INTRODUCTION

Pancreatic acinar cells produce most of the enzymes required for the digestion of complex food substances in the small intestine. Underlying this specialized function is a cellular organization predominantly devoted to the production of protein. Considerable attention has been given to the process of protein metabolism in the pancreas (6, 10, 11, 19, 26, 29, 30). As a result, the major steps in the synthesis and secretion of protein by the acinar cells have been worked out in some detail (Fig. 1). In contrast, practically nothing is known about the formation and release of non-protein substances by pancreatic acinar cells.

One of the first applications of the technique of radioautography (18) revealed an incorporation of inorganic sulfate-35S by acinar cells in the rabbit pancreas. This was subsequently confirmed in the mouse (17). It was suspected that the sulfate uptake might reflect the synthesis of sulfated mucopolysaccharides, for this had proved to be the case in other tissues, such as cartilage. However, nothing further was reported on this topic for many years.

Very recently, Berg (4) reopened the question of inorganic sulfate use by pancreatic acinar cells. He observed that 1 hr after injection of sulfate-35S in the mouse, radioactivity was concentrated in the general region of the Golgi complex. At later intervals (8-12 hr) the labeled material appeared in zymogen granules. Because these granules contain the enzymes and enzyme precursors which are destined to be secreted into the pancreatic juice, this suggested that there might be sulfated materials (perhaps mucopolysaccharides) in the pancreatic secretion. Also, it appeared that the production of these substances could involve intracellular supply lines similar to those previously demonstrated for proteins.

We decided to explore this process in greater detail, using electron microscope radioautography.
FIGURE 1 Schematic diagram of a pancreatic acinar cell. Digestive enzymes are synthesized on the ribosomes of the granular endoplasmic reticulum, then released into the cisternae of the reticulum. These converge upon the Golgi complex. The proteins are conveyed through the Golgi complex in small vesicles which appear to fuse into larger condensing vacuoles within which the enzymes are progressively concentrated. This process converts the condensing vacuoles into zymogen granules. These are shifted towards the apical surface of the cell (above). At the surface, the membrane of the granule becomes continuous with the cell membrane, and the contents of enzyme and enzyme-precursors are secreted into the acinar lumen. The present report shows that a sulfated material, probably a mucopolysaccharide, undergoes sulfation in the Golgi complex, and is then added to the contents of the condensing vacuoles. Thus it comes to be incorporated into zymogen granules and is released with the other contents of the granules into the pancreatic secretion.

to analyze the distribution of radioactivity in pancreatic acinar cells taken from mice at different times after the injection of sulfate-$^{35}$S.

MATERIALS AND METHODS

Six female C57 BL/6J mice, 7-8 weeks old, were anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.), then were injected via the external jugular vein with 80-200 mCi of carrier-free sulfate-$^{35}$S in aqueous solution (Schwarz Bio Research Inc., Orangeburg, N.Y.) at a concentration of $0.5-1.0$ Ci/ml. The animals were killed by intracardiac perfusion of a solution containing $17c$ formaldehyde and $1\%$ glutaraldehyde in phosphate buffer, pH 7.1, at 10 and 30 min, 1, 4, 8, and 12 hr after injection. The mice, fed Purina Lab Chow ad libitum before the injection, did not eat during the experimental interval. After perfusion of the animal at 80 mm Hg pressure for about 10 min, the pancreas was removed and placed in the same fixative overnight. The specimens were next cut into smaller pieces, which were postfixed in $1\%$ osmium tetroxide in the same buffer for 1 hr, dehydrated in ethanol, and embedded in Araldite. Electron microscope radioautograms were then prepared by the dipping technique using Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England), according to procedures reported elsewhere (34). The preparations were exposed for 40-50 days in the dark at 4°C under low humidity, developed in Phenidon developer (14) for 1 min at 15°C, fixed in $307$ sodium thiosulfate, and examined in a Siemens Elmiskop IA electron microscope.

Quantitative analysis of the distribution of radioactivity was carried out by counting developed silver grains in the emulsion. Sections of comparable thickness (silver color) which had been coated with emulsion, exposed, and developed together were used. Photographs of cells which had been sectioned so that all of the major cellular components were visible were prepared at a final magnification of 13,000. Silver grains overlying the nucleus, mitochondria, condensing vacuoles, Golgi complex, zymogen granules, and granular endoplasmic reticulum were counted at each of the six intervals after injection. The counts for each cell component were then expressed as a percentage of the total counts recorded over all cell components at that interval. The area occupied by each cell component was then determined by planimetry of photographs from 18 different cells. These results were also expressed as percentages of the total. This made it possible to estimate the concentration of radioactivity in the several components at each interval by relating the percentage of total radioactivity to the percentage of total area.

