three frogs killed 24 hours after treatment with corresponding volumes of 0.7% saline solution served as controls.

In the second part of the experiments exactly the same treatment was repeated using 28 winter (December) animals. The pancreatic pieces were fixed for light microscopy in Helly's solution for 1 hour and embedded in paraffin. The sections were stained for basophilia using 2% toluidine blue dissolved in pH 2.7 veronal acetate-HCl buffer solution. For electron microscopy, tissue pieces were fixed for 2 hours in 5% glutaraldehyde buffered with 0.1 M (pH 7.4) sodium cacodylate-HCl solution. This was

followed by a postfixation in buffered 2% osmium tetroxide and embedding in Araldit. The ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a UEMV – 100B electron microscope.

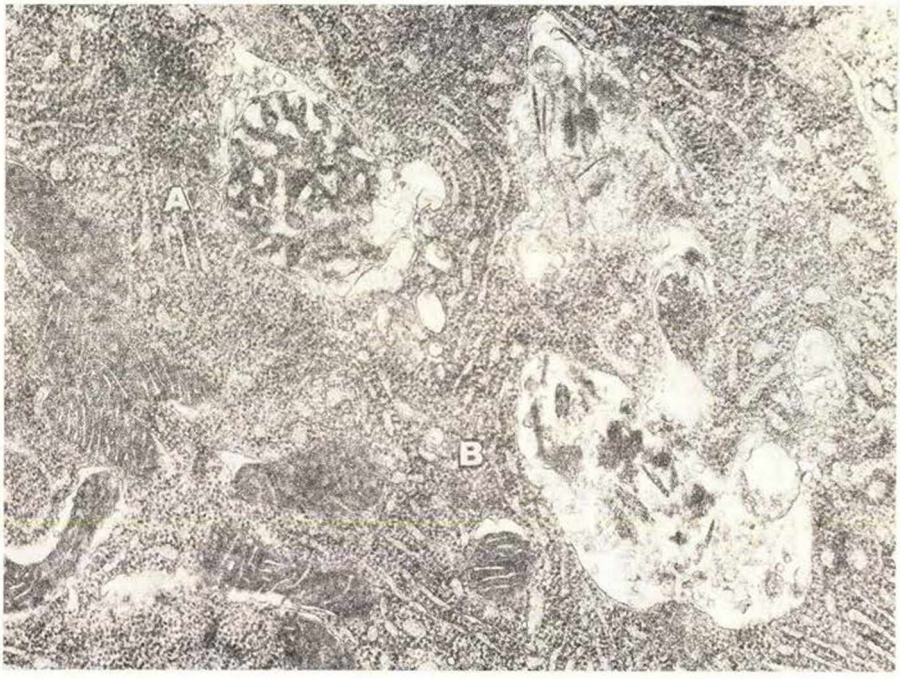

Fig. 1. Autophagic vacuoles in the cytoplasm 24 hours after the neutral red injection. Vacuoles of increased density (A) and light-type residual bodies (B) are demonstrated. Note the presence of Golgi-like vesicles round the vacuoles. (x 27000)

Results and discussion

The autophagic vacuoles appear in the cytoplasm of the acinar cells 8 hours after the neutral red-treatment. Later on, the number of the vacuoles increases and 24 hours after the injection numerous krinom granules (light microscopic forms of autophagic vacuoles, see Réz and Kovács 1973) can also be observed in the cells.

The fine structure of the encapsuled organelles (largely rough-surfaced ER) can clearly be recognized in the interior of the double membrane-limited early forms of autophagic vacuoles (Figs 4 and 5). Round them, a large number of Golgi-like vesicles can be observed, many of

