

Differences in Glucose Recognition by Individual Rat Pancreatic B Cells Are Associated with Intercellular Differences in Glucose-induced Biosynthetic Activity

Rita Kiekens, Peter In 't Veld,* Tania Mahler,* Frans Schuit,[‡] Marnix Van De Winkel, and Daniel Pipeleers
Department of Metabolism and Endocrinology, *Department of Experimental Pathology, and [‡]Department of Biochemistry, Vrije Universiteit Brussel, B-1090 Brussels, Belgium

Abstract

In vitro incubated rat islet B cells differ in their individual rates of protein synthesis. The number of cells in biosynthetic activity increases with the glucose concentration. Flow cytometric monitoring of the cellular redox states indicated that islet B cells differ in their individual metabolic responsiveness to glucose. A shift from basal to increased NAD(P)H fluorescence occurred for 18% of the cells at 1 mM glucose, for 43% at 5 mM, and for 70% at 20 mM. The functional significance of this metabolic heterogeneity was assessed by comparing protein synthesis in metabolically responsive and unresponsive subpopulations, shortly after their separation by autofluorescence-activated cell sorting. The glucose-sensitive subpopulation exhibited four- to fivefold higher rates of insulin synthesis during 60-min incubations at 2.5–10 mM glucose. Its higher biosynthetic activity was mainly caused by recruitment of cells into active synthesis and, to a lesser extent, by higher biosynthetic activity per recruited cell. Cells from the glucose-sensitive subpopulation were larger, and presented a threefold higher density of a pale secretory vesicle subtype, which is thought to contain unprocessed proinsulin. It is concluded that intercellular differences in metabolic responsiveness result in functional heterogeneity of the pancreatic B cell population. (*J. Clin. Invest.* 1992; 89:117–125.) **Key words:** cellular heterogeneity • insulin synthesis • islet cells • endocrine pancreas • metabolic redox state

Introduction

Glucose exerts a tight control on the release of insulin from the endocrine pancreas. The sugar has been found to stimulate several steps in the formation of insulin and its release by pancreatic B cells (1–3). Not all cells appear, however, equally involved in the process of glucose-induced insulin biosynthesis (4). At low glucose levels, only a minority of islet B cells is actively involved in protein synthesis (4). Glucose dose-dependently increases the number of active cells and hence the rate at which insulin is synthesized by the total population (4). The sigmoidal shape of the dose response curves in glucose-exposed

B cell preparations may thus result, at least in part, from a dose-dependent recruitment of cells into a biosynthetic activity (4). The recognition of intercellular differences in the biosynthetic activity of glucose-exposed B cells also suggests that the cells markedly differ in the sensitivity of their glucose-driven metabolic pathways. The present study examines this possibility and assesses whether variations in cellular thresholds for glucose metabolism can explain the intercellular differences in glucose-induced protein synthesis. The experiments were conducted on purified rat islet B cells that are isolated by autofluorescence-activated cell sorting (5, 6). This model offers the possibility of measuring the glucose-induced changes in the metabolic redox state of individual cells (7). It can be used to separate B cell (sub)populations which differ in their metabolic handling of glucose and which can then be compared for their respective biosynthetic activities.

Methods

Preparation of rat pancreatic beta cells. Pancreatic islets were isolated from male adult Wistar rats (200–300 g) according to a modification of the collagenase digestion method of Lacy and Kostianovsky (6, 8). They were dissociated in a Ca²⁺-free medium containing trypsin and DNase (9). The islet cell suspensions were then submitted to autofluorescence-activated cell sorting, using cellular light scatter and flavin adenine dinucleotide autofluorescence as separation parameters (5, 6). This technique allows purification of single insulin-containing B cells with a yield of 2–3.10⁵ cells per adult rat pancreas (6).

Flow analysis of metabolic alterations in glucose-exposed B cells. We have previously described how the metabolic redox state can be measured in purified B cells (7). Samples of 5.10⁴ cells were incubated for 15 min at 37°C in the presence of different glucose concentrations. They were then analyzed for their light scatter and NAD(P)H-autofluorescence, using a FACS[®]-IV flow cytometer (Becton Dickinson & Co., Mountain View, CA) equipped with an argon laser (Spectra Physics Inc., Mountain View, CA). Excitation occurred at 351–363 nm, emission was measured between 400 and 470 nm (7). The FACS[®]-data are represented as dot plots with the relative cellular autofluorescence intensity in the ordinate and the relative cellular light scatter in the abscissa (Fig. 1). The two internal controls consisted of islet B cells incubated at 1 mM or at 20 mM glucose; their respective dot plots were used to select the glucose-unresponsive and the glucose-responsive cells according to their distinct autofluorescence intensities (see Results). Windows were set on the basis of mean autofluorescence intensities ± 2 SD and on the basis of the forward light scatter of single islet B cells (see Results). FACS[®] analysis determined the percent particles that were collected in each of the two preselected windows. A total of 5.10⁴ cells was analyzed per experiment, each condition being examined in at least six independent experiments. Results were expressed as mean ± SD, and statistically analysed using paired Student's *t* testing.

Comparative studies on islet B cells with different metabolic responsiveness to glucose. Purified B cells were incubated for 15 min at 37°C in the presence of 7.5 mM glucose. They were then analyzed for their light scattering properties and cellular NAD(P)H-autofluorescence intensity. Cells appearing in the preset windows of high and low NAD(P)H cells were isolated and considered as subpopulations of, re-

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Address correspondence to Daniel Pipeleers, Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium.

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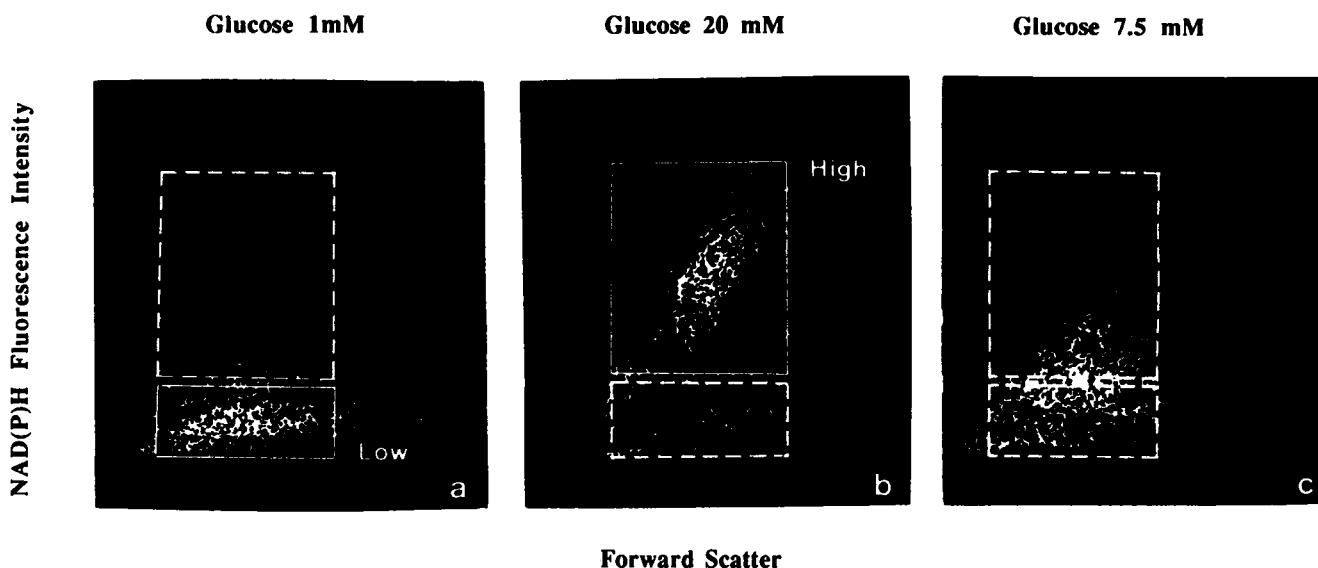