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RESULTS

10 min after injection of sulfate-35S, radioactivity was concentrated in the Golgi complex (Figs. 2-4). Nearly 50% of the silver grains were situated over the small vesicles and agranular membranes of this organelle, which accounted for only 6% of the area of the cell sections (Table I). Some of the condensing vacuoles associated with the Golgi complex were also labeled. These contained about 6% of the total radioactivity, a significant quantity in relation to their relatively small size. Scattered grains were present over the zymogen...
FIGURE 3  10 min after injection. Radioactivity is largely confined to the Golgi complex (g). A few of the condensing vacuoles (arrows) appear to be labeled, but the zymogen granules and other components of the cell are not significantly radioactive. The acinar lumen (lu) is devoid of radioactivity. × 16,400.
FIGURE 4  10 min after injection. Most of the silver grains are situated over the vesicles and membranes of the Golgi complex, demonstrating that this is the site at which sulfation occurs. Several condensing vacuoles are indicated by arrows. $\times$ 28,000.

FIGURE 5 Concentration of radioactivity (per cent total silver grains/per cent total cell area) in different components of pancreatic acinar cells between 10 min and 4 hr after injection of sulfate-$^{35}$S. The highest concentration is initially in the Golgi complex, but labeling of this organelle progressively declines after 10 min. Labeling of the condensing vacuoles increases between 10 and 30 min, then decreases. Radioactivity appears in the zymogen granules at 1 hr, and increases between 1 and 4 hr. (The concentration of radioactivity in the labeled granules was greater than it appears to be here because the concentration value is based upon granules, nucleus, mitochondria, and granular endoplasmic reticulum. However, when the area occupied by these components was taken into account (Fig. 5), it was apparent that none of them was preferentially labeled.

During the following 20 min, there was a dramatic shift in the distribution of radioactive material in the acinar cells. Labeling of the Golgi complex declined by nearly one-half. This was accompanied by a remarkable increase in labeling of the condensing vacuoles. These structures now contained 35% of the radioactivity, although they comprised less than 2% of the area in the sections (Table I, Figs. 6, 7). None of the other components of the cell contained significant concentrations of radioactivity at this time (Fig. 5).

This decline in the amount of radioactivity in the Golgi complex continued during the next 30 min. An even greater decrease in labeling of the condensing vacuoles took place (Table I). Nevertheless, most of the vacuoles were still intensely radioactive (Figs. 8, 9) at a concentration which continued to exceed that in any other part of the cell (Fig. 5). For the first time, significant amounts of the entire zymogen granule population, and not all of them were labeled.) The concentration of radioactivity in the nucleus (o-o), mitochondria (e-•-•), and granular endoplasmic reticulum (x-x) is low or negligible and stable. These organelles do not appear to participate directly in sulfate metabolism.
Figure 6  30 min after injection. The Golgi complex (g) still contains radioactive material, but now the most intensely labeled structures are the condensing vacuoles (arrows). The zymogen granules remain free of radioactivity. × 24,750.
FIGURE 7  30 min after injection. Much of the labeled material which was present in the Golgi complex during the first few minutes after injection has now been transferred to the condensing vacuoles (arrows). \( \times 16,000. \)
TABLE I
Distribution of Radioactivity* among Different Components of Pancreatic Acinar Cells

| Cell component and relative size | Time after injection of sulfate-35S |
|----------------------------------|------------------------------------|
|                                  | 10 min | 30 min | 1 hr | 4 hr | 8 hr | 12 hr |
| Golgi complex (6.3)              | 47.4   | 25.6   | 19.4 | 7.1  | 8.0  | 12.0  |
| Condensing vacuoles (1.4)        | 6.2    | 35.2   | 19.6 | 3.3  | 6.9  | 3.5   |
| Zymogen granules (16.2)          | 9.3    | 13.1   | 35.1 | 68.6 | 49.2 | 43.0  |
| Nucleus (12.2)                   | 4.1    | 5.1    | 4.6  | 3.6  | 4.4  | 6.3   |
| Mitochondria (4.9)               | 6.8    | 3.9    | 3.1  | 2.8  | 5.9  | 4.5   |
| Granular endoplasmic reticulum   | 26.2   | 17.1   | 18.2 | 14.6 | 25.6 | 30.7  |

* Percentage of total silver grains overlying individual cell components at each interval.
† Percentage of total cell area occupied by each component.

