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The Stimulus–Secretion Coupling of Glucose-Induced Insulin Release

EFFECT OF EXOGENOUS PYRUVATE ON ISLET FUNCTION

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1. In isolated pancreatic islets, pyruvate causes a shift to the left of the sigmoidal curve relating the rate of insulin release to the ambient glucose concentration. The magnitude of this effect is related to the concentration of pyruvate (5–90mM) and, at a 30mM concentration, is equivalent to that evoked by 2mM-glucose. Pyruvate also enhances insulin release in the presence of fructose, leucine and 4-methyl-2-oxopentanoate. 2. In the presence of glucose (8mM), the secretory response to pyruvate is an immediate process, displaying a biphasic pattern. 3. The insulinotropic action of pyruvate coincides with an inhibition of ^{45}Ca efflux and a stimulation of ^{45}Ca net uptake. The relationship between ^{45}Ca uptake and insulin release displays its usual pattern in the presence of pyruvate. 4. Exogenous pyruvate rapidly accumulates in the islets in amounts close to those derived from the metabolism of glucose. The oxidation of [2-^{14}C]pyruvate represents 64% of the rate of [1-^{14}C]pyruvate decarboxylation and, at a 30mM concentration, is comparable with that of 8mM-[U-^{14}C]glucose. 5. When corrected for the conversion of pyruvate into lactate, the oxidation of 30mM-pyruvate corresponds to a net generation of about 314pmol of reducing equivalents/120min per islet. 6. Pyruvate does not affect the rate of glycolysis, but inhibits the oxidation of glucose. Glucose does not affect pyruvate oxidation. 7. Pyruvate (30mM) does not affect the concentration of ATP, ADP and AMP in the islet cells. 8. Pyruvate (30mM) increases the concentration of reduced nicotinamide nucleotides in the presence but not in the absence of glucose. A close correlation is seen between the concentration of reduced nicotinamide nucleotides and the net uptake of ^{45}Ca. Menadione inhibits the effect of pyruvate on insulin release, without altering its rate of oxidation. 9. Pyruvate, like glucose, modestly stimulates lipogenesis. 10. Pyruvate, in contrast with glucose, markedly inhibits the oxidation of endogenous nutrients. The latter effect accounts for the apparent discrepancy between the rate of pyruvate oxidation and the magnitude of its insulinotropic action. 11. Dichloroacetate fails to affect glucose oxidation and glucose-stimulated insulin release. 12. It is concluded that the effect of pyruvate to stimulate insulin release depends on its ability to increase the concentration of reduced nicotinamide nucleotides in the islet cells.

In the preceding papers in this series (Malaisse *et al.*, 1978*b*,*c*), we have proposed that the generation of reduced nicotinamide nucleotides plays an essential role in the process of glucose-induced insulin release, by coupling metabolic to subsequent cationic events in the secretory sequence. We have also postulated that any nutrient that is able, as a result of its metabolism, to increase the concentration of NAD(P)H in islet cells should be able to mimic the effect of glucose on insulin release. The present investigation, parts of which have been already reported in abstract form (Kawazu *et al.*, 1977; Sener *et al.*, 1978), was primarily undertaken to verify the validity of the latter postulate for exogenous pyruvate. By analogy, our results also suggest a possible role for the mitochondrial oxidation of endogenous pyruvate in the process of glucose-induced insulin release.

Experimental

Insulin release by isolated islets

Groups of eight islets each, removed from female albino rats, were incubated for 90min in 1.0ml of a bicarbonate-buffered medium containing albumin (5mg/ml), the release of insulin being measured as described elsewhere (Malaisse *et al.*, 1970). Whenever sodium pyruvate (Sigma Chemical Co., St. Louis, MO, U.S.A.) was added to the incubation medium, the NaCl concentration was decreased accordingly to maintain a normal Na^+ concentration (139mM).

Other agents added to the medium included menadione (Sigma) and cytochalasin B (Aldrich Chemical Co., Milwaukee, WI, U.S.A.).

