Amylase Activity of Acinar Cells Separated from Guinea Pig Submaxillary Gland* (33850)

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It has long been thought from histological studies on the cellular distribution of the zymogen granules and changes in their numbers and form during various phases of the secretory cycle that amylase in salivary gland or pancreas is primarily localized in the acinar cells (1). More direct evidence for this localization has been provided recently by biochemical and histochemical investigations. Thus, analysis of amylase activity in isolated zymogen granules, derived from pancreas (2) or parotid gland (3), clearly showed that this activity is greatest in these primarily acinar cell components. In addition, histochemical evidence was recently provided (4–6) which directly demonstrates that amylase activity in frozen sections of salivary or pancreatic gland is generally confined to acinar cells and ductal lumina.

While histochemical methods demonstrated the cellular localization of amylase to acinar components of salivary gland and pancreas, they provided virtually no quantitative data (4–6). The present study was undertaken to obtain a quantitative estimate for the level of amylase activity from acinar cells. For this, acinar cells of guinea pig submaxillary gland were separated from other glandular elements, using the cell-separation procedure of Schneider and Person (7). It was found that the acinar cells provide levels of amylase activity approximately 60% higher than those of whole gland.

Methods. Guinea pig was used in these experiments. Rabbit, which had been used in previously reported work (7) on separation of cells from submaxillary gland, was found in early experiments to have levels of amylase too low for satisfactory analysis. Adult male or female guinea pigs were fasted for 24 hr and anesthetized with Nembutal prior to removal of the submaxillary glands. After removal, usually from four animals for each of 9 experiments, a small piece was cut from each gland; these pieces were pooled, weighed, and then frozen for subsequent analysis for amylase in whole tissue. The remaining glandular tissue was pooled for use in the cell-separation procedure.

The method of Schneider and Person (7), slightly modified, was used to separate acinar from ductal cells. The glandular tissue was minced by scissors and placed in a sack of nylon bolting cloth (Nitex, No. 153, Tobler, Ernst and Traber, New York, N. Y.). The sack was securely closed, placed in a glass mortar containing 10 ml of 0.15 *M* NaCl, and gently squeezed with a glass pestle to express acinar cells. This procedure was repeated twice in fresh 10-ml aliquotes of 0.15 *M* NaCl. The three aliquots of expressate were combined, filtered (Nitex, No. 73) and centrifuged at 1300*g* for 20 min. The resulting pellet was washed with normal saline, drained, and divided into two fractions. One fraction was homogenized and analyzed for amylase activity while the other was used to obtain the dry/wet weight ratio. Amylase activity and dry weight were also determined on the supernatant fluid resulting from centrifugation of the acinar cell fraction and on homogenate of the tissue remaining in the nylon sack. In calculating the dry weight for supernatant fluid, the weight of the dissolved NaCl was subtracted. Dry/wet weight ratios were also determined on intact submaxillary tissue from several guinea pigs. Dry weight was obtained from the change in weight after 24 hr at 100°. Amylase activity was determined by the method of Myers *et al.* (8) and expressed as milligrams of reducing substance, as glucose, formed per milligram of dry weight of tissue, or tissue extract. Micro-