Figure 8 1 hr after injection. Labeled zymogen granules (z) begin to be a prominent feature of the acinar cells at this interval. Significant amounts of radioactivity are still present in many of the condensing vacuoles (arrows). × 14,000.
FIGURE 9 1 hr after injection. Although weaker than before, labeling is still observed in the Golgi complex (g). Condensing vacuoles (arrows) and nearby zymogen granules are heavily radioactive. × 17,000.

FIGURE 10 4 hr after injection. Most of the radioactive material is now located in the zymogen granules. × 6875.
of labeled material appeared in some of the zymogen granules. Although only a few of the granules were labeled, they contained about 35% of the radioactivity in the cell. Thus, at 1 hr, three components were highly radioactive: Golgi complex, condensing vacuoles, and a few of the zymogen granules (Fig. 9).

3 hr later, only the zymogen granules retained significant quantities of radioactivity (Figs. 5, 10, 11; Table I). Nearly 70% of the radioactivity in the cell was now contained in about half of the zymogen granules. The other zymogen granules appeared to contain no radioactive material whatsoever. Some labeled granules had been displaced to the apical end of the cell and were observed in the process of emptying their radioactive contents into the acinar lumen (Fig. 12). Labeled material also was present in the duct system of the gland at this time (Fig. 13).

By 8 hr, and increasingly at 12 hr, the continued secretion of labeled zymogen granule material depleted the acinar cells of most of the radioactive sulfur. Less than 20% of the granules were labeled at 8 hr, and these were not heavily reactive (Fig. 14). By 12 hr, only 10% of the granules contained radioactive material. Throughout the period studied, the concentration of radioactivity in the nucleus, granular endoplasmic reticulum, and mitochondria was very low or negligible and showed no tendency to either increase or decrease with time (Fig. 5).

**DISCUSSION**

Within 10 min after the intravenous injection of inorganic sulfate tagged with $^{35}$S, acinar cells in the pancreas had incorporated significant amounts of the radioactive precursor. Perfusion, prolonged aqueous fixation, and subsequent extraction procedures presumably remove free sulfate from the tissues, so that the residual radioactivity must represent sulfate which had been bound to large molecules retained in the sections. This process, sulfation, takes place in the Golgi complex of the pancreatic acinar cells, as shown by the predominance of labeling, 10 min after injection, in that organelle. The incorporation of sulfate directly into the Golgi complex has been observed previously by radioautography in goblet cells (12, 13, 15, 16), chondrocytes (9, 16), and developing eosinophilic leukocytes (32). In some of these studies (9, 13) cells were examined as early as 2 or 3 min after exposure to the sulfate-$^{35}$S, and the...
Golgi complex was already preferentially labeled. None of this earlier work provided any evidence that the nucleus, granular endoplasmic reticulum, or mitochondria participate directly in sulfate metabolism. Similarly, although we observed scattered silver grains over these three cell components in pancreatic acinar cells at all intervals after injection, when the grains were counted and related to the area of the components, it could be seen that labeling was negligible and did not vary with time. This is spurious labeling which can be accounted for by beta particles emanating from adjacent, heavily labeled cell components, and by background grains in the emulsion.

The metabolism of sulfate in pancreatic acinar cells is restricted to the Golgi complex, con-

Figure 12. 4 hr after injection. Radioactive material is present in the acinar lumen (lu). Two zymogen granules can be seen in the process of releasing their radioactive contents (arrows). × 12,800.
densing vacuoles, and zymogen granules. As the concentration of radioactivity in the Golgi complex declined between 10 and 30 min, labeling of the condensing vacuoles rose sharply. This means that material which had been sulfated in the Golgi complex was being transferred to the condensing vacuoles. (A small amount of transfer may have occurred by 10 min, as there was a slight labeling of some condensing vacuoles at that time.) By 1 hr, labeling in the condensing vacuoles had also begun to decrease, and radioactivity began to appear in a few of the zymogen granules. These must have been new granules, formed by maturation of the condensing vacuoles which had been so heavily labeled 30 min earlier.

By 4 hr, the Golgi complex and condensing vacuoles were free of significant labeling, indicating that metabolism of the radioactive sulfate introduced by the single injection was essentially complete. Practically all of the bound radioactivity in the cell was by then concentrated in about half of the zymogen granules. Most of the unlabeled granules present at this time must have been formed before the injection of labeled sulfate. Radioactive granules were observed releasing their contents into the acinar lumen, and labeled material was present in the pancreatic duct system. This demonstrates that material which is sulfated in the Golgi complex ultimately finds its way into the pancreatic secretion.