Insulin release by the isolated perfused pancreas

The method used for the measurement of insulin release by the isolated perfused pancreas was previously described (Van Obberghen *et al.*, 1973). The perfusate contained albumin (5mg/ml) and dextran (40mg/ml), as outlined elsewhere (Leclercq-Meyer *et al.*, 1976). Its calcium concentration was 2.0mM.

^{45}Ca handling by the islets

For the measurement of ^{45}Ca efflux, groups of 100 islets each were prelabelled with ^{45}Ca and placed in a perifusion chamber, as described in detail elsewhere (Malaisse *et al.*, 1973). In one series of experiments, verapamil (Knoll A. G., Ludwigshafen, Germany) was used to inhibit Ca entry into islet cells (Malaisse *et al.*, 1977). For the measurement of net uptake of ^{45}Ca, the same technique was used as that proposed for the net uptake of ^{42}K (Boschero *et al.*, 1977). In that method, the islets are rapidly separated from the radioactive incubation medium by passing the latter through a layer of di-n-butyl phthalate. The net uptake of ^{45}Ca is corrected for extracellular contamination, with [^{3}H]sucrose as an extracellular marker.

Uptake of [^{14}C]pyruvate by the islets

Groups of ten islets each were placed in polythene microcentrifuge tubes, preincubated for 30min in the absence of exogenous nutrients, and eventually incubated for various times in 0.05ml of a medium containing [1-^{14}C]- or [U-^{14}C]-pyruvate (10–25μCi/ml) and [6,6′(*n*)-^{3}H]sucrose (1.0mM; 20–25μCi/ml). At the end of the incubation period, the islets were deposited in the bottom of the tube during a first centrifugation (5s; Beckman Microfuge, model 152). Di-*n*-butyl phthalate (0.1ml; BDH Chemicals, Poole, Dorset, U.K.) was then layered on the top of the incubation medium, and a second centrifugation (10s) performed to separate the islets pellet from the medium. The lowest 2mm of the tube was removed with a scalpel, transferred to a scintillation vial containing a solution of EGTA (1.0ml, 2.0mM, pH7.0), mixed with 10ml of scintillation fluid (Insta-Gel; Packard, Downers Grove, IL, U.S.A.), and examined for ^{14}C and ^{3}H content. The net uptake of [^{14}C]pyruvate was corrected for extracellular contamination.

Endogenous pyruvate concentration in the islets

Groups of ten islets each were placed in polythene microcentrifuge tubes and incubated for 30min in media (0.1ml) containing various concentrations of glucose. The islets were separated from the medium, as described above, except that silicone oil (0.2ml; Versilube F50, General Electric, Bergen-Op-Zoom, The Netherlands) was used instead of di-*n*-butyl phthalate. A solution (5μl) of NaOH (0.02M) and sucrose (20%, w/v) was immediately injected with aid of a microsyringe in the bottoms of the tubes, which were then placed for 10min in a water bath maintained at 80°C. Samples (4μl) either removed from the tube or taken from pyruvate standard solutions were then mixed with 10μl of an imidazole buffer (50mM, pH7.4) containing bovine albumin (0.3mg/ml), ascorbate (3mM), NADH (10μM) and lactate dehydrogenase (0.2μg/ml; EC 1.1.1.27), and incubated for 20min at room temperature. The remaining NADH was destroyed during a 30min incubation at room temperature, after addition of 10μl of HCl (0.5M). The media were neutralized by addition of 5μl of NaOH (1.0M). An enzymic cycling reagent (0.1ml) consisting of a Tris buffer (100mM; pH8.4) containing lactate (100mM), ADP (0.3mM), 2-oxoglutarate (5mM) and both lactate dehydrogenase (50μg/ml) and glutamate dehydrogenase (400μg/ml; EC 1.4.1.3) was then added to the tubes, which were incubated for 60min at 37°C. The reaction was stopped by placing the tubes for 5min in a water bath maintained at 95°C. After addition of 1.0ml of a Tris buffer (50mM, pH8.5) containing bovine albumin (0.1mg/ml), NAD^+ (1.0mM), ADP (0.1mM) and H_2O_2 (9.0mM) and incubation for 20min at room temperature to destroy the remaining 2-oxoglutarate, a first measurement by microfluorimetry was performed. Glutamate dehydrogenase (5μl; 10mg/ml) was then added to the tube and a second measurement of NADH fluorescence performed after a last 30min incubation at room temperature.

