Glucose exerts opposite effects upon glucagon and insulin release from the endocrine pancreas. Glucose uptake and oxidation were therefore compared in purified A- and B-cells. In purified B-cells, the intracellular concentration of glucose or 3-O-methyl-D-glucose equilibrates within 2 min with the extracellular levels, and, like in intact islets, the rate of glucose oxidation displays a sigmoidal dose-response curve for glucose. In contrast, even after 5 min of incubation, the apparent distribution space of D-glucose or 3-O-methyl-D-glucose in A-cells remains much lower than the intracellular volume. In A-cells, both the rate of 3-O-methyl-D-glucose uptake and glucose oxidation proceed proportional to the hexose concentration up to 10 mM and reach saturation at higher concentrations. Addition of insulin failed to affect 3-O-methyl-D-glucose or D-glucose uptake and glucose oxidation by purified A-cells.

Glucose releases 30-fold more insulin from islets than from single B-cells, but this marked difference is not associated with differences in glucose handling. The rate of glucose oxidation is virtually identical in single and reaggregated B-cells and is not altered after addition of glucagon or somatostatin. It is concluded that the dependency of glucose-induced insulin release upon the functional coordination between islet cells is not mediated through changes in glucose metabolism.

The regulation by circulating nutrients of insulin and glucagon release from the endocrine pancreas plays an essential role in the hormonal control of fuel homeostasis. The observation that glucose exerts opposite effects upon insulin and glucagon release (1–7) raises questions as to the mechanisms involved in such opposite actions on neighboring cells. Glucose recognition by the pancreatic B-cell has been extensively studied in rat and mouse islets, which contain 60–90% B-cells (13). The analysis of A-cells has been hampered by their low occurrence in normal rodent islets (10–30% of islet cells); they have been examined predominantly in other experimental models such as the A-cell-rich islets from birds (19). Glucose releases 30-fold more insulin from islets than from single B-cells, but this marked difference is not associated with differences in glucose handling. The rate of glucose oxidation is virtually identical in single and reaggregated B-cells and is not altered after addition of glucagon or somatostatin. It is concluded that the dependency of glucose-induced insulin release upon the functional coordination between islet cells is not mediated through changes in glucose metabolism.

**Experimental Procedures**

Materials—Only siliconized (silicone solution, Serva, Heidelberg, Federal Republic of Germany) glassware or disposable plastics were used. Collagenase, trypsin, DNase, antymycin A, and rotenone were purchased from Boehringer (Mannheim, FRG); poly-l-lysine (M, > 180,000) from Sigma, HEPES and neutral red from Merck (Darmstadt, FRG); bovine serum albumin fraction V from Armour (Armour, Bucks, United Kingdom); CMRL-1066 tissue culture medium from Flow (Irvine, Scotland); heat-inactivated fetal calf serum, Eagle’s minimal essential medium with Earl’s salts, and penicillin G from Gibco (Paisley, Scotland); Percoll and Dextran T70 from Pharmacia (Uppsala, Sweden); streptomycin sulfate from Pfizer (Brussels, Belgium); hydroxide of Hyamine 10x from Packard Instrument Co.; di-n-butyl phthalate oil (d 1.045 g/ml) from BDH Chemicals (Poole, Dorset, UK); lyophilized highly purified rat insulin and recrystallized highly purified porcine glucagon from Novo Industries (Bagvaard, Denmark); ovine somatostatin from Beckman Instruments; D-[U-14C]glucose, [14C]urea, and 3-O-methyl-D-[U-14C]glucose from Amer sham (Amersham, Bucks, UK); and Dyna-Gel scintillation mixture from Baker (Devter, The Netherlands). Unless otherwise stated, metabolic studies were carried out in Krebs-Ringer medium buffered with bicarbonate and HEPES at pH 7.4 (37 °C) and containing 0.6% (w/v) bovine serum albumin (17). Cell counts were performed in Burker counting chambers.

Preparation of Purified Islet A- and B-cells—Islets of Langerhans were prepared by collagenase digestion of pancreata from fed adult male Sprague-Dawley rats and were dissociated by trypsin treatment in calcium-free KRBB (17). Single B-cells (more than 95% B-cells) were isolated from non-B-cells (more than 85% A-cells and less than 3% B-cells) by fluorescence-activated cell sorting (18). Non-B-cell fractions will also be referred to as A-cell fractions. The purified islet cells were used either immediately after their separation or following a 16-h culture period at 37 °C and in humidified 95% air, 5% CO2 (19).

Reaggregated B-cells were obtained by culturing single B-cells at 30,000 cells/ml in a rotatory shaker incubator (30 rpm; Braun, Melsungen, FRG); after 16 h in basal culture medium (CMRL-1066, 5 mM glucose), clusters of 10–100 cells were collected from the culture flasks. After filtering the cultured cells through a Percoll layer of density 1.045 g/ml (17), the cells were washed and finally taken up in the media used for uptake or oxidation experiments.

