Diabetologia (1981) 20: 654–663

Diabetologia

A Method for the Purification of Single A, B and D Cells and for the Isolation of Coupled Cells from Isolated Rat Islets

D. G. Pipeleers[1] and M. A. Pipeleers-Marichal[2]

[1]Department of Metabolism and Endocrinology and [2]Department of Pathology, Fakulteit Geneeskunde en Farmacie, Vrije Universiteit Brussel, Belgium

Summary. A method has been developed for the purification of single A, B and D cells and for the isolation of coupled islet cells. Isolated rat islets were dissociated by repeated pipetting in the presence of trypsin and ethylene glycol bis (β-aminoethyl ether) N, N' tetra-acetic acid (EGTA), and then filtered through nylon and Percoll; the cell preparation consisted of 70% single and 30% coupled cells. Sizing of the cells led to the recognition of a small-islet cell population (35%; cell volume 200–600 μm^3) composed of A and D cells, and a large-islet cell population (65%; cell volume 600–1500 μm^3) identified as B cells. Differences in sedimentation velocity formed the basis for the islet cell separation by counterflow elutriation. Single islet cells eluted prior to coupled cells and were distributed over A and D cell-enriched fractions I and II (65% A, 25% B, 10% D) and the B cell-enriched fraction III (93% B). The slightly different densities of A ($d = 1.068$), B ($d = 1.065$) and D ($d = 1.070$) cells allowed a subsequent purification by density gradient centrifugation resulting in a final 10- to 30-fold enrichment in either A, B or D cells. Most coupled islet cells were recovered in fraction IV, occurred mainly as doublets and were composed of 90% B cells and 7% D cells; the multiple pseudopods, which characterize isolated D cells, might contribute to the coupling tendency of the D cells. It is concluded that the purified A, B and D cell fractions and the coupled islet cell preparations offer a direct approach to the study of individual islet cell types and their intercellular communication.

Key words: Islets, cells, elutriation, insulin, glucagon, somatostatin, coupling

The mammalian endocrine pancreas represents a heterogeneous cell population in which several cell types have been described on the basis of histological, immunocytochemical and ultrastructural characteristics [1]. Immunofluorescent studies led also to the recognition of a precise topography of islet cells, with the A, D and pancreatic polypeptide cells mostly located at the islet periphery and surrounding the B cells [2, 3], and with the existence of an inverse relationship between the number of A and pancreatic polypeptide cells in different parts of the pancreas [3, 4]. The factors regulating pancreatic hormone release might thus vary for each islet cell type, depending on its location in the pancreas; intercellular junctions, heterogeneously distributed within the islets [5], may also be involved in the release process [6–9]. The study of individual islet cell types and their functional role in cell coupling thus requires the purification of single islet cells and the isolation of coupled cells. The method we developed therefore, consists in the dissociation of isolated rat islets, counterflow centrifugation [10] of the dissociated cells, and isopycnic gradient centrifugation [11] of the cells separated by elutriation.

Materials and Methods

Materials

Crude collagenase (150 U/mg) and trypsin (33 U/mg) were obtained from Worthington Biochemicals, Freehold, New Jersey, and bovine pancreatic desoxyribunuclease (DN-ase, 1000 U/mg) from Boehringer, Mannheim, Federal Republic of Germany. Bovine serum albumin fraction V (BSA), calf thymus DNA (type I) and ethylene glycol bis (β-amino-ethyl ether), N, N'-tetra-acetic acid (EGTA) were Sigma products (Sigma Chemicals, St. Louis); 2-(4–2-hydroxy-ethyl)-piperazinyl-(1)-ethan sulphonic acid (Hepes) was from Merck, Darmstadt, Federal Republic of Germany and 3,5-diamino benzoic acid (DABA) from Union Chimique Belge, Brussels, Belgium; Percoll and density marker beads were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.

Gibco (Glasgow, Scotland) provided tissue culture medium CMRL-1066 (1x, without glutamine) and fetal calf serum. All glass and plastic ware was treated with silicone oil, purchased from Serva, Heidelberg, Federal Republic of Germany.

0012-186X/81/0020/0654/$02.00

Media

Islet isolation and dissociation were carried out in Krebs-Ringer solution buffered with Hepes (KR-Hepes) [12] (115 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l $MgSO_4$. 7 H_2O, 2.56 mmol/l $CaCl_2$, 1.2 mmol/l KH_2PO_4, 20 mmol/l $NaHCO_3$ and 16 mmol/l Hepes at pH 7.4); omission of $CaCl_2$ is indicated as KR-Hepes-no Ca. Islet cell separation by elutriation occurred in calcium-free Krebs-Ringer bicarbonate (KR-no Ca). All media contained 0.5 g/l glucose and 5 g/l BSA, and were filtered through a Millipore 0.5 μm filter.

Higher density media for cell filtration and gradient centrifugation were prepared by diluting iso-osmotic Percoll, containing one part 10 × concentrated KR-Hepes-no Ca and nine parts Percoll, with KR-Hepes-no Ca, to produce mixtures of density (g/ml) 1.045, 1.065 and 1.085, respectively; all Percoll solutions contained 1000 U/ml aprotinin (Trasylol, Bayer, Leverkusen, Federal Republic of Germany).

Before use, the CMRL-1066 medium was supplemented with 0.1 g/l L-glutamine, 0.1 g/l streptomycin, 0.1 g/l penicillin and 10% (v/v) heat-inactivated fetal calf serum. The insulin assay was carried out in 0.15 mol/l NaCl, 20 mmol/l phosphate buffer, 5 g/l BSA (SPA buffer – pH 7.6), the glucagon assay in 0.2 mol/l glycine, 25 g/l dextran, 2.5 g/l BSA and 1000 U/ml aprotinin (GDA buffer – pH 8.8) and the somatostatin assay in 0.2 mol/l glycine, 25 mmol/l EDTA, 50 g/l dextran, 1 g/l BSA and 1000 U/ml aprotinin (GED buffer – pH 8.8) [13].

