Physicochemical Characteristics of Insulin Secretion Granules

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β -Granules were prepared from micro-dissected pancreatic islets of obese-hyperglycaemic mice. This fraction contained 60% of the insulin, 30% of the cytochrome oxidase, 16% of the acid phosphatase activity and 20% of the protein present in whole islets. The isolated granules retained a heavy metal during fractionation. Optimum conditions for granule stability were low ionic strength and pH 6, the granules being unexpectedly fragile at pH 7.4. The stability of the granules was unaffected by sucrose in the concentration range 50–320 mM, but 1% (w/v) sodium deoxycholate released all insulin. A solubilizing effect was also noted with ATP and citrate. Spinning through 1.6 M-sucrose yielded a further purification in relation to mitochondria and acid-phosphatase-carrying particles but virtually no purification in relation to protein. Electron microscopy revealed that the major contaminants were rough-surfaced vesicles and membranes. A separation of granules from acid phosphatase was achieved by phase distribution in polyethylene glycol and dextran. The location of the enzyme to the interphase was so pronounced in systems buffered with lithium phosphate that the technique may be used for future purification of acid-phosphatase-carrying particles from the β -cells.

A major part of the insulin content of the pancreatic β -cells is normally stored in cytoplasmic granules surrounded by smooth membranes. Information about the biochemical characteristics of these granules can therefore be expected to contribute to our understanding of insulin release. In the present study, micro-dissected islets containing more than 90% of β -cells (Hellman, 1965) were subjected to various fractionation procedures. This approach made possible systematic analyses of the granule stability under various conditions *in vitro*.

MATERIALS AND METHODS

Materials. About 100 5–6-month-old obese-hyperglycaemic mice (gene symbol *ob ob*; Ingalls, Dickie & Snell, 1950; Hellman, 1965) of both sexes were starved overnight and killed by decapitation. After rapid excision of the pancreas, 30–60 islets were isolated by micro-dissection (Hellerström, 1964) in a solution of 320 mM-sucrose adjusted to pH 6.0. The islets were pooled and homogenized by means of a Perspex micro-homogenizer in 100 μ l. of a solution containing 304 mM-sucrose and 8 mM-potassium phosphate or -tris-HCl buffer, pH 6.0. All preparative handling was performed at about 3°.

Unless otherwise stated, the reagents were of analytical grade. Three-times-distilled water was used throughout the experiments.

Differential centrifugation. The routine preparation of a partially purified granule fraction was achieved by

differential centrifugation in a Sorvall refrigerated centrifuge (RC2-B). The islet homogenates were spun in small polythene micro-tubes (height 45 mm.; maximal capacity 400 μ l.; Milian Instruments, Geneva, Switzerland), which were placed in Perspex inserts shaped to fit the ordinary rotor SS-34. The given gravitational forces refer to the middle of these micro-tubes.

Debris, whole cells and nuclei were removed by two repeated centrifugations at 600g for 10 min. A granule-rich fraction was then obtained by centrifugation at 6000g for 10 min. After a washing in 320 mM-sucrose adjusted to pH 6.0, the fraction was once more sedimented at 6000g for 10 min. When used for analyses of granule stability (see below) the sediment was suspended in 500 μ l. of 320 mM-sucrose, pH 6.0.

Centrifugation through dense sucrose. Centrifugation through dense sucrose was tested as a means for further purification of granules. The crude fraction twice sedimented at 6000g (see above) was suspended in 100 μ l. of a solution containing 304 mM-sucrose and 8 mM-potassium phosphate buffer, pH 6.0, and subsequently layered on to 300 μ l. of 1.6 M-sucrose, pH 6.0, in a polythene micro-tube. Centrifugation was performed at 100000g in a Spinco L50 ultracentrifuge equipped with Perspex tube holders fitting the rotor SW 39. After 90 min. the supernatant was discarded and the pellet resuspended either for repeated sedimentation through 1.6 M-sucrose or for direct chemical or electron-microscopical analyses.

