INTERMEDIATE CELLS OF THE PANCREAS

II. THE EFFECTS OF DIETARY SOYBEAN TRYPsin INHIBITOR ON ACINAR-β CELL STRUCTURE AND FUNCTION IN THE RAT

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SUMMARY

The well known hypertrophy of the rat exocrine pancreas caused by dietary soybean trypsin inhibitor (STI) has now been shown to be accompanied by an increased prominence of acinar-β intermediate cells and a reduction in the size of the islets of Langerhans. The acinar-β cells are situated in the exocrine tissue surrounding the islets where the acinar cells in STI-fed rats show a greater number of zymogen granules than in acinar cells elsewhere in the pancreas or in peri-islet acinar cells of control rats.

Consistent with the reduction in islet size in STI-fed rats is a lowered insulin content of the pancreas and a lowered insulin secretion following the intravenous administration of glucose. However, the release of insulin into the blood and pancreatic juice after intravenous administration of the hormone secretin is similar in both STI-fed and control rats.

The results of these morphological and functional studies are discussed in relation to the possible contribution of the acinar-β cell to the functional insulin pool.

INTRODUCTION

The occurrence of intermediate cells in the normal pancreas of several species has recently been investigated (Melmed, Benitez & Holt, 1972) and of the various types described, acinar-β cells, i.e. exocrine acinar cells containing β-granules in addition to zymogen granules† are of particular biological importance as their occurrence points to a possible insulin-producing potentiality in the exocrine pancreas. These acinar-β cells are found in areas adjacent to the islets at points of close contact between the acinar and islet cells ('acinar-β cell areas'). The structure and function of the acinar-β cell in the mammalian pancreas is of interest, not only as an example of a naturally occurring differentiated cell involved in the synthesis and storage of 2 distinct secretory products, but because a better understanding of this cell type may help determine whether it makes a significant contribution to the total functional body insulin pool.

Rats fed on a diet containing raw soybean meal with a naturally high trypsin inhibitor content have been shown to develop hypertrophy of the acinar cells of the exocrine pancreas (Booth, Robbins, Ribelin & De Eds, 1960; Rackis, 1965; Konijn

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† The nomenclature used to describe intermediate cells in this and the following article is that suggested in the first paper of this series (Melmed et al. 1972).
As acinar-β cells are topographically part of the exocrine pancreas, it was thought that their response to trypsin inhibitor might be similar to that of the acinar cell and thus provide an experimental system of possible use in assessing their functional potentiality for insulin production. Although difficult to quantitate accurately, we are now able to report that acinar-β cells are more prominent in rats fed raw soybean. Use has been made of this finding to extend the ultrastructural observations reported earlier (Melmed et al. 1972) and to study pancreatic insulin content and release in rats fed on a diet containing soybean trypsin inhibitor (STI).

MATERIALS AND METHODS

Animals and diet

All studies were performed on adult male albino Wistar rats (Courtauld Institute inbred strain) fed on a high STI-containing diet consisting of equal weights of raw soybean flour ('Diasoy') and standard Oxoid 41B diet (W. Lillico & Son, Wonham Mill, Betchworth, Surrey, U.K.). All control observations were made on rats fed a similar diet, but containing heated soybean flour ('Soyolk') in which the trypsin inhibitor is inactivated. Both Soyolk and Diasoy were obtained from Soya Foods Ltd., 30 Mincing Lane, London, E.C.3, U.K.

In all experiments the rats were put on the diet at 90-g body weight and fed ad libitum from 8 to 10 weeks before study.

Light microscopy

Light-microscopic observations were made on cyanine-dye-stained (S. J. Holt &; C. J. Benitez, in preparation) 0.25-0.5 μm sections of Epon-embedded tail of the pancreas (see below).

The incidence of acinar-β cell areas was quantitated in 4 control and 5 STI-fed rats by taking consecutive sections through each block of pancreas and examining a random section of each islet for areas of close contact between exocrine and islet tissue where they are normally situated (Melmed et al. 1972). In addition, islet size was determined with a calibrated eye-piece graticule. The mean cross-sectional area of islets was determined in 5 control and 6 STI-fed rats and was calculated from their mean diameter. This was taken as the mean of the longest and shortest diameter measured for each islet in a section. This was done to examine the relationship between islet size and insulin content in the pancreases of the 2 groups of rats.