Islet Cell Isolation

Islets were isolated from pancreases of male Sprague-Dawley rats (250–300 g) by the collagenase technique of Lacy and Kostianovsky [14]. After collection of 1000–1500 islets, they were washed with KR-Hepes and resuspended in 4 ml cell isolation medium (KR-Hepes-no Ca supplemented with 1 mmol/l EGTA, 0.01 g/dl trypsin and 0.0002 g/dl DN-ase – pH 7.4). Islet disruption was achieved by gentle aspiration for 10–12 min at 30°C. When most cells occurred as single particles, the suspension was filtered through a 63-μm nylon screen to remove any filamentous material and collected in 10 ml ice-cold KR-no Ca (to stop the enzymatic activity). After centrifugation (150 × g, 7 min) through a Percoll layer (2 ml, density 1.045 g/ml) to replace the dissociation medium by KR-no Ca and to remove cell debris, the cells were aspirated from the bottom of the Percoll cushion and incubated for 20 min at 37 °C in 8 ml CMRL-1066 under O_2/CO_2 (95/5%). The islet cells were finally centrifuged at 100 × *g* for 4 min and resuspended in 7 ml CMRL-1066.

Centrifugal Elutriation

Islet cells were separated by counterflow centrifugation (centrifugal elutriation), as first described by Lindahl [15] and later adapted for biological applications [16–18]. The elutriation was carried out in a Beckman JE-6 rotor, driven by a Beckman J-21B centrifuge and equipped with a stroboscope (Beckman Instruments, Palo Alto, California). At a rotor speed of 2050 rev./min the separation chamber was perfused continuously with KR-no Ca containing 10 g/l BSA (10 °C), the flow rate being controlled by a Vario Perpex pump (LKB Instruments, Uppsala, Sweden), a three-way bypass valve between pump and centrifuge allowing the temporary use of a mixing chamber (volume 30 ml) [19].

The islet cell suspension was slowly injected into the mixing chamber, which was subsequently rinsed with 90 ml perfusion fluid at a flow rate of 6 ml/min; the fraction collected during this period contained no islet cells and was discarded. The mixing chamber was then excluded from the perfusion circuit, and the flow rate was increased stepwise to 16, 25, 34 and 50 ml/min. For each flow rate, 120 ml fractions were collected in three polycarbonate tubes (Beckman, volume 45 ml), containing a cushion of 3 ml Percoll density 1.045 g/ml (fractions I → IV). After centrifugation at 150 × *g* for 7 min, the Percoll cushions were transferred to conical centrifuge tubes containing 10 ml KR-Hepes-no Ca and spun at 100 × g for 4 min. The cell pellets corresponding to the same elutriation fraction were combined, resuspended in 0.5 ml KR-no Ca and examined for their cellular and hormonal content. In one series of experiments, the elutriated cell fractions were further purified by isopycnic density gradient centrifugation using continuous Percoll gradients.

Density Gradient Centrifugation

Linear density gradients were prepared using a gradient maker (LKB, Bromma, Sweden) and Percoll media of density 1.065 and 1.085 g/ml. The 6.5 ml gradients were generated in polyallomer tubes (Beckman, ¾" × 2 ⅛") on top of Percoll (0.5 ml, density 1.10 g/ml). The cell preparations (suspended in 2 ml Percoll, density 1.045 g/ml) were carefully layered on top of the gradient, which was then centrifuged in a swing-out rotor (Heraeus-Christ, Digifuge) at 2000 × *g* for 20 min; a control gradient tube contained coloured density marker beads (density 1.049, 1.063, 1.075 and 1.085 g/ml). After centrifugation, the gradients were fractionated with a density gradient fractionator (model 640, Isco, Lincoln, Nebraska) into 15 fractions of 0.6 ml and each fraction was examined for its cellular and hormonal content. Purified islet cell fractions were obtained less than 3 h (elutriation alone) or 4 h (elutriation + gradient centrifugation) after decapitation of the rats.

Characterisation of the Cell Fractions

Evaluation of Cell Dissociation, Cell Number and Cell Size: After fixation in 2% (v/v) formaldehyde, each cell fraction was photographed in phase contrast and at least 10^3 cells per fraction were examined in the evaluation of single and clustered cells; to minimize the effect of superimposition, counting was carried out at two different dilutions. After dilution of the cell samples in 0.154 mol/l saline supplemented with 5 g/l BSA, total cell numbers were determined with a particle counter equipped with a 100 μm probe (Model ZBI, Coulter Electronics, Harpenden, England). A multichannel analyser (Channelyzer-C 1000, Coulter Electronics), integrated cell sizes of minimally 10^4 cells per sample, with an XY recorder II (Coulter Electronics) plotting the cell volume distribution curves simultaneously. Counter and channelyser were calibrated with Latex particles of known size (diameter 13.7 μm).

A distribution curve of islet cell sizes was also plotted with data obtained from semi-automatic cell surface analysis (Manual Optical Planimeter, Kontron, Switzerland); only the cells with a visible nucleus were included in this study, carried out on a minimum of 10^3 cells per fraction.

The DNA content of each fraction was determined by the fluorometric methods of Kissane and Robbins [20] and Parman [21].

The percentage viable cells was counted after staining with 0.2% (w/v) trypan blue [22].