Continued secretion of the labeled zymogen granules gradually depleted the acinar cells of their radioactivity. By 12 hr very few of the granules were labeled. These were only weakly reactive, indicating that they had probably been formed relatively late after the injection, when the level of available sulfate-35S had declined.

What is the nature of the material which is sulfated in the Golgi complex? It is unlikely to be protein, for the participation of inorganic sulfate in protein synthetic pathways is negligible or absent (5, 8, 27, 28). Both lipids and mucopolysaccharides become labeled in the brain after injection of sulfate-35S (1, 24). Sulfated lipids, however, are mainly, perhaps exclusively, found in myelin (25), and, to our knowledge, have not been conclusively demonstrated outside the central nervous system. It therefore appears likely that the sulfated material synthesized by pancreatic acinar cells will prove to be a sulfated mucopolysaccharide. The addition of such a compound to the contents of the zymogen gran-
Figure 14  8 hr after injection. A few of the zymogen granules are still labeled. Otherwise, most of the radioactivity has left the cell. Golgi complex, g; acinar lumen, lu. × 9600.
ules might aid somehow in packaging the digestive enzymes, or in subsequently releasing them into the pancreatic juice.

In several types of cells certain kinds of complex carbohydrates are synthesized in the Golgi complex, as shown by radioautographic studies on the incorporation of labeled glucose, galactose, and fucose (2, 3, 7, 15, 16, 20, 21, 31, 33, 35). This could be the site of origin of the carbohydrate portion of the glycoprotein enzymes which are secreted by the acinar cells (22, 23). In addition, if our speculations about the nature of the sulfated component are borne out, it may prove that the mucopolysaccharide is synthesized as well as sulfated in the Golgi complex.

Whether or not this proves to be the case, our work with sulfate-35S demonstrates that the Golgi complex in pancreatic acinar cells does more than simply concentrate and wrap in a membrane the digestive enzymes which are delivered to it. This organelle adds to the granules materials of its own manufacture which are later released along with the enzymes into the pancreatic secretion.

The technical assistance of Mrs. Zoja Trirogoff and Mr. Roger Witucki is gratefully acknowledged.

This work was supported by Atomic Energy Commission Contract AT(11-1)-34, PA No. 176, and by a United States Public Health Service Postdoctoral Fellowship (to Dr. Berg) 1 F02, NS-43871-01 VSN.

Received for publication 16 October 1970, and in revised form 13 January 1971.

REFERENCES

1. BALASUBRAMANIAN, A. S., and B. K. BACHHAWAT. 1970. Sulfate metabolism in brain. Brain Res. 20:341.
2. BENNETT, G. 1970. Migration of glycoprotein from Golgi apparatus to cell coat in the columnar cells of the duodenal epithelium. J. Cell Biol. 45:660.
3. BENNETT, G., and C. P. LEBLOND. 1970. Formation of cell coat material for the whole surface of columnar cells in the rat small intestine, as visualized by radioautography with L-fucose-3H. J. Cell Biol. 46:409.
4. BERG, N. B. 1970. Inorganic sulfate incorporation into the zymogen granules of pancreatic acinar cells. Anat. Rec. 166:278. (Abstr.)
5. BOSTRÖM, H., and S. AQVIST. 1952. Utilization of S35-labeled sodium sulphate in the synthesis of chondroitin sulphuric acid, taurine, methionine and cystine. Acta Chem. Scand. 6:1557.
6. CARO, L. G., and G. E. PALADE. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. J. Cell Biol. 20:473.
7. DROZ, B. 1966. Élaboration de glycoprotéines dans l'appareil de Golgi des cellules hépatiques chez le rat; étude radioautographique en microscopie électronique après injection de galactose-3H. C. R. Acad. Sci. Ser. D. 262:766.
8. DZEWIATKOWSKI, D. D. 1954. Utilization of sulfate sulfur in the rat for the synthesis of cystine. J. Biol. Chem. 207:181.
9. GODMAN, G. C., and N. LANE. 1964. On the site of sulfation in the chondrocyte. J. Cell Biol. 21:333.
10. JAMIESON, J. D., and G. E. PALADE. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. J. Cell Biol. 34:577, 596.
11. JAMIESON, J. D., and G. E. PALADE. 1968. Intracellular transport of secretory proteins in the pancreatic exocrine cell. J. Cell Biol. 39:580, 589.
12. JENNINGS, M. A., and H. W. FLOREY. 1956. Autoradiographic observations on the mucous cells of the stomach and intestine. Quart. J. Exp. Physiol. Cog. Med. Sci. 41:151.
13. LANE, N., L. CARO, L. R. OTERO-VILARDEBO, and G. C. GODMAN. 1964. On the site of sulfation in colonic goblet cells. J. Cell Biol. 21:339.
14. LETTRÉ, H., and N. PAWELETZ. 1966. Probleme der elektronenmikroskopischen Autoradiographie. Naturwissenshaften. 53:268.
15. NEUTRA, M., and C. P. LEBLOND. 1966. Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope radioautography of goblet cells from rats injected with glucose-3H. J. Cell Biol. 30:119.
16. NEUTRA, M., and C. P. LEBLOND. 1966. Radioautographic comparison of the uptake of galactose-3H and glucose-3H in the Golgi region of various cells secreting glycoproteins or mucopolysaccharides. J. Cell Biol. 30:137.
17. NORHAGEN, A., and E. ODEBLAD. 1955. Uptake of radioasulale in the islets of Langerhans of mice. Arch. Biochem. Biophys. 54:562.
18. ODEBLAD, E., and H. BOSTRÖM. 1952. An autoradiographic study of the incorporation of S35-labeled sodium sulfate in different organs of adult rats and rabbits. Acta Pathol. Microbiol. Stand. 31:339.
19. PALADE, G. E., P. SIEKEVITZ, and L. G. CARO. 1962. Structure, chemistry and function of...
the pancreatic exocrine cell. In The Exocrine Pancreas. A. V. S. de Reuck and M. P. Cameron, editors. J. and A. Churchill, Ltd., London. 23.