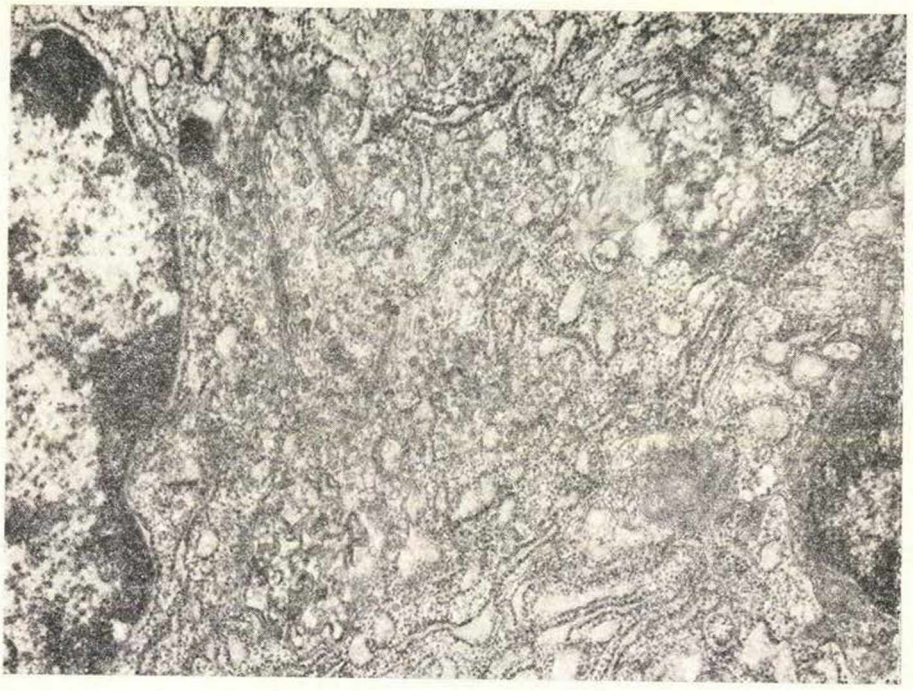

Fig. 2. Autophagic vacuoles (arrows) segregating Golgi material are shown 8 hours after the dye injection. (x 18000)

which being in close contact with the limiting membrane of the autophagic vacuoles (Figs. 1, 4, 5 and 6). The vacuoles are frequently located in the immediate vicinity of the Golgi complex, and even in the Golgi-area itself where they mainly segregate elemets of the Golgi apparatus (Fig 2).

The advanced forms of autophagic vacuoles can be classed with two main morphologic types. Of the *first type* an increasing density of the segregated material is characteristic (Figs. 1 and 6), with a single membrane-limited residual body containing highly dense material remaining

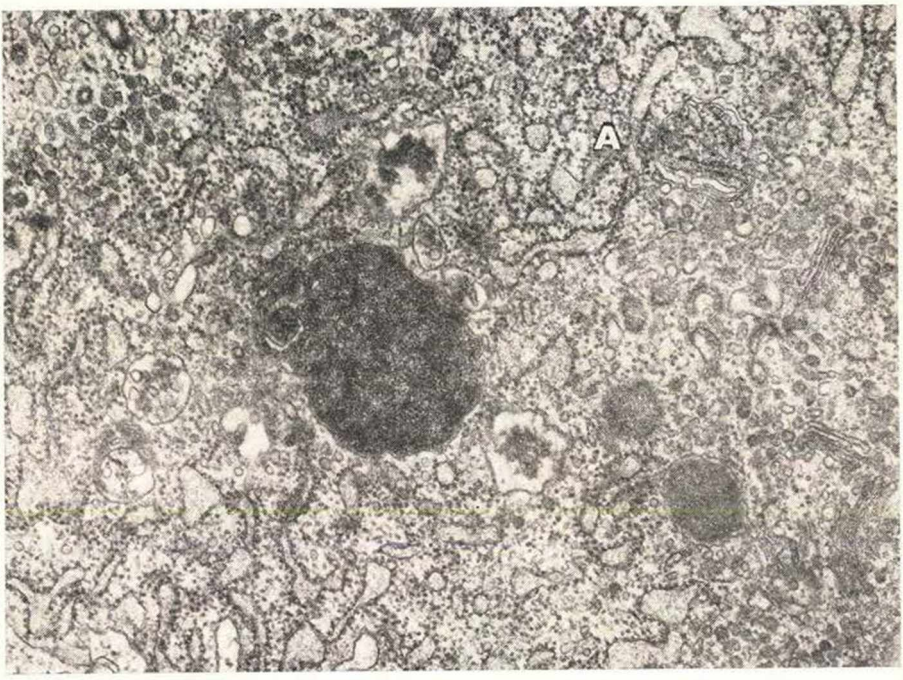

Fig. 3. A residual body filled with highly dense material and a newly formed, double membrane-limited autophagic vacuole (A) can be seen in the vicinity of the Golgi-complex 24 hours after the dye treatment. (x 26400)

