Effect of glucose on the intracellular pH of pancreatic islet cells

Per LINDSTRÖM and Janove SEHLIN

Department of Histology and Cell Biology, University of Umeå, S-901 87 Umeå, Sweden

(Received 3 November 1983/Accepted 23 November 1983)

To characterize the effect of glucose on the intracellular pH (pH_i) of pancreatic islet cells, we measured the accumulation of ^{14}C -labelled 5,5-dimethyloxazolidine-2,4-dione ($[^{14}\text{C}]\text{DMO}$) in β -cell-rich islets from *ob/ob* mice. D-Glucose (20 mM) stimulated insulin release and enhanced the $[^{14}\text{C}]\text{DMO}$ equilibrium uptake corresponding to an increase of pH_i by about 0.15 unit. The glucose effect on DMO uptake was concentration-dependent, with half-maximal effect at about 4 mM-glucose and maximum effect at about 10 mM-glucose. It was inhibited by 20 mM-mannoheptulose and potentiated by 4 mM-L-5-hydroxytryptophan, but not affected by 2 mM-theophylline. Mannoheptulose is an inhibitor and L-5-hydroxytryptophan and theophylline are potentiators of glucose-stimulated insulin release. The glucose-induced increase in pH_i appeared rapidly (7 min) and persisted for at least 30 min and it was observed both in bicarbonate/ CO_2 -buffered and in Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]-buffered media. Addition of extracellular bicarbonate buffer lowered the pH_i , but did not affect basal insulin release, whereas 5 mM- NH_4^+ increased pH_i and induced a 4-fold increase of basal insulin release. We conclude that, in contrast with previous assumptions, glucose increases intracellular pH in the islet cells. This effect may be coupled to the glucose metabolism and associated with triggering of insulin release.

The intracellular H^+ activity (pH_i) is important for the regulation of many cellular activities (see Roos & Boron, 1981; Nuccitelli & Deamer, 1982). It has been suggested that a decrease in pH_i in the pancreatic β -cells could be an important step in the stimulus-secretion coupling in glucose-induced insulin release (Malaisse *et al.*, 1980; Pace *et al.*, 1983). However, in previous direct attempts to measure pH_i in islet cells, no such glucose-induced decrease in pH_i has been found (Hellman *et al.*, 1972; Malaisse *et al.*, 1979a; Lebrun *et al.*, 1982; Deleers *et al.*, 1983). In fact, in all those studies there was some tendency for glucose to increase pH_i . We have re-investigated the effects of glucose on the pH_i in pancreatic β -cells by measuring the accumulation of $[^{14}\text{C}]\text{DMO}$ in β -cell-rich pancreatic islets of *ob/ob* mice. A preliminary account of these results has been presented elsewhere (Lindström & Sehlín, 1983).

Abbreviations used: pH_i , intracellular pH; $[^{14}\text{C}]\text{DMO}$, 5,5-dimethyl[2- ^{14}C]oxazolidine-2,4-dione; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Materials and methods

Materials

$[^{14}\text{C}]\text{DMO}$ and D-[5- ^3H]glucose were from Amersham International, Amersham, Bucks., U.K. L-[1- ^3H]glucose and Aquasol were from New England Nuclear, Dreieich, Germany. Hyamine 10-X and Instafluor were from Packard Instrument Co., Downers Grove, IL, U.S.A. L-5-Hydroxytryptophan, mannoheptulose and Hepes were bought from Sigma Chemical Co., St. Louis, MO, U.S.A. Theophylline was from Mann Research Laboratories, New York, NY, U.S.A. Bovine serum albumin (Fraction V) was from Miles Laboratories, Stoke Poges, Bucks., U.K. Other chemicals were commercially available reagents of analytical grade.

Animals

Islets microdissected from non-inbred *ob/ob* mice (Umeå *ob/ob*) were used throughout. Islets from these animals are large and contain an unusually high proportion of insulin-producing β -cells (>90%; Hellman, 1965). They are therefore useful in studies of pancreatic β -cell function.