Electron Microscopy: The various cell fractions were fixed in 2.5% (v/v) glutaraldehyde (0.1 mol/l phosphate buffer, pH 7.4) and postfixed in 1% (w/v) OsO_4 in 0.1 mol/l phosphate buffer. After washing in the same buffer, the samples were dehydrated in successively 25, 50, 75 and 100% (v/v) ethanol and propylene oxide before embedding in araldite. Ultra-thin sections stained with lead

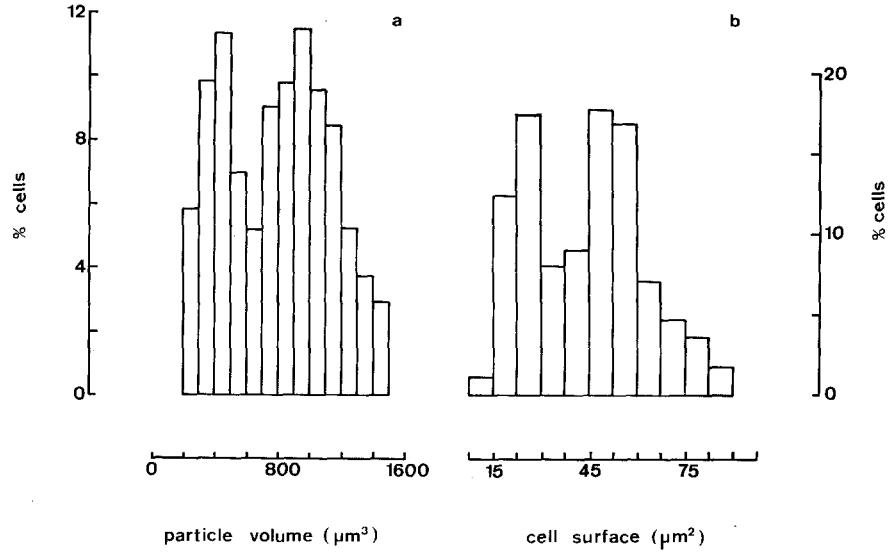

Fig. 1 a and b. Size distribution of unpurified islet cells. Particle volumes were measured on suspended islet cells in a Coulter counter (**a**), whereas the cell surfaces were obtained from semi-automatic image analysis of electron micrographs (**b**). Results express the mean of three experiments

citrate and uranyl acetate, were examined in a Zeiss transmission electron microscope. Photographs were taken from the total section which was then reconstituted by mounting the prints. The surface analysis was carried out on total sections.

Hormone Assays: The distribution of A, B and D cells over the various cell fractions was evaluated by determination of their glucagon, insulin and somatostatin content. Pancreatic polypeptide (PP) was barely detectable in rat islet homogenates assayed with human PP standard, bovine PP tracer and rabbit antihuman PP serum (PP and anti-PP serum were kindly provided by Dr. R. E. Chance, Eli-Lilly Research, Indianapolis, Indiana).

The hormone assays were carried out on dried samples from cell extracts in 2 mol/l acetic acid – 2.5 g/l BSA; the assay samples were resuspended in the corresponding buffers. Each radioimmunoassay consisted of a two step incubation at 4 °C. During the first incubation period, 100 μl samples were exposed to 200 μl antibody during 24 h for glucagon and insulin, and 16 h for somatostatin; the guinea-pig anti-insulin serum (final dilution 1:3 $\times 10^5$) was a gift from Dr. P. H. Wright (when at Indiana University, Indianapolis), the rabbit anti-glucagon serum (30 K – final dilution 1:75 $\times 10^3$) was purchased from Dr. R Unger (University of Texas, Southwestern Medical School, Dallas, Texas) and the rabbit anti-somatostatin serum (final dilution 1:6 $\times 10^3$) was a gift from Dr. De Mey (when at Free Brussels University). After addition of 100 μl ^{125}I-labelled hormone (~ 7000 cpm), the incubation was continued for 16 h (insulin), 64 h (glucagon) or 4 h (somatostatin). ^{125}-I-insulin (sp. act. 100–150 μCi/μg) was purchased from the Institut de Radio-Eléments, Fleurus, Belgium, and ^{125}I-glucagon (sp. act. 400–550 μCi/μg) from the Centre National de Transfusion Sanguine, Orsay, France; ^{125}I-somatostatin (sp. act. 150–200 μCi/μg) was prepared as described earlier [13]. Bound and free hormones were separated by dextran-coated charcoal (Norit, Fisher Scientific Co., Springfield, New Jersey) and the washed pellets counted in a Rackgamma II counter (LKB, Bromma, Sweden) which calculated the sample's hormone content from standard curves for porcine glucagon, rat insulin and synthetic somatostatin. The extraction procedure resulted in hormone recoveries of > 95%. The insulin, glucagon and somatostatin assays were sensitive to respectively 5, 2.5 and 5 pg; intra-assay coefficients of variation averaged 5–10% for the three hormones, whereas the interassay coefficients of variation were 15% for insulin and 20% for glucagon and somatostatin.

The hormonal content of each cell fraction was calculated as a percentage of the total eluted hormone, and as μg (ng) per 10^3 islets or per μg DNA. The results were expressed as the mean ± SEM.

Results

Islet Cell Preparation

After dissociation, many islet cells displayed an irregular surface, sometimes with cytoplasmic extrusions. A 20-min incubation in CMRL-1066 medium, at 37 °C, however, restored the cell's smooth and spherical appearance. Filtration of the cell suspension through a nylon screen and a Percoll layer removed material that could provoke cell clumping and, hence hamper the elutriation process. The islet suspension thus obtained contained mostly single cells (more than 70%) with less than 30% of the cells incorporated in clumps of two (15–20%), three (5–10%) or four (0–5%) cells. When islet dissociation was carried out in the absence of trypsin, more than 70% of the cells remained aggregated, whereas the absence of DNase resulted in clumping of the dissociated cells.