20. Peterson, M. R., and C. P. Lefebvre. 1964. Uptake by the Golgi region of glucose labeled with tritium in the 1 or 6 position, as an indicator of synthesis of complex carbohydrates. Exp. Cell Res. 34:420.

21. Peterson, M. R., and C. P. Lefebvre. 1964. Synthesis of complex carbohydrates in the Golgi region, as shown by radioautography after injection of labeled glucose. J. Cell Biol. 21:143.

22. Plummer, T. H., Jr. 1968. Glycoproteins of bovine pancreatic juice. Isolation of ribonucleases C and D. J. Biol. Chem. 243:5961.

23. Reinhold, V. N., F. T. Dunne, J. C. Wriston, M. Schwart, L. Sarda, and C. H. W. Hirs. 1968. The isolation of porcine ribonuclease, a glycoprotein, from pancreatic juice. J. Biol. Chem. 243:5482.

24. Robinson, J. D., Jr., and J. P. Green. 1962. Sulfomucopolysaccharides in brain. Yale J. Biol. Med. 35:248.

25. Rouger, G., and A. Yamamoto. 1969. Lipids. In Handbook of Neurochemistry. A. Lajtha, editor. Plenum Publishing Corporation, New York. 1:121.

26. Siekevitz, P., and G. E. Palade. 1958. A cytochemical study on the pancreas of the guinea pig. J. Biophys. Biochem. Cytol. 4:203, 309, 557.

27. Tarver, H., and C. L. A. Schmidt. 1939. The conversion of methionine to cystine. Experiments with radioactive sulfur. J. Biol. Chem. 130:67.

28. Tarver, H., and C. L. A. Schmidt. 1942. Radioactive sulfur studies. J. Biol. Chem. 146:69.

29. Van Heyningen, H. E. 1964. Secretion of protein by the acinar cells of the rat pancreas, as studied by electron microscope radioautography. Anat. Rec. 148:485.

30. Warshawsky, H., C. P. Lefebvre, and B. Droz. 1963. Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radioautographs. J. Cell Biol. 16:1.

31. Whirr, P., A. Herscovics, and C. P. Lefebvre. 1969. Radioautographic visualization of the incorporation of galactose-3H and mannose-3H by rat thyroids in vitro in relation to the stages of thyroglobulin synthesis. J. Cell Biol. 43:289.

32. Young, R. W. 1969. Utilization of S35-sulfate in the formation of azurophil granules in the Golgi complex of developing eosinophil leucocytes. Anat. Rec. 163:534. (Abstr.)

33. Young, R. W., and D. Bok. 1970. Autoradiographic studies on the metabolism of the retinal pigment epithelium. Invest. Ophthalmol. 9:524.

34. Young, R. W., and B. Droz. 1968. The renewal of protein in retinal rods and cones. J. Cell Biol. 39:169.

35. Zagury, D., J. W. Uhr, J. D. Jamieson, and G. E. Palade. 1970. Immunoglobulin synthesis and secretion. II. J. Cell Biol. 46:52.