A Cytochemical Study on the Pancreas of the Guinea Pig

II. Functional Variations in the Enzymatic Activity of Microsomes

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ABSTRACT

Microsomes were isolated from the pancreas of starved and fed guinea pigs. In the first case, the gland was removed from animals starved for 48 hours; in the second, the pancreas was excised 1 hour after the beginning of a meal that ended a fast of 48 hours. These are referred to below as fed animals. In both cases the tissue was homogenized in 0.88 M sucrose and the microsomes obtained by centrifuging the mitochondrial supernatant at 105,000 g for 60 minutes.

In starved animals the content of the endoplasmic reticulum of the exocrine cells and the content of the microsomes were found to be of low or moderate density. In fed guinea pigs the cavities of the reticulum frequently contained dense intracisternal granules and the microsomes were distinguished by a content of high density sometimes in the form of recognizable intracisternal granules.

In starved animals, the microsomes were found to account for 5 to 20 per cent of the trypsin-activatable proteolytic activity and ribonuclease activity of the whole cell, whereas in fed animals they contained uniformly almost 30 per cent of these activities.

In fed animals the dense, cohesive content of the microsomes (intracisternal granules) could be isolated by breaking up the microsomes with dilute (0.1 per cent) deoxycholate solutions and separating microsomal subfractions by differential centrifugation. The specific enzymatic activities of a heavy microsomal subfraction rich in intracisternal granules were almost equal to those of isolated purified zymogen granules. The ribonucleoprotein particles attached to the microsomal membranes could be isolated by the same technique and found also to exhibit some of the same enzymatic activities. Corresponding subfractions isolated from the microsomes of starved animals were considerably less active.

The relevance of these findings for the synthesis and intracellular transport of protein in the exocrine cell of the pancreas is discussed.

INTRODUCTION

We attempt to answer in this paper a number of questions raised by results recorded in two previous publications. In one of these (1) we have shown that microsomal fractions isolated from the pancreas of starved guinea pigs are endowed with ribonuclease (RNase)- and trypsin-activatable protease (TAPase) superscript 1 activities that vary from one experiment to another and reach in some cases 30 per cent of the corresponding activities of the companion zymogen fractions. The latter mainly consist of zymogen granules and are distinguished by the highest total and specific RNase and TAPase activities of all the cell fractions. Evidence presented and reviewed in reference 1 indicates that the zymogen granules are primarily comprised of stored digestive enzymes and enzyme precursors (zymogens). With these findings in mind, one may ask whether the variability noted in microsomal enzymatic activities has functional significance or is merely due to vagaries in the separation technique.

1Proteolytic activity, due primarily to chymotrypsinogen and trypsinogen, and elicited by the addition of trypsin.
In another paper (2) it was shown that in certain cases relatively large (0.05 to 0.3 μ) dense granules are found within the cisternae of the endoplasmic reticulum in the pancreatic exocrine cells of the guinea pig. A comparison of these intracisternal granules with the usual zymogen granules of the exocrine cell has shown that the two are similar in density and fine texture, but different in size and intracellular location. More recent feeding experiments, to be reported in detail in another paper, have indicated that the intracisternal granules are temporary structures which are present in large numbers between 1 and 2 hours after feeding and disappear by 3 to 4 hours. Since the endoplasmic reticulum becomes the microsome fraction in the usual cell fractionation procedure (3), it can be expected that many microsomal vesicles isolated 1 hour after feeding will contain intracisternal granules or related material. In relation, then, to these granules, one may ask whether they have TAPase and RNase activities, whether they represent a stage in the formation of zymogen granules, and finally, whether the variation in microsomal enzymatic activities is due to a concomitant variation in the number of such intracisternal granules within the microsomal fraction.

To answer these questions, we carried out a number of experiments in which we compared the TAPase and RNase activities of microsomal and zymogen fractions, as well as the pellets of the zymogen and microsomal fractions, after refeeding. In the case of fed animals, the glands were collected 1 hour after feeding, a time at which we expected a high concentration of intracisternal granules. In addition we tried to obtain a preparation rich in intracisternal granules and with this intent we subfractionated, by differential centrifugation, microsomal fractions treated with low concentrations of Na deoxycholate. We found that pancreatic microsomes have a denser content (sometimes in the form of distinct intracisternal granules) and possess a higher total and specific TAPase activity when isolated from fed guinea pigs than when obtained from starved animals. The same applies for RNase activity. Heavy microsomal subfractions isolated 1 hour after feedings were found to contain intracisternal granules mixed with various microsomal detritus. The specific TAPase and RNase activities of these subfractions were higher than those of the parent microsomes, and as high or higher than the corresponding values for companion zymogen fractions.

The results strongly suggest the active participation of the microsomes in the synthesis of new digestive enzymes and indicate that the intracisternal granules, like the zymogen granules, are masses of segregated enzymes and enzyme precursors.

**Experimental**

**Cell Fractionation.**—As already shown by light microscope studies (4, 5) the acini of the pancreas are usually found in different functional stages. Complete synchronization is difficult if not impossible to achieve. Long fasting followed by feeding brings, however, a large part of the exocrine cell population into functional synchrony. For this reason the adult (~400 gm.) guinea pigs used in these experiments were starved for 48 hours, and then given a meal of cabbage. 1 hour after the beginning of refeeding, the glands were removed under ether anesthesia after being separated by dissection from most of the surrounding adipose and connective tissues. Glands from 2 to 4 animals were pooled, minced, and homogenized at 4°C. in cold 0.88 M sucrose solution, the final dilution of the homogenate being adjusted to 1:10 (weight to volume). The homogenate was fractionated either by the usual differential centrifugation or by centrifugation in a discontinuous density-gradient. In some experiments the zymogen and mitochondrial fractions were washed as previously described. All the details and qualifications pertaining to fractionation and washing procedures can be found in reference 1.