Incubation media

Two different basal media were used. One medium (KRBH) was gassed with O_2/CO_2 (19:1) and had the following composition: 115 mM-NaCl, 4.7 mM-KCl, 2.56 mM- $CaCl_2$, 1.2 mM- KH_2PO_4 , 1.2 mM- $MgSO_4$, 25 mM- $NaHCO_3$ and 20 mM-Hepes, adjusted with NaOH to pH 7.40. In the other medium (KRH) the bicarbonate was omitted, only Hepes was used as buffer and the gas phase was ambient air. The composition of this medium was: 130 mM-NaCl, 4.7 mM-KCl, 2.56 mM- $CaCl_2$, 1.2 mM- KH_2PO_4 , 1.2 mM- $MgSO_4$ and 20 mM-Hepes; pH was adjusted to 7.40 with NaOH. Unless otherwise stated both basal media were supplemented with 1 mM-D-glucose and 3 mg of bovine serum albumin/ml.

Incubation procedures

The rationale for the [^{14}C]DMO-uptake method for determining pH_i has been described by Waddell & Butler (1959). The technique used here is a slight modification of that described previously for studies on isolated pancreatic islets (Hellman *et al.*, 1972). Groups of five microdissected islets were first preincubated for 30 min at 37°C. Then they were transferred to new vials and incubated for 7–40 min with additives as indicated in the Tables and in Fig. 4. Control experiments revealed that [^{14}C]DMO equilibrated in the islet cells within the initial 7 min (see Tables 2 and 3 and Fig. 1). The incubation media were supplemented with 89 μ M- [^{14}C]DMO (2 TBq/mol) and 0.9 μ M-L-[1- 3H (n)]glucose (400 TBq/mol) as a marker for the extracellular space.

After the incubation, the islets were placed on a piece of aluminium foil and rapidly freed of extracellular fluid with a micro-pipette under a stereo microscope. Then they were transferred to liquid-scintillation vials and dissolved in 100 μ l of Hyamine, and their radioactivity was measured in a liquid-scintillation spectrometer with Instafluor as scintillation liquid. Samples from the radioactive incubation media were used as external standards.

In studies of insulin release, groups of two or three micro-dissected islets were first preincubated for 30 min at 37°C in either basal KRH or KRBH buffer and then incubated for 60 min in either of these media with additions as indicated in Tables 1 and 2. For technical reasons we used longer incubation periods in the insulin-release studies (60 min) than in the [^{14}C]DMO studies (7 or 30 min) in order to increase the precision and reproducibility in the measurements. After incubation, the islets were freeze-dried overnight (0.1 Pa; -40°C) and weighed on a quartz-fibre balance. The incubation media were frozen for subsequent insulin determination by radioimmunological assay, with crystalline mouse insulin as standard.

Free and antibody-bound insulin were separated by precipitation with 81% (v/v) ethanol (Heding, 1966).

Production of 3H_2O from D-[5- 3H]glucose was measured as an index of the combined metabolic fluxes in the glycolytic and pentose phosphate pathways (see Ashcroft *et al.*, 1972). The experimental procedure strictly followed that described in detail previously (Hellman *et al.*, 1975). The contents of 3H_2O were measured in a liquid-scintillation spectrometer with Aquasol as scintillation liquid. Samples from the radioactive incubation media were used as external standards. The incubated islets were freeze-dried and weighed as described above.

Calculations of [^{14}C]DMO uptake and intracellular pH

Islet-cell uptake of [^{14}C]DMO in excess of the extracellular space was estimated by assuming that the mean dry weight of five microdissected islets was 35 μ g. In separate sets of experiments performed during the same period, the mean weight of batches of five freeze-dried islets was $35.8 \pm 0.9 \mu$ g ($n = 48$). Data are presented as mean values $\pm$ S.E.M. throughout the paper. The pH_i was calculated from the [^{14}C]DMO-uptake values after correction for [^{14}C]DMO in the L-glucose (extracellular) space, by assuming an intracellular water volume of 1.2 litres/kg dry wt. (Hellman *et al.*, 1971).