Figure 1. Dot plots after FACS®-analysis of single purified B cells which have been exposed for 15 min to 1, 20, and 7.5 mM glucose. The cells were examined for their NAD(P)H autofluorescence intensity (ordinate) and their forward scatter (abscissa). In the 1 and 20 mM condition, the windows of, respectively, low and high NAD(P)H autofluorescence were set as described under Results (solid line). These windows were then used to determine the percent unresponsive (low) and responsive (high) cells in each condition (broken line).

spectively, 7.5 mM glucose-responsive and unresponsive cells. The two subpopulations were compared for their structural and functional properties. Cell viability was examined by vital staining with neutral red (6), the percent living cells being counted immediately after isolation as well as after a 10-d culture period in polylysine-coated microtiter cups. Structural integrity was also assessed by electron microscopy on glutaraldehyde-fixed cells (6). The mean insulin content of the isolated cells was measured in acetic acid extracts of samples of 10^4 cells (6). The biosynthetic activity of the cells was determined over 60-min incubations, immediately after their isolation.

Analysis of protein biosynthesis in isolated islet B cells. Samples of 5.10^4 islet B cells were incubated for 60 min at different glucose concentrations in 200 μ l Earle's-Hepes medium containing 1% (wt/vol) BSA (fraction V, RIA grade; Sigma Chemical Co., St. Louis, MO) and 50 μ Ci L-(3,5- 3 H)-tyrosine (sp act 50 Ci/mmol; total tyrosine concentration 5 μ M; Amersham International, Amersham, Bucks, UK) (10). Under these conditions the rate of insulin biosynthesis can be expressed as fmol/h (10). At the end of the incubation, the cells were washed in Earle's-Hepes buffer containing 1 mM unlabeled L-tyrosine and then extracted in acetic acid or processed for autoradiography.

Acid extracts were prepared in 2 M acetic acid containing 0.25% BSA. Samples were dried and assayed for 3 H-protein as well as for total and 3 H-labeled insulin immunoreactivity (4). The content in 3 H-protein was obtained by counting the TCA precipitable radioactivity. The 3 H-labeled insulin immunoreactivity was assayed by adding an excess of guinea pig antiinsulin serum and precipitating the immune complexes with protein A-Sepharose; nonspecific binding was measured in samples receiving normal guinea pig serum instead of antiinsulin serum (4). The 3 H-labeled insulin immunoreactivity was extracted from the immunoprecipitated complexes with 2 M acetic acid and counted. The acid cell extracts were also further analyzed by gel chromatography. Samples were eluted on Biogel P-10 columns (Bio-Rad Laboratories, Richmond, CA) with 2 M acetic acid, 0.25% BSA as running solution (11). Elution fractions were counted and plotted against 125 I-insulin standards; the two 3 H-labeled peaks were completely immunoprecipitable with an insulin antibody and the second peak coincided with the insulin immunoreactive fraction of the cell extract.

Autoradiographs were developed as described by Schuit et al. (4). Briefly, the 3 H-labeled cell preparations were fixed for 30 min in 2%

p-formaldehyde, washed with PBS containing 0.1% BSA, and dried overnight on poly(L-lysine)-coated glass slides. The cells were then postfixed for 15 min in 2% glutaraldehyde and washed with distilled water. The slides were exposed for 90 min to an autoradiographic emulsion (L-4; Ilford Ltd., Basildon, Essex, UK), which was developed for 7 min in ID-11 (Ilford) at 20°C and then fixed for another 7 min in Hypam (Ilford). The cell preparations were counterstained with hematoxylin. A total of at least 125 cells were analyzed per condition. Analysis consisted in counting the number of silver grains per cell at a screen magnification of 5,800 using contrast-enhanced video microscopy. As it was not possible to count the grains in cells with a high grain density, cells with more than 40 grains were considered as one group. Within an experiment a cell was considered to be biosynthetically activated when

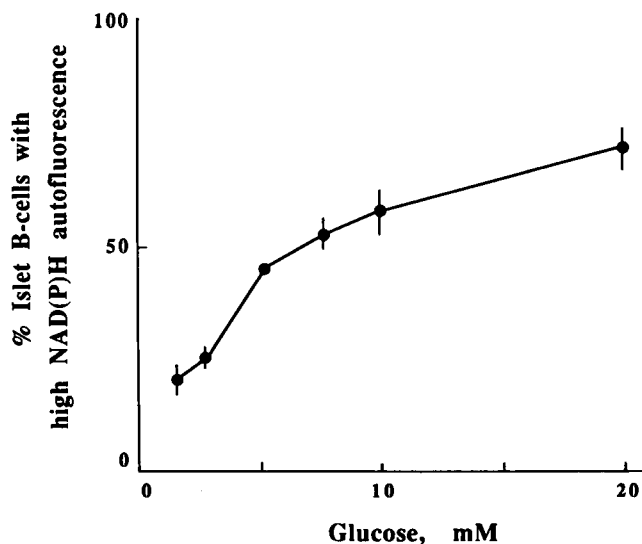


Figure 2. Dose-response curve for the effect of glucose on the percent B cells with high NAD(P)H autofluorescence. Data represent mean values \pm SEM of 6 to 18 experiments.