No detectable loss of pyruvate occurred during 10-min incubation at 80°C in the presence of NaOH (0.02–0.04M). In calculating the concentration of pyruvate in the islets, correction was made for the blank values found with incubation media containing no islets. In arbitrary units such a blank averaged 135.0 ± 5.7 ($n = 4$) as distinct from experimental values ranging between 156.4 ± 7.7 ($n = 11$) and 219.1 ± 7.4 ($n = 11$). By using the same mode of expression and by combining the S.E.M. on both the blank of the standard curve (117.0 ± 5.6; $n = 4$) and the lowest standard amount (3.13pmol/4μl) of pyruvate (136.0 ± 4.4; $n = 4$), the limit of sensitivity of the assay was found to amount to 1.17pmol/4μl (corresponding to 0.15pmol/islet).

Oxidation of exogenous ^{14}C-labelled nutrients by the islets

All ^{14}C-labelled nutrients were purchased from The Radiochemical Centre (Amersham, Bucks.,

U.K.). Before incorporation into the incubation medium, the solution of [^{14}C]pyruvate was placed in a tube that was either maintained for 5min in boiling water or, after acidification of the solution with HCl (final concn. 10mM), incubated at 37°C for 60min in a scintillation vial itself containing outside the tube 0.5ml of Hyamine hydroxide (Packard Instrument Co., Downers Grove, IL, U.S.A.). Groups of 15 islets each were incubated for 120min in 75μl of incubation medium containing the labelled substrate (2–10μCi/ml). The incubation was stopped by addition of 0.1ml of phosphate buffer (400mM, pH6.0) containing antimycin A (10μM; Sigma), and the $^{14}CO_2$ trapped in Hyamine hydroxide, as previously described (Malaisse *et al.*, 1974). The islet content of amylase usually represents less than 1% of that found in the same amount (μg of protein) of acinar tissue (A. Sener & W. J. Malaisse, unpublished work). Occasional contamination of the islets by acinar tissue would not represent an important source of error in estimating the rate of pyruvate oxidation. Indeed, in the presence of 20mM-[1-^{14}C]pyruvate, the rate of oxidation (expressed as pmol/120min per μg of protein) by the acinar tissue represented 94.2± 14.1% ($n = 9$) of the mean value found within the same experiment with isolated islets.

Conversion of pyruvate into lactate in the islets

Groups of 50 islets each were incubated for 120min at 37°C in 0.1ml of the usual medium. The lactate present in the islets and incubation medium was assayed by an enzymic procedure described in detail elsewhere (Sener & Malaisse, 1976).

Concentration of adenine nucleotides in the islets

Groups of eight islets each were incubated for 30min in 0.06ml of medium. The determination of ATP, ADP and AMP was carried out as described elsewhere (Malaisse *et al.*, 1978*b*).

Concentration of reduced nicotinamide nucleotides in the islets

Groups of ten islets each were incubated for 30min in 0.06ml of medium. The total concentration of reduced nicotinamide nucleotides (NADH + NADPH) was assayed by the luciferase technique, as previously reported (Malaisse *et al.*, 1978*b*).