**Microsomal Subfractionation.**—The microsomes were subfractionated as follows: the top loosely packed layer of a microsome pellet derived from 1 gm. wet weight of tissue was removed by adding a few drops of sucrose solution, swirling, and discarding the ensuing suspension. The tightly packed pellet left behind was resuspended in 10 ml. 0.88 M sucrose. Enough 2 per cent Na deoxycholate (DOC) solution, pH 7.5 to 7.7, was added (with rapid mixing) to the microsomal suspension to bring the final DOC concentration to 0.1 per cent. The partially cleared preparation was then centrifuged for 30 minutes at 105,000 g. The resulting supernatant was decanted and the corresponding pellet resuspended in 0.88 M sucrose. The pellet (MS90; microsomal subfraction90) and the supernatant (SS90; supernatant subfraction90) were taken to represent respectively a heavy and light microsomal subfraction. In some experiments, an intermediary subfraction was obtained by centrifuging the light subfraction either for 60 minutes (MS60) or 120 minutes (MS120) at 105,000 g and separating the ensuing pellet and supernatant fluid.

The controls for all the fractions obtained from fed guinea pigs were represented by corresponding fractions isolated from animals starved for 48 hours and sacrificed without refeeding.

**Electron Microscopy.**—The pellets of the zymogen and microsomal fractions, as well as the pellets of microsomal subfractions, were fixed in toto in 2 per
as indicated in references 1 and 6.

Chemistry.—Protein N was determined as described in reference 6; proteolytic activity and ribonuclease activity as given in reference 1.

RESULTS

Morphology

Exocrine Cells.—The pancreatic exocrine cells of starving guinea pigs were distinguished by an endoplasmic reticulum, the cisternae of which showed minimal lumina and extensive preferential orientation (Fig. 1). Intracisternal granules were absent or only exceptionally present. The apical regions of the cells were heavily loaded with numerous zymogen granules, while the acinar lumina were narrow and appeared frequently occupied by an amorphous material similar in density to the content of the zymogen granules (Fig. 2). The finding is in agreement with the view that small discharges of zymogen occur continuously even during prolonged starvation (5, 7). By contrast, many of the exocrine cells of the guinea pigs killed 1 hour after feeding showed large accumulations of intracisternal granules in an endoplasmic reticulum characterized by distended cavities and little or no preferred orientation (Fig. 4). The number of zymogen granules in the apical region of the cells varied to a certain extent from acinus to acinus but was, in general, smaller than in starved animals. The acinar lumina were distended and frequently occupied by large, irregular, and ill defined masses of dense material, in all probability discharged zymogen (Fig. 3). Similar masses were found in the lumina of the pancreatic ducts (Fig. 5).

Microsome Fractions.—The microsome pellets obtained from starved animals consisted, as already described (3), of small (50 to 150 mμ), spherical vesicles limited by a thin, continuous membrane which bore small (~15 mμ) attached particles on its outer surface (Fig. 6). The microsomal content varied in density from one vesicle to another, being relatively dense in the small vesicles and light in the large ones, possibly on account of swelling and partial extraction. It is noteworthy that in general the content of the microsomal vesicles appeared denser than that of the endoplasmic reticulum in situ. The increase in density could be explained by water loss and possibly membrane contraction during the isolation procedure. The microsomal content was generally amorphous with formed bodies of the size and shape of the intracisternal granules only exceptionally encountered (Fig. 6, insert). The pellets contained as minor components a few smooth surfaced vesicles and a few free, dense granules of ~50 mμ diameter. Damaged mitochondria contaminated in small numbers the bottom layers of the pellets.

The microsome fractions isolated from animals killed 1 hour after feeding were of similar composition and their main component, the microsomal vesicles, showed comparable morphological features (Fig. 7). The only difference was represented by the higher density of their content which frequently approached or matched the density and the fine texture of the intracisternal granules seen in situ. Bodies fitting entirely the description of these granules, i.e. dense, well defined masses surrounded by a light halo, were also found more frequently within the microsomal vesicles isolated from fed animals. They were however, less numerous than expected from the situation encountered in situ. The discrepancy can be explained by assuming that the light halo around many intracisternal granules disappears through water loss and membrane contraction during the homogenization and fractionation of the pancreas.

Microsomal Subfractions.—The pellet of the heavy microsomal subfraction (MSω) was distinctly stratified with the bottom layer formed by a large number of dense bodies similar in size, shape, and density to the intracisternal granules (Fig. 8). Some of them appeared entirely free, others were partially or entirely surrounded by remnants of microsomal membranes still bearing a few attached particles (Figs. 11 and 12). On account of the features described, these dense bodies were identified as intracisternal granules partially or totally freed from their microsomal vesicles as a result of the dissolution of the microsomal membranes by deoxycholate. The middle layer contained, in addition to intracisternal granules, numerous microsomal vesicles damaged to a varied extent by DOC treatment (Fig. 9). Detached ribonucleoprotein particles mixed with a few damaged vesicles appeared in a thin upper layer (Figs. 10 and 13). Taken as a whole, the heavy microsomal subfraction appeared to represent a concentrate of intracisternal granules.