In both cultured and uncultured cell preparations, more than 90% of the cells accumulated neutral red.

Preparation of Fibroblasts—Fibroblasts were prepared by trypsin treatment of finely minced 14-day-old rat embryos (20); they were grown to confluency at 37 °C and 10% CO2 in 25-cm2 Falcon tissue culture flasks. After filtering the cultured cells through a Percoll layer of density 1.045 g/ml (17), the cells were washed and finally taken up in the media used for uptake or oxidation experiments.

In both cultured and uncultured cell preparations, more than 90% of the cells accumulated neutral red.
culture flasks (1-2 days) containing Eagle's minimal essential medium with Earl's salts and 10% (v/v) heat-inactivated fetal calf serum. After four cycles of trypsin treatment of the cultured cells and subsequent reculturing, they were distributed in 0.1-ml culture medium with Earl's salts and 10% (v/v) heat-inactivated fetal calf serum for uptake experiments after confluency was reached (approximately 10⁵ cells/well).

Glucose Uptake—After preincubating the purified islet cells for 30 min at 37 °C in glucose-free KRBH, they were distributed in 0.1-ml samples containing 75,000 cells and submitted to a further 1-30 min incubation at 37 °C in the presence of 0.6 mM [6,6'-3H]sucrose (25 μCi/ml) and 0.6 mM [1-14C]glucose (12.5 μCi/ml), 0.6-20 mM 3-O-methyl-d-[U-14C]glucose (12.5 μCi/ml), or 0.6-20 mM d-[U-14C]glucose (12.5 μCi/ml). The uptake experiments were stopped by filtering the cells through a 4-s centrifugation (8000 × g; Microfuge B, Beckman Instruments) and submitted to a further 1-30 min incubation and counted in 10 ml of Dyna-Gel. Calculations were carried out as described above for islet cells.

Glucose Oxidation—Glucose oxidation experiments were performed in siliconized glass tubes containing 75,000 islet cells in 0.06 ml of KRBH and 0.6-30 mM d-[U-14C]glucose (10 μCi/ml). Each tube was placed in a sealed 15-ml scintillation vial, and oxidation experiments were carried out as previously described (22). Incubation time was 1 h for B-cells and 2 h for non-B-cells.

Statistical Analysis—Results are expressed as mean ± S.E. for the number of experiments stated in parentheses. The statistical significance of differences between experimental groups was assessed by the Student t test for unpaired data.

RESULTS

Glucose Uptake—Raising the concentration of d-glucose or its nonmetabolizable analogue, 3-O-MG results in a rapid equilibration across the B-cell membrane. Within 2 min, the apparent distribution space of both sugars equals the cellular water space as estimated by urea distribution (Fig. 1). This phenomenon is observed at both low (0.6 mM) and high (20 mM) glucose concentrations (Table I). After this first initial rise, a further but much slower accumulation of radioactivity is noted with [14C]glucose, but not with labeled 3-O-MG (Fig. 1). A modest increase of the urea space in non-B-cells is noted between the 5th and 30th minute of incubation.

D-Glucose and 3-O-MG uptake proceeds at a much slower rate in islet non-B-cells than in B-cells. Even after 30 min, their apparent distribution space does not exceed 70-80% of the corresponding urea space (Fig. 1). At 0.6 mM, d-glucose uptake is slightly, although not significantly, higher than that of 3-O-MG (Fig. 1). The initial rate of 3-O-MG uptake remains constant over the first 5 min and proceeds proportionally to the prevailing 3-O-MG levels (Fig. 2) up to at least 10 mM. Eadie-Hofstee analysis suggests a $K_m$ of 15-25 mM and a $V_{max}$ of 20-30 fmol of 3-O-MG/h/cell for the putative carrier-mediated glucose transport in non-B-cells. Preincubation and incubation of non-B-cells in the presence of insulin (0.5-4.0 μg/ml) does not significantly alter glucose or 3-O-MG uptake (Table II).

All presented uptake results were collected on uncultured islet cells; identical results were obtained for cultured B- and non-B-cells (results not shown). In contrast to non-B-cells, fibroblasts incorporated 2-fold more glucose when exposed to 4 μg/ml of insulin (Table II).

Glucose Oxidation—The dose-response curve of glucose oxidation in uncultured B-cells (Fig. 3) displays a sigmoidal shape with an apparent $K_m$ of 8 mM and a $V_{max}$ of 85 fmol of CO₂ produced per h/cell. From 1.4 mM glucose on, the curve proceeds smoothly to higher oxidation rates, reaches its steepest slope between 5 and 12 mM, and approaches towards $V_{max}$ near 16 mM. Cultured B-cells exhibit a comparable dose-response curve (results not shown).