Results

Table 1 shows the effects of sugars on the islet-cell uptake of [^{14}C]DMO and insulin release. The [^{14}C]DMO uptake at 1 mM-D-glucose corresponded to a pH_i of 7.18 ± 0.04 ($n = 17$). When the islets were exposed to 20 mM-D-glucose during the incubation period for 7 min, the [^{14}C]DMO uptake increased by 39% and reached values corresponding to a pH_i of 7.33 ± 0.03 ($n = 18$). The difference, 0.16 ± 0.03 , is highly significant ($P < 0.001$; $n = 17$). Table 1 also shows that 20 mM-D-mannoheptulose, well known as an inhibitor of β -cell glucose metabolism and insulin release (Ashcroft *et al.*, 1973), completely counteracted the glucose-induced increase in pH_i . Control experiments with the same type of islets and basal media as used in Table 1 showed that the glucose utilization at 20 mM-D-glucose, measured as the formation of 3H_2O from D-[5- 3H]glucose, was 81.9 ± 5.0 mmol of glucose equivalents/h per kg dry wt. of islets. D-Mannoheptulose (20 mM) decreased the utilization ($P < 0.001$) to 34.0 ± 2.1 mmol of glucose equivalents/h per kg dry wt. of islets ($n = 8$). As shown in Table 1, 20 mM-D-glucose stimulated insulin release many fold, and this stimulation was almost completely blocked by 20 mM-mannoheptulose.

Table 1. *Effect of sugars on [14 C]DMO uptake and insulin release in bicarbonate-buffered medium*

Islets were preincubated for 30 min and then incubated for 7 min ([14 C]DMO uptake) or 60 min (insulin release) in KRBH medium. Data are presented as mean values $\pm$ S.E.M. for the numbers of experiments stated in parentheses. ^a $P < 0.001$ for difference between (1) and (2); ^b $P < 0.02$ for difference between (2) and (3) ([14 C]DMO uptake); ^c $P < 0.001$ for difference between (2) and (3) (insulin release). The comparisons were made by Student's *t* test for paired data.

Preincubation medium	Incubation medium	Uptake of [14 C]DMO (μ mol/kg dry wt. of islets)	Insulin release (ng/ μ g dry wt. of islets)
(1) KRBH + 1 mM-D-glucose	KRBH + 1 mM-D-glucose	69.5 $\pm$ 5.6 (17)	0.17 $\pm$ 0.03 (8)
(2) KRBH + 1 mM-D-glucose	KRBH + 20 mM-D-glucose	96.5 $\pm$ 6.4 (18) ^a	5.36 $\pm$ 0.85 (8) ^a
(3) KRBH + 1 mM-D-glucose	KRBH + 20 mM-D-glucose + 20 mM-mannoheptulose	59.5 $\pm$ 7.9 (7) ^b	0.31 $\pm$ 0.09 (8) ^c

Table 2. *Effects of bicarbonate buffer and NH $_4^+$ on islet [14 C]DMO uptake and insulin release*

Batches of isolated islets were preincubated for 30 min in KRBH medium and then incubated for 7 min ([14 C]DMO uptake) or 60 min (insulin release) in KRBH or in KRBH with NH $_4$ Cl added as indicated. Data are presented as mean values $\pm$ S.E.M. for the numbers of experiments stated in parentheses. ^a $P < 0.001$ for difference between (1) and (2); ^b $P < 0.01$ for difference between (1) and (3). The comparisons were made by Student's *t* test for paired data.