Phase distribution in polyethylene glycol and dextran. The crude fraction (6000g sediment, see above) was thoroughly suspended in 390 μ l. of an aqueous polymer system (Albertsson, 1960) containing 4.0% (w/w) polyethylene

glycol (Carbowax 6000; Union Carbide, New York, N.Y., U.S.A.), 5.0% (w/w) Dextran 500 (lot no. To 8689; Pharmacia Fine Chemicals, Uppsala, Sweden) and 8–10 mm-potassium or -lithium phosphate buffer, pH 6.0. In some cases 10 mM-LiCl was also included in the system. Phase separation was performed at 3° in horizontally oriented polythene micro-tubes. After complete settling, the tube was gently raised and a known volume of the top and bottom phases carefully removed and transferred to other tubes. These fractions, as well as the residual fraction containing the interphase, were made up to 400 μ l. with 304 mM-sucrose and 8 mM-potassium or -lithium phosphate buffer, pH 6.0. After centrifugation at 40 000g for 30 min. (Spinco L50 ultracentrifuge), the upper and lower halves (200 μ l. each) of the tube contents were collected for further chemical or electron-microscopical analyses. The distribution of investigated activities to the top, bottom and intermediate phases of the polymer system was calculated and expressed as percentages of the gross recovery.

Insulin assay. For the determination of insulin activity in subcellular fractions, 150 μ l. of the sample was carefully transferred to a 15 ml. test tube containing 150 μ l. of 5% (w/v) bovine serum albumin (fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) dissolved in distilled water. Insulin was extracted at 3° by the addition of 0.72 ml. of acid alcohol [1.42% (v/v) H_3PO_4 in ethanol]. After 30 min. insulin and albumin were precipitated by the addition of 5.75 ml. of acetone. The precipitate was collected by centrifugation, washed with 2 ml. of acetone and sedimented once again. The supernatant was carefully poured off and the precipitate left to dry at room temperature for 2–12 hr. The precipitate was subsequently dissolved in 10.0 ml. of iso-osmotic phosphate buffer and, after appropriate dilution, assayed by the radioimmunological procedure of Hales & Randle (1963), with crystalline mouse insulin as standard and commercially available reagents (The Radiochemical Centre, Amersham, Bucks.). To evaluate the efficiency of the employed extraction procedure, crystalline ox insulin (Vitrum, Stockholm, Sweden) was dissolved in 30 mM-HCl and then diluted 100-fold with a solution containing 40 mM-tris-HCl buffer, pH 6.0 or 7.4, and 240 mM-sucrose. When 150 μ l., containing 7.5 μ g. of insulin, was used to replace the unknown sample, approx. 85% of the insulin was recovered after extraction with acid alcohol and precipitation with acetone.

Enzyme and protein assays. For the assay of acid phosphatase activity hydrolysis of *p*-nitrophenyl phosphate (Hopkin and Williams Ltd., Chadwell Heath, Essex) was recorded in triplicate samples by means of a previously described procedure (Bessey, Lowry & Brock, 1946; Hellerström, Täljedal & Hellman, 1964). Measurements of liberated *p*-nitrophenol were performed at 410 m μ in a Zeiss PMQII spectrophotometer equipped with micro-cells (10 mm. optical pathway).

Cytochrome oxidase activity was assayed by the technique of Pearl, Cascarano & Zweifach (1963). Triplicate samples (3.0 μ l. each) of homogenate or subcellular fractions were incubated with 15.0 μ l. of substrate-reagent medium at 37°. The latter solution was freshly prepared each day according to the following procedure: 100 mg. of phenyl-*p*-phenylenediamine (Sigma Chemical Co., St Louis, Mo., U.S.A.) was dissolved in 7.5 ml. of ethanol. After dilution to 50.0 ml. with water, the solution was left at room temperature for 30 min. and then any precipitate was removed by centrifuga-

tion. A 2.0 ml. sample of the supernatant was mixed with 1.0 ml. of 0.01% (w/v) cytochrome *c* (horse heart; Sigma Chemical Co.) in 30 mM-potassium phosphate buffer, pH 7.4. After centrifugation, the clear supernatant was used as incubation medium. After 60 min. of incubation the tubes were centrifuged at 10 000g for 5 min. and the E_{510} values of the supernatants determined in a Zeiss microscope photometer equipped with an interference filter. For this purpose about 8 μ l. was used to fill a fine capillary drilled into a Perspex rod, which fitted into an ordinary glass cuvette of 10 mm. optical pathway. An objective lens (4 $\times$ magnification) with a diaphragm was used as a condenser to produce a beam of narrow angle (Fischer, 1966). Under microscopic control the cuvette containing the sample could be arranged on the microscope stage so as to let this narrow light-beam pass the filled capillary without touching its walls. It was checked that E_{510} was proportionally related to the amount of enzyme present during incubation.

Protein determinations were carried out with triplicate samples (10 μ l. each) by the method of Lowry, Rosebrough, Farr & Randall (1951). Bovine serum albumin (see above) served as reference.