Electron micrographs of the islet cell preparation illustrated well granulated A, B and D cells, mostly with regular and spherical shape. The cells appeared well preserved with a well-developed endoplasmic reticulum and no swelling of the cytoplasmic constituents. The preparation consisted of 67% B cells, 24% A cells and 6% D cells; necrotic cells and pancreatic exocrine cells were seldom observed. Morphometric analysis of 10^3 islet cells revealed a bimodal distribution in cell size, with 35% of the cells

located in the small-size population, and 65% corresponding to larger cell surfaces (Fig. 1).

A similar distribution was obtained for islet cell volumes, as measured by a Coulter counter (Fig. 1).

The surface distribution curves plotted separately for A, B and D cells indicated that A and D cells are smaller than B cells, and thus correspond to the smaller islet cell population (Fig. 2).

Centrifugal Elutriation of Islet Cells

Cell Distribution: Elutriation fractions I, II and III comprised mainly single cells, whereas 70% of the cells in fraction IV were clusters of two to four cells. The cell counts and DNA measurements showed a 75% yield in the elutriation fractions, with approximately 7% eluting in fraction I, 22% in fraction II, 39% in fraction III and 32% in fraction IV (Table 1). Cell counts may be underestimated since the Coulter counter may not completely differentiate between clumped and single cells; the DNA content per cell might therefore be over-estimated, although parallel distributions were noted in cell number, DNA and hormone content (Table 1).

The degree of islet cell separation was examined by sizing the cells in the elutriation fractions. More than 70% of the cells in fractions I and II measured less than 600 μm^3, and thus correspond to the small-sized islet cell population (Fig. 3). The particles in fractions III and IV were larger than 600 μm^3, indicating an enrichment in single large-sized islet cells (fractions III and IV) or in coupled cells (fraction IV).

Hormone Distribution: The hormonal data demonstrates clearly that glucagon- and somatostatin-containing cells were eluted mainly in fractions I and II, whilst most insulin-containing cells were recovered in fractions III and IV (Table 1, Fig. 4). This purification pattern is compatible with the observed size difference of A, B and D cells. The elutriation of somatostatin cells was, however, not identical to that of the glucagon cells, although both cells exhibit similar cell sizes: elutriation fractions I and II were indeed twofold less enriched in somatostatin than in glucagon, and the somatostatin/glucagon ratio in fraction IV was tenfold higher than in unpurified islet cells (Table 1). Part of the somatostatin cells thus migrated with the aggregated cell fraction.

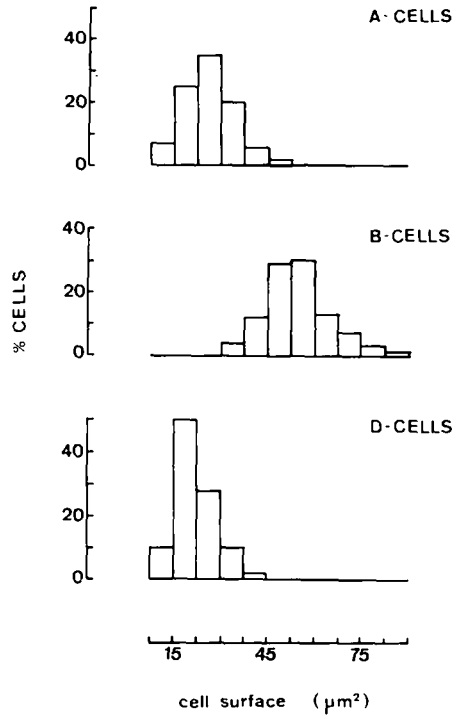

Fig. 2. Size distribution of A, B and D cells, as determined morphometrically on electron micrographs of unpurified islet cells. Results express the mean of four experiments

Table 1. Cell and hormone distribution after centrifugal elutriation of islet cells[a]

Fractions	Cell count[b] (10^3 cells)	DNA content (μg)	Insulin (μg)	Hormone content	
				Glucagon (ng)	Somatostatin (ng)
Isolated islets	–	42.9 ± 5.9	95.2 ± 7.5	2086 ± 185	222 ± 17
Dissociated islet cells	550 ± 65	14.7 ± 1.9	29.5 ± 3.8	1351 ± 75	112 ± 7
Elutriation fraction I	28 ± 4	0.7 ± 0.2	0.5 ± 0.1	105 ± 7	5 ± 1
II	85 ± 10	2.1 ± 0.3	1.4 ± 0.1	641 ± 41	25 ± 2
III	150 ± 20	3.7 ± 0.6	8.9 ± 0.3	152 ± 8	14 ± 1
IV	127 ± 17	4.0 ± 0.6	10.3 ± 0.5	32 ± 4	30 ± 2

[a] All parameters are calculated for 10^3 isolated islets; the results are expressed as mean ± SEM ($n = 6$ for cell count, $n = 13$ for DNA and hormone content)

[b] determined in Coulter counter

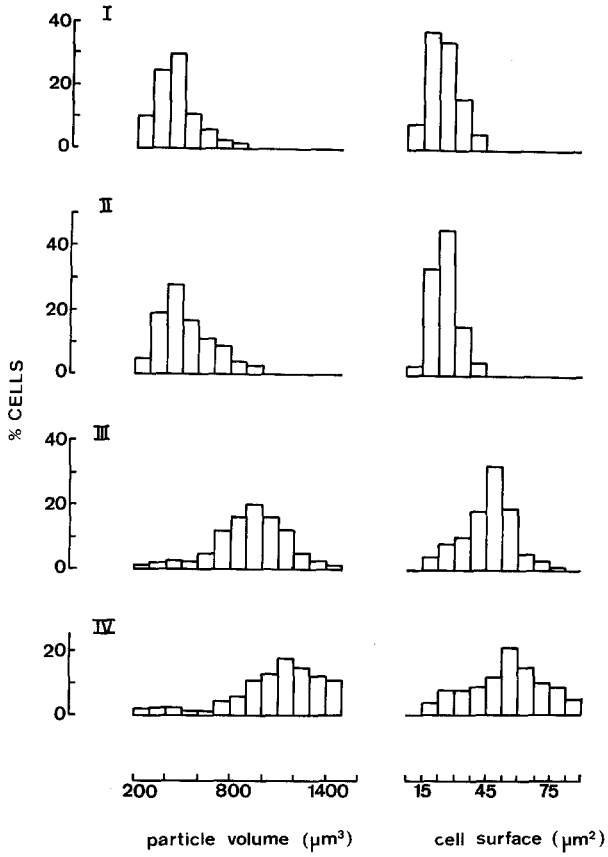

Fig. 3. Size distribution of the islet cells in elutriation fractions I, II, III and IV. Sizing was performed as previously described (Fig. 1), and for each fraction, the percentage cells corresponding to a given range in particle volume or cell surface was determined. Results express the mean of four experiments