at the end of the degrative process (Figs. 3 and 6). In the case of the *second* type the density of the encapsuled material decreases (Figs. 1 and 5) which leads to the formation of usually large-sized vacuoles containing membrane whorls and fragments, vesicles, rod-like crystals and electron-lucent matrix (Fig. 1). These two morphologic types of autophagy possibly indicating the existence of vacuoles different in their enzyme con-

tent have been described in mouse pancreas, too (Réz and Kovács 1971, 1972).

Therefore, we conclude that neutral red-induced autophagocytosis in amphibian pancreas is similar to that described in mammals. However,

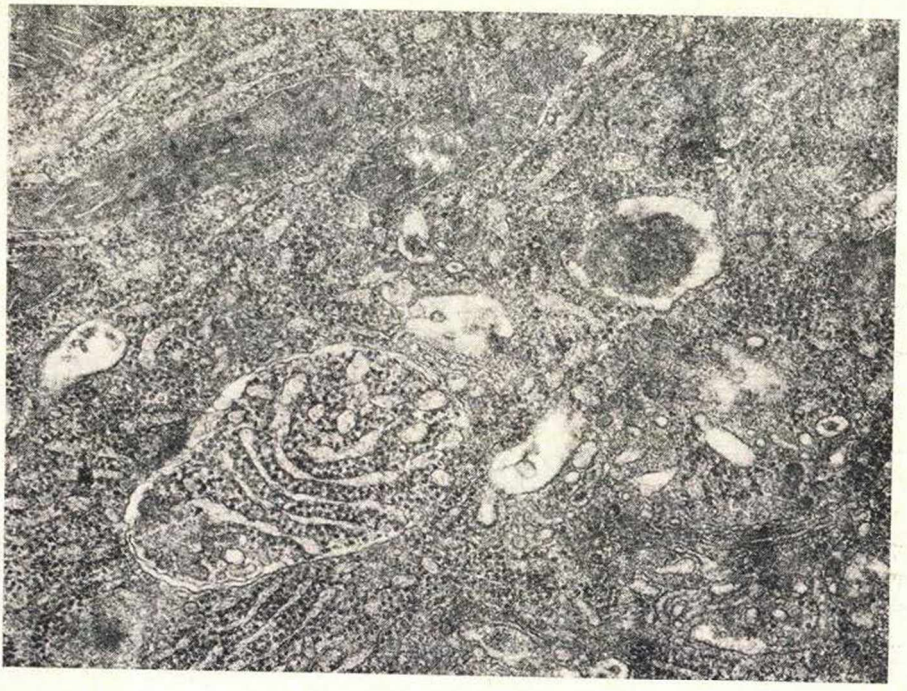

Fig. 4. Neutral red for 24 hours. Double membrane-limited autophagic vacuole segregating rough-surfaced ER and Golgi vesicles are demonstrated near the Golgi-complex. (x 33400)

while autophagic response to the dye in pancreatic acinar cells of mice and chickens (unpublished data) occurs within 30 – 60 minutes after the injection, it takes much more time (*i. e.* 8 hours) in the case of frogs.

The reason for this longer time lag is not understood at present. We could not find any difference between summer and winter animals in reactivity to neutral red-treatment.

Summary

Neutral red (0.4 mg/g body weight) was shown to induce massive autophagy in pancreatic acinar cells of the frog (*Rana esculenta* L.) within 8 hours after a single intraperitoneal injection. In the course of the autophagic process, parts of rough-surfaced ER and of the Golgi-complex were segregated and degraded. The morphology of this phenomenon was similar to that described earlier in mammalian pancreas.