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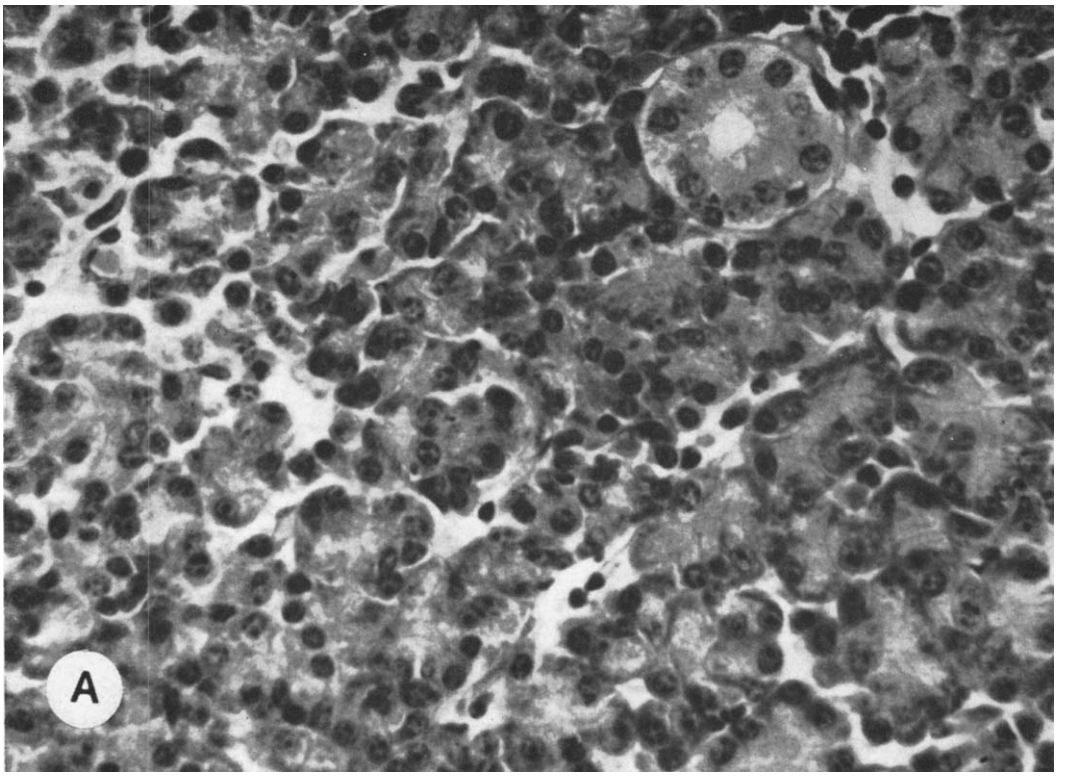

FIG. 1(A). Histologic preparations of intact submaxillary gland of guinea pig, ×400,