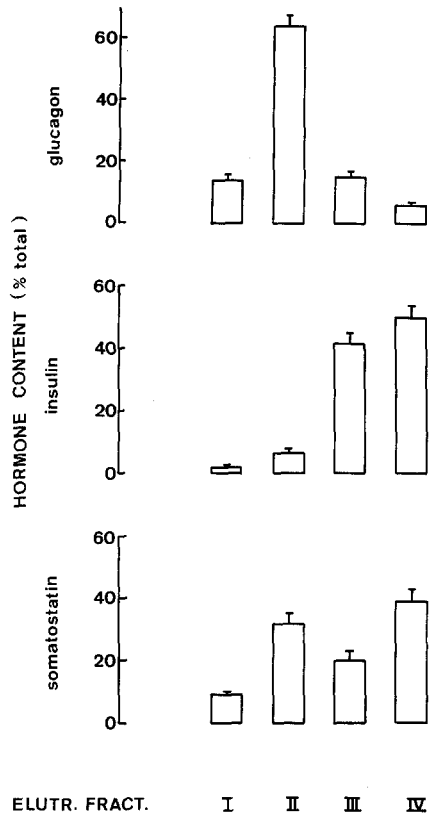

Fig. 4. Hormone distribution in the elutriated fraction I–IV. The glucagon, insulin and somatostatin content of each fraction are expressed as a percentage of the total hormone elutriated. Results express the mean ± SEM of 13 experiments

Electron Microscopy: The ultrastructure of the purified islet cells was well preserved, which is consistent with the small percentage of trypan blue positive cells (less than 4%). The cellular composition of the elutriated fractions was in accord with the hormonal data: fractions I and II were significantly enriched in A cells, and, to a lesser extent, in D cells, whereas fractions III and IV contained much lower numbers of non-B cells than unpurified islet cell preparations (Table 2, Fig. 5). The separation technique did not affect the size of the islet cells. Surface analysis of the elutriated cells also demonstrated that the small islet cells eluted in fractions I and II and the large islet cells eluted in fractions III and IV (Fig. 3). More than 90% of the cells in fraction IV corresponded to B cells with a wider surface variation and a larger average cell size than those in the single B cell fraction III.

Electron micrographs of fraction IV showed many cells closely attached to other islet cells (Fig. 6); the presence of many B/B and B/D cell couples

Table 2. Cell composition of elutriated islet cell fractions[a]

Fraction	Single cells (%)	A cells (%)	B cells (%)	D cells (%)
Dissociated islet cells	70	24	67	6
Elutriation fraction I	>90	61	25	9
II	>90	65	25	8
III	>90	5	93	2
IV	<30	<1	95	4

[a] The results are expressed as a percentage of each fraction's cell content, and represent the mean of three counts, carried out on at least 10^3 cells per fraction

was readily noted. As suggested by the hormone data, fraction IV contained more D cells than A cells (Table 2). The D cells in fraction IV displayed the same cell size as those in fraction II, and were also characterised by multiple long microvilli (Fig. 7); their presence in the larger particle fraction might