The intermediary subfraction MSω consisted of damaged microsomes, and remnants of disintegrated microsomes, primarily small (~15 mμ) ribonucleoprotein particles (Figs. 14 and 15). Intracisternal granules occurred infrequently in
The guinea pigs were fed, after a fast of 48 hours, and the glands removed and homogenized 1 hour after the beginning of the meal. Methods are given in the Experimental Section. The homogenate and fractions from 1 gm. wet weight pancreas were diluted to 100 ml. with 0.88 M sucrose solution. The enzymatic determinations tabulated below were carried through on appropriate aliquots of these diluted fractions. The zymogen and mitochondrial fractions were washed as described previously (1).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein N</th>
<th>RNase activity</th>
<th>Proteolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/gm $\dagger$</td>
<td>$\mu$g/mg protein N</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td>$\mu$g/gm</td>
<td>$\mu$g/mg protein N</td>
<td>Per cent</td>
</tr>
<tr>
<td>Homogenate</td>
<td>19.00</td>
<td>6200</td>
<td>324</td>
</tr>
<tr>
<td>Nuclear fraction</td>
<td>3.79</td>
<td>700</td>
<td>184</td>
</tr>
<tr>
<td>Zymogen fraction</td>
<td>2.79</td>
<td>1300</td>
<td>466</td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>1.27</td>
<td>480</td>
<td>377</td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>2.33</td>
<td>2000</td>
<td>860</td>
</tr>
<tr>
<td>Supernatant fraction</td>
<td>7.66</td>
<td>2050</td>
<td>269</td>
</tr>
<tr>
<td>Zymogen fraction (head and tail washed)</td>
<td>0.67</td>
<td>350</td>
<td>523</td>
</tr>
<tr>
<td>Mitochondrial fraction (head and tail washed)</td>
<td>0.65</td>
<td>150</td>
<td>231</td>
</tr>
<tr>
<td>All fractions incubated together</td>
<td>—</td>
<td>6700</td>
<td>—</td>
</tr>
<tr>
<td>All fractions minus supernatant incubated together</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Additive recovery, all fractions</td>
<td>17.80</td>
<td>6500</td>
<td>—</td>
</tr>
<tr>
<td>Additive recovery, all fractions minus supernatant</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$\dagger$ A, activity in the absence of trypsin in the preincubation medium.

$\dagger$ B, trypsin-activatable protease activity = (total proteolytic activity in the presence of trypsin in the preincubation medium) minus (A).

The RNase activity of the homogenate, higher than in previous experiments (1), was satisfactorily recovered in the cell fractions. Again as already shown (1), the washing of the zymogen fraction caused an increase, whereas the washing of the mitochondrial fractions resulted in a decrease, in the specific activities of the enzymes assayed.

An interesting difference, which concerns the enzymatic activities of the microsome fraction,
TABLE II

A Comparison of the Enzymatic Activities of the Zymogen Fraction with Those of the Microsomal Fraction and Subfractions Obtained from the Pancreas of Fed Guinea Pigs

The guinea pigs were fed, after a fast of 48 hours, and the glands removed and homogenized 1 hour after the beginning of the meal.

Methods are given in the Experimental Section. General notations are similar to those in Table I. Z, unwashed zymogen fraction obtained by differential centrifugation, except for Z in Experiment 4; M, microsomal fraction isolated by differential centrifugation, except for M in Experiment 4; MS90, pellet obtained by centrifuging for 30 minutes at 105,000 g a microsomal fraction (M) treated with 0.1 per cent deoxycholate, SS90, supernatant fluid of MS90; MS60, pellet obtained by centrifuging SS90 for 60 minutes at 105,000 g; SS60, supernatant fluid of MS60; MS120, pellet obtained by centrifuging SS90 for 120 minutes at 105,000 g; SS120, supernatant fluid of MS120.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Fraction</th>
<th>Protein N (mg./gm.)</th>
<th>TAPase activity*</th>
<th>RNase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\Delta E_{280}/\mu g.)</td>
<td>(\Delta E_{280}/\mu g.) protein N</td>
</tr>
<tr>
<td>1</td>
<td>Z</td>
<td>1.90</td>
<td>126</td>
<td>66.2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.76</td>
<td>102</td>
<td>58.0</td>
</tr>
<tr>
<td></td>
<td>MS90</td>
<td>0.28</td>
<td>35</td>
<td>125.0</td>
</tr>
<tr>
<td></td>
<td>SS90</td>
<td>1.40</td>
<td>75</td>
<td>53.6</td>
</tr>
<tr>
<td></td>
<td>Additive recovery</td>
<td>1.68</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Z</td>
<td>2.73</td>
<td>157</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.90</td>
<td>152</td>
<td>80.3</td>
</tr>
<tr>
<td></td>
<td>MS90</td>
<td>0.43</td>
<td>60</td>
<td>139.5</td>
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<tr>
<td></td>
<td>MS120</td>
<td>0.21</td>
<td>24</td>
<td>134.3</td>
</tr>
<tr>
<td></td>
<td>SS90</td>
<td>1.14</td>
<td>80</td>
<td>70.2</td>
</tr>
<tr>
<td></td>
<td>Additive recovery</td>
<td>1.78</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>3‡</td>
<td>Z</td>
<td>2.73</td>
<td>163</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2.07</td>
<td>121</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>MS90</td>
<td>0.41</td>
<td>41</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>MS120</td>
<td>0.17</td>
<td>17</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>SS120</td>
<td>1.40</td>
<td>72</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>Additive recovery</td>
<td>1.98</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>4§</td>
<td>Z</td>
<td>—</td>
<td>—</td>
<td>423.0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>—</td>
<td>—</td>
<td>179.0</td>
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<tr>
<td></td>
<td>MS90</td>
<td>—</td>
<td>—</td>
<td>303.0</td>
</tr>
<tr>
<td></td>
<td>SS90</td>
<td>—</td>
<td>—</td>
<td>142.0</td>
</tr>
</tbody>
</table>