Incorporation of ^3H_2O into islet lipids

Groups of 20 islets each were incubated for 120min in 0.05ml of medium containing ^3H_2O (250mCi/ml). At the end of the incubation and after removal of most of the incubation medium, the residual radioactive medium was diluted by addition of 0.2ml of ice-cold medium, and the islets were separated from this medium by the oil-centrifugation technique previously described. The tip of the tube was transferred to a tube containing 2.5ml of chloroform/methanol (2:1, v/v). Rat serum (20μl) was added to each tube to provide carrier lipids. The content of the tube was sonicated, and the extraction of lipids performed by the procedure described by Folch *et al.* (1957). Anhydrous Na_2SO_4 was added to the final extract, which was then transferred to a scintillation vial and evaporated at 60°C. Final traces of water were removed by vacuum desiccation overnight and by azeotropic evaporation with acetone at 40°C. After reconstitution in 0.15ml of toluene, a portion (0.1ml) of the extract was examined for its radioactive content. The pooled remainder of four such extracts was evaporated to a small volume, and separated into its principal components by t.l.c. on silica-gel G in a light petroleum (60–80°C b.p.)/ether/acetic acid (65:35:1, by vol.) solvent system. The areas of the chromatogram corresponding to each lipid class were identified by using appropriate standards, made visible with iodine vapour, scraped from the chromatographic plate, and suspended in 10ml of scintillation fluid (Lipoluma; Brussels, Belgium) for determination of their radioactivity. Control experiments, in which a sample of the incubation medium (1.0μl) was extracted in an identical manner, yielded no detectable amount of ^{3}H-labelled lipids. The results obtained with the islets (pmol of ^3H_2O incorporated into lipids) were multiplied by 1.15 to express them as pmol of acetyl-CoA residues (Jungas, 1968; Brunengraber, 1976).

Oxidation of endogenous nutrients

For measuring the oxidation of endogenous nutrients, the islets were prelabelled with [U-^{14}C]palmitate (Berne, 1975). After evaporation of solvent under a stream of N_2, the [U-^{14}C]palmitate (250μCi; 928mCi/mmol) was mixed overnight at room temperature with KOH (10μl; 0.1M), and a solution (0.7ml; pH7.4) containing 20mg of fatty acid-poor bovine albumin (fraction V; Pentex, Miles Laboratories, Elkhart, IN, U.S.A.), 0.1mg of streptomycin and 0.05mg of penicillin. This mixture was then brought up to a final volume of 1.0ml with a solution containing glucose and the usual salts of our incubation medium to reach appropriate final concentrations (e.g. palmitate 0.27mM and glucose 8.3mM). Batches of 600–800 islets each were preincubated for 120min in the latter medium, and then washed five times at room temperature with a non-radioactive medium also containing 20mg of albumin/ml. The islets were redistributed in groups of 20 islets each and eventually incubated for 120min in 0.1ml of the usual incubation medium for measurement of $^{14}CO_2$ output, the

tube containing the islets being placed in a scintillation vial. At the end of the incubation, Hyamine hydroxide (0.5ml) was introduced in the scintillation vial, and 0.1ml of a phosphate buffer (400mM; pH6.0) containing antimycin A (10μM) placed in the incubation medium. After 60min shaking at room temperature, the islets were sonicated together with their surrounding medium and examined for their radioactive content, by liquid-scintillation counting.

Control experiments indicated (i) that the recovery of $^{14}CO_2$ was the same whether 0.1M-HCl (0.1ml) or the phosphate buffer was used to acidify the incubation medium; (ii) that, in the absence of islets, less than 0.05% of the radioactivity ([U-^{14}C]-palmitate being purposely added to the incubation medium) was recovered in the Hyamine hydroxide; (iii) that only 10–20% of the $^{14}CO_2$ output measured when the islets were incubated for 120min in the presence of metabolic poisons (see Table 10) could be accounted for as radioactive CO_2 or bicarbonate already present in the islets at the onset of the final incubation period.

In a limited series of experiments, the islet homogenate obtained after the final incubation was extracted by the method of Folch *et al.* (1957) and separated into lipid classes by the procedure described above. The fraction containing the phospholipids and monoacylglycerols was scraped from the chromatographic plate, loaded dry into a column (0.5cm×3.0cm), and subsequently eluted with 5ml of chloroform/methanol (2:1, v/v) followed by 5ml of methanol. This extract was evaporated to dryness under N_2 at 40°C and rechromatographed on silica-gel G in a solvent system of chloroform/methanol/25% (v/v) NH_3 (14:6:1, by vol.). The lipid components of each chromatogram were made visible with iodine vapour and identified by using appropriate standards. The radioactivity contained in each band was determined by a chromatogram scanner, and quantified by planimetry of the trace so obtained.

Presentation of results

All results are expressed as the mean (±S.E.M.) together with the number of individual determinations (n). The statistical significance of differences between mean control and experimental values was assessed by use of the Student's t test.