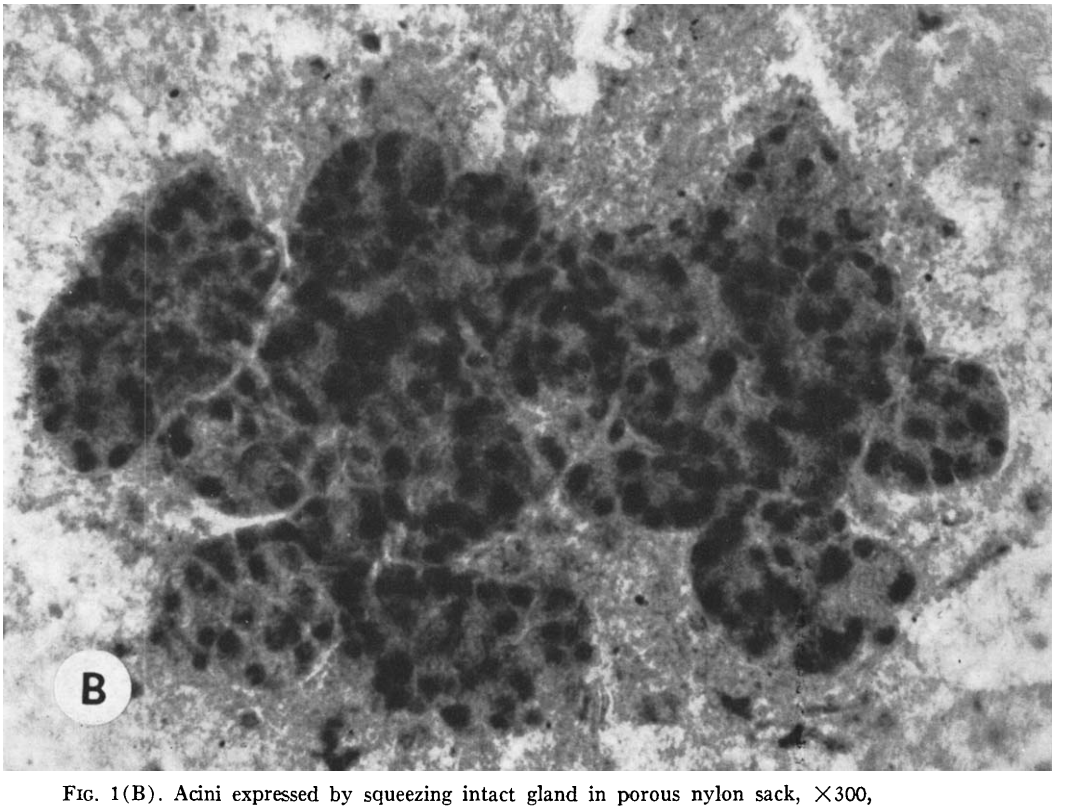

FIG. 1(B). Acini expressed by squeezing intact gland in porous nylon sack, ×300,

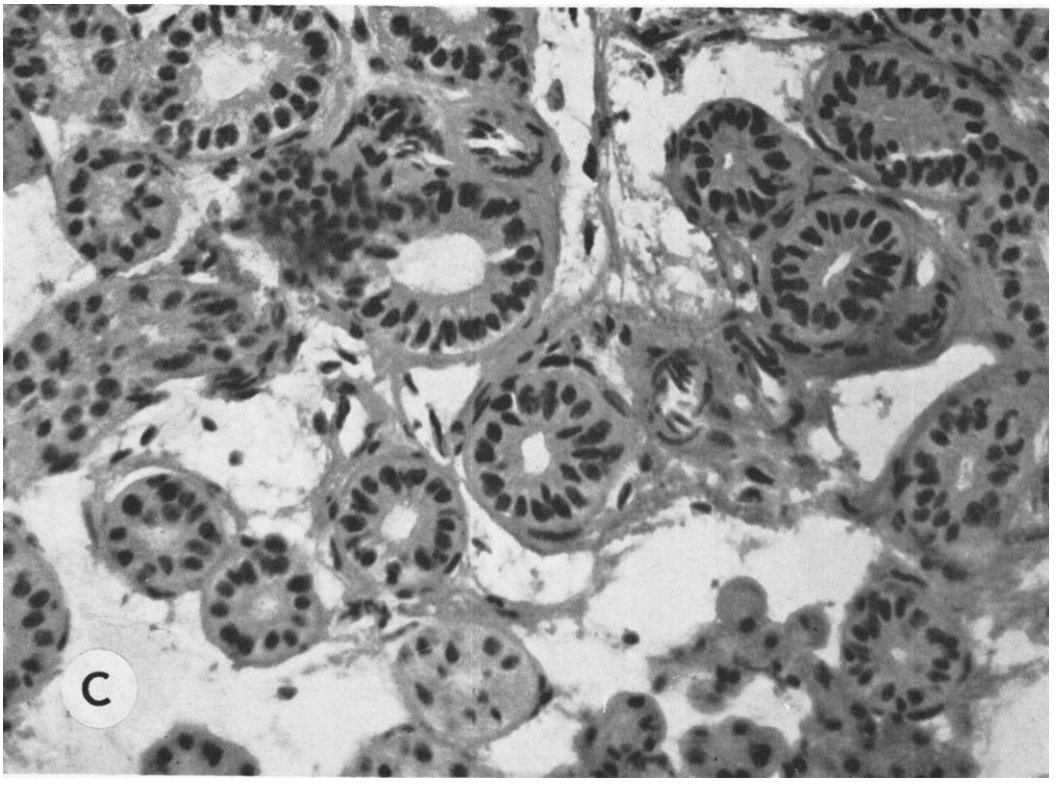

FIG. 1(C). Remainder, consisting mainly of ducts and connective tissue, in the sack after acinar cells were expressed, ×400,.

scopic sections and smears were prepared, respectively, from intact gland and cell suspensions. For sections, tissue was fixed in Bouins' solution, while smears were heat fixed. Hematoxylin and eosin were used for staining.