* Same as B of Table I.
‡ In this experiment the guinea pigs were injected (for a different purpose) with a 0.5 ml. solution of radioactive leucine in isotonic saline into the heart 15 minutes after starting the feeding.
§ In this experiment the Z and M fractions were isolated by centrifugation in a discontinuous density-gradient as described previously (1). No attempts were made to obtain quantitative recovery.

Emerges when the experiments on fed guinea pigs recorded in this paper are compared with those previously carried through (1) on starved animals. In the case of fed guinea pigs, more than 30 per cent of the total TAPase and RNase activities of the whole homogenate (Table I) were recovered in the microsome fraction, a percentage equal to, or higher than, that found in any other cell fraction, zymogen fraction included. The specific TAPase activity of the microsomes (Tables I and II) was equal to that of unwashed zymogen fractions, whereas the specific RNase activity was higher.
Since the specific activities of unwashed zymogen fractions are lowered by mitochondrial contamination, the microsomes were also compared to more homogeneous zymogen fractions obtained by washing (Table I), or by separation in a discontinuous density-gradient (Table II, experiment 4). In such cases, a difference was usually found in favor of the purified zymogen preparations. The TAPase activity of the microsomes amounted to 40 to 80 per cent, whereas their specific RNase activity varied from 50 to 150 per cent of the corresponding value for purified zymogen fractions. As can be seen, the two enzymatic activities were not equally concentrated in the microsome fraction: the concentration being higher for RNase, than for TAPase activity. In the case of the starved animals previously investigated (1), only ~10 per cent of the proteolytic activity, and ~20 per cent of the RNase activity of the homogenates were recovered in the microsomal fractions. The specific TAPase activity of the microsomes reached 5 to 30 per cent of that of washed zymogen fractions, whereas their specific RNase activity amounted to 10 to 40 per cent of the corresponding zymogen value. The discrepancy between the concentration of TAPase and that of RNase activity in the microsomal fractions was less pronounced than in the case of fed animals. From a comparison of these figures, it follows that 1 hour after feeding the amounts and concentrations of TAPase and RNase activities rise noticeably in the microsome fraction, so much in fact that they approach or equal the total amounts found in unwashed zymogen fractions and the concentrations encountered in purified zymogen preparations.

The biochemical variations described could be correlated with simultaneous changes in the morphology of the microsomes and of the endoplasmic reticulum of the acinar cells of the pancreas. Indeed, 1 hour after feeding, at the time of the high enzymatic activity recorded, the cavities of the endoplasmic reticulum contained numerous intracisternal granules and the microsomal vesicles derived from the reticulum were distinguished by a content of noticeably high density and by the relatively frequent presence of typical intracisternal granules.

The results thus far obtained could be satisfactorily explained by assuming that the intracisternal granules are masses of segregated enzymes, comparable in this respect to the zymogen granules of the acinar cell. To test this assumption, an attempt was made to separate intracisternal granules from microsomes treated with 0.1 per cent deoxycholate. This reagent was used because it is known to "solubilize" the membranes of microsomal vesicles (3, 6), and a low concentration was chosen to prevent the disruption of the intracisternal granules. The DOC-treated microsomal suspensions were fractionated as described under Methods and the microsomal subfractions obtained were analyzed morphologically (see page 311) and biochemically (Table II). The results showed that the heavy microsomal subfraction (MS\textsubscript{H}), which morphologically appeared to be a concentrate of intracisternal granules, accounted for one-fourth to one-third of the enzymatic activities of the microsomes. Its specific TAPase activity was 70 to 100 per cent higher than that of the original microsomal fraction; its specific RNase activity was also higher by 70 to 80 per cent. The enzymes assayed were found to be 2 to 4 times more concentrated in the heavy microsomal subfraction than in the unwashed zymogen fractions isolated from the same glandular tissue (Table II, Experiments 1 to 3). Even when compared to more homogeneous zymogen fractions obtained by centrifugation in a discontinuous density-gradient (Table II, Experiment 4), the specific activities of MS\textsubscript{H} appeared remarkably high: they reached 70 to 80 per cent of the corresponding zymogen values.