Results

Effect of pyruvate on insulin release by isolated islets

In the absence of glucose, pyruvate up to a 30mM concentration failed to affect insulin release. An increase in insulin output above basal value was observed, however, in the presence of 60mM- and 90mM-pyruvate (Table 1). In the presence of 8.3mM-glucose, pyruvate caused a dose-related increase in insulin output (Fig. 1). Pyruvate, at the lowest concentration here examined (5mM), already provoked a significant enhancement of glucose-stimulated insulin release. The effect of pyruvate (30mM) in augmenting glucose-stimulated insulin release was most marked at a 8.3mM-glucose concentration, and progressively faded out as the glucose concentration was either decreased below or increased above such a value. As Fig. 2 shows, this behaviour indicates that pyruvate

Table 1. *Effect of pyruvate on insulin release in the absence of glucose*

Mean values (±S.E.M.) are shown together with the number of individual observations.

Pyruvate (mM)	Insulin output (μ-i.u./90min per islet)
0	16.2±3.9 (40)
10.0	18.1±4.8 (28)
20.0	17.4±1.6 (6)
30.0	15.6±2.3 (74)
60.0	28.1±6.0 (19)
90.0	44.0±3.3 (19)

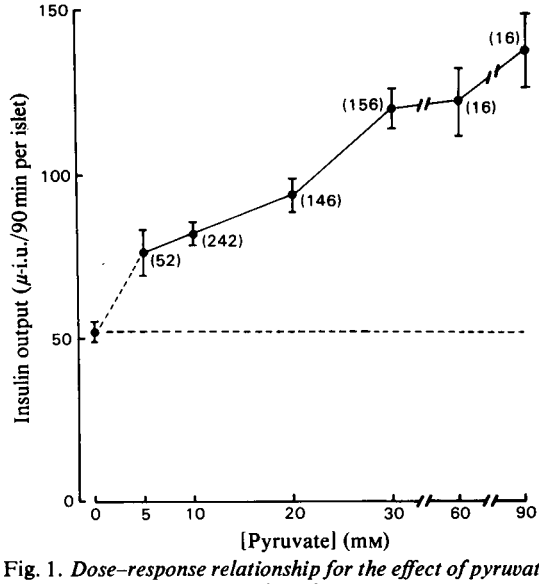

Fig. 1. *Dose–response relationship for the effect of pyruvate on insulin release*

The isolated islets were incubated in the presence of 8.3mM-glucose; ---- indicates the mean insulin output evoked by glucose alone. Within each experiment, the increment in secretion rate attributable to pyruvate was estimated from close-to-equal numbers of individual observations performed in the presence and absence of the substrate. The results are shown together with the degrees of freedom (in parentheses).

shifts to the left the sigmoidal curve normally relating the rate of insulin release to the ambient glucose concentration. The magnitude of such a shift indicates that the insulinotropic capacity of 30mM-pyruvate is equivalent to that of glucose itself at a concentration of approximately 2mM.

Various substrates and drugs were tested for their ability to replace glucose as an apparent permissive factor for the insulinotropic action of 30mM-pyruvate (Table 2). In this respect, 2-deoxyglucose, 3-*O*-methylglucose, xylitol and malate were unable to replace glucose. Theophylline, cytochalasin B and ouabain also failed to enhance significantly the close-to-basal rate of insulin release found in the presence of 30mM-pyruvate. However, in the presence of fructose, glyceraldehyde, leucine or 4-methyl-2-oxopentanoate, pyruvate significantly enhanced insulin output. The results obtained with fructose are especially striking in that the hexose itself failed to stimulate insulin release.

Dynamics of pyruvate-induced insulin release in the isolated perfused pancreas

In the isolated perfused pancreas, the administration of glucose (8.3mM) after 25min of perfusion evoked a typical secretory response, an early peak being followed by a slow and progressive build-up in insulin output (Fig. 3). When pyruvate (30.0mM) was administered during exposure of the pancreas to glucose (8.3mM), a biphasic secretory response was again observed that was superimposed on the release pattern seen in the sole presence of glucose (Fig. 3). Over the 20min of exposure to pyruvate (41–60min of perfusion), the integrated output of insulin averaged 4.34±0.49m-i.u./pancreas, as distinct from a control value (glucose alone) of 2.48±0.42m-i.u./pancreas ($P<0.02$; $n=6$ in each case). In the absence of glucose, pyruvate failed to stimulate insulin release (Fig. 3).

