

## DUCTAL EXCRETION OF NEUTRAL RED

## LYSOSOMES IN THE MOUSE PANCREAS

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## INTRODUCTION

In earlier studies of the mouse pancreas (1), it was found microscopically in saline preparations of living tissue that, within 30 min after the injection of an optimum dose of neutral red, small dye granules appeared in the cytoplasm of the acinar cells. With time, the granules became larger, reached a peak development at about 7 hr, then gradually diminished in size and number until, at about 14 hr, the cell was clear of dye and again normal in appearance. Throughout this "neutral red granule cycle," the dye granules appeared to be confined to supra- and paranuclear positions in the cell.

In an effort to shed some light on the possible role of the Golgi apparatus in this process, the Aoyama silver impregnation method was applied to pieces of the same pancreas examined in the fresh at each of the intervals throughout the cycle (2). With this procedure, the acinar cell showed a gradual loss of the typical Golgi network after dye injection and the development of argentophil granules having many of the properties of the neutral red granules seen in the fresh tissue. The argentophil bodies were not observed in the acinar cell unless the animal had been injected with neutral red. The position, number, general configuration, and relative size of the argentophil bodies were those of the neutral red granules. Finally, the time-relationships in terms of the appearance, development, and disappearance of the argentophil bodies were identical with those of the neutral red granules (2, 3). The neutral red granules as seen in a cryostat-frozen section of

pancreas 5 hr after dye injection (Fig. 1) may be compared with the argentophil bodies shown by the Aoyama technique applied to the same pancreas (Fig. 2).

It was concluded from this study that the argentophil bodies demonstrated by the Aoyama method were the fixed-tissue counterparts of the neutral red granules seen in living cells. Recent electron microscope studies of similarly timed sequences after injection of neutral red have indicated that the dye granules are lysosomal in nature (4, 5), and are positive for acid phosphatase (6).

The fate of the neutral red granules formed in the cytoplasm of pancreatic acinar cells has never been resolved. The possibilities have been considered that the dye is broken down within the granule, or is merely held temporarily in these saclike structures and then passes back into the serum for final excretion by another organ, e.g. the liver.

In relation to this question, Covell in an early cytological study (7) reported that after pilocarpine stimulation the neutral red granules pass into the lumen of the pancreatic acinus and then into the ductules. In our studies of bits of fresh pancreas examined microscopically in saline, the dye granules always appeared confined to supra- and paranuclear positions in the acinar cell. They were never seen mixed amongst the zymogen granules or near the apex of the cell. It was our conclusion, therefore, that the dye granules did not pass into the ducts.

While our preparations of bits of fresh pancreas

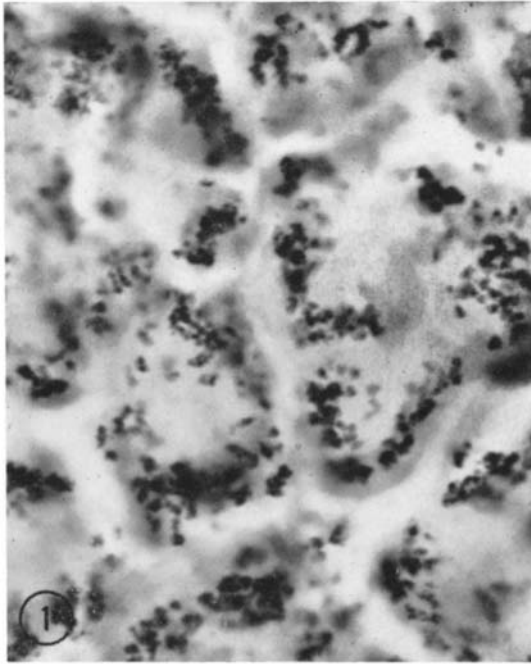


FIGURE 1 20- $\mu$ -thick cryostat-frozen section of pancreas from mouse given neutral red 5 hr earlier. The neutral red granules appearing as black bodies in the photomicrograph outline the acinar structure.  $\times 600$ .

from an animal given neutral red still fail to reveal passage of the dye granules into the ducts, it must be admitted that in such preparations few ducts are visualized. Recently, cryostat-frozen sections of pancreas have been made, and in these preparations many ducts could be seen. It is of much interest that such sections of pancreas from an animal injected with neutral red do not reveal dye granules in the ducts although many such granules are seen in the parenchymal cells (Fig. 1). The reason for our inability to confirm the observations of Covell (7) is not apparent.