In all spectrophotometric assays blank values were obtained by substituting the tissue homogenate or subcellular fraction with an equal volume of homogenization or suspension fluid throughout the analytical procedure.

Electron microscopy. The composition of isolated fractions as well as the effects of various factors on granule stability were checked by electron microscopy. The sediments were resuspended in 200 μ l. of 2.5% (w/v) glutaraldehyde adjusted to pH 6.0 with 7.2 mM-potassium phosphate buffer. After centrifugation at 8000g for 20 min. in a Sorvall refrigerated centrifuge equipped with swing-out rotor HB-4, the tubes were kept cold for 1–2 hr. The glutaraldehyde was replaced by 304 mM-sucrose and 8 mM-potassium phosphate buffer, pH 6.0, overnight. The pellet was post-fixed in osmium tetroxide and subsequently processed for embedding in Epon (Luft, 1961) and sectioning on an LKB ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead.

Histochemical demonstration of heavy metals at the ultrastructural level was accomplished mainly as described by Pihl (1967) with glycol methacrylate (Leduc & Bernhard, 1967) as embedding medium.

Analyses of granule stability. The stability of isolated insulin granules in media of various compositions was assessed by the following procedure. A 50 μ l. sample of the granule suspension was carefully mixed with 250 μ l. of test solution in a polythene micro-tube. After incubation for 15 min. at 4° or 37° the test tube was spun at 6000g for 20 min. The upper half (150 μ l.) of the tube contents was gently withdrawn and transferred to a 15 ml. test tube for later extraction and insulin assay (see above), and the bottom half (150 μ l.) was carefully washed out with 150 μ l. of 5% (w/v) bovine serum albumin (see above) and similarly transferred to a test tube for insulin assay. The relative amount of particle-bound insulin after incubation was calculated as the percentage excess of insulin in the lower fraction as compared with the upper one. Thus percentage of sedimentable insulin = $100 \times (\text{insulin in bottom half} - \text{insulin in upper half}) / \text{total insulin}$. Control experiments with crystalline insulin and isolated liver mitochondria showed that neither adsorption of insulin on the tube wall

Fig. 1. Percentage recovery of insulin (I), protein (P), cytochrome oxidase (CO) and acid phosphatase (AP) in the granule fraction isolated at pH 6.0 and 6000g as compared with the whole homogenate.

or on mitochondria nor precipitation of soluble insulin contributed to the results. Further, only 2-3% of the insulin granules remained unsedimented in the upper half of the incubation media after centrifugation at 6000g for 20 min., as demonstrated by repeated centrifugation of the supernatant at 40000g for 30 min.

RESULTS

Properties of the crude granule fraction

Yield and purity. In 18 separate experiments 30 homogenized islets yielded $65 \pm 5 \mu\text{g.}$ of insulin, $65 \pm 2\%$ of which was retained in the granule fraction isolated at pH 6.0 and 6000g. The purity of this fraction was estimated by relating its insulin content to the amount of protein and to the cytochrome oxidase and acid phosphatase activities present. As shown in Fig. 1, there was a doubling of the purity in relation to cytochrome oxidase and a threefold or higher purification of granules in relation to protein and acid phosphatase. The electron-microscopic observations on the individual granule fractions were consistent with these chemical analyses and the effect on granule stability noted after exposure to various factors (see below). The ultrastructural appearance of the crude granule fraction is shown in Plate 1(a).

Effect of pH. The stability of isolated granules was markedly affected by the pH of the incubation medium (Fig. 2). The granules were rather fragile in both tris and phosphate buffers of pH 7.4. Maximal stability was obtained at pH 6.0. This effect of pH is also shown in Table 1, where the value obtained for 8mm-tris buffer, pH 7.4, is based on five different experiments.

Effect of buffer concentration. The results in Fig. 2 suggest that the granule stability is inversely related to the buffer concentration. This was

Fig. 2. Percentage of granule-bound insulin after incubation for 15 min. at 37° in media of different pH: ○, 8mm-tris-HCl buffer; □, 8mm-potassium phosphate buffer; ●, 40mm-tris-HCl buffer. An osmoticity of 320m-osmolar was obtained in all media by the addition of sucrose. Each point represents the mean of two to seven determinations; in cases of more than four determinations S.E.M. is also indicated.

confirmed in a separate series of experiments presented in Fig. 3. Fig. 3 also shows that there was no obvious difference between tris and phosphate buffers with regard to granule stability at pH 6.0.