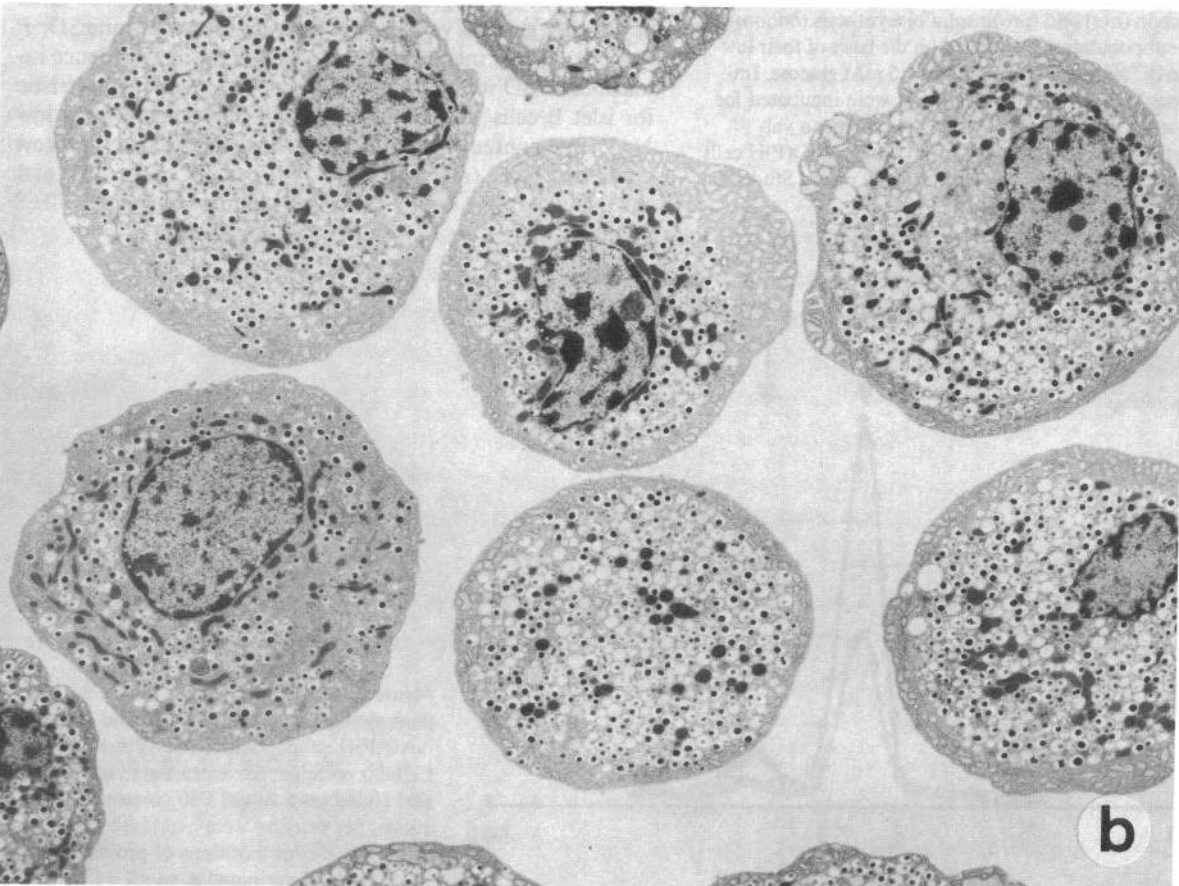
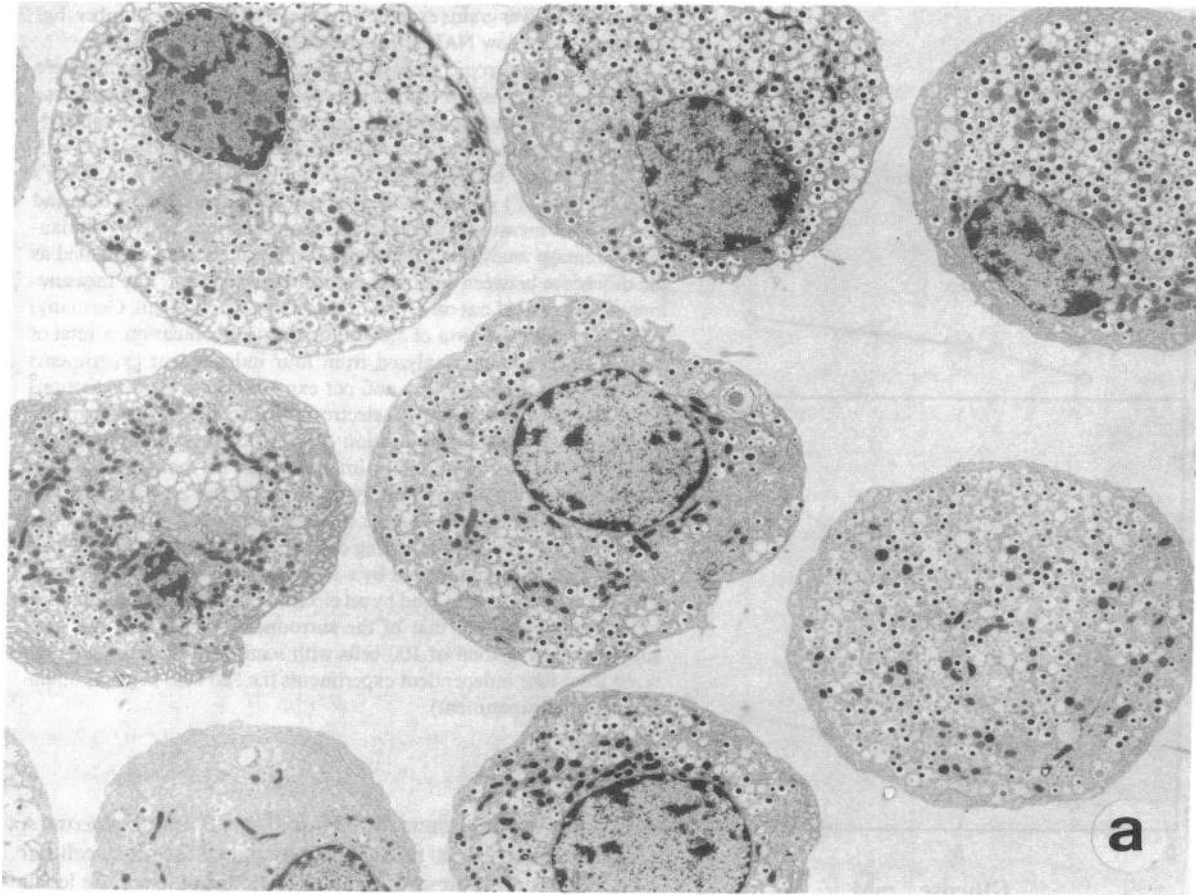


Figure 3. Electron micrographs of B cells recovered in the high (a) and low (b) NAD(P)H autofluorescence windows after 15 min of exposure to 7.5 mM glucose. The cells from both subpopulations exhibit an intact ultrastructure.