However, the application of the Aoyama method, a silver impregnation technique employed to demonstrate the Golgi apparatus, gives strong evidence that excretion through the pancreatic ducts is the major, if not the only, way that neutral red in the form of granules is removed from the pancreas. Other studies have shown that neutral red is not changed chemically in the pancreas, although a process of reduction does occur in the liver (4).

## MATERIALS AND METHODS

Male albino mice weighing 30–35 g were used in these studies. Neutral red chloride (British Drug House, Ltd., London) was dissolved in normal saline as a 1% solution and was injected subcutaneously over the back without anesthesia. The optimum dosage was considered to be 1 ml (10 mg dye)/25-g body weight (1). Pairs of mice were sacrificed at hourly intervals, after injection of dye, up to 9 hr. Part of the pancreas was examined fresh for the degree of formation of the neutral red granules, and the rest was placed in Aoyama's fixative. Control noninjected animals were sacrificed at the same time points.

The Aoyama procedure used was that described by Baker (8) and used by us earlier (2).

## RESULTS

In the pancreatic acinar cell from normal animals, the Aoyama method demonstrates or produces an

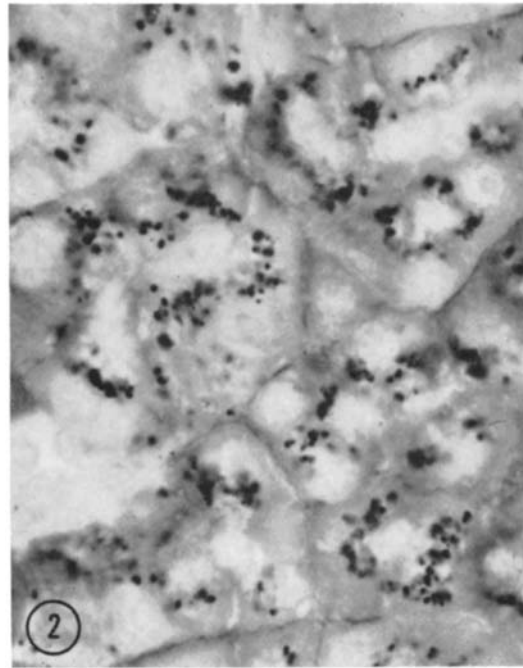
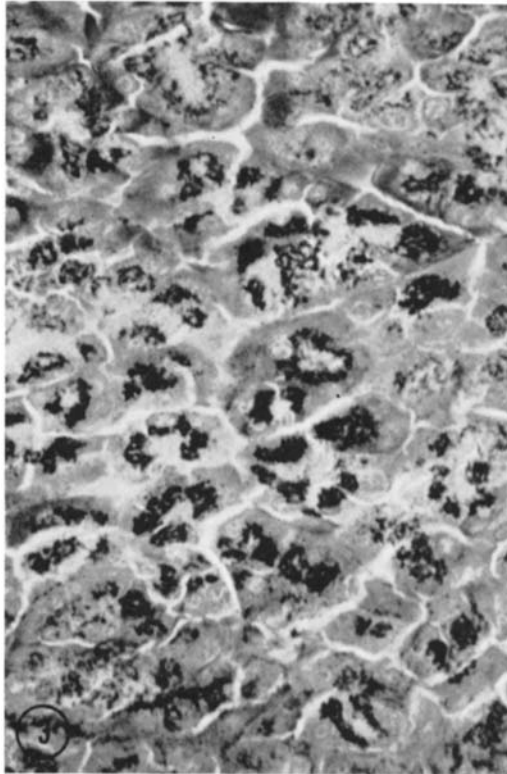


FIGURE 2 7- $\mu$ -thick paraffin section, prepared by the Aoyama technique, from the same tissue as in Fig. 1. The argentophil granules are of similar size and distribution in the acinus, as were the neutral red granules.  $\times 900$ . Note: The black bodies appear more numerous in Fig. 1 because the section is almost three times the thickness of that shown in Fig. 2. An increase of 300 magnification was made in Fig. 2 to adjust for the 30% shrinkage due formalin fixation and processing.

argentophil Golgi network interwoven among the more basal zymogen granules (Fig. 3). However, in the advanced stages of neutral red granule formation no Golgi network is seen, and the argentophil material in the cell is confined to bodies having the size, shape, number, and location of the neutral red granules (2) (Fig. 4).