In non-B-cells, the glucose oxidation rate increases linearly
with the external glucose concentration up to 10–15 mM and levels off at higher concentrations (Fig. 3). The estimated $K_m$ is comparable to that observed for glucose transport, whereas $V_{max}$ approximates 25–35 fmoles of CO$_2$/h/cell. The presence of insulin (0.5 μg/ml) has no significant effect on the amount of glucose oxidized by non-B-cells in the presence of 16.7 mM glucose (Table IV). A 16-h culture period at 5.5 mM glucose levels off at higher concentrations (Fig. 3). The estimated $K_m$ with the external glucose concentration up to 10–15 mM and levels off at higher concentrations (Fig. 3). The estimated $K_m$ is comparable to that observed for glucose transport, whereas $V_{max}$ approximates 25–35 fmoles of CO$_2$/h/cell. The presence of insulin (0.5 μg/ml) has no significant effect on the amount of glucose oxidized by non-B-cells, nor did it induce responsiveness to insulin (results not shown).

Neither glucagon (1 μg/ml) nor somatostatin (50 ng/ml) alters the rate of glucose oxidation by B-cells (Table IV). Glucose oxidation rates in cultured single B-cells were similar to those measured in reaggregated B-cells (Table IV). Furthermore, the glucose oxidation rate of freshly isolated islets compared favorably with that of uncultured purified B- and non-B-cells isolated from the same batch of islets. In this experiment, islets were found to contain in average 1500 B-cells and 600 A-cells as judged from the insulin and glucagon content of the different cell preparations. Taking into account the measured rates of glucose oxidation of the purified B- and non-B-cells at 8.3 and 16.7 mM glucose together with the above mentioned calculated cell numbers/islet, the predicted glucose oxidation rate/islet averaged 125 pmol of CO$_2$/h at 16.7 mM glucose and 75 pmol of CO$_2$/h at 8.3 mM, which agreed well with our measured values of 112 ± 4 (n = 3) and 69 ± 9 (n = 3), respectively. It thus appears that the amount of glucose oxidized per islet at 8.3 or 16.7 mM glucose is comparable to the sum of the quantities oxidized by an equivalent number of single islet cells.

**Discussion**

In basal media containing no amino acids, glucose uptake proceeds much slower in A-cells than in B-cells; Eadie-Hofstee analysis of glucose uptake by non-B-cells indicates the existence of a low capacity glucose transport system with a high $K_m$ value. This glucose uptake process probably represents a rate-limiting step in glucose metabolism, as it exhibits almost an identical dose-response curve as glucose oxidation.

An increase in extracellular glucose lowers circulating glucagon levels in normal man and animals (5, 13, 23); it also decreases glucagon release from perfused pancreas of normal rodents (5, 24). It is far unknown whether this suppressed A-cell function is the result of a direct interaction with glucose or the consequence of glucose-induced changes in the behavior of the adjacent B- or D-cells (3, 5). The investigation of glucose handling by A-cells has been hampered by the lack of purified A-cell preparations from normal pancreas. A-cell-enriched models have been developed from animals with experimental diabetes but have not clarified the issue (5). Using islets from streptozotocin diabetic guinea pigs, Östenson (25) demonstrated that glucose metabolism in A-cells increases with the extracellular glucose levels and with the presence of insulin; a reduced glucagon release was only observed after addition of both insulin and glucagon. In studies on diabetic ducks and dogs, glucagon suppression by glucagon required the presence of insulin both before and during the exposure to high glucose (13, 26). On the other hand, Pagliara et al. (24) and Matschinsky et al. (27) that insulin was unable to elevate glucose transport in islets from streptozotocin diabetic rats and was, in addition, not necessary for glucagon suppression of glucagon release. The major question emerging from the current, sometimes conflicting, information concerns the effect of insulin upon normal A-cells, both in terms of their glucose handling and their hormone release. The availability of A-cell suspensions, prepared from normal rats and almost devoid of insulin-containing B-cells, makes it

| Cell type | ³C-labeled compound (mM) | Incubation time (min) | Distribution space (fl/cell) |
|-----------|-------------------------|----------------------|-----------------------------|
| **Non-B-cells** | | | |
| | Urea (0.6) | 5 | 201 ± 8 (5) | 54 ± 8 (5) |
| | 3-O-MG (0.6) | 5 | 62 ± 9 (5) | 135 ± 23 (3) |
| | d-Glucose (0.6) | 15 | 65 ± 3 (3) | 65 ± 4 (3) |
| | d-Glucose (1.4) | 30 | 201 ± 23 (3) | 212 ± 5 (3) |
| | d-Glucose (20.0) | 15 | 110 ± 13 (3) | 118 ± 19 (3) |
| **Fibroblasts** | d-Glucose (0.6) | 15 | 1689 ± 83 (3) | 3588 ± 217 (3) |

Values are means ± S.E. followed by the number of individual observations (n).
exclude, it is unlikely that the cell dissociation procedure has
the same conditions, Van Schravendijk et al.2 were unable to
detect insulin receptors on non-B-cells. Although difficult to
exclude, it is unlikely that the cell dissociation procedure has
possible to examine this question directly.