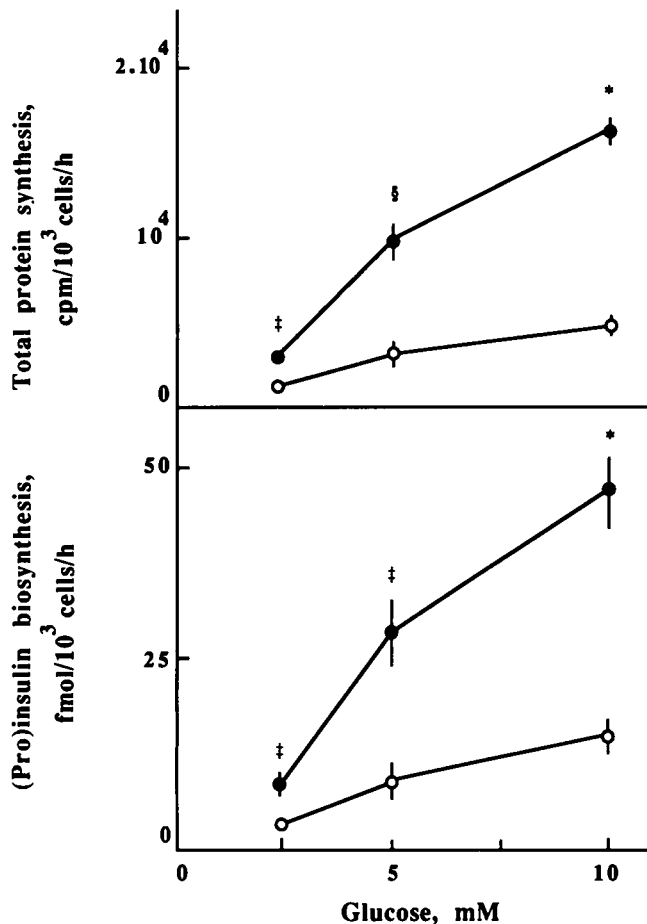


Figure 4. Total protein (top) and (pro)insulin biosynthesis (bottom) in the two islet B cell subpopulations separated on the basis of their low (○) and high (●) NAD(P)H autofluorescence at 7.5 mM glucose. Immediately after separation, the two preparations were incubated for 60 min at 2.5, 5, or 10 mM glucose. Data represent mean values \pm SEM of eight to nine experiments. Low and high NAD(P)H cells were compared at the same glucose concentration by paired Student's *t* test. **P* < 0.001; †*P* < 0.005; ‡*P* < 0.01.

its number of silver grains exceeded the mean + 2 SD of the number that was found in the low NAD(P)H cells labeled at 1.4 mM glucose.

Electron microscopic analysis. The two subpopulations that were separated on the basis of their (un)responsiveness to 7.5 mM glucose were fixed in 2.5% glutaraldehyde (prepared in 0.1 M sodium cacodylate pH 7.4), postfixed in 1% osmium tetroxide, and stained en bloc with 1% uranylacetate before being dehydrated and embedded in Spurr's resin. Sections of 1 μ m thickness were stained with methylene blue and then used for measuring total and nuclear cell surface areas by semiautomatic image analysis. Cytoplasmic surface areas were calculated as the difference between total and nuclear surface areas. The measurements were carried out on a Videoplan (Zeiss, Oberkochen, Germany) at a screen magnification of 5,800. For each subpopulation, a total of 400 cell profiles were analyzed from four independent experiments (100 cells per subpopulation and per experiment). Ultrathin sections were examined in a Zeiss 9S2 electron microscope, and micrographs were taken at a final magnification of 17,300. The micrographs were also submitted to semiautomatic image analysis for the counting of the number of secretory vesicles per cytoplasmic surface area. Analysis was restricted to vesicles with a sectioned granule core. A distinction was made between vesicles containing dark and pale granule cores. A pale secretory granule was defined by a large core, occupying at least two-thirds of the vesicle area, and by an electron density that was comparable to, or lower than, that of the surrounding cytoplasm. For each subpopulation, a total of 100 cells with sectioned nucleus were analyzed from four independent experiments (i.e., 25 cells per subpopulation and per experiment).

Results

Metabolic responsiveness of individual islet B cells to glucose. A 15-min exposure to 20 mM glucose increased the mean cellular NAD(P)H-autofluorescence intensity threefold over the levels measured at 1 mM glucose (78 ± 14 relative fluorescence units at 20 mM glucose vs. 26 ± 6 at 1 mM glucose; mean \pm SD, *P* < 0.001). These mean cellular NAD(P)H autofluorescence intensities \pm 2 SD were taken to delineate the areas characteristic for islet B cells with a high (Fig. 1 *b*, high) and with a low NAD(P)H content (Fig. 1 *a*, low). The low NAD(P)H window contained 82 ± 7 (mean \pm SD, *n* = 18) percent of the cells at 1 mM glucose, the high NAD(P)H window collected 70 ± 8

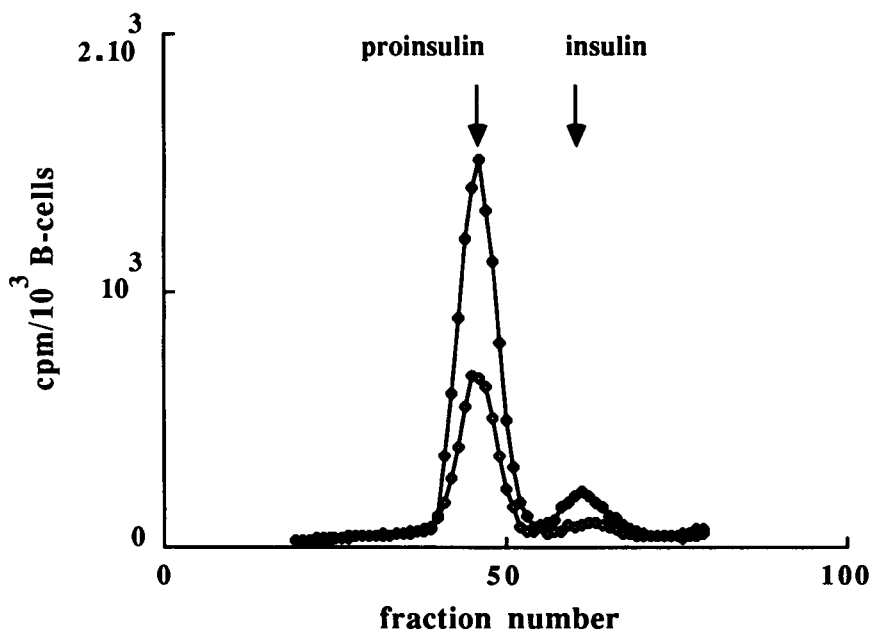


Figure 5. Elution pattern of ³H-labeled proteins synthesized in the low (○) and high (●) NAD(P)H subpopulations at 10 mM glucose. Cellular proteins were extracted in acetic acid and eluted on a Biogel P10 column. The counts per fraction were expressed per 10³ cells. The elution positions of proinsulin and insulin were determined as in ref. 11.