Electron microscopy

All materials for electron microscopy were obtained from TAAB Laboratories, 52 Kidmore End Road, Emmer Green, Reading, U.K.

Small pieces of the tail of the pancreas were fixed overnight at room temperature in a mixture of 2% glutaraldehyde and 3% formaldehyde (after Karnovsky, 1965) buffered at pH 7.2 with 67 mM cacodylate. They were briefly rinsed in the same buffer and then postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.2, after which they were dehydrated and embedded in Epon 812 by standard procedures (Luft, 1961).

Acinar-β cell areas were first located by light microscopy as described above, after which ultrathin sections were cut from them and examined in a Philips EM 200 electron microscope at 60 kV.

Isolation of islets

An attempt was made to separate the islets in control and STI-fed rats using the collagenase technique of Lacy & Kostianovsky (1967). In the STI-fed rats this proved unusually difficult because the density and microscopic appearance of the islets were similar to that of the hypertrophied exocrine pancreas.
Intravenous glucose tolerance tests

The general experimental procedure was as follows: Under light Fluothane anaesthesia, catheters were inserted through a midline neck incision into both the right carotid artery and left external jugular vein of each of 7 control and 7 STI-fed rats. The free ends of the catheters were then passed subcutaneously, with the help of a trocar, to emerge through the skin of the upper abdomen. After careful suture of the neck incision the catheters were fixed with a stay-suture in the skin at the point of exit. This prevented the rats from biting through the catheters. The rats were put in restraining cages with access to water only overnight, the catheters being flushed by a constant infusion of sterile 0.9% saline containing heparin (5 units/ml) and ampicillin (1 mg/ml) at a rate of approximately 1 ml/12 h. After the overnight fast, blood samples (ca. 0.2 ml each) were collected from the carotid cannula into small heparinized polypropylene tubes and were immediately centrifuged. The plasma was removed and stored at −20 °C pending insulin and glucose assay (see below).

In detail, 2 fasting samples were collected with a 10-min interval between them, the second being immediately before the intravenous injection of glucose (0.5 g/kg body weight), after which blood samples were taken at timed intervals up to 30 min. After each sample the arterial catheter was flushed with saline and 0.2 ml of saline was injected through the venous catheter. The rats remained conscious and were mostly undisturbed throughout the procedure. They were then killed by an intravenous injection of Nembutal.

Extraction of insulin from whole pancreas

Twelve control and 10 STI-fed rats were killed by cervical dislocation and the whole pancreas was removed, weighed and immediately homogenized in 80% ethanol containing 16% concentrated hydrochloric acid (5 ml/g of pancreas) (Scott & Fisher, 1938) with the use of a homogenizer fitted with a Teflon pestle tipped with a cutting blade (‘Disintegrinder’, Kontes Glass Co., Vineland, New Jersey 08360, U.S.A.). A further similar volume of ethanol/acid was used to rinse the homogenizer and was added to the homogenate. The mixture was kept overnight at 4 °C after which it was centrifuged at 6000 g for 15 min, the extract decanted and the precipitate resuspended in ethanol/acid (to give a 20% (w/v) homogenate, based on the original weight of the pancreas) for a further 3 h. The mixture was centrifuged and the supernatant kept separate from the first. An aliquot of each extract was diluted in phosphate buffer, pH 7.4, containing 0.3% crystalline bovine serum albumin for subsequent immunoassay (Albano, Ekins, Maritz & Turner, 1972). The 2 extracts from each pancreas were assayed separately, the final insulin content of the organ being the sum of the insulin contents of the 2 extracts. This procedure allowed the efficiency of the extraction procedure to be checked for each pancreas.

Separation of proinsulin

The content of proinsulin was determined in 5 control and 5 STI-fed rats using a similar extraction procedure as described above. Proinsulin was separated from insulin on a Biogel P 30 (100–200 mesh) column (Melani, Rubenstein & Steiner, 1970) and immunoassayable insulin in the effluent measured with Burroughs Wellcome anti-insulin serum MR 41.