Effect of osmoticity and ionic strength. Increasing concentrations of electrolytes other than the buffer substances investigated also produced decreased granule stability in iso-osmotic media (Fig. 4). Whereas more than 80% of the insulin remained particle-bound in 320mm-sucrose alone, only 10-20% was sedimentable after incubation in media of ionic strength 0.08. It is evident from Fig. 5 that the granule stability was unrelated to the sucrose concentration. At pH 6.0, 80% of the insulin was still sedimentable in non-ionic media with an osmotic pressure of only 50m-osmolar.

Effect of temperature. The effect of temperature was studied both at pH 6.0 and pH 7.4. No difference in the relative amount of sedimentable insulin was observed at 4° as compared with 37° in media of the former pH. At pH 7.4 the amount of granule-bound insulin was lower than at pH 6.0 and this effect of pH seemed to be potentiated by the higher temperature (Table 1).

Effects of specific compounds. Table 2 summarizes the screening of a number of substances for their effects on granule stability. Iso-osmoticity and equal ionic strength in test and control tubes were maintained throughout by appropriate adjustments of buffer and sucrose concentrations. The bound insulin was completely liberated by 1% (w/v) sodium deoxycholate. Treatment with 1mm-ATP and 2mm-sodium citrate also resulted in decreased granule stability. Significant ion-specific effects were not, however, observed with potassium chloride, calcium chloride or magnesium chloride.

Table 1. *Effect of temperature on granule stability*

The values denote percentage of sedimentable insulin (mean values $\pm$ S.E.M.) after incubation of granules for 15 min. in 8 mM-tris-HCl buffer, pH 6.0 or 7.4. The numbers of experiments performed are given in parentheses.

pH	Sedimentable insulin (%)	
	4°	37°
6.0	69-74 (2)	70-73 (2)
7.4	41 $\pm$ 4 (5)	16 $\pm$ 6 (5)

Fig. 3. Granule stability in tris-HCl (●) and potassium phosphate (○) buffers, pH 6.0, of various concentrations. Each medium was adjusted to 320 m-osmolar with appropriate amounts of sucrose. The incubation conditions were the same as for Fig. 2. Means of three experiments are shown.

Concn. of NaCl or sodium acetate in iso-osmotic media (mM)

Fig. 4. Effect of different concentrations of sodium acetate (●) and NaCl (○) on granule stability in solutions adjusted to 320 m-osmolar with appropriate amounts of sucrose. Buffer substances were omitted, but pH 6 was obtained by equilibration with ambient air. The incubation conditions were the same as for Fig. 2. Means of two experiments are shown.

The addition of zinc chloride in low concentrations resulted in an increased proportion of sedimentable insulin after incubation (Table 3).

Control experiments with pure ox insulin. The behaviour of pure ox insulin in our analytical system

Fig. 5. Granule stability in sucrose solutions of different concentrations. Incubation was for 15 min. at 37° in media adjusted to pH 6 as for Fig. 4. Means of two experiments are shown.

Table 2. *Classification of various compounds with respect to their effect on granule stability*

When not otherwise stated the tests were performed at 37° in tris-HCl buffer, pH 6.0. Approximately equal osmoticity (320 m-osmolar) and ionic strength (0.04) in test and control tubes were maintained throughout by appropriate adjustments of buffer and sucrose concentrations. For compounds with significant effects, the percentage of insulin sedimentable is given as mean values $\pm$ S.E.M. Unless otherwise indicated within parentheses, each compound was tested in two to four experiments.

Substances with apparent effect

Test substance	Sedimentable insulin (%)
ATP (disodium salt) (1 mM)	8.0 $\pm$ 0.8 (5)
None (control)	30.7 $\pm$ 5.9 (5)
Sodium citrate (2 mM)	7.6 $\pm$ 2.3 (5)
None (control)	30.7 $\pm$ 5.9 (5)
Sodium deoxycholate (1%)	2* (3)
None (control)	50* (3)

Substances without apparent effect

3',5'-(cyclic)-AMP (1 mM)	EDTA (1 mM)
Glucose 6-phosphate (1 mM)	CaCl ₂ (5 mM)
Serotonin (1 mM)	KCl (20 mM)
Tolbutamide (0.2 mg./ml.)	MgCl ₂ (5 mM)

* pH 7.4, 4°.