Results and Discussion. Histological examination of the intact submaxillary gland (Fig. 1A) and of the suspension of cells expressed from the squeeze-sack (Fig. 1B), as well as of the tissue remaining in the sack (Fig. 1C), showed that the expressate consisted mainly of intact acinar cells, with some naked nuclei and cytoplasmic fragments. Intact ductal cells were not present. Examination of the tissue left behind in the sack after expressing acinar cells showed this to consist primarily of a mixture of ductal and connective tissue, with some degree of contamination by acinar cells. A pure preparation of ductal tissue could not be achieved.

Amylase activities of the acinar fraction, supernatant fluid and remainder in the sack are shown in Fig. 2. The amylase activity of the acinar fraction, at a mean value of 341

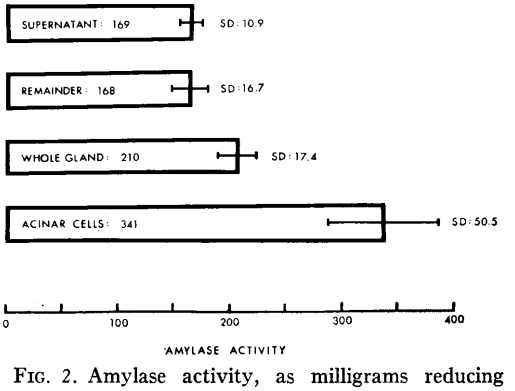

FIG. 2. Amylase activity, as milligrams reducing substance per milligram of dry weight, for whole submaxillary gland, expressed acinar cells, supernatant fluid from centrifugation of saline suspension of material expressed from bolting cloth, and tissue remaining in the sack. Values are means ± SD, from 9 experiments.

mg of reducing substance/mg of dry wt, appreciably exceeded the activity of whole gland (210 mg of reducing substance/mg of dry wt) and this difference was significant ($p<.001$). The mean amylase activity of the acinar fraction was also much greater (by at least 2-fold) than the activity of the remainder in the sack or than the activity in the supernatant fluid from the separation procedure. The average "recovery" of amylase activity was about 92%, *i.e.*, the sum for all fractions of the product: (activity/mg of dry wt) × (dry weight), accounted for 92% of the amylase activity for the whole gland. While the extent of leakage of amylase from acinar cells during the cell separation procedure cannot be gauged, it is evident from the data that the level of activity of amylase in acinar cells is at least 60% greater, on a dry weight basis, than the level in whole guinea pig submaxillary gland. Although it was not possible to determine directly the level of activity of amylase in ductal cells because neither a pure preparation of ductal cells nor a separation of all the acinar cells in the gland could be obtained, it can be shown that the amylase level in ductal cells is probably low. Thus, from the amylase data it appears that acinar cells would occupy at least 62% of the mass of the gland, on a dry weight basis, if amylase were present only in acini. Examination of representative histologic sections of whole gland indicated that approximately 15% of the volume of the gland consists of ductal elements. From other reported data (9), it appears that 15–20% of the volume of submaxillary gland may be attributable to intralobular and extralobular stroma. Hence, the fraction of the whole gland actually represented by acini probably does approximate 62%. On this basis, therefore, it appears likely that amylase activity in ductal cells is low compared to that of acini and that very little of the total amylase activity of the whole gland is contributed by ductal cells.

Summary. Amylase activity was measured in whole tissue and in separated acinar cells from submaxillary gland of guinea pig. Acinar cells showed amylase levels which were approximately 60% higher, on a dry weight basis, than levels in whole gland.

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