In the intermediary microsomal subfractions (Table II), the concentration of proteolytic activity was lower than, or equal to, that found in the heavy subfraction; the concentration of RNase activity was, however, noticeably higher, especially in the case of MS\textsubscript{S}. As already mentioned, this last subfraction consisted almost entirely of detached RNP particles. In the light microsomal subfraction (supernatant of preceding preparations) both specific TAPase and RNase activities dropped below the level encountered in the original microsomal fractions.\footnote{We also attempted to investigate the structural connections of the microsomal enzymes by other means than membrane solubilization through DOC. To this intent, the microsomes were resuspended either in 0.88 M sucrose, or in distilled water, or in 0.15 M NaCl, or finally in 0.07 M phosphate buffer pH 7.6 and incubated for 30 minutes at 0°C. The suspensions were subsequently centrifuged for 2 hours at 105,000 g and the enzyme activity determined in the ensuing pellets and supernatant fluids. Losses from the pellets to the supernatant, presumably by the extraction and solubilization of microsomal contents, were negligible after}
TABLE III

A Comparison of the Enzymatic Activities of the Zymogen Fraction with Those of the Microsomal Fraction and Subfractions Isolated from the Pancreas of Starved Guinea Pigs

The glands were removed after the animals had been starved for 48 hours. Methods are given in the Experimental Section. The general notation is similar to that used in Tables I and II. No attempt to obtain quantitative recovery was made in Experiment 2.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Fraction or subfraction</th>
<th>Protein N (mg./gm.)</th>
<th>TAPase activity (mg./mg. protein N)</th>
<th>RNase activity (mg./mg. protein N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Z</td>
<td>2.80</td>
<td>196</td>
<td>1620</td>
</tr>
<tr>
<td></td>
<td>Z, tail-washed once</td>
<td>1.62</td>
<td>191</td>
<td>1390</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2.72</td>
<td>119</td>
<td>1180</td>
</tr>
<tr>
<td></td>
<td>MS$_{30}$</td>
<td>0.52</td>
<td>18</td>
<td>3712</td>
</tr>
<tr>
<td></td>
<td>SS$_{30}$</td>
<td>2.05</td>
<td>96</td>
<td>579</td>
</tr>
<tr>
<td>2</td>
<td>Z, head and tail washed</td>
<td></td>
<td>197.5</td>
<td>2655</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>32.7</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>MS$_{30}$</td>
<td></td>
<td>33.2</td>
<td>665</td>
</tr>
<tr>
<td></td>
<td>SS$_{30}$</td>
<td></td>
<td>28.4</td>
<td>540</td>
</tr>
</tbody>
</table>

As a control, similar microsomal subfractions were obtained from starved guinea pigs. Their analysis (Table III) showed that, in contrast with the situation found after feeding, the specific TAPase activity of the heavy subfraction remained equal to, or lower than, that of the original microsome fraction and only amounted to ~16 per cent of the corresponding value for a purified zymogen preparation (Table III, Experiment 2). The concentration of RNase activity also appeared low in the subfractions tested.

In addition to the main finding, which concerns an appreciable difference between the microsomal activities of fed and starved animals, some other points in our experiments deserve to be noticed. In the case of the intermediary subfraction MS$_{30}$ isolated from fed guinea pigs, we found relatively high concentrations of RNA and RNase activity in the same structural element: the attached RNP particles. This respect it could be recalled that difficulty in dissociating RNase activity from pancreatic RNA preparations has already been reported by Bacher and Allen (8). Although substrate and enzyme appeared to be carried by the same structure, no appreciable digestion of the RNA of the particles was detected in our experiments. It may be assumed that, as in certain incubation in 0.88 M sucrose or H$_2$O. Extraction of both enzymatic activities amounted, however, to ~50 per cent during incubation in saline (cf. 10) and reached 60 to 80 per cent in phosphate buffer.

viruses, the RNA was protected by an appropriate combination with the proteins of the particles.

DISCUSSION

The results of these experiments indicate clearly that 1 hour after feeding, the pancreatic microsomes of the guinea pig contain TAPase and RNase activity in higher concentrations and in relatively larger amounts than before feeding. The increase which follows food intake is so substantial that, at the time mentioned, the microsome fraction approaches in total and specific activity the crude zymogen fraction known to consist primarily of zymogen granules (1); i.e., granules in which enzyme and enzyme precursors (zymogens) are stored in concentrated form (9, 1) for release during digestion. The change in microsomal activity coincides in time with noticeable modifications in the morphology of the microsomes and of the endoplasmic reticulum of the acinar cells, the most important of these modifications being the appearance of intracisternal granules. A concentrate of such granules, isolated as a heavy microsomal subfraction from DOC-treated microsomes, proved to have higher specific activities for TAPase and RNase than the original microsomes. As far as the concentration of these enzymes is concerned, the heavy microsomal subfraction came close to purified zymogen preparations. On the basis of these findings it can be assumed that the intracisternal granules, like the zymogen granules, are
masses of segregated digestive enzymes and enzyme precursors. The negative results obtained with microsomal subfractions isolated from starved animals are in good agreement with this view. It follows that the increase in enzymatic activities recorded in the microsomal fraction 1 hour after feeding could be due, at least in part, to the presence of these intracisternal granules within the cavities of the endoplasmic reticulum and, after fractionation, within the cavities of the microsomal vesicles. A comparable but smaller increase in the activity of another digestive enzyme, namely amylase, was recently reported by Laird and Barton (10) in the pancreatic microsomes of pilocarpine-treated rats. These authors recovered ~25 per cent of the amylase activity of the rat pancreas in the microsome fraction. The situation appears to be different in the dog (9) and in the mouse pancreas (11) whose microsomes were found to have little amylolytic activity. The reason for this discrepancy is not known, but it might be due either to laboratory differences in fractionation technique, or to species differences in the rate of intracellular transport, or to differences in the nutritive state of the animals.