EXPLANATION OF PLATE 1

Electron micrographs of isolated granules. (a) The appearance of the granule fraction sedimenting at pH 6.0 and 6000 g. Magnification $\times$ 9625. (b) Detail of a granule fraction obtained by layering on 1.6 M-sucrose and centrifuging at 100 000 g for 90 min. There is still some contamination by rough-surfaced vesicles and membranes. In this particular case membranous sacs appear around the granules. Magnification $\times$ 21875. (c) Isolated granules treated with the sulphide-silver method. The presence of heavy metal is seen in the granules as distinct dark spots. Magnification $\times$ 64 750.

Table 3. *Insulin sedimentable after exposing isolated granules to Zn²⁺ ions*

The same incubation conditions and denotation as for Table 2 were used. The numbers of experiments are given in parentheses.

Test substance	Sedimentable insulin (%)	
	pH 6.0	pH 7.4
Control	48 (4)	33 (2)
1 mM-ZnCl ₂	84 (2)	—
5 mM-ZnCl ₂	87 (4)	71 (2)
10 mM-ZnCl ₂	92 (2)	—

Table 4. *Behaviour of pure ox insulin (0.003–0.005%, w/v) when introduced in the system for studying granule stability*

The values denote the percentage of sedimentable insulin (mean values $\pm$ S.E.M.) in tris-HCl buffers of different pH and molarity. The numbers of experiments performed are given in parentheses.

Medium	Sedimentable insulin (%)
240 mM-Sucrose–40 mM-tris, pH 7.4	1 $\pm$ 4 (4)
240 mM-Sucrose–40 mM-tris, pH 6.0	0 $\pm$ 3 (4)
240 mM-Sucrose–8 mM-tris, pH 7.4	0 (2)
240 mM-Sucrose–8 mM-tris, pH 6.0	8 $\pm$ 1 (4)

is illustrated in Table 4. No crystalline insulin could be sedimented after incubation in 40 mM-tris buffer, pH 7.4, nor was significant precipitation of insulin produced by lowering the pH to 6.0 or by decreasing the buffer concentration to 8 mM. Most of the insulin was, however, recovered in the bottom fraction after the addition of 1–5 mM-zinc chloride to the media. The proportion of Zn²⁺-precipitated insulin was at least as large at pH 7.4 as at pH 6.0.

Electron-microscopical demonstration of heavy metals. The presence and distribution of heavy metals in the granule fraction is shown in Plate 1(c). There was a distinct localization of silver grains with the secretory granules. The intensity of the 'staining' reaction appeared to be of the same magnitude as in similarly treated pancreatic islets.

Attempts to purify β -granules further

Centrifugation. By using the available stability data, attempts were made to purify the granule fraction further. After layering of the 6000g fraction on 1.6 M-sucrose and subsequent centrifugation at 100 000g for 90 min. about 40% of the insulin initially present in the whole-islet homogenate was recovered in the sediment. This fraction

Fig. 6. Purification obtained by centrifugation through 1.6 M-sucrose at 100 000g for 90 min. The results are expressed as the ratio between insulin (I) on the one hand and protein (P), cytochrome oxidase (CO) and acid phosphatase (AP) on the other. The corresponding ratios in the whole homogenate were taken as unity. The ratios are also given for the crude granule fraction sedimenting at 6000g.

displayed improved purification of granules in relation to cytochrome oxidase and acid phosphatase but not in relation to protein (Fig. 6). The result was consistent with electron-microscopical observations that the major contaminant was not mitochondria or lysosomes but rough-surfaced vesicles and membranes (Plate 1b). Sedimentation by initial forces below 6000g or the addition of heparin did not prevent contamination by endoplasmic reticulum. Increasing the pH to 7 resulted in a low recovery of sedimentable insulin.

Phase distribution in polyethylene glycol and dextran. The relative distribution of insulin, cytochrome oxidase, acid phosphatase and protein in a system of 4.0% polyethylene glycol and 5.0% dextran is shown in Fig. 7. With potassium phosphate buffer both insulin and enzyme activities were predominantly localized to the bottom and intermediate phases. When the K⁺ in the buffer was replaced by Li⁺, most of the granules were recovered in the top phase, as were cytochrome oxidase and protein. Acid phosphatase, however, was mainly localized to the interphase in this system. The addition of lithium chloride resulted in a slight displacement of all the four activities under study towards the intermediate and bottom phases.