When this technique is applied to pancreas from control animals, no argentophil bodies are observed in the ducts (Fig. 5), but in the pancreatic ducts of an animal given neutral red, many bodies are present which have the size and shape of the argentophil granules observed in the acinar cells themselves (Fig. 6).

It is of interest that, in a 30-g mouse given subcutaneously 1.2 ml of a 1% solution of the dye, few argentophil granules are seen in the ducts until



FIGURES 3-6 demonstrate mouse pancreas after application of the Aoyama technique. All,  $\times 400$ .

FIGURE 3 Control pancreas from noninjected animal. The argentophil Golgi "apparatus" or "network" is shown.

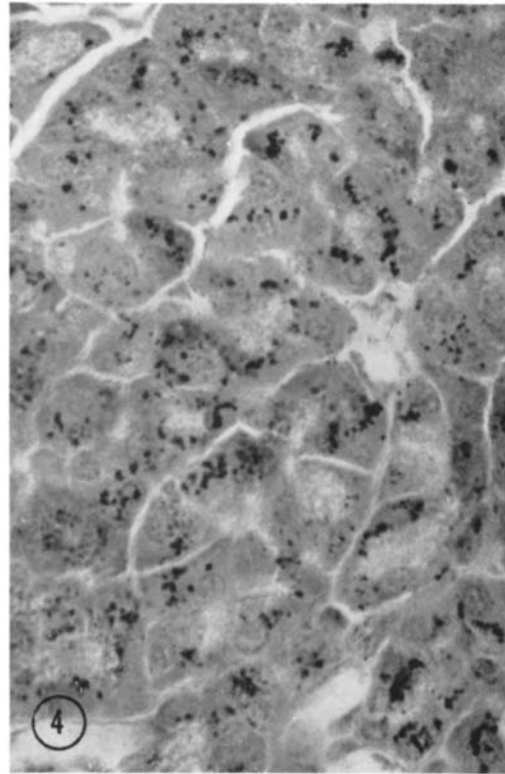


FIGURE 4 Pancreas from animal given neutral red 5 hr earlier. The argentophil network is no longer demonstrated, and the argentophilia of the cytoplasm is confined to the granules.

about 4 hr after injection, but during the 5th through 7th hr they are very numerous. Animals uninjected, but fasted up to 18 hr did not show similar bodies in the ducts. The presence of the argentophil granules in the ducts of animals given neutral red was a highly reproducible finding, seen in every mouse given the dye.

#### DISCUSSION

The question must be asked why, if the argentophil bodies observed in the pancreatic ducts represent the fixed-tissue counterparts of the neutral red granules, the latter cannot be observed in the ducts in the cryostat sections. Since in such preparations dye granules are present in the adjacent acinar cells, it seems unjustified to attribute this discrepancy to some aspect of technique. The most probable explanation is that the pancreatic duct secretion, by its alkaline pH or some other chemi-

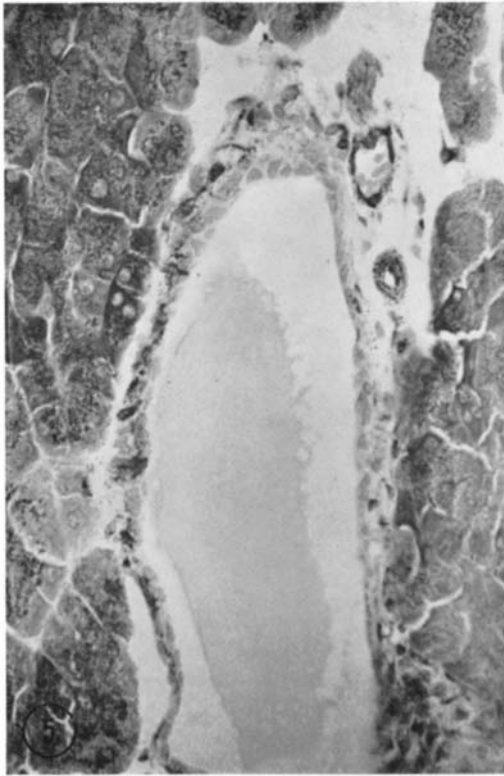


FIGURE 5 Control pancreas from noninjected animal. Secretion appears in duct.