(mean \pm SD, $n = 7$) percent at 20 mM. Analysis of the individual cell responses to 20 mM glucose indicated that not all glucose-exposed B cells showed an increase in NAD(P)H content: 30 \pm 8 percent (mean \pm SD) remained in the window of cells with low cellular NAD(P)H levels (Fig. 1 *b*, broken line). On the other hand, 18 \pm 7 (mean \pm SD) percent of the cells analyzed at 1 mM glucose appeared within the window of cells with high cellular NAD(P)H content (Fig. 1 *a*, broken line). These results indicate the existence of a heterogeneity in the metabolic redox state of individual B cells, whether these cells are examined at 1 or at 20 mM glucose. They also demonstrate that glucose increases the number of islet B cells with high NAD(P)H content. To determine the dose responsiveness of this glucose effect, we measured the percent of pancreatic B cells with high NAD(P)H autofluorescence after 15 min exposure to four intermediate glucose concentrations. It was found that glucose increased, dose-dependently, the number of cells falling within the high NAD(P)H window, the sharpest rise being noticed between 2.5

and 5 mM glucose (Fig. 2). At 7.5 mM glucose, \sim 50% of the cells were recovered within the high NAD(P)H window. This percentage was slightly higher at 10 mM glucose (56 \pm 7) and rose to 70 \pm 3 percent at 20 mM. It was not tested whether glucose concentrations above 20 mM were able to further increase this percentage. In addition to increasing the number of cells with elevated NAD(P)H level, glucose also raised the NAD(P)H fluorescence intensity of metabolically responsive cells. This effect was most marked above 7.5 mM glucose as evidenced by the higher mean autofluorescence intensity of the "high" cells at 20 mM than those at 1 or 7.5 mM glucose (Fig. 1).

Preparation of islet B cells subpopulations with different metabolic responsiveness to glucose. To compare the functional properties of islet B cells with different metabolic responsiveness to glucose, we separated the cells according to their NAD(P)H-autofluorescence intensity at 7.5 mM glucose. This concentration was selected in view of the equal size of the sub-

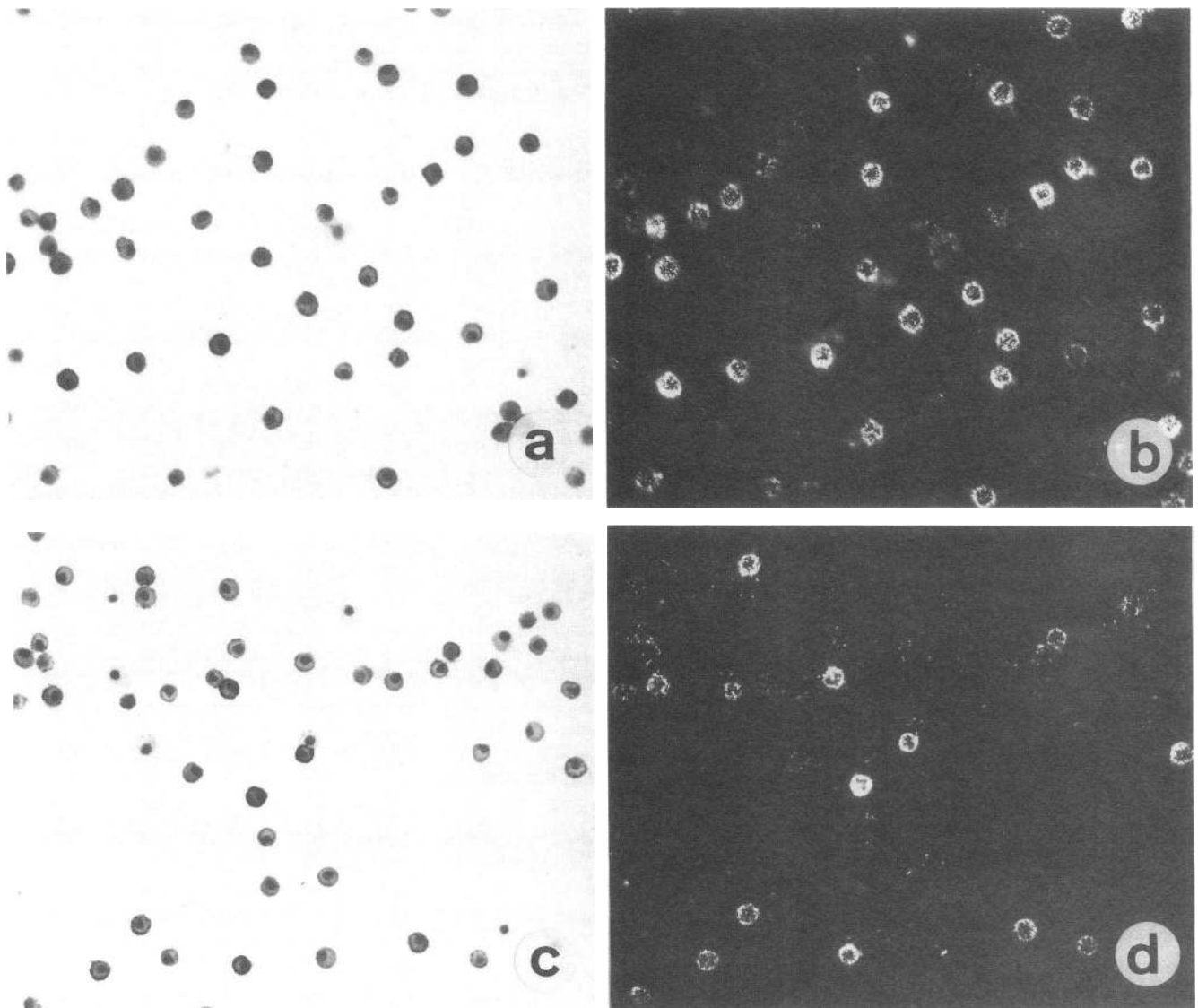


Figure 6. Autoradiographs of high (*a* and *b*) and low (*c* and *d*) NAD(P)H cells incubated with ^3H -tyrosine for 60 min at 7.5 mM glucose. The cells were photographed in normal (*a*-*c*) and epipolarization (*b*-*d*) microscopy. The high NAD(P)H subpopulation contained a higher percent densely labeled cells than the low NAD(P)H subpopulation.

populations collected in the low and high NAD(P)H windows (Figs. 1 and 2). Under this condition, sufficient cell numbers were collected from the low and high windows to undertake the proposed experiments. This would not have been possible in the 1 mM or 20 mM condition. On the other hand, the two subpopulations, separated at 7.5 mM and further denoted as low and high NAD(P)H cells, differ less in their mean fluorescence intensity, than the low and high subpopulation at 20 mM. This is less optimal for detecting functional differences that are associated to cellular differences in redox state.