Fig. 7. Relative distribution of insulin (I), protein (P), cytochrome oxidase (CO) and acid phosphatase (AP) in a phase system of 4.0% polyethylene glycol and 5.0% dextran. The effects of substituting K^+ for Li^+ in the phosphate buffer as well as of the addition of LiCl are shown: (a) 8 mM-potassium phosphate buffer; (b) 10 mM-lithium phosphate buffer; (c) 10 mM-lithium phosphate buffer + LiCl (10 mM).

DISCUSSION

Fractions rich in insulin secretion granules have previously been obtained by differential centrifugation of fish islets (Lazarow, Bauer & Lindall, 1964) and whole ox pancreas (Randall & Shaw, 1964). High purification with a low yield of granules has also been achieved with the aid of free zone electrophoresis (Hjertén, Höglund & Hellman, 1964). In the present study more convenient techniques of centrifugation were used for the rapid isolation of comparatively large fractions of granules from mammalian islets to permit analyses of granule stability under various physicochemical conditions.

The mobilization of stored insulin is generally thought to involve transport of granules up to the cell membrane, rupture of the granule sac, and extrusion and extracellular dissolution of the granular contents (Lacy, 1967). The present results do not elucidate the first two events, for which the properties of the membrane forming the granule sac are probably relevant. The electron-microscopic appearance of the granule sac was capricious and varied between good preservation and absence without any obvious relation to the observed granule stability. We therefore believe that the present observations principally reflect properties of the granule contents and wish to emphasize four

major conclusions that might throw some light on their dissolution *in vivo*. First, the granules proved very fragile at pH 7.4, optimum stability being obtained at about pH 6. Secondly, at the latter pH they seemed by contrast strikingly stable to various agents promoting the secretion of insulin, e.g. glucose, tolbutamide and 3',5'-(cyclic)-AMP. Thirdly, the electrolyte concentration in the medium was correlated to granule fragility. Fourthly, stable granules retained a heavy metal.

Apart from the fact that this information should facilitate future purification attempts, it might have some immediate relevance for the study and understanding of insulin secretion. For example, it seems necessary to pay increased attention to the concentration of hydronium and salt ions in the histological processing of pancreatic islet material for electron microscopy. Further, the rapid dissolution of granules ejected from the β -cell can well be explained by the pH of blood, which in mice is about 7.4. The intracellular dissolution of granules, as proposed by Lever & Findlay (1966), is more difficult to explain on such a simple physicochemical basis, but it cannot be excluded that the immediate surroundings of the granules in the β -cells are characterized by rapid pH and ionic fluctuations. In this context attention may also be drawn to the observed solubilizing effect of ATP

and citrate. An increased formation of citrate with chelation of Zn^{2+} in the β -cell has previously been suggested as a possible mechanism of insulin release (Maske, 1957).

Studies by Lazarow *et al.* (1964) showed that insulin granules and mitochondria from fish islets could be separated by a discontinuous sucrose gradient, the granules layering at the interface between 1.55M- and 2.0M-sucrose layers. In good agreement with this observation, repeated centrifugation through 1.6M-sucrose proved to be a means for the purification of mammalian granules from cytochrome oxidase. However, purification in relation to protein was not consistent, and electron microscopy showed that the major contaminant consisted of microsomes. This somewhat surprising result may be explained by the relatively low pH necessary for granule stability (cf. de Duve, Berthet, Hers & Dupret, 1949) and by the large amounts of endoplasmic reticulum present in β -cells of obese-hyperglycaemic mice (Björkman, Hellerström & Hellman, 1963). On the other hand, these animals provide the advantage of easily dissected islets containing more than 90% of β -cells (Hellman, 1965). In this context it should be emphasized that there is no evidence for an abnormal mechanism of insulin secretion in the obese-hyperglycaemic mice. On the contrary, their β -cells display a normal response to glucose *in vitro* (Å. Lernmark & B. Hellman, unpublished work). The high concentrations of circulating insulin in these mice are at present best explained as an adaptation of the islet organ to an impaired glucose metabolism in peripheral tissues (Stauffacher, Lambert, Vecchio & Renold, 1967).

Several techniques were tried for the elimination of microsomes from the granules. Satisfactory results were obtained neither by centrifugation at lower speeds, at higher pH, in the presence of heparin and glycogen, nor by phase distribution in polyethylene glycol and dextran. However, the latter technique gave good separation of granules from acid phosphatase activity. The concentration of this enzyme to the interphase was in fact so pronounced in systems buffered with lithium phosphate that this method may be useful for the future purification of acid-phosphatase-carrying particles from pancreatic β -cells.

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