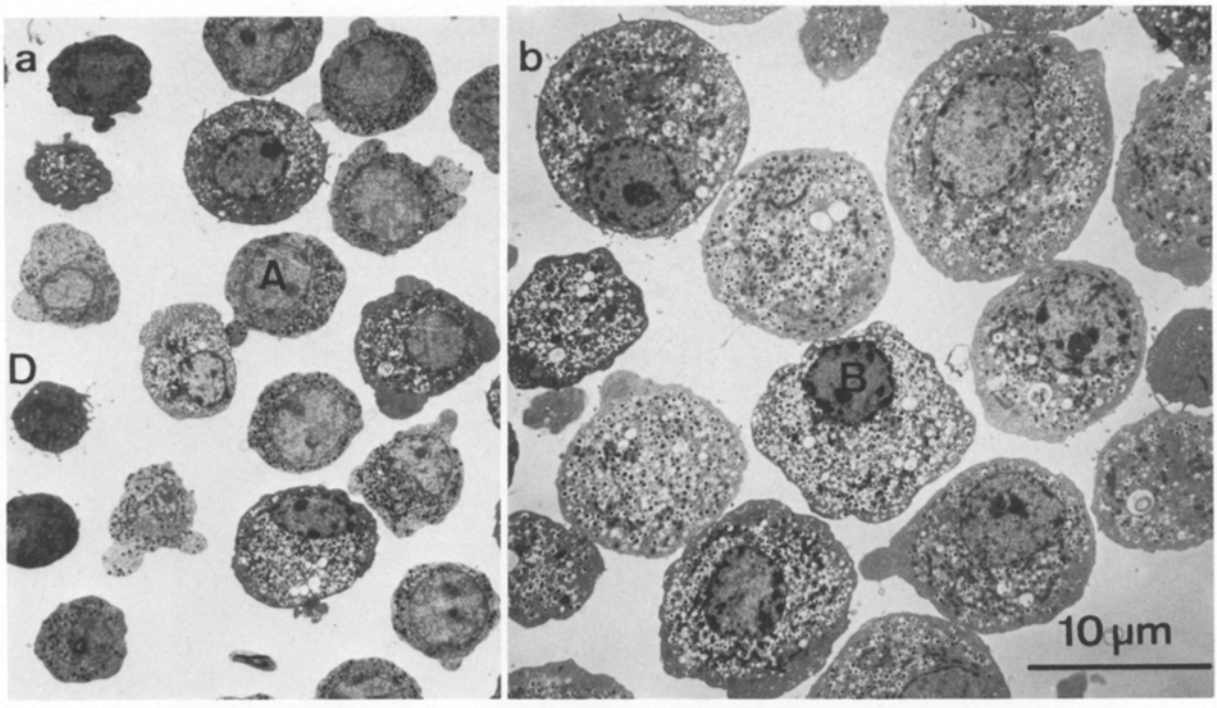

Fig. 5. Electron micrographs of islet cells collected in elutriation fraction II (**a**) and III (**b**). The cells in fraction II were significantly smaller than those in fraction III. Fraction II contained mainly A cells and was enriched in D cells; fraction III was almost entirely composed of B cells (× 3100)

thus be related to their occurence in cell couples (Fig. 6c).

Isopycnic Density Gradient Centrifugation of Islet Cells

The applicability of isopycnic density gradient centrifugation to the separation of islet cells was tested by measuring the density of A, B and D cells. This parameter was obtained by determining the densities at which the peak hormone levels are measured after isopycnic density gradient centrifugation of islet cells. The small differences in densities (g/ml) of A (1.068), B (1.065) and D (1.070) cells are consistent with the considerable overlap in hormone distribution and with a low degree of islet cell purification; single cells were not isolated from coupled cells. Part of these limitations were overcome by applying the purified single cell fractions II and III to density gradients; under these conditions 50–70% of the insulin content was recovered between density 1.060 and 1.067 g/ml, whereas > 65% of the glucagon and somatostatin content had migrated into higher densities (Fig. 8). Comparison of the hormone ratios insulin/glucagon and insulin/somatostatin in the various gradient fractions with those in the initial islet cell suspension indicates that isopycnic density gradient centrifugation of elutriation fraction II produced fractions which were 27- and 16-fold enriched in respectively A (density 1.067–1.077) and D (density 1.070–1.077) cells (Table 3). A similar degree of purification is indicated by the increase in glucagon or somatostatin content per µg DNA (Table 3). However, the purified A and D cell fractions contained only 7 and 3% of the initial islet number, which is certainly a limiting factor for further analytical studies. The hormone ratios indicated similarly that elutriation alone produced a single B-cell fraction III which was threefold enriched in insulin, whereas the subsequent density gradient centrifugation of fraction III resulted in a final sixfold enrichment in insulin. The purified B cell fractions contained 29% (elutriation alone) and 19% (combination procedure) of the initial cell number (Table 3).

Discussion

Although the first method for the preparation of islet cells was described in 1966 [23], no techniques have so far been reported for the purification of the various islet cell types. Consequently, most in vitro studies on the endocrine pancreas have been carried out on heterogeneous cell populations such as the