Extraction of insulin from pancreatic juice

The insulin content of the pancreatic juice was determined in 6 control and 4 STI-fed rats. Under light Fluothane anaesthesia, the pancreatic duct was cannulated at its entry into the duodenal wall. The duct was ligated in the region of the porta hepatis to prevent continuous contamination of the pancreatic juice with bile, which in some species, has been shown to contain insulin (Daniel & Henderson, 1970; Lopez-Quijada & Goni, 1967). After the catheter had been completely cleared of bile-stained pancreatic juice, a free flow of juice was established by 2 consecutive injections of secretin and 3 of secretin plus pancreozymin, each injection being given at 10-min intervals, between which the total output of secreted juice was collected. The dose of both hormones (Boots Pure Drug Company Ltd., Nottingham, U.K.) was 0.1 unit/100 g body weight. The pancreatic juice was collected in polypropylene tubes containing 300 μl of
the ethanol/acid mixture (see above) and the amount secreted was determined by weighing. The tubes were centrifuged as before and the supernatants were brought to a pH reading of 5.6 by addition of 1 N sodium hydroxide and then 1.5 vol. of ethanol and 2.5 vol. of ether were added to the extract. After standing overnight at 4 °C, the tubes were centrifuged, the supernatant discarded and each insulin-containing pellet dissolved in a solution containing 100 µl of the protease inhibitor Trasylol (10000 units/ml, Bayer, Germany) and 500 µl of phosphate buffer, pH 7.4 (Albano et al. 1972), for subsequent immunoassay (see below).

Plasma insulin levels after intravenous secretin and secretin/pancreozymin injections

Eight control and 5 STI-fed rats with arterial and venous catheters were placed in restraining cages with access to water only (see above). After an overnight fast the rats were given an injection of secretin (0.1 unit/100 g body weight), 2 blood samples being collected before the injection and others at timed intervals up to 20 min afterwards. A combined secretin/pancreozymin injection (both at 0.1 unit/100 g body weight) was administered immediately after the 20-min blood sample and further samples were taken at 1, 2, 3 and 5 min afterwards. The insulin content was determined for all blood samples.

Insulin and glucose assays

Insulin was assayed at suitable dilution against a rat insulin standard (Novo R 169), using Burroughs Wellcome MR 41 anti-insulin serum, by the radio-immunoassay technique of Albano et al. (1972). The sensitivity of the technique permits assay of insulin in 10-µl samples of plasma.

In the case of the pancreatic tissue and juice extracts, ethanol/acid precipitation of digestive enzymes and the subsequent addition of Trasylol to the incubation solution eliminated the possibility of errors resulting from proteolytic activity (Pruitt, Boshell & Kreisberg, 1966). In addition, stepwise dilution of the pancreatic juice extract prepared as above, and the use of anti-insulin antisera from 2 different guinea-pigs, established that the reactive substance behaved in a manner immunologically indistinguishable from insulin (Fig. 1), for the presence of interfering substances is likely to cause a substantial deviation from linearity.

Plasma glucose was assayed by a glucose oxidase method adapted for use on the Technicon Autoanalyser (Marks & Lloyd, 1963).

RESULTS

The effect of a high STI-containing diet on the exocrine pancreas

Light-microscopic observations. In rats on a high STI-containing diet, the acini of the exocrine pancreas are larger than in the normal pancreas (Beswick et al. 1971) and have many more zymogen granules. It has now been observed that the 'zymogen-halo' effect occurring in the normal pancreas (Ferner, 1958; Kramer & Tan, 1968), due to greater numbers of zymogen granules around the islets, is considerably enhanced by this diet. The difference in the numbers of zymogen granules in some peri-islet exocrine tissue of an STI-fed rat, and in areas further removed from the islets is shown in Figs. 5 and 6.

The results of the survey to determine the relative incidence of acinar-β intermediate cell areas in control and STI-fed rats are given in Table 1 and show that although the incidence of acinar-β cell areas is similar in both groups, the acinar-β cells tend to be more prominent in the STI-fed rats (Fig. 7) than in control rats (Fig. 8) due to the presence of more endocrine granules in the former and the fact that the acinar-β cells occupy more extensive areas than in the control rats. An addi-
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Fig. 1. The effect of dilution on the immunoassay of insulin in pancreatic juice extract utilizing antibodies to insulin from 2 different guinea-pigs (○, ×).