Preincubation medium	Incubation medium	Uptake of [14 C]DMO (μ mol/kg dry wt. of islets)	Insulin release (ng/ μ g dry wt. of islets)
(1) KRBH + 1 mM-D-glucose	KRBH + 1 mM-D-glucose	114.9 $\pm$ 10.0 (10)	0.12 $\pm$ 0.01 (8)
(2) KRBH + 1 mM-D-glucose	KRBH + 1 mM-D-glucose (containing HCO $_3^-$ /CO $_2$)	63.6 $\pm$ 6.2 (10) ^a	0.17 $\pm$ 0.03 (8)
(3) KRBH + 1 mM-D-glucose	KRBH + 1 mM-D-glucose + 5 mM-NH $_4$ Cl	133.8 $\pm$ 10.8 (10) ^b	0.47 $\pm$ 0.09 (8) ^b

Exposing cells to HCO $_3^-$ /CO $_2$ has been shown to decrease pH $_i$, and exposure to NH $_4$ Cl causes a transient increase in pH $_i$ (Roos & Boron, 1981). Table 2 shows that this applied also to isolated pancreatic islets. Incubation for 7 min in basal medium without addition of bicarbonate buffer resulted in a [14 C]DMO uptake of 114.9 $\pm$ 10.0 μ mol/kg dry wt. of islets ($n = 10$). This corresponds to a pH $_i$ of 7.41, which is significantly higher than that obtained with medium containing added HCO $_3^-$ /CO $_2$ (cf. Tables 1 and 2). In line 2 of Table 2 it is shown that exposure to extracellular bicarbonate buffer led to a marked decrease in the [14 C]DMO uptake (apparent pH $_i$ 7.13), and addition of 5 mM-NH $_4^+$ (line 3) induced an increase in the [14 C]DMO uptake (apparent pH $_i$ 7.49). Table 2 also shows that the basal insulin release at 1 mM-D-glucose was not significantly changed by adding bicarbonate buffer to the external medium. However, 5 mM-NH $_4$ Cl induced a 4-fold increase in the insulin release. In the absence of external bicarbonate buffer, 20 mM-D-glucose increased the insulin release from 0.12 $\pm$ 0.01 to 3.48 $\pm$ 0.46 ng/ μ g dry wt. of islets ($n = 8$). This increment is 35% less than the insulin release in the presence of extracellular bicarbonate buffer (cf. Table 1).

Table 3 shows experiments performed with Hepes as the extracellular buffer and with no bicarbonate buffer added. The control values at 1 mM-D-glucose correspond to a pH $_i$ of 7.32. Elevation of

the D-glucose concentration to 20 mM increased the [14 C]DMO uptake by 35%, to an apparent pH $_i$ value of 7.46. The [14 C]DMO uptake at high glucose was further increased by 4 mM-L-5-hydroxytryptophan to an apparent pH $_i$ of 7.53. We have previously shown that L-5-hydroxytryptophan is a strong potentiator of glucose-induced insulin release in experiments using bicarbonate-buffered media (Lindström & Sehlin, 1983). In separate experiments we found that this is the case also in media devoid of bicarbonate buffer. L-5-Hydroxytryptophan (4 mM) potentiated the glucose-induced (20 mM-D-glucose) insulin release ($P < 0.001$) from 3.48 $\pm$ 0.46 to 7.48 $\pm$ 0.66 ng/ μ g dry wt. of islets ($n = 8$). Theophylline (2 mM), another potentiator of insulin secretion (Malaisse *et al.*, 1967; Hellman *et al.*, 1974), did not change the glucose-stimulated [14 C]DMO uptake.

The stimulation of [14 C]DMO uptake by 20 mM-D-glucose and the further potentiation by 4 mM-L-5-hydroxytryptophan were retained after 30 min of incubation (Table 3), indicating that these effects reflected a change in the equilibrium distribution of [14 C]DMO (pH $_i$).

Since the Hepes-buffered medium devoid of extracellular bicarbonate buffer had also not been gassed with O $_2$, we tested whether equilibration with O $_2$ (100%) changed the basal or glucose-stimulated [14 C]DMO uptake. Table 3 shows that this was not the case.