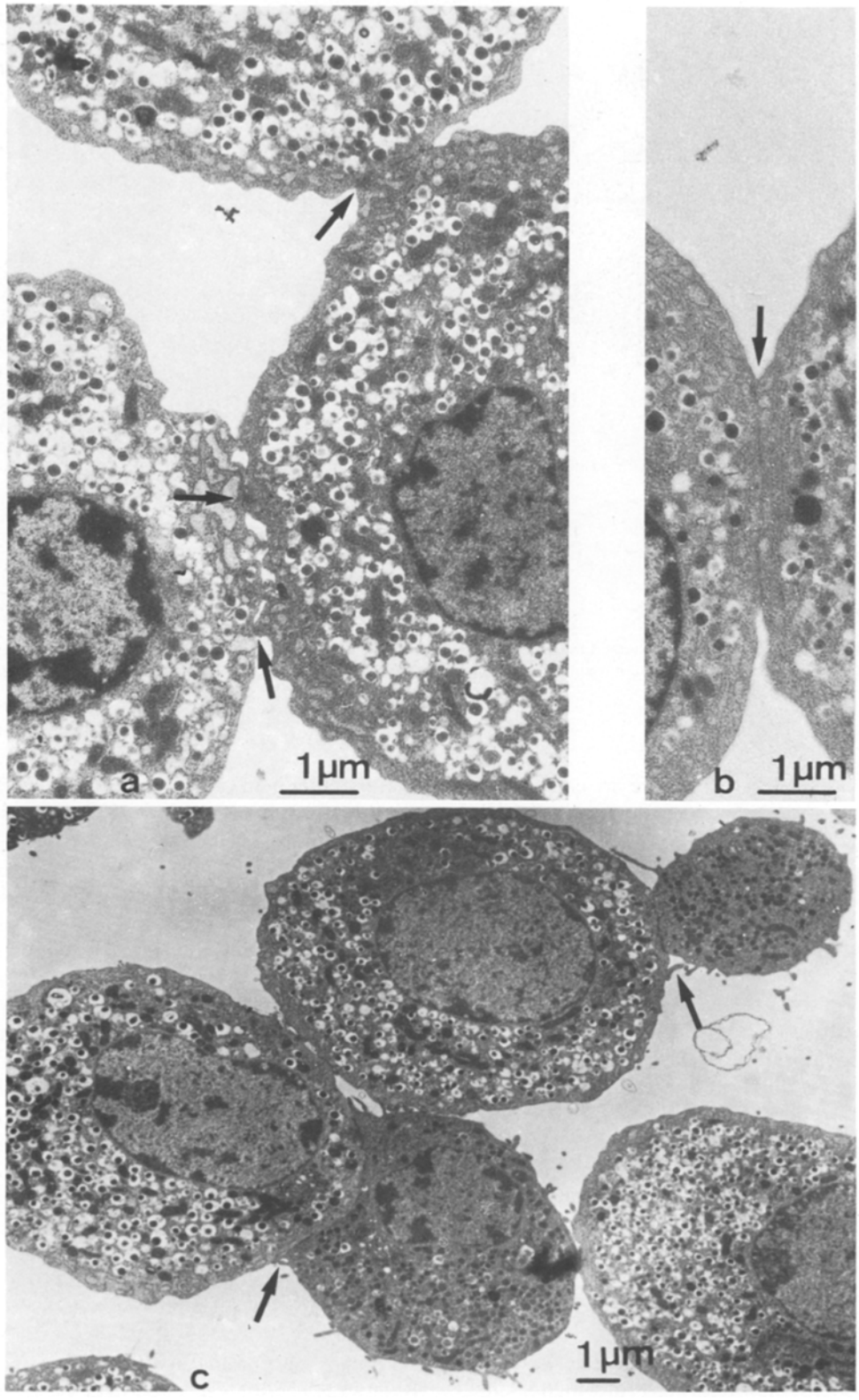

Fig. 6. Electron micrographs of elutriation fraction IV, illustrating the existence of cell contacts (arrows) between B cells (a, b – × 11400) and between B and D cells (c – × 6125)

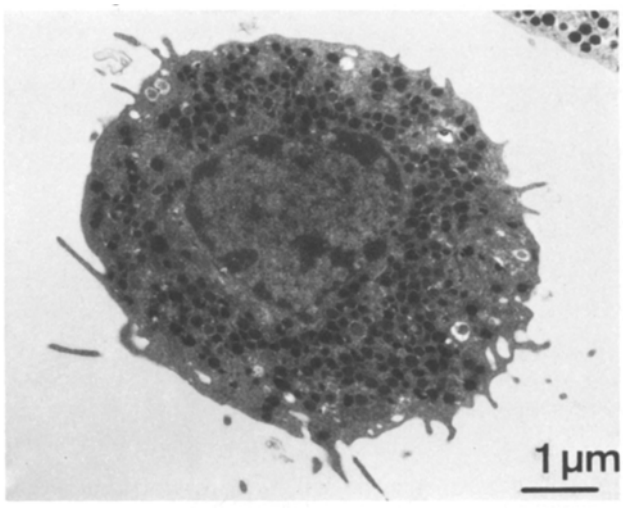

Fig. 7). Isolated D cell with its characteristic multiple microvilli (× 10^4)

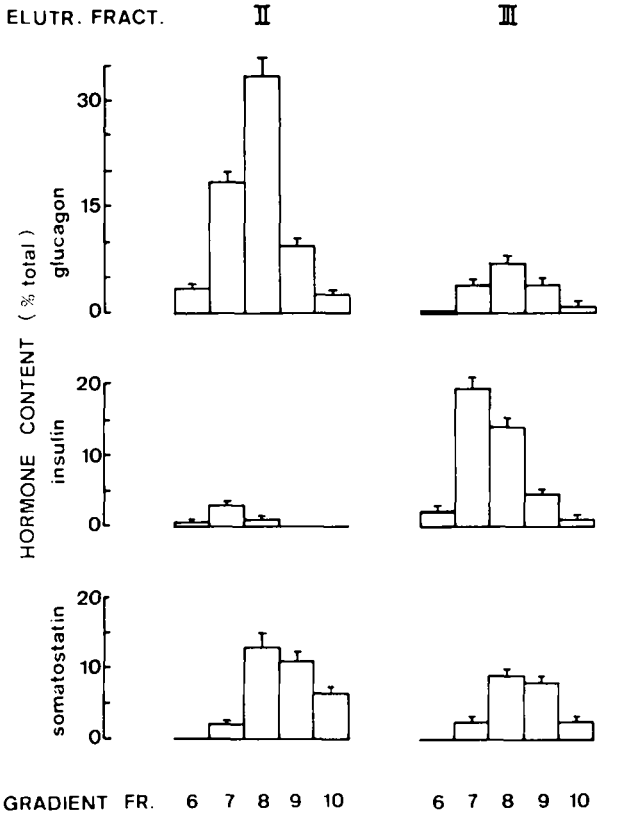

Fig. 8. Hormone distribution after density gradient centrifugation of the A and D cell enriched fraction II and the B cell enriched fraction III. The glucagon, insulin and somatostatin content of each gradient fraction were expressed as a percentage of the total hormone applied onto each density gradient. The results express the mean ± SEM of five experiments. The gradient fractions corresponded to the following densities (g/ml): 6 (1.060–1.064), 7 (1.064–1.067), 8 (1.067–1070), 9 (1.070–1.073), 10 (1.073–1.077)

perfused pancreas [24], pancreatic fragments [25], isolated islets [14, 26] and dissociated islet cells [12, 27–30]. The need for homogeneous islet cell preparations was, however, early recognized, leading to the use of B cell rich islets from obese hyperglycaemic (ob/ob) mice [21, 31] and of A cell enriched islets from streptozotocin or alloxan diabetic rats [32, 33]. Whereas the islets from ob/ob mice represent a suitable model for the study of the B cell and the insulin release mechanism, they are less appropriate for examining the role of other islet cell types, especially in intercellular communication, and are, in addition, less representative for the normoglycaemic conditions. The usefulness of B cell destroying agents such as streptozotocin or alloxan to prepare A cell enriched islets is, on the other hand, questionable in view of their effects upon A cells and glucagon release [34].