Table 1. Acinar-β cell incidence in the pancreas of control and STI-fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. examined</th>
<th>Islets with acinar-β cell areas</th>
<th>Prominence of acinar-β cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rats Islets</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4 35</td>
<td>7 20</td>
<td>Cells difficult to see by light microscopy.</td>
</tr>
<tr>
<td>STI-fed</td>
<td>5 39</td>
<td>9 23</td>
<td>(i) Acinar-β cells more prominent. (ii) Areas more extensive.</td>
</tr>
</tbody>
</table>

Additional observation was the more frequent presence of zymogen-like granules in islet β cells (β-acinar cells) in the STI-fed rats compared to those of the control group.

Ultrastructural observations. In STI-fed rats the acinar-β cells were similar to those described in normal animals (Herman, Sato & Fitzgerald, 1964; Melmed et al. 1972), such as that illustrated in Fig. 9. However, as revealed by light microscopy, they have more zymogen and β-granules than in acinar-β cells of control pancreases. Moreover, the unusual papillary type of rough-surfaced endoplasmic reticulum in these cells (Melmed et al. 1972) was particularly prominent in the STI-fed rats.

Of particular interest was the presence in the same Golgi region of an acinar-β cell from an STI-fed rat of both condensing vacuoles and β-granules (Figs. 10, 11).

There appeared to be no increase in the numbers or prominence of other acinar intermediate cell types in the STI-fed rats.

The effects of a high STI-containing diet on the endocrine pancreas

Comparison of islet size. The data comparing the mean islet cross-sectional area in control and STI-fed rats are presented in Table 2 and show that the STI-fed rats have a highly significant reduction in islet area compared to those in the control group. This observation was confirmed by examination of isolated islets, but because of the considerable difficulty in separating the islets from exocrine tissue in STI-fed rats, further quantitation was not feasible.
Table 2. Comparison of islet size in control and STI-fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>No. of islets examined</th>
<th>Mean cross-sectional area of islets, /μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>70</td>
<td>4900 ± 5508</td>
</tr>
<tr>
<td>STI-fed</td>
<td>6</td>
<td>86</td>
<td>2900 ± 3500 (P &lt; 0.001)</td>
</tr>
</tbody>
</table>

The mean cross-sectional area is given together with the standard deviation. \(P\) is the probability level of significance by Student's \(t\) test.

Table 3. Body and pancreas weight and insulin content in control and STI-fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Mean weight, g</th>
<th>Insulin content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body Pancreas</td>
<td>Units/pancreas</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>210 ± 21 0.86 ± 0.17</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>STI-fed</td>
<td>10</td>
<td>160 ± 22 1.07 ± 0.14</td>
<td>1.88 ± 0.4</td>
</tr>
</tbody>
</table>

\(P < 0.001\) \(P < 0.01\) \(P < 0.001\) \(P < 0.005\)

The mean values are given together with the standard deviations.

Table 4. Mean body and pancreas weights of rats used in the intravenous glucose tolerance tests

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Mean weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body Pancreas</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>193 ± 30 0.66 ± 0.09</td>
</tr>
<tr>
<td>STI-fed</td>
<td>7</td>
<td>176 ± 15 1.01 ± 0.12</td>
</tr>
</tbody>
</table>

\(P > 0.05\) \(P < 0.001\)

Mean values are given together with the standard deviations.

Insulin content of the pancreas. Table 3 summarizes data for the control and STI-fed groups of rats and records the insulin content of their pancreases. The findings of reduced body weight and considerably increased pancreas size in the STI-fed rats is consistent with the previously reported effects of STI in the rat (Booth et al. 1960; Rackis, 1965). However, it can be seen that the insulin content of the STI-fed rat pancreas is significantly reduced.

Pancreas proinsulin content. The proportion of proinsulin ('Big' insulin) immunoassayable in the pancreatic extract was 1.3 ± 0.55% (mean ± S.D.) of the total insulin in the control group and 1.9 ± 0.75% in the STI-fed group \(P < 0.05\).

Intravenous glucose tolerance tests. The mean body and pancreas weights of the control and STI-fed rats used for these tests are given in Table 4. This shows that the
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Reduction in the mean body weight of the STI-fed rats is not statistically significant. However, these rats do have a significantly enlarged pancreas.

The plasma glucose and insulin response in both groups to the body weight related glucose load is shown in Figs. 2 and 3. The most striking difference between the groups is the significantly reduced fasting insulin levels and insulin response in the STI-fed rats (Table 5). This seems to be associated with an enhanced glucose tolerance, although it is only at the 15-min mark that this attains statistical significance.