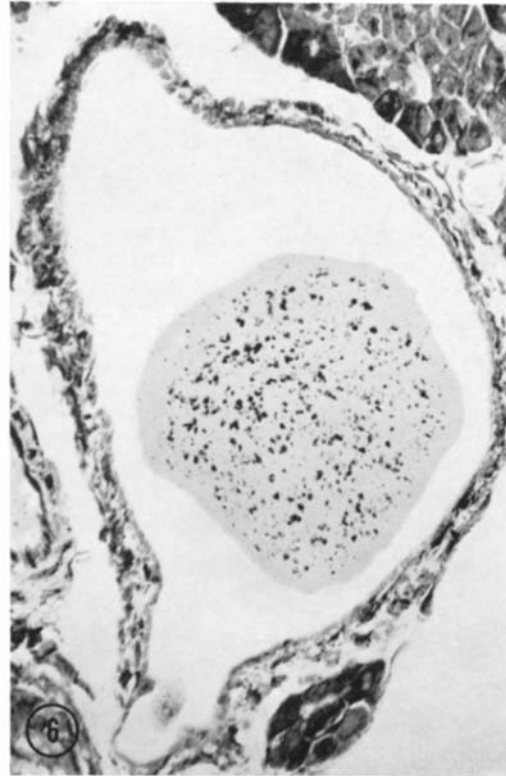


FIGURE 6 Pancreas from animal given neutral red 5 hr earlier. Argentophil granules in the duct are similar to those in acinar cells.

cal means, alters the dye granules and makes them no longer recognizable as such.

In view of the great numbers of argentophil granules in the ducts, it is also difficult to understand why, in fresh preparations, some dye granules are not seen near the apical part of the cell prior to their passage through the cell membrane into the lumen of the acinus. Byrne has confirmed the basal localization of the neutral red granules in the acinar cells (5). A possible explanation is that movement of the granules from the paranuclear area to the apex of the acinar cell and out into the ductule lumen may be rapid and complete, once such movement is initiated.

The process of lysosome formation is frequently interpreted as a means by which the cell may rid itself of worn-out parts and reutilize their valuable constituents, (9-11). In other cases, e.g. the neutrophil granules, lysosomal structures serve to package enzymes for subsequent release from the

cell (9). In still other instances, similar structures may contain metals or other substances for extended periods of time (12). Tanaka has shown that a number of dyes are segregated in animal tissues by lysosomal structures (13). It has become apparent that the role of lysosome formation must be viewed in a very broad sense because, although ultrastructurally similar, lysosomal bodies may differ in their function and controlling mechanisms (9).

The observation is of interest that in the advanced stages of neutral red granule formation the Golgi network is no longer demonstrated by the Aoyama technique. In recent electron microscope studies of pancreas<sup>1</sup> at similarly timed intervals af-

<sup>1</sup> Alousi, M. A., R. J. Stenger, and W. S. Morgan. The fine structure of pancreatic and hepatic parenchymal cells after neutral red dye injection. Data to be published.

ter injection of dye, the Golgi cisternae were observed but were relatively small in the advanced stages of neutral red granule formation. This suggests that the Golgi apparatus may serve as a repository or source for material making up the membrane of the dye granules.

The observations here reported indicate that the pancreatic acinar cell has the capacity to segregate a foreign substance which has entered the cell, in this case, neutral red, and to eliminate it, packaged as lysosomes, via the ducts, in a relatively brief period of about 12–14 hr. With an optimum dose of dye (1) such excretory activity is especially intense 5–7 hr after injection, and 6–8 hr later, the acinar cells are cytologically normal. There are indications that a similar process may occur in the liver (4).

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#### SUMMARY

The application of a silver impregnation method (Aoyama) to pancreatic tissue from mice given neutral red demonstrates the presence of large numbers of argentophil bodies in the pancreatic ducts. These bodies are interpreted as the fixed-tissue counterparts of the dye granules observed in acinar cells in fresh tissue. It would appear that ductal excretion is the means used by the cell to rid itself of neutral red lysosomes.

This work was supported by United States Public Health Service Grant CA 07766. The author is grateful to Miss Linda Woltman for technical assistance.

Received for publication 3 March 1967, and in revised form 28 September 1967.