The preparation of single rat islet cells required the combination of mechanical shear, trypsin and EGTA, which is comparable to the dissociation conditions for pancreatic exocrine cells [35]; the addition of DN-ase reduced cell clumping, as has been observed in other systems [36], whereas the trypsin concentration was five- to 50-fold lower than in other islet dissociation methods [27, 28, 30]. With the present method, 5 × 10^5 cells were prepared from 10^3 isolated islets, which corresponds to a 35% cell yield. Higher cell yields have been reported for dissociations without enzymes [12], which are however less successful in producing single cell preparations. After filtering and incubating the dissociated cells, clean cell suspensions were obtained with mainly spherical and single cells: these characteristics fulfill the criteria for both the measurement of islet cell volumes and densities and the purification of islet cells.

As the densities of A, B and D cells differ only slightly, this parameter does not form the basis for major differences in sedimentation velocity. The recognition of two islet cell populations differing in cell size and corresponding to small-sized A and D cells and large-sized B cells indicates however that cell separation techniques, based upon differences in sedimentation velocity, can be used for the separation of A and D cells from B cells.

Among the methods which separate cells according to their sedimentation velocity, centrifugal elutriation has been described as a gentle, sensitive and rapid technique with high cell recovery and minimal cell aggregation [10, 19, 36, 37]. This is particularly attractive for islet cells, in view of their relatively long preparation time (2–2.5 h) and the low initial cell number. The usefulness of counterflow centrifugation in islet cell separation is evident from its short duration (35 min), its high cell recovery (75%) and

Table 3. Parameters expressing the degree of islet cell purification

Fraction	Cell yield	Hormone ratio		Hormone content (ng/μg DNA)		
		INS/GLU	INS/SOM	Insulin	Glucagon	Somatostatin
	(%)					
Dissociated islet cells	100	21.5 ± 2.4	263 ± 38	2075 ± 210	85 ± 10	7.6 ± 0.9
Elutriation fraction II	16 ± 2	2.3 ± 0.2	57 ± 4	663 ± 49	375 ± 34	12.1 ± 1.3
Elutriation fraction II after gradient centrifugation						
density 1.067–1.077 g/ml	7 ± 1	0.8 ± 0.1	33 ± 3	< 500	> 1500	> 150
density 1.070–1.077 g/ml	3 ± 1	0.8 ± 0.1	16 ± 2	< 500	> 1500	> 150
Elutriation fraction III	29 ± 3	58.5 ± 4.2	564 ± 70	2450 ± 275	41 ± 5	3.8 ± 0.4
Elutriation fraction III after gradient centrifugation						
density 1.060–1067 g/ml	19 ± 2	130.2 ± 9.4	1120 ± 195	2495 ± 275	20 ± 3	1.8 ± 0.4

INS/GLU = insulin/glucagon; INS/SOM = insulin/somatostatin. Results are expressed as the mean of five experiments ± SEM

minimal cell clumping, and from its ability to separate single from coupled cells, and A and D cells from B cells. The viability of the purified islet cells was demonstrated by the low percentage trypan blue positive cells (< 5%), by their ultrastructural integrity and their secretory responsiveness after a culture period (unpublished observations).

The availability of islet cell preparations with different cell composition would allow comparative studies which could clarify each cell type's impact on neighbouring cells' functions. A more profound (sub)cellular analysis of A or D cells requires, however, additional purification steps, such as isopycnic density gradient centrifugation which results in a final 27-fold enrichment in glucagon and 16-fold enrichment in somatostatin; the low cell yield of this procedure (< 10% of the unpurified islet cell population), however, makes further separations of A or D cells dependent on techniques for the large scale isolation of islets [38]. Density gradient centrifugation of the single B cell fraction III increases the percentage B cells from 93 to 98%, while reducing the cell yield by almost 40%; conditions should therefore be selected whereby further purification of the B cell enriched elutriation fraction is essential.

The coupled islet cells were recovered almost entirely in elutriation fraction IV, which also contained 30% single cells. Both hormone data and electron micrographs described fraction IV as a B cell enriched preparation (95% B cells) and thus identified the majority of its single and coupled cells as B cells. The elution of small somatostatin cells in fraction IV might be attributed to their participation in cell coupling, whereas the concomitant absence of A cells indicates a higher coupling tendency of D cells. The notion of avidly coupling D cells would be consistent with earlier reports on a close topographic inter-relationship between somatostatin-containing cells and proximally or distally located cells [2, 39, 40]. It is tempting to visualize their multiple long microvilli as an anatomical basis for facilitated cell contacts. Further experiments should however determine the nature and physiological significance of these cell couplings and should examine the dependency of the B-cell secretory activity on junctional activity. The present purification method provides a useful model for such studies as it results in the preparation of both single and coupled B cells.

Acknowledgements. We thank C. Arijs, M. Berghmans, S. Duys, J. C. Hannaert, N. Harnie, L. Heylen and M. Jacobs for their excellent technical assistance; A. M. Kiggen and A. Vanhaelewijck for their appreciated secretarial help. We are also grateful to Professor W. Gepts for his continuous support, to the late Professor J. C. Wanson and to Dr. G. Smets for help and advice with the electron micrographs. This work was supported by grant Nr. 3.0014.76 from the Nationaal Fonds voor Wetenschappelijk Onderzoek (NFWO) and a research grant from the Nationale Bank van België. D. G. Pipeleers was 'bevoegdverklaard navorser' of the NFWO. Part of this work has been carried out in the Queen Elisabeth Foundation, Director Professor P. P. Lambert) and was presented at the 1979 World Congress of the International Diabetes Association [41].

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Received: 18 September 1980
and in revised form: 24 February 1981

Dr. D. G. Pipeleers
Department of Metabolism and Endocrinology
Fakulteit Geneeskunde en Farmacie
Vrije Universiteit Brussel
Laarbeeklaan 103
B-1090 Brussels
Belgium