Table 3. [^{14}C]DMO uptake in the absence of extracellular bicarbonate buffer

Islets were first preincubated in KRH supplemented with 1 mM-D-glucose for 30 min at 37°C. Then they were transferred to new vials and incubated with 89 μM -[^{14}C]DMO present as indicated in the Table. Values are given as means $\pm$ S.E.M. (numbers of experiments given in parentheses) of the uptake of [^{14}C]DMO with the same specific radioactivity as in the incubation medium. ^a $P < 0.001$ for difference between (1) and (2); ^b $P < 0.05$ for difference between (2) and (3); ^c $P < 0.01$ for difference between (5) and (6); ^d $P < 0.005$ for difference between (6) and (7); ^e $P < 0.025$ for difference between (9) and (10). All comparisons were made by Student's *t* test for paired data.

Incubation medium	Incubation time (min)	Uptake of [^{14}C]DMO ($\mu\text{mol/kg}$ dry wt. of islets)
(1) KRH + 1 mM-D-glucose	7	89.4 $\pm$ 8.1 (13)
(2) KRH + 20 mM-D-glucose	7	121.1 $\pm$ 8.0 (13) ^a
(3) KRH + 20 mM-D-glucose + 4 mM-L-5-hydroxytryptophan	7	143.7 $\pm$ 10.1 (5) ^b
(4) KRH + 20 mM-D-glucose + 2 mM-theophylline	7	120.0 $\pm$ 12.0 (8)
(5) KRH + 1 mM-D-glucose	30	108.2 $\pm$ 13.2 (9)
(6) KRH + 20 mM-D-glucose	30	156.5 $\pm$ 22.5 (9) ^c
(7) KRH + 20 mM-D-glucose + 4 mM-L-5-hydroxytryptophan	30	178.5 $\pm$ 24.3 (9) ^d
(8) KRH + 1 mM-D-glucose	7	103.0 $\pm$ 8.2 (7)
(9) KRH + 1 mM-D-glucose, gassed with O ₂ (100%)	7	101.4 $\pm$ 8.0 (7)
(10) KRH + 20 mM-D-glucose, gassed with O ₂ (100%)	7	131.5 $\pm$ 14.9 (7) ^e

The effect of glucose on the [^{14}C]DMO uptake in a HEPES/bicarbonate-buffered medium was concentration dependent (Fig. 1). The apparent k_m for glucose was around 4 mM, and the maximum stimulation was reached at about 10 mM-glucose. The inset in Fig. 1 shows that equilibration of [^{14}C]DMO in the islet cells was reached at the shortest incubation time used (7 min).

Discussion

The present results indicate that D-glucose increases pH_i of pancreatic islet cells. The dose-dependence of this effect is shifted to the lower concentration range, in comparison with the effect of glucose on insulin release (Coore & Randle, 1964; Lernmark, 1971; Malaisse *et al.*, 1979b). Malaisse *et al.* (1979a) showed that D-glucose induces an increased outflow of H^+ from islets and suggested that glucose activates specific acid extrusion mechanisms (Lebrun *et al.*, 1982; Deleers *et al.*, 1983). The dose-dependence of the glucose-induced H^+ outflow resembles that we have now observed for pH_i . It cannot be excluded that these observations reflect the same phenomenon. However, since the extracellular-medium volume was large and well buffered to pH 7.40 and the diffusion distances within the islets are short, it is hard to believe that the increased uptake of [^{14}C]DMO is caused by extracellular acidification.

The [^{14}C]DMO-uptake method estimates the mean pH_i , but, of course, cannot detect heterogeneity in pH between different intracellular compartments (Roos & Boron, 1981; Nuccitelli & Deamer, 1982). Therefore the present results give no clue as to whether glucose can enhance proton accumulation by insulin granules, which has been

suggested (Abrahamsson & Gylfe, 1980; Pace & Sachs, 1982). This method requires that [^{14}C]DMO reaches an equilibrium distribution, and therefore transient changes in pH can escape detection. We do not know what happens with the pH_i during the first few minutes after change to a medium with a stimulatory glucose concentration. However, since the same increase was recorded after 30 min of incubation as after 7 min, we believe that glucose causes a sustained net increase in pH_i .