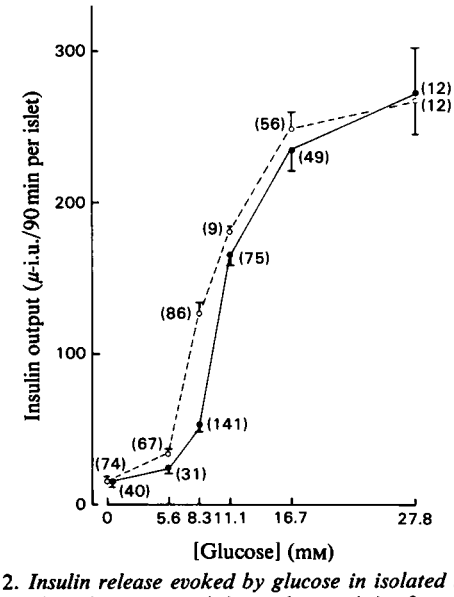

Fig. 2. *Insulin release evoked by glucose in isolated islets incubated in the presence* (○) *or absence* (●) *of pyruvate* (30.0*mM*)
The numbers of individual observations are given on the Figure (in parentheses).

Table 2. *Effect of pyruvate on insulin release in the presence of various agents*
Mean values (±S.E.M.) for insulin output (μ-i.u./90min per islet) are shown together with the number of individual determinations (in parentheses). In one series of experiments (shown by the asterisk) the incubation time was decreased to 60min. The statistical significance ($P<0.05$ or less) of differences in secretory rate refer either to the effect of the agent, as tested by vertical comparison with the data shown on the first line in the absence[a] or presence[b] of pyruvate, or to the effect of pyruvate[c], as tested by horizontal comparison.

Agent(s) (mM)	Insulin output	
	No pyruvate	Pyruvate (30.0mM)
None	16.2±3.9 (40)	15.6±2.3 (74)
Glucose (8.3)	52.0±3.0 (141)[a]	119.9±5.4 (86)[b,c]
Glucose (11.1)+menadione (0.01)	64.8±7.2 (19)[a]	99.0±9.1 (19)[b,c]
2-Deoxyglucose (20.0)	—	7.1±10.2 (8)
3-*O*-Methylglucose (8.3–30.0)	6.1±3.9 (13)	11.4±2.2 (25)
Fructose (20.0–30.0)	7.8±8.6 (8)	51.0±10.8 (16)[b,c]
Xylitol (19.7)	14.6±2.7 (7)	17.7±2.1 (7)
Glyceraldehyde (7.5)*	30.9±5.7 (19)[a]	60.2±5.2 (19)[b,c]
Leucine (15.0)	44.8±4.8 (28)[a]	59.8±5.6 (29)[b,c]
4-Methyl-2-oxopentanoate (10.0–12.5)	133.2±14.6 (28)[a]	181.1±13.5 (28)[b,c]
Malate (1.0–5.0)	21.7±8.4 (6)	17.1±3.8 (25)
Theophylline (1.4)	—	8.7±1.2 (12)
Cytochalasin B (0.02)	—	27.2±15.2 (8)
Ouabain (0.1)	19.6±6.1 (10)	8.3±10.4 (10)