The biochemical and morphological events described could be explained by assuming that the old intracellular stock of enzymes is redistributed after feeding. Alternatively, the events could represent steps in a new secretory cycle by which the enzymatic stock of the cell is renewed. We favor the second alternative because the changes described occur at a time when a new secretory cycle is reasonably expected to begin (cf. reference 12). We recognize, however, that further evidence is needed to support this interpretation, and for this reason we have carried out a series of experiments in which the time course of the incorporation of isotopically labelled leucine into the proteins of the various cell fractions has been studied. The results, to be published in a subsequent paper, support the view that the events under consideration are part of a new secretory cycle.

If it is admitted that the increase in microsomal enzymatic activity is due to the synthesis of new enzymes by the microsomes, i.e. by the rough surfaced part of the endoplasmic reticulum, then a number of findings deserve further comment. It appears, for instance, that by the time of their segregation into intracisternal granules the newly synthesized enzymes are already in a finished state, at least as far as enzymatic activity for RNase and TAPases (trypsinogen and chymotrypsinogen) is concerned. On account of this finding, the intracisternal granules could be considered as precursors of the zymogen granules, but it should be pointed out that further work is required to establish their exact position in the secretory cycle and to clarify the series of transformations by which these assumed precursors become mature zymogen granules. The late events known to take place in the secretory cycle, such as the filling of relatively large vacuoles in the Golgi zone with dense material (13, 2) and the accumulation of zymogen granules in the apical region of the cell, appear to be secondary operations which represent the intracellular transport, packing, and storage of already finished products. It follows that the significant steps in the synthetic process precede the formation of intracisternal granules and could be reasonably connected with more stable structures in the endoplasmic reticulum (microsomes) such as the limiting membrane and the attached ribonucleoprotein particles. In this respect it should be noted that not only the heavy microsomal subfractions, which is a concentrate of intracisternal granules, but also the intermediary and light subfractions have relatively high proteolytic and RNase activities. The finding may be explained by assuming that only part of the enzymes produced during the cycle is segregated into distinct intracisternal granules, whereas the balance remains “in solution” in the fluid that fills the cavities of the endoplasmic reticulum. Upon disruption of the microsomal membranes, enzymes “in solution” are expected to find their way into the light microsomal subfraction. Moreover a number of intracisternal granules may disintegrate during DOC treatment and their dispersed enzymes may eventually separate in the same light subfraction or become adsorbed on other structural elements that survive the treatment (cf. below). What appears to be tenta-
tively more intriguing is the finding that even the intermediary subfractions (MS₆₀ and MS₁₀₀), which consist primarily of ribonucleoprotein particles mixed with relatively few microsomal vesicles, have high proteolytic activity and especially pronounced RNase activity. Further work is necessary to decide whether the particles and subjacent membrane are the loci of enzyme formation or whether such enzymatic activity as is found associated with these structures is due to preparatory artifacts, and represents relocation by enzyme adsorption (cf. reference 14).

The difference between the concentrations of RNase and TAPase activities in the microsomes and their various subfractions remains an unexplained finding. Particularly noteworthy is the high RNase activity in the subfractions rich in ribonucleoprotein particles. Here again further work will decide whether the finding is or is not representative of the situation in situ and whether the RNase is only a secretory product or is also a part of the cellular mechanism for protein synthesis.

A perusal of the results reported in this study and in a preceding paper (1), shows clearly that there is considerable variation in TAPase and especially in RNase activity from one animal to another. Such individual variations affect both the total amount and concentration of the activities assayed. A similar situation is apparently encountered in other small laboratory animals (15, 16). Our experience has shown, however, that under standardized physiological conditions the intracellular distribution of enzyme activity, as well as the ratios between the specific activities of the various cell fractions, varies much less from animal to animal. For this reason absolute values were considered in this study only when related to fractions derived from the same pancreas or the same pool of pancreatic tissue. Relative values were generally used in comparing results obtained in different experiments. Because of this situation, our data cannot contribute towards a solution of the controversy between those advocating (15, 17) and those denying (18, 19) the existence of a "steady state" for the digestive enzymes of the acinar cell. According to the steady state hypothesis any excretion of enzyme during the secretory cycle of the cell is compensated by synthesis of new protein. The simultaneous and, to a certain extent, complementary variations shown by the enzymatic activities assayed in the zymogen and microsomal fractions, would apparently favor this hypothesis. One may wonder, however, whether with the variability mentioned, this controversial question could be actually put to test in experiments on small animals.

BIBLIOGRAPHY

FIG. 1. Pancreatic exocrine cell. Specimen collected from a guinea pig starved for 48 hours.

The micrograph shows a relatively large field in the basal region of the cell with the cell membrane appearing at cm and two mitochondria at m₁ and m₂. The mitochondrion marked m₂ is in intimate contact with a lipid inclusion (l), an association frequently encountered in starving guinea pigs.

The numerous rough surfaced elements of the endoplasmic reticulum (rs) which fill this region of the cell body are characterized by relatively narrow cavities, light content (l), and preferred orientation. Their profiles appear disposed in parallel rows at more or less regular intervals. Note that groups of such rows are in turn oriented parallel to the surface of adjacent structures, e.g., the cell membrane in the lower half of the figure and the mitochondrion m₂ in the upper right quarter.