Immunocytochemistry of the low and high NAD(P)H cells showed that both fractions contained > 95% insulin-positive cells (data not shown). This degree of purity was confirmed by electron micrographs illustrating ultrastructurally intact and well granulated cells in both preparations (Fig. 3). Staining with neutral red indicated a comparable viability (> 90%) of both subpopulations immediately after their isolation; the two cell groups survived equally well during a 10-d culture period (data not shown).

Comparison of glucose-induced protein biosynthesis in islet B cell subpopulations with different metabolic responsiveness to glucose. The two islet B cell subpopulations that were separated according to their distinct NAD(P)H-autofluorescence at 7.5 mM glucose, were compared for their protein biosynthetic activity during a 60-min incubation at different glucose concentrations. At 2.5 mM glucose, the high NAD(P)H cells incorporated fourfold more ³H-tyrosine into total protein than the low NAD(P)H cells (Fig. 4). Their rate of insulin biosynthesis was almost fivefold higher (7.2 ± 1.5 fmol/ 10^3 cells per h vs. 1.5 ± 0.4 fmol/ 10^3 cells per h in the low NAD(P)H preparation, mean \pm SEM, $n = 9$, $P < 0.005$) (Fig. 4). Raising the glucose concentration to 5 and 10 mM resulted, in both preparations, in a dose-dependent increase in the respective rates of protein and proinsulin biosynthesis (Fig. 4). The biosynthetic rates in high NAD(P)H cells remained three to fourfold higher than those in low NAD(P)H cells. In both subpopulations, 60% of the ³H-total protein fraction at 10 and 20 mM glucose, corresponded to newly formed (pro)insulin. When the ³H-labeled material was eluted over a Biogel P-10 column, most of the newly formed proteins were recovered in the proinsulin peak (Fig. 5). No difference was observed in the respective proportions of the insulin peaks: $11 \pm 2\%$ of ³H-IRI in the low NAD(P)H cells and $12 \pm 3\%$ in the high NAD(P)H cells (Fig. 5).

The ³H-labeled B cell preparations were also examined in autoradiographs to determine the percent cells which have contributed to the measured biosynthetic activities (Fig. 6). At 1.4 mM glucose, $20 \pm 4\%$ of high NAD(P)H-cells were involved in protein synthesis, versus only $7 \pm 2\%$ of low NAD(P)H cells (mean \pm SEM, $n = 4$, NS) (Fig. 7). Addition of glucose dose-dependently increased the number of active cells in both preparations. This glucose-induced recruitment occurred almost linearly between 1.4 and 7.5 to 10 mM glucose. The sharpest rise was observed in high NAD(P)H cells, where the addition of 3.6 mM glucose to the basal medium (1.4 mM) recruited $54 \pm 6\%$ of the cells into protein synthesis; in low NAD(P)H cells, this rise in glucose recruited only $28 \pm 10\%$ of the cells (mean \pm SEM, $n = 4$, $P < 0.05$). The maximal level of cell recruitment was obtained at 7.5 mM glucose for the high NAD(P)H cells. This resulted in a total of $87 \pm 3\%$ active cells. In low NAD(P)H cells, a glucose concentration of 10 mM was required for maximal cell recruitment, which resulted in $65 \pm 6\%$ active cells

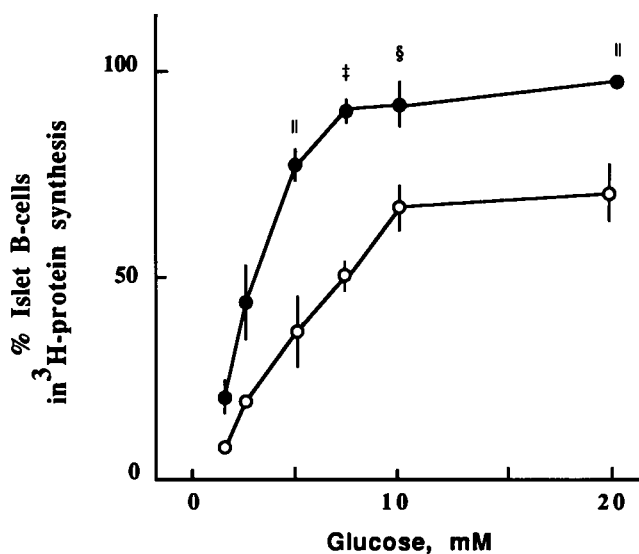


Figure 7. Effect of glucose on the percent biosynthetically activated cells in autoradiographs of low (○) and high (●) NAD(P)H subpopulations which had been separated at 7.5 mM glucose. Data represent mean values \pm SEM of four experiments. Low and high NAD(P)H-cells were compared at the same glucose concentration by paired Student's *t* testing. † $P < 0.005$; § $P < 0.01$; || $P < 0.05$.

(mean \pm SEM, $n = 4$), a significantly lower number than in the high NAD(P)H subpopulation ($P < 0.01$) (Fig. 7).

Comparison of hormone stores in islet B cell subpopulations with different metabolic responsiveness to glucose. The observation of a lower biosynthetic activity in cells with a low metabolic responsiveness to glucose led us to compare the hormone stores in the two separated B cell subpopulations. By immunoassay, the high NAD(P)H cells were found to contain a higher amount of insulin immunoreactive material (68 ± 6 ng/ 10^3 cells) than the low NAD(P)H cells (49 ± 4 ng/ 10^3 cells, mean \pm SEM, $n = 11$, $P < 0.01$). The high NAD(P)H cells presented also a larger cytoplasmic surface area than the low NAD(P)H cells (Table I), while the number of secretory vesicles per unit cytoplasmic area, was similar in both subpopulations (Table I). However, when the relative amount of a pale secretory vesicle subtype was compared in both preparations, markedly higher numbers per unit cytoplasmic area were counted in high NAD(P)H cells than in low NAD(P)H cells ($P < 0.05$, Table I and Fig. 8). Pale granules were noticed in 12% of the secretory vesicles in the high NAD(P)H subpopulation, versus only 4% in the low NAD(P)H subpopulation (Table I).