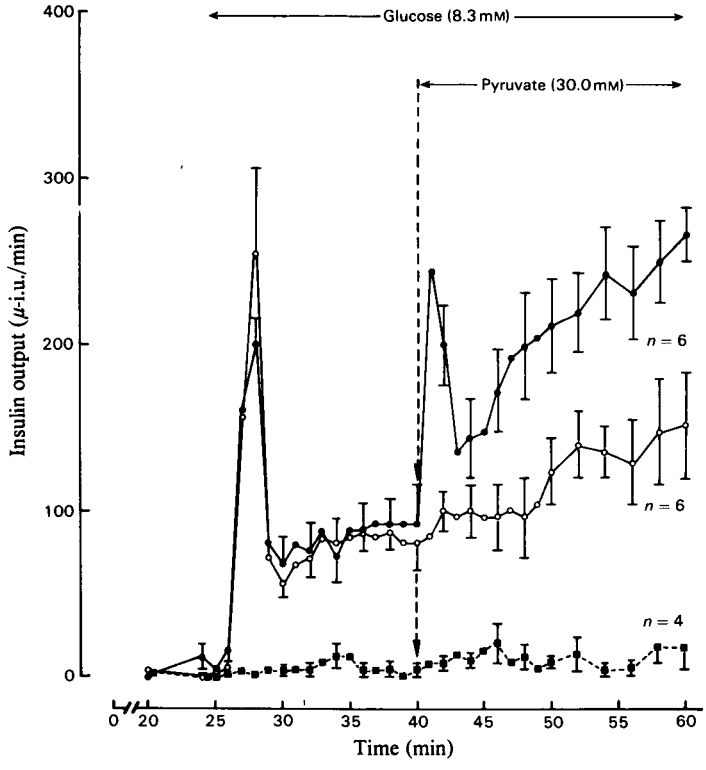

Fig. 3. *Insulin release by the isolated perfused rat pancreas*
The perfusate contained no nutrient up to 25 min. In two groups of experiments (○, ●), glucose (8.3 mM) was administered from 25 min onwards. Pyruvate (30.0 mM) was added at 40 min, either in the absence (■) or presence (●) of glucose.

When the pancreas was first exposed to pyruvate (30.0 mM), which was delivered throughout the experimental procedure, the administration of glucose (at 25 min) evoked a more rapid release of insulin than that normally seen in response to the sugar (i.e. in the absence of pyruvate). Thus in the presence of pyruvate, the values for insulin output at 26 min averaged 92 ± 28 μ-i.u./min per pancreas ($n = 7$), as distinct from only 12 ± 4 μ-i.u./min per pancreas ($n = 12$) in the control experiments ($P < 0.005$). The integrated output of insulin provoked by glucose (8.3 mM) between 25 and 39 min was also significantly higher ($P < 0.05$) in the presence (1.97 ± 0.32 m-i.u./pancreas; $n = 7$) than in the absence (1.26 ± 0.11 m-i.u./pancreas; $n = 12$) of pyruvate (results not shown).

Alteration of pyruvate-induced insulin release

In the presence of menadione (0.01 mM), the secretory response to 11.1 mM-glucose is severely decreased, but not abolished (Malaisse *et al.*, 1978*c*), so that the actual rate of insulin release is comparable with that normally seen at 8.3 mM-glucose in the absence of menadione (Table 2, lines 2 and 3). Despite such a similarity in secretory rate, the increment in insulin output attributable to pyruvate (30 mM) was about half as much ($P < 0.05$) in the presence of 11.1 mM-glucose and 0.01 mM-menadione (pyruvate-induced increment in insulin output $+34.2 \pm 11.6$ μ-i.u./90 min per islet) as that seen in the sole presence of 8.3 mM-glucose (pyruvate-induced increment $+67.9 \pm 5.7$ μ-i.u./90 min per islet). The latter observation suggests that menadione also impairs the proper insulinotropic action of pyruvate. And indeed, comparison with previously reported data (see Fig. 2 in Malaisse *et al.*, 1978*c*) indicates that the fractional decrease in insulin output attributable to 0.01 mM-menadione was the same whether glucose alone or a combination of glucose and pyruvate was used to stimulate insulin release at a given secretory rate.