The cytoplasmic matrix is relatively dense and contains numerous, dense, apparently free particles (p). X 45,000.
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Fig. 2. Lumen of a pancreatic acinus. Specimen collected from a guinea pig starved for 48 hours.

The lumen marked $lm$ is completely filled by a dense homogeneous material against which the fine villi of the cell surface, sectioned longitudinally (long arrow) or transversely (short arrow), appear in sharp contrast. The lumen is surrounded by parts of three exocrine cells. Their apical cytoplasm is occupied by zymogen granules ($z$), mitochondria ($m$), elements of the endoplasmic reticulum with attached particles ($p_z$), and free particles which appear freely scattered in the cytoplasmic matrix. Note that the density and texture of the content of the lumen match those of the zymogen granules. The finding suggests the existence of a small, continuous discharge of zymogen from the acinar cells. $\times$ 40,000.

Fig. 3. Lumen of a pancreatic acinus. Guinea pig fed after a fasting of 48 hours. Specimen collected 1 hour after refeeding.

The large, apparently distended lumen appears surrounded by parts of two acinar cells and two centroacinar cells ($ca$) and is occupied by a light substance ($f$), supposedly fluid in vivo, in which are scattered irregular masses of dense material ($dz$). Note that the latter match in density the content of the zymogen granules ($z$) present in some of the surrounding acinar cells. The dense material in the lumen ($dz$) represents discharged and disintegrating zymogen. Note also that fine villi cover the free surface of both the acinar ($v_3$) and centroacinar ($v_2$) cells. $\times$ 40,000.
(Sickevitz and Palade: Cytochemistry of pancreas. II)
Fig. 4. Pancreatic exocrine cell. Specimen collected 1 hour after the refeeding of a guinea pig previously starved for 48 hours.

The micrograph shows part of the basal region of the cell with the cell membrane at cm, a few mitochondria at m, and the nucleus at n. The basal cytoplasm is occupied as usual by numerous elements of the endoplasmic reticulum bearing dense particles attached to their outer surface (rs). In contrast with the situation encountered in resting cells (see Fig. 1), however, the elements of the endoplasmic reticulum appear randomly disposed and their cavities are noticeably distended. Large, dense, intracisternal granules (g) can be seen within the light, supposedly fluid content of the distended cisternae. The intracisternal granules have a dense, apparently homogeneous texture. They are not bound by a proper membrane, but appear to have an outer, relatively thick shell of different density (arrows). The cytoplasmic matrix contains clusters of smooth surfaced, vesicular elements (ss) and numerous, apparently free, dense particles. × 45,000.
FIG. 5. Small pancreatic duct. Guinea pig fed after a 48 hour fast; specimen collected 1 hour after the beginning of the meal.

The lumen of the duct is filled with a light material (f), presumably fluid in vivo, and contains a large, irregular mass of a dense substance (ds) assumed to be discharged zymogen.

Parts of five epithelial cells surround the lumen. Their apical surfaces bear a few microvilli (v). Their cytoplasm contains numerous mitochondria (m), a lipid inclusion (l), relatively few elements of the endoplasmic reticulum (er), and clusters of small dense particles (p). Parts of the nuclei (n) of three cells are included in the field. Small adhesion plates (ap), or terminal bars, and a few interdigitations (id) can be seen along the cell boundaries. The outer surface of the duct is covered by a thin layer of dense material presumably a basement membrane (bm).

X 26,000.
(Siekevitz and Palade: Cytochemistry of pancreas. II)
Representative Field in a Pancreatic Microsomal Pellet.—Guinea pig starved for 48 hours.

The microsomes (m₁ to m₄) appear as vesicular bodies limited by a thin membrane (long arrow) which bears small dense particles (short arrow) attached to its outer surface. These morphological details indicate clearly that the microsomes are fragments of the rough surfaced part of the endoplasmic reticulum.

Morphologically the fraction appears relatively homogeneous, most of the apparent individual variation in size and appearance being due to sectioning. For instance m₁ corresponds to a median section through a microsomal vesicle, m₂ to a medial section, and m₃ to lateral or extremely lateral sections which afford a full faced view of the particle-dotted microsomal membranes.

Note that the content of the microsomes also varies from light (m₁) to dense (m₄) with a whole spectrum of intermediates (m₄). Note also that the content is homogeneous in some vesicles and finely granular or filamentous in others (m₃). In general the content of isolated microsomes is higher in density than the content of the endoplasmic reticulum in situ. × 43,000.

The insert shows a dense, relatively large, intracisternal granule (g) within a microsomal vesicle. Although the latter appears ruptured (arrow), the intracisternal granule retains its integrity. × 55,000.

Representative Field in a Pancreatic Microsomal Pellet.—Guinea pig fed after a 48 hour starvation period. Specimen collected 1 hour after feeding.

As in the case of the starved animals, the microsomes appear to be vesicles bounded by a thin membrane that bears small, dense particles attached to its outer surface. Most of the variation in size and appearance is due, as in Fig. 6, to sectioning.

The content of the microsomal vesicles, though variable in density, is generally higher than in starved animals. Microsomes with a light content (m₁₁) are less numerous and microsomes with a content of high density (m₁₂, m₁₃) are quite frequently encountered. Note that in the case of the latter the limiting membrane cannot be clearly visualized because the density of the content matches the density of the membrane. A dense body with a narrow light halo appears at m₁₄. It represents in all probability an intracisternal granule. × 43,000.
(Siekevitz and Palade: Cytochemistry of pancreas. II)
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Figs. 8 to 10 illustrate three different levels in the pellet of a heavy microsomal subfraction (MS30). The pellet was obtained by centrifuging at 105,000 g for 30 minutes a microsome suspension treated with 0.1 per cent deoxycholate.