Discussion

We have previously reported that *in vitro* incubated islet B cells do not exhibit an identical state of protein synthesis (4). Glucose increased, dose-dependently, the percent B cells with activated biosynthetic activity (4). These results indicate that islet B cells differ in their individual responsiveness to glucose. The molecular basis of this cellular heterogeneity has not yet been identified. It could be located at the level of glucose handling or at the site where glucose-induced signals control translation. The present study points to the existence of intercellular differences in glucose handling. The cellular redox state was taken as

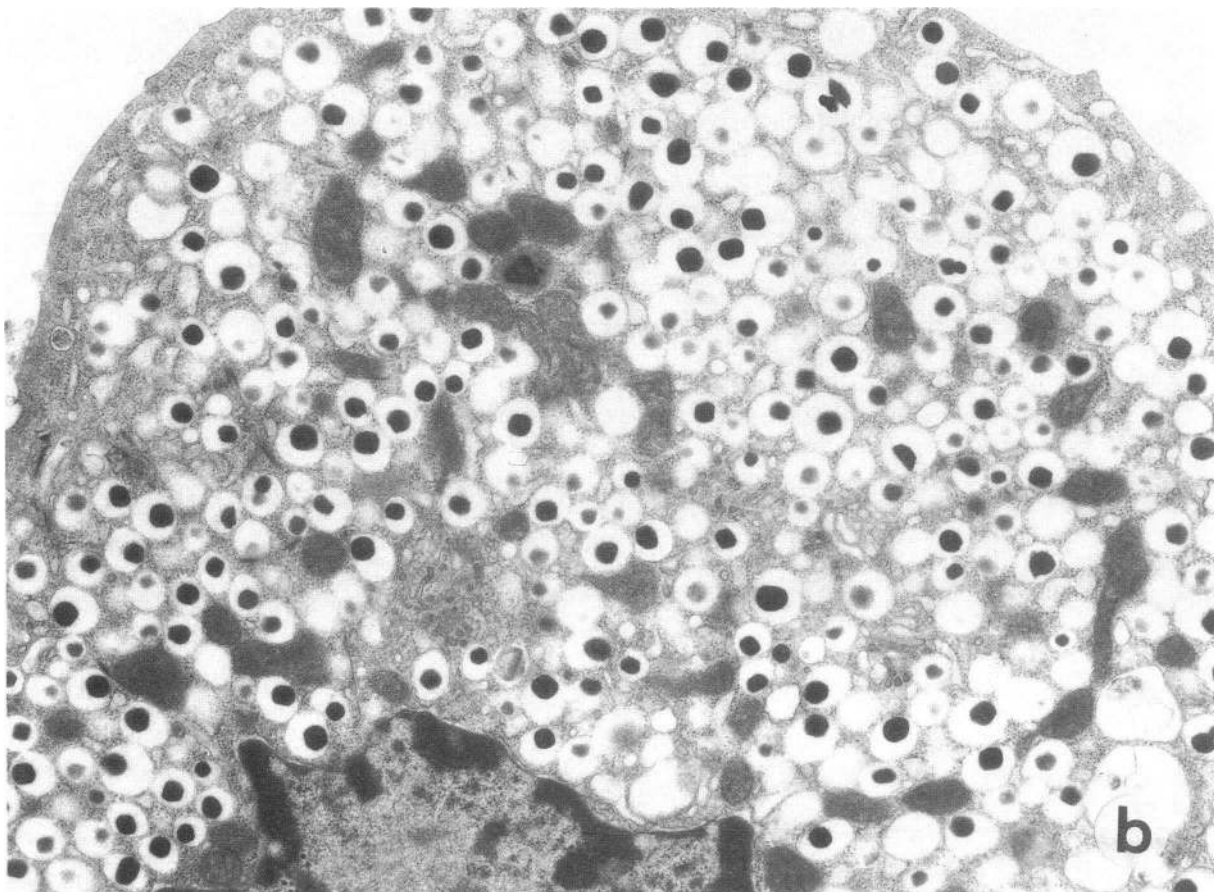
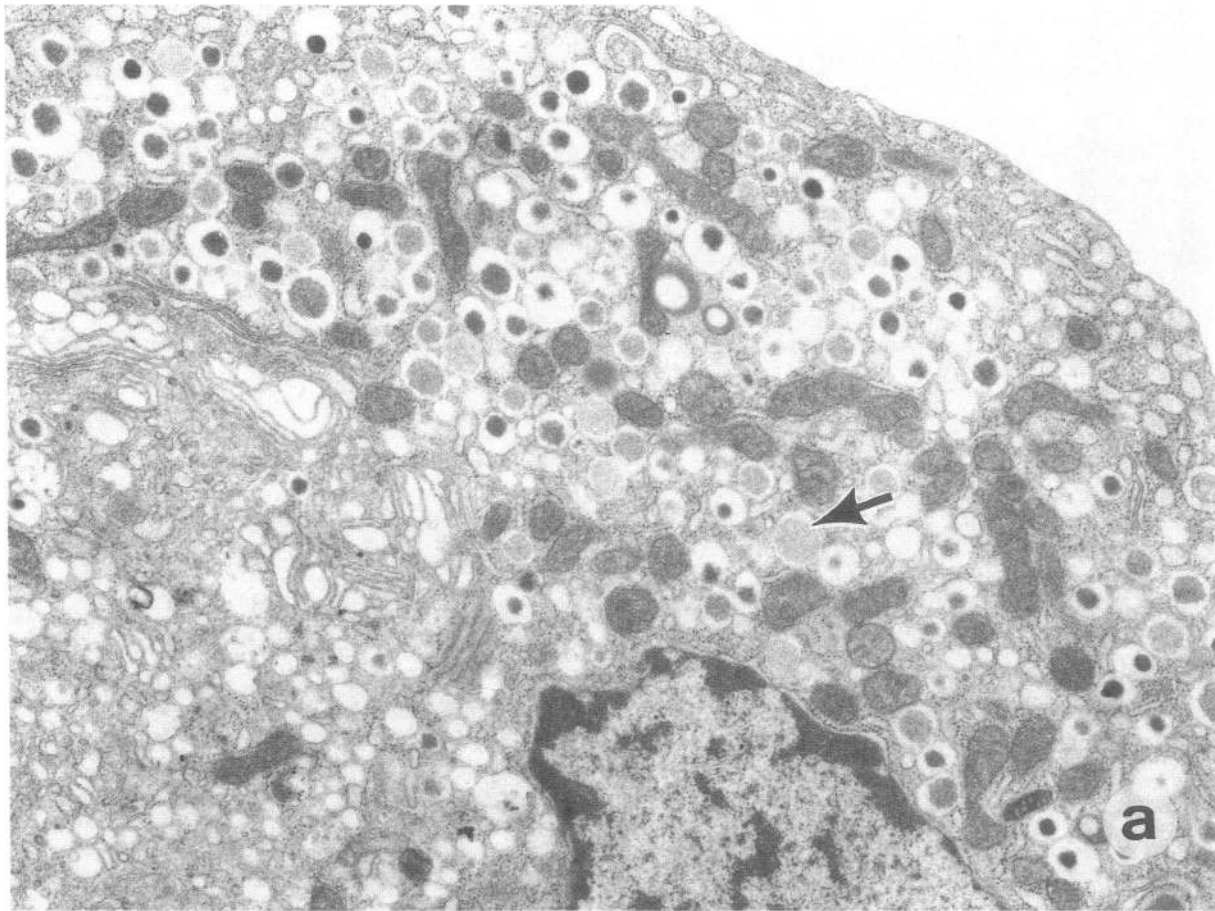


Figure 8. Electron micrographs of B cells collected in the high (a) and low (b) NAD(P)H-window after exposing the cells to 7.5 mM glucose. High NAD(P)H cells display similar levels of granulation as low NAD(P)H cells, but contain a higher amount of a pale secretory vesicle subtype (arrow).