Fig. 5. A group of autophagic vacuoles from acinar cell of a frog treated with the dye for 24 hours. Double membrane-limited newly formed vacuoles (A) and advanced forms are to be seen. Note the presence of numerous Golgi-like vesicles near the vacuoles. (x 31300)

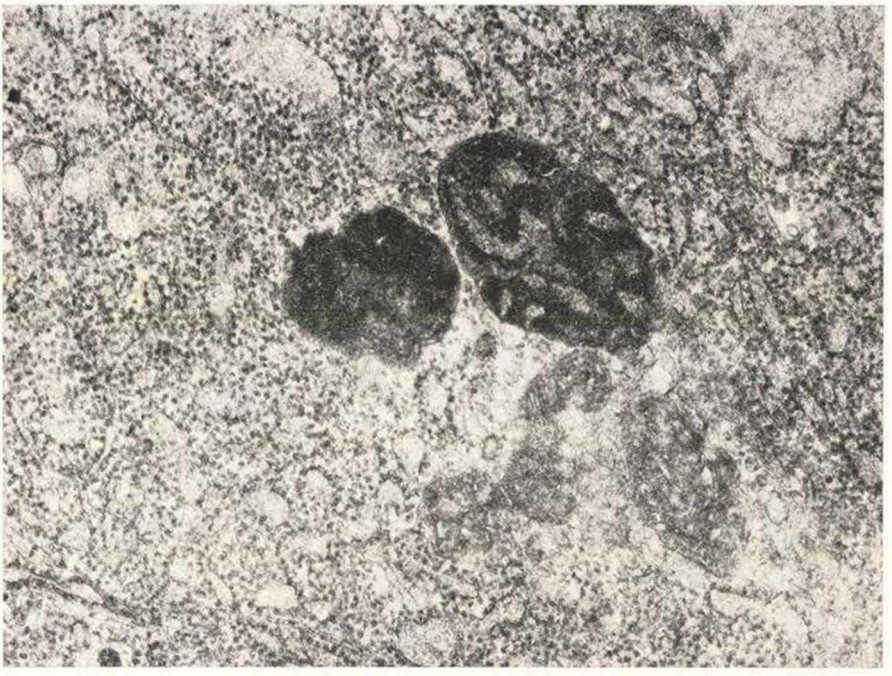

Fig. 6. Advanced vacuole of increased density, segregating ER cisternae and dense-type residual body, 24 hours after the dye injection. (x 29500)

REFERENCES

Alousi, M. A. – Stenger, R. J. – Morgan, W. S. 1968. The fine structure of pancreatic acinar and hepatic parenchymal cells after neutral red dye injection. Exp. molec. Path. **9**: 97 – 109.

Byrne, J. M. 1964. An electronmicroscopical study of neutral red granules in mouse exocrine pancreas. Quart. J. micr. Sci. **105**: 219 – 225.

Ericsson, J. L. E. 1969. Mechanism of cellular autophagy. In: Lysosomes in biology and pathology, **1**: 345 – 384. Amsterdam – London: North Holland.

Essner, E. 1970. Cytochemical studies of neutral red-induced granules in mouse pancreatic exocrine cells. VII-éme Congr. internat. micr. electr. Grenoble, **3**: 497 – 498.

Pfeifer, U. 1971. Probleme der cellulären Autophagie. Morphologische, enzymzytochemische und quantitative Untersuchungen an normalen und alterierten Leberepithelien der Ratte. Ergebn. Anat. Entwickl.-Gesch. **44**: 1 – 74.

Réz, G. – Kovács, J. 1967. A light and electron microscopic study of the focal cytoplasmic degradation of the cells of mouse exocrine pancreas. Mikroskopie **22**: 347 (Abstr.)

Réz, G. – Kovács, J. 1971. Morphological examination of autophagic vacuoles formed in mouse pancreatic exocrine cells under the effect of neutral red. Ann. Univ. Sci. Budapest, Sect. Biol. **13**: 315 – 326.

Réz, G. – Kovács, J. 1972. Electron microscopic examination of autophagy and cytoplasmic degradation induced by cadmium chloride and hyperosmotic sucrose in exocrine pancreatic cells of mice. Ann. Univ. Sci. Budapest, Sect. Biol. **14**: 219–234.

Réz, G. – Kovács, J. 1973. Prevention by cyloheximide of neutral red-induced formation of autophagic vacuoles and krinom granules in mouse pancreatic acinar cells. Virchows Arch. Abt. B, Zellpath. **12**: 123–132.

Weiss, J. M. 1955. Intracellular changes due to neutral red as revealed in the pancreas and kidney of the mouse by the electron microscope. J. Exptl. Med. **107**: 213–224.