Fig. 8. Bottom Layer of MS30.—At this level the preparation consists almost exclusively of relatively large granules (g) of high density and fine texture which, in size and general appearance, are identical with the intracisternal granules of the intact exocrine cell. Like the latter, they are not bound by a limiting membrane but appear to have a peripheral shell of slightly different density (arrows). Dense detritus (x) of unknown origin (dust? hemosiderin from macrophages?) contaminates the bottom layer. X 31,000.

Fig. 9. Middle Layer of MS30.—The middle layer mainly consists of microsomal vesicles (mv) of various sizes. Many of them bear dense particles attached to the outer surface of their limiting membrane (arrows), but the number of such particles is considerably smaller than in untreated microsomes (Fig. 7). Two microsomal vesicles contain typical intracisternal granules (g). A few apparently free particles (p) are scattered among the microsomes X 31,000.

Fig. 10. Upper Layer of MS30.—At this level the preparation consists almost exclusively of small dense particles (p), most of them in chains (ch) or clusters (c). A few small vesicles (mv) are scattered among the particles. X 31,000.

The bottom layer (Fig. 8) makes up one-third of the whole pellet. The next one-third is intermediate in appearance between Figs. 8 and 9. The following layer (Fig. 9) reaches almost to the top of the pellet and is covered only by a thin superficial layer of small particles (Fig. 10).
**Fig. 11. Limited Field in the Bottom Layer of MS30.**—The micrograph illustrates a situation occasionally encountered in the bottom layer of heavy microsomal subfractions.

Small dense particles (p), presumably RNP particles formerly attached to the surface of microsomal vesicles, appear scattered at the surface of intracisternal granules (g). In most cases no distinct microsomal membrane is visible although some suggestions of membrane remnants are encountered (arrows). \( \times 48,000 \).

**Fig. 12. Limited Field in the Middle Layer of MS30.**—In addition to many deformed and apparently distended vesicles of microsomal (m) or possible mitochondrial (m) origin, the field contains five intracisternal granules (g₁ to g₅). The first ones (g₁ and g₂) are completely free of microsomal remnants, whereas the last three are still partially (g₃, g₄) or entirely (g₅) surrounded by microsomal membranes which in the case of g₄ and g₅ still bear a few dense particles attached to their outer surface (arrow). Note the light halo (clearly visible in the case of g₅), that separates the granules from the investing membranes. \( \times 60,000 \).

Morphological details like the ones illustrated by Figs. 8, 9, 11, and 12, e.g. the existence of an outer shell, the association with small dense particles, and the occurrence of granules still invested by microsomal membranes bearing attached particles, indicate clearly that the dense relatively large granules of the heavy microsomal subfraction (MS30) are, at least in their majority, intracisternal granules released from the exocrine cells of the pancreas.

Another possible source of granular material might be represented by the dense granules (insulin?) of the islet cells of the pancreas. In their case, however, there is more variety in size and more heterogeneity in texture. In addition the granules of the islet cells are bound \textit{in situ} by smooth surfaced membranes. Their fate during tissue fractionation is unknown.

**Fig. 13. Limited Field in the Top Layer of MS30.**—The micrograph, which is an enlargement of part of Fig. 10, shows to advantage the relatively dense particles which constitute this layer. They are of more or less uniform size, measure \( \sim 150 \) \( \text{A} \) in diameter, and appear either isolated (p) or, more frequently, associated in chains (ch) or small clusters (c). \( \times 96,000 \).
(Siekevitz and Palade: Cytochemistry of pancreas. II)
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Figs. 14 and 15. **Intermediary Microsomal Subfraction MS60.**—The pellet is a duplicate of MS60 in Experiment 2, Table II, and was obtained by centrifuging the supernatant of MS30 for 60 minutes at 105,000 g.

**Fig. 14.** Bottom layer of MS60. The micrograph shows that this layer consists of microsomal vesicles (mv1 to mv3) which have lost all (mv1), or a large part (mv2) of their attached particles. Note that some of these vesicles still retain a content of noticeable density (mv3). Free, presumably detached particles are scattered amidst the microsomal vesicles.

**Fig. 15.** Upper layer of MS60. The upper and middle layers, which represent more than three-fourths of this pellet, consist entirely of small dense particles scattered in chains (arrows), or in small clusters (c).

**Figs. 16 and 17.** **Intermediary Microsomal Subfraction MS120.**—The subfraction was obtained by centrifuging the supernatant of MS10 for 120 minutes at 105,000 g. It is a companion pellet of MS120 in Experiment 3, Table II.

**Fig. 16.** Bottom layer of MS120. A few distended and extracted microsomal vesicles (mv) appear in this layer together with small clusters (c) of dense particles. Note that some of the microsomal vesicles still bear a few attached particles (arrow).

**Fig. 17.** Upper layer of MS120. Small dense particles disposed in chains (arrows) or in small clusters (c) constitute more than four-fifths of this pellet. In the middle layer they appear more tightly packed than in this micrograph. A fine, punctate contamination can be noticed in some parts (x) of this preparation. Figs. 14 to 17, X 70,000.