The alkalinizing effect of glucose appears not to be secondary to increased insulin release. Both theophylline (Malaisse *et al.*, 1967; Hellman *et al.*, 1974) and L-5-hydroxytryptophan (Lindström & Sehlin, 1983; see the Results section above) are strong potentiators of glucose-induced insulin release. Yet L-5-hydroxytryptophan increased pH_i at high glucose concentration, whereas theophylline showed no effect. The possibility that L-5-hydroxytryptophan interacts with fuel metabolism in the β -cells in a way that could result in alkalization requires further study. The question arises whether increased pH_i is in fact a step in the triggering of β -cells to secrete insulin. That 5 mM- NH_4^+ , under conditions where it acutely increased the pH_i , also induced a 4-fold increase in basal insulin release would support an involvement in the stimulation. It has been reported that NH_4^+ inhibits glucose-induced insulin release (Feldman & Lebovitz, 1971; Sener *et al.*, 1978; Smith & Pace, 1983). However, this inhibition is probably not mediated by a change in pH_i , because [^{14}C]DMO uptake at 16.7 mM-glucose was not affected by 5 mM- NH_4^+ (Sener *et al.*, 1978).

That metabolic inhibition by mannoheptulose blocked the glucose effect on pH_i may indicate that this effect is somehow linked to glucose meta-

Fig. 1. Concentration-dependent increase by glucose of islet $[^{14}\text{C}]\text{DMO}$ uptake

Islets were preincubated for 30 min in KRBH with no glucose added. They were then incubated for 7 min in KRBH with glucose added as indicated and with $[^{14}\text{C}]\text{DMO}$. Values are given as means $\pm$ S.E.M., for seven or eight experiments, except when 1 mM-D-glucose was tested. This value is the mean of several sets of experiments and includes 31 observations. In the inset is shown the time-dependence for accumulation of $[^{14}\text{C}]\text{DMO}$ in KRBH medium with 1 mM-glucose added (mean $\pm$ S.E.M. for the numbers of observations indicated).

bolism in the islet cells. The complexity of the metabolic pattern, including production and extrusion of lactate (Ashcroft *et al.*, 1970; Hellerström & Gunnarsson, 1970; Sener & Malaisse, 1976) and net production of ATP (Hellman *et al.*, 1969), makes it difficult to estimate the acute net effect of metabolism on the pH_i . It cannot be ruled out that increased metabolism of D-glucose by itself leads to an acute decrease in the proton activity. Alternatively, alkalization could be effected by acceleration of an acid-extruding $\text{HCO}_3^-/\text{Cl}^-$ exchange mechanism (Lebrun *et al.*, 1982; Deleers *et al.*, 1983), similar to the mechanism proposed for other cells (for review, see Roos & Boron, 1981). Indeed,

D-glucose increases $^{36}\text{Cl}^-$ efflux from β -cells (Sehlin, 1978), and there is evidence for $\text{HCO}_3^-/\text{Cl}^-$ exchange (Sehlin, 1980). However, the fact that the glucose-induced $^{36}\text{Cl}^-$ efflux is not dependent on (Sehlin, 1978), but rather suppressed by, extracellular bicarbonate buffer (J. Sehlin, unpublished work) makes it unlikely that glucose accelerates a $\text{HCO}_3^-/\text{Cl}^-$ exchange mechanism. Also, since glucose showed the same effect on the $[^{14}\text{C}]\text{DMO}$ uptake, whether extracellular bicarbonate buffer was added or not, it would appear that transmembrane uptake of HCO_3^- is not primarily responsible for the glucose-induced increase in pH_i .

This work was supported by grants from The Swedish Medical Research Council (12X-4756), the Swedish Diabetes Association, and the Medical Faculty, University of Umeå.