Table 5. Statistical analysis of plasma insulin and glucose levels in control and STI-fed rats (see Figs. 2 and 3)

<table>
<thead>
<tr>
<th>Sample time, min</th>
<th>P value</th>
<th>Insulin</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (fasting)</td>
<td>&lt;0.005</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>2</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>3</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.01</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>15</td>
<td>&lt;0.005</td>
<td>&lt;0.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>20</td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>30</td>
<td>&lt;0.02</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S., not significant.

Figs. 2, 3. Plasma glucose and insulin responses to a standard intravenous glucose load in control (●——●) and STI-fed rats (○——○). Each value represents the mean ± S.E.M. for 7 rats in each group.
Fig. 4. Plasma insulin response to intravenous secretin (at A) and secretin/pancreozymin (B) injections in 8 control (●) and 5 STI-fed (○) rats. Mean values ± S.E.M. are shown.

Table 6. Secretion of insulin in the pancreatic juice following secretin and secretin/pancreozymin injections

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Volume and microunits of insulin secreted/10-min collection following injection of secretin and secretin/pancreozymin injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Secretin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sample no.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>Vol., µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin, units</td>
</tr>
<tr>
<td>STI-fed</td>
<td>4</td>
<td>Vol., µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin, units</td>
</tr>
</tbody>
</table>

Mean values are given together with the standard deviations. (*P < 0.02) (*P < 0.01)

Plasma and pancreatic juice insulin levels after secretin and secretin/pancreozymin injections

The release of insulin into the plasma and the pancreatic juice after the successive secretin and secretin/pancreozymin injections is shown respectively in Fig. 4 and Table 6. In contrast to the significantly reduced plasma insulin response to glucose stimulation in STI-fed rats (Fig. 3), the rates of release of insulin into the blood and pancreatic juice after secretin and secretin/pancreozymin injection are similar in
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both control and STI-fed rats. However, the volume of pancreatic juice secreted in the STI-fed animals was greater than in controls, particularly in later samples (Table 6).

DISCUSSION

Factors influencing acinar-β cell function and the effects of dietary STI

Prominent acinar-β cells have previously been described in various species almost exclusively in situations of relative or absolute insulin deficiency, such as after chronic glucose infusions (Woerner, 1938), partial destruction of islet tissue with alloxan (Johnson, 1950; Hughes, 1956; House, 1958), partial pancreatectomy (Marx, Schmidt, Herrmann & Gobena, 1970) and in certain of the hereditary diabetic syndromes in mice (Picket et al. 1967; Shino & Iwatsuka, 1970). In such cases, the observed prominence of acinar-β cells presumably reflects their potentiated function in response to a greater insulin need and suggests that the endocrine function of the acinar-β cell is controlled principally by the normal homeostatic factors that regulate β-cell function. Thus, in raw soybean fed rats, with reduced islet size, lowered pancreatic insulin and proinsulin content and reduced insulin requirement, it might be expected that acinar-β cells would be correspondingly less prominent. However, their conspicuous presence in the considerably hypertrophied pancreases of STI-fed rats suggests that the endocrine component of the intermediate cell, like the exocrine, is stimulated by the dietary STI.

The presence of insulin in pancreatic juice, as indicated by our observation and that of others (Satake et al. 1972) is most simply explained as being due to secretion of the endocrine granules from the apex of the intermediate cell where they occur mixed with zymogen granules (Melmed et al. 1972). The fact that similar levels of insulin were found in the pancreatic juice of both STI-fed and control rats, in spite of reduced quantities of insulin in the pancreas and the diminished insulin response to glucose injection, is compatible with a relatively greater output of insulin from the acinar-β cells, an observation consistent with their greater prominence in STI-fed rats.