The addition of insulin to cultured rat A-cells remained
without effect upon the rate of glucose transport and oxida-
tion, at least during exposure times not exceeding 2 h. Under
the same conditions, Van Schravendijk et al.2 were unable to
detect insulin receptors on non-B-cells. Although difficult to
exclude, it is unlikely that the cell dissociation procedure has
2 C. Van Schravendijk, E. Hooge-Peters, P. De Meyts, and D.
Pipeleers, unpublished observations.

damaged the putative insulin receptors and therefore masked
the insulin effects. Indeed, fibroblasts prepared by a similar
dissociation method were characterized by an insulin-depend-
ent glucose uptake, comparable to that observed in previous
reports (28-30); furthermore, a 16-h culture period, which is
assumed to restore at least partially the membrane of trypsin-
exposed cells (31), was not followed by an insulin responsive-
ness. Our results are therefore compatible with those of Mat-
schinsky et al. (27), where the addition of insulin to islets
from diabetic rats did not result in an increased glucose
transport (27). It appears unlikely that chronic exposure to
insulin is a prerequisite for the cells to respond acutely to the
hormone, since insulin failed to affect glucose uptake or
oxidation in freshly isolated A-cells.

The rate of glucose uptake by purified single B-cells indica-
tes the existence of a high capacity glucose transport sys-
tem, which adjusts almost instantaneously the intracellular
concentration to that in the extracellular space; these
results confirm the conclusions obtained from studies on
unpurified islet preparations (22). In addition to the rapid
equilibration phase, a second slower and continuous phase
was recognized in the glucose transport curves of B-cells; as
this component was not observed with the nonmetabolized 3-
O-MG, it is associated to the rate of glucose metabolism in
single B-cells (33). It should be noted that glucose uptake by
single insulin-containing B-cells was measured at insulin
concentrations of 0.1-0.2 μg/ml, which corresponds to the
hormone discharge during the preincubation period (19).

The dose-response curve of glucose oxidation by purified
single B-cells displays the same sigmoidal shape as in intact
islets (34); at the different glucose concentrations tested, CO2
production by single B-cells was comparable to that calculated
for B-cells which are incorporated into intact islets. The
sigmoidal aspect of the curve is therefore a characteristic of
glucose handling by individual B-cells, rather than an expres-
sion of metabolic coupling between islet cells or a result from
paracrine effects of locally released glucagon or somatostatin.
This view is also illustrated by the unaltered rate of glucose
oxidation after aggregation of single cells or during exposure
to glucagon or somatostatin.

In contrast to the similarity in glucose handling between

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### Table III

**Glucose oxidation by purified islet non-B-cells at 37 °C**

| Medium            | Glucose concentration | Glucose oxidation |
|-------------------|-----------------------|-------------------|
| KRBH              | 16.7 mM               | 11.7 ± 0.7 (3)    |
| KRBH + insulin    | 16.7 (0.5 μg/ml)      | 11.6 ± 1.8 (3)    |

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### Table IV

**Glucose oxidation by purified cultured (16 h) islet B-cells in various media at 37 °C**

| Cell preparation | KRBH medium | Glucose concentration | Glucose oxidation |
|------------------|-------------|-----------------------|-------------------|
|                  | 16.7 mM     | 88 ± 3 (8)            |
|                  | 8.3         | 53 ± 3 (8)            |
|                  | 1 μg/ml glucagon | 16.7 ± 8 (8)    |
|                  | 50 ng/ml somatostatin | 16.7 ± 9 (5)     |
|                  | Control     | 8.3 ± 4 (4)          |
|                  | 50 ± 6 (4)  |

*Statistically not significantly different from oxidation by single cells in KRBH with 16.7 mM glucose.

*Statistically not significantly different from oxidation by single cells in KRBH with 8.3 mM glucose.
single B-cells and intact islets, single B-cells release 30-fold less insulin in response to glucose than intact islets (19). The poor secretory activity of single B-cells is thus not caused by some metabolic defect in glucose catabolism. These results also indicate that glucose metabolism alone is not sufficient for appropriate regulation by glucose of insulin release. The secretory response is markedly amplified when single B-cells are incubated in the presence of A-cells or glucagon or when they are allowed to reaggregate (35). No alteration in glucose handling appears involved in this amplification, suggesting that the functional coordination between islet cells (19) depends on other factors, possibly messengers such as calcium or cAMP, which are known to participate in the process of insulin release (11, 11, 36).

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