Table I. Morphometric Comparison of Low and High NAD(P)H Cells Separated at 7.5 mM Glucose

		Low	High	P
Total cell surface area	$\mu\text{m}^2/\text{cell}$	106±6	127±5	$P < 0.001$
Cytoplasmic surface area (CSA)	$\mu\text{m}^2/\text{cell}$	81±7	99±6	$P < 0.01$
Number of secretory vesicles				
Total	$n/\mu\text{m}^2$ C.S.A.	1.85±0.26	2.00±0.35	NS
"Pale"	$n/\mu\text{m}^2$ C.S.A.	0.07±0.05	0.25±0.14	$P < 0.05$
	% Total	4±2	12±4	$P < 0.01$

Results are expressed as mean±SD of four independent experiments. The number of sectioned cells per condition are indicated in Results. Differences between the values of low and high NAD(P)H cells are calculated by paired Student's *t* test.

an index for the level of glucose metabolism. It was monitored through the cellular NAD(P)H-autofluorescence intensity, which is known to increase during the fluxes of glycolysis, the Krebs cycle, and the pentose phosphate shunt.

When pancreatic B cell populations were examined at 1 mM glucose, cellular NAD(P)H levels were not normally distributed, with almost 20% of the cells exhibiting higher NAD(P)H levels than the majority of cells which were clustered around values considered as basal. Short exposure to glucose dose-dependently increased the number of B cells with a higher NAD(P)H autofluorescence. This glucose-induced shift in cellular metabolic redox state occurred for 5% of the cells after a rise from 1 to 2.5 mM glucose; an additional 20% of cells responded to an increase from 2.5 to 5 mM, while 25% was only responsive between 5 and 20 mM. Pancreatic B cells thus differ in their individual thresholds for glucose-induced changes in the cellular redox state. Since glucose metabolism in islet B cells regulates the opening or closing of ionic membrane channels (12–14), the intercellular differences in metabolic threshold are likely to be associated with parallel differences in glucose-induced depolarization. The present observations can thus account for the earlier described heterogeneity in electrically recorded signals from glucose-exposed islet cells (15). In view of the well known parallelism between the rate of glucose metabolism and that of insulin synthesis (11, 16), the cellular heterogeneity in metabolic activity can be held responsible for the earlier observed intercellular differences in protein synthesis (4). The glucose-induced recruitment of islet B cells into a protein synthetic activity seems thus to depend on the sugar's ability to overcome the cellular thresholds in the metabolic pathways which regulate B cell function. This recruiting effect was mainly observed between 1 and 7.5 mM glucose. Increasing the glucose concentration above 7.5 mM rather resulted in a further rise of NAD(P)H levels in metabolically activated cells, and may thus be responsible for an amplification of functions in recruited cells.

It is presently unknown where the sites of metabolic variability are located within the insulin-producing B cells. The ability to prepare islet B cell subpopulations which differ in metabolic responsiveness to glucose, makes it feasible to compare glucose responsive and unresponsive subpopulations for the expression and activities of potential key regulators such as the liver-type glucose transporter (17), glucokinase (18, 19), or other glucose-induced signals (20, 21). Such type of investigation may also indicate why a minority of adult rat islet B cells remain unresponsive to all tested glucose concentrations. Although it is difficult to exclude that this subpopulation corre-

sponds to cells which have undergone some damage during the isolation procedure, we have not found any experimental evidence to support this possibility. Most cells of this fraction appear structurally intact by vital staining and electron microscopy, and survive a 10-d culture period. It is to be examined whether the glucose unresponsiveness of these cells results from the in vitro conditions during their preparation or whether it expresses the in situ metabolic state of a particular islet B cell subpopulation.

The correlation between the cellular metabolic responsiveness to glucose and its biosynthetic activity became evident by comparing glucose responsive and unresponsive subpopulations. Metabolically different subpopulations were prepared on the basis of the cellular redox state at 7.5 mM glucose. At this concentration, the distribution of cells is mainly the result of the sugar's recruiting action, i.e., a shift from basal to increased NAD(P)H levels. The majority of cells with a rapid metabolic response to 7.5 mM glucose were also recruited into biosynthetic activity during a subsequent 60-min incubation at this glucose concentration. Despite this relatively long incubation time, markedly less cells were recruited in the subpopulation which had failed to respond to short 7.5 mM glucose exposure. Similar differences in recruitment were noticed when both subpopulations were incubated at lower glucose concentrations. This observation can explain the markedly lower rates of protein synthesis in the 7.5-mM unresponsive B cell preparation. When this subpopulation was incubated at higher glucose concentrations (10 mM), its percent of recruited cells increased, but its rate of protein synthesis did not rise proportionally. Higher glucose levels can thus succeed in the recruitment of cells with poor glucose sensitivity but induce only a relatively small increase in their protein synthetic activity. One explanation for this finding could be a shortage in preproinsulin mRNA, possibly as a consequence of a chronic state of reduced glucose metabolism in these cells. It is thus conceivable that, under basal physiologic circumstances, only a subpopulation of islet B-cells is metabolically responsive to glucose and hence involved in hormone synthesis. Other islet B cells, which are not responsive to physiologic glucose concentrations, seem to participate only marginally to the process of hormone production under these circumstances, but could be activated by conditions of high metabolic demand. This view is supported by morphometric comparison of the 7.5 mM responsive and unresponsive cells. This analysis strongly suggested that the two subpopulations had been in different states of biosynthetic activity before their isolation. High responsive cells were larger, and contained, both in absolute and relative terms, a higher

amount of a pale secretory vesicle subtype which is shown to contain unprocessed hormone (22, 23). We have, so far, not detected any inhibition in the rate of proinsulin conversion in the high responsive cells, so that their higher content in pale granules may be related to the higher state of biosynthetic activity in these cells. The high responsive cells were, on the other hand, found to be more sensitive to glucose stimuli for release (24). Assuming that a higher secretory activity implies a higher release of dark, "mature" granules, this could contribute to a higher proportion of pale over dark granules in the high responsive cells. These observations favor the hypothesis that the frequency of pale granules may serve as a marker for the glucose-responsive state of islet cells in situ. Such study may help answering the question whether the in situ pancreatic B cell population is also composed of cells with different sensitivity to glucose, and hence of cells in a different state of functional activity.

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