Selective packaging of the different secretory granules in the acinar-β cell

The evidence presented here and elsewhere (Melmed et al. 1972; Melmed, Benitez & Holt, 1973) provides strong evidence that the endocrine-like granule of the acinar-β cell is indistinguishable, by several criteria, from the insulin-containing granule of the β-cell of the pancreatic islet. This raises the question as to how the 2 granule types in the intermediate cell are separately packaged. This is partially answered by the observation that β-granules are present in sacules of a Golgi complex, other areas of which appear to be involved in the production of condensing vacuoles. This segregation is consistent with the existence of a functional polarity in the Golgi complex of the acinar-β cell analogous to that observed by Bainton & Farquar (1966) in the packaging of the different granule types of the polymorphonuclear leucocyte.
The physiological role of the acinar-β cell

It has been demonstrated that in contrast to its effects on slices of normal pancreatic tissue, secretin fails to elicit insulin release from isolated islets or from slices of pancreas in which exocrine atrophy has been induced, although glucose is effective in all cases (Guidoux-Grassi & Felber, 1968; Vannotti et al. 1969; Hinz et al. 1971; Goberna et al. 1971; Raptis et al. 1971). To account for this dependence of the insulin-releasing action of secretin on an intact exocrine pancreas, these authors postulated some unknown influence of the exocrine tissue on islet cell function. However, the existence of a population of insulin containing acinar-β cells in the exocrine pancreas, selectively responsive to secretin, could provide an explanation for the action of this hormone on the intact pancreas. The observed reduction in glucose-mediated insulin release in STI-fed rats coupled with the reduced islet size suggests a reduced contribution of insulin from the islets. Consequently, the observed normal stimulation of insulin release by secretin in STI-fed rats, in which acinar-β cells are unusually prominent, favours the possibility that these cells are able to make a contribution to the functional insulin pool. That this contribution may be quite considerable is supported by the observation that the rapid recovery of the guinea-pig from alloxan diabetes is associated with the so-called 'transformation' of numerous acinar cells, on the periphery of islets, into cells with the light-microscope characteristics of acinar-β cells: it is postulated that this mechanism accounts for the alloxan resistance of this species (Johnson, 1950). In addition, the observed prominence of acinar-β cells under conditions of relative or absolute insulin deficiency in various species (see above) suggests that these cells may contribute to the functional hormone pool. Although the extent of their contribution has not yet been determined it seems probable that in certain species, the acinar-β cell system may represent a reserve capacity for insulin production which is only mobilized in time of need.

We thank Mrs C. J. Benitez, Mrs M. Jordan and Miss B. Schneeloch for valuable assistance and we are grateful to Dr J. D. N. Nabarro for his interest and encouragement. The financial support of the Wellcome Trust (R.N.M.) and the British Diabetes Association (R.C.T.) is gratefully acknowledged, and the London University Central Research Fund is thanked for an equipment grant.

REFERENCES


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Figs. 5–8. Light micrographs of cyanine dye-stained thin sections of Epon-embedded rat pancreas.

Fig. 5. STI-fed rat. This field shows the distribution of zymogen granules in the acini of the exocrine pancreas in an area removed from islet tissue. × 720.

Fig. 6. An islet (i) and adjacent exocrine cells from the same pancreas as in Fig. 5, showing the increased numbers of zymogen granules in this region. The area enclosed in the rectangle contains acinar-β cells and is shown at higher magnification in Fig. 7. × 720.

Fig. 7. Two prominent acinar-β cells (arrows) are seen in exocrine tissue adjacent to islet cells (i) on the left. Also note the unusually large numbers of zymogen granules in the acinar cells on either side of the upper acinar-β cell. × 2500 (oil immersion).
Figs. 5–7. For legend see p. 291.
Figs. 8 and 9. For legend see p. 294.
Fig. 8. Control rat. This shows an area of intimate contact between exocrine and endocrine cells. The enclosed area contains part of the cytoplasm of several cells in the exocrine pancreas immediately adjacent to the islets. Although these are acinar-β cells it requires electron microscopy to identify them (see Fig. 9). ×1200 (oil immersion).

Fig. 9. Electron micrograph of an ultrathin section close to the enclosed area of the semithin section used for Fig. 8. The presence of β-granules (b) intermingled with zymogen granules (z) identifies these cells as intermediate cells. The papillary rough-surfaced endoplasmic reticulum (p) is frequently seen in such acinar-β cells. ×10000.

Figs. 10, 11. Serial sections of part of an acinar-β cell in an STI-fed rat showing a Golgi complex and associated secretory granules. In addition to the presence of condensing vacuoles (cv) and zymogen granules (z), the fields also contain a mature β-granule (b) with its characteristic ’umbilical’ connexion to its membranous sac and another (b’) which appears to be forming from a distended Golgi saccule (s). Fig. 10, ×28000; Fig. 11, ×32000.
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