References

- Abrahamsson, H. & Gylfe, E. (1980) *Acta Physiol. Scand.* **109**, 113–114
- Ashcroft, S. J. H., Hedeskov, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Ashcroft, S. J. H., Weerasinghe, L. C. C., Bassett, J. M. & Randle, P. J. (1972) *Biochem. J.* **126**, 525–532
- Ashcroft, S. J. H., Weerasinghe, L. C. C. & Randle, P. J. (1973) *Biochem. J.* **132**, 223–231
- Coore, H. G. & Randle, P. J. (1964) *Biochem. J.* **93**, 66–78
- Deleers, M., Lebrun, P. & Malaisse, W. J. (1983) *FEBS Lett.* **154**, 97–100
- Feldman, J. M. & Lebovitz, H. E. (1971) *Am. J. Physiol.* **221**, 1027–1032
- Heding, L. (1966) in *Labelled Proteins in Tracer Studies* (Donato, L., Milhaud, G. & Sirchis, G., eds.), pp. 345–351, Euratom, Brussels
- Hellerström, C. & Gunnarsson, R. (1970) in *On the Pathogenesis of Diabetes Mellitus* (Luft, R. & Randle, P. J., eds.), pp. 127–151, Casa Editrice 'Il Ponte', Milano
- Hellman, B. (1965) *Ann. N.Y. Acad. Sci.* **131**, 541–558
- Hellman, B., Idahl, L.-Å. & Danielsson, Å. (1969) *Diabetes* **18**, 509–516
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971) *Diabetologia* **7**, 256–265
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1972) *Endocrinology* **90**, 335–337
- Hellman, B., Idahl, L.-Å., Lernmark, Å. & Täljedal, I.-B. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3405–3409
- Hellman, B., Idahl, L.-Å., Sehlin, J. & Täljedal, I.-B. (1975) *Diabetologia* **11**, 495–500
- Lebrun, P., Malaisse, W. J. & Herchuelz, A. (1982) *Biochim. Biophys. Acta* **721**, 357–365
- Lernmark, Å. (1971) *Acta Diabetol. Lat.* **8**, 649–679
- Lindström, P. & Sehlin, J. (1983) *Diabetologia* **24**, 52–57
- Malaisse, W. J., Malaisse-Lagae, F. & Mayhew, D. (1967) *J. Clin. Invest.* **46**, 1724–1734
- Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I. & Sener, A. (1979a) *Diabetologia* **16**, 331–341

- Malaisse, W. J., Sener, A., Herchuelz, A. & Hutton, J. C. (1979b) *Metab. Clin. Exp.* **28**, 373–386
- Malaisse, W. J., Herchuelz, A. & Sener, A. (1980) *Life Sci.* **26**, 1367–1371
- Nuccitelli, R. & Deamer, D. W. (1982) *Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Functions: Kroc Found. Ser.* vol. 15, Alan R. Liss, New York
- Pace, C. S. & Sachs, G. (1982) *Am. J. Physiol.* **242**, C382–C387
- Pace, C. S., Tarvin, J. T. & Smith, J. S. (1983) *Am. J. Physiol.* **244**, E3–E18
- Roos, A. & Boron, W. F. (1981) *Physiol. Rev.* **61**, 296–434
- Sehlin, J. (1978) *Am. J. Physiol.* **235**, E501–E508
- Sehlin, J. (1980) *Horm. Metab. Res. Suppl. Ser.* **10**, 73–80
- Sener, A. & Malaisse, W. J. (1976) *Biochem. Med.* **15**, 34–41
- Sener, A., Hutton, J. C., Kawazu, S., Boschero, A. C., Somers, K., Devis, G., Herchuelz, A. & Malaisse, W. J. (1978) *J. Clin. Invest.* **62**, 868–878
- Smith, J. S. & Pace, C. S. (1983) *Diabetes* **32**, 61–66
- Waddell, W. J. & Butler, T. C. (1959) *J. Clin. Invest.* **38**, 720–729