When the islets are preincubated for 90 min in the absence of glucose, the capacity of the sugar to stimulate insulin release is apparently somewhat impaired (Matschinsky *et al.*, 1972). The insulino-

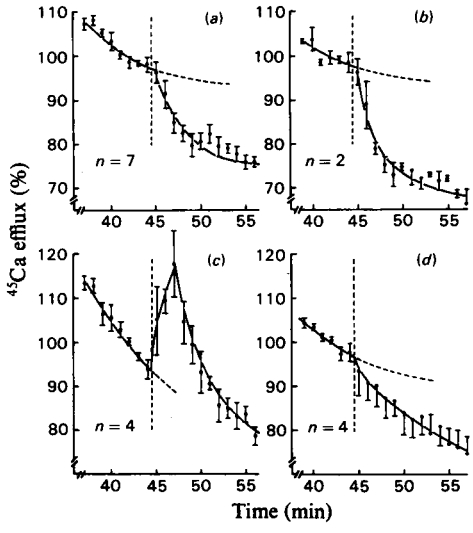

Fig. 4. *Effect of pyruvate on ^{45}Ca efflux from perifused islets*
Pyruvate (30.0mM) was invariably administered from 45min (vertical broken line) onwards. The perifusate circulated throughout the experiment either represented a glucose-free solution (*a*, *b*) or contained 8.3mM glucose (*c*, *d*). At each glucose concentration the experiments were performed at a normal Ca^{2+} concentration (1.0mM; *a* and *c*) or under conditions known to prevent insulin release, namely in the absence of extracellular Ca^{2+} and presence of EGTA (0.5mM; *b*) or at a normal Ca^{2+} concentration, but in the presence of verapamil (0.02mM; *d*). The efflux of ^{45}Ca is expressed as percentage of the mean control value found within each experiment between 40 and 44min, and is shown together with the number of individual observations (*n*).

tropic potency of pyruvate appeared to be less affected than that of glucose by such a prior deprivation. Thus after preincubation in glucose-free medium, glucose (8.3mM) modestly increased insulin output from a basal value of 9.6 ± 3.6 ($n = 36$) to 20.0 ± 3.6 ($n = 58$) μ-i.u./islet over the ensuing 90min incubation. However, after the same period of glucose deprivation, pyruvate (30.0mM) still markedly enhanced insulin release; the secretory rate observed in the presence of both glucose and pyruvate averaged 69.0 ± 5.7 μ-i.u./90min per islet ($n = 58$).

Effect of pyruvate on the handling of ^{45}Ca

Pyruvate mimicked the effect of glucose on ^{45}Ca efflux (Fig. 4). In the absence of glucose, pyruvate (30mM) provoked a fall in the efflux of ^{45}Ca, whether at normal Ca^{2+} concentration (1.0mM) or in islets deprived of extracellular Ca^{2+} (Figs. 4*a* and 4*b*). The effect of pyruvate was detectable during the second minute of exposure to this nutrient. In the presence of glucose (8.3mM) and at normal Ca^{2+} concentration,

Table 3. *Effect of glucose and pyruvate on net uptake of ^{45}Ca*
Mean values (±S.E.M.) were measured at 90min of incubation, and are shown together with the number of individual observations (in parentheses).

Glucose (mM)	Pyruvate (mM)	^{45}Ca net uptake (pmol/islet at 90min)
—	—	8.45 ± 0.85 (22)
—	30.0	11.02 ± 0.87 (23)
8.3	—	14.00 ± 0.92 (22)
8.3	30.0	16.73 ± 0.77 (24)
16.7	—	20.28 ± 1.42 (16)

a fraction of the effluent ^{45}Ca is thought to correspond to a release of the cation occurring at the time and site of exocytosis (Malaisse *et al.*, 1973). Under such an experimental condition, the addition of pyruvate resulted in an immediate increase in ^{45}Ca efflux (Fig. 4*c*). This behaviour could be due to a rapid enhancement of insulin secretion and concomitant exocytotic release of ^{45}Ca. Indeed, when the organic calcium-antagonist verapamil (0.02mM) was used to prevent glucose-induced insulin release (Malaisse *et al.*, 1977), the addition of pyruvate failed to enhance ^{45}Ca efflux, but, instead, caused a partial decrease in effluent radioactivity (Fig. 4*d*). The latter effect of pyruvate was somewhat less marked than that seen in the absence of glucose. It was also observed when pyruvate was administered to islets perifused with a medium containing glucose (8.3mM) but deprived of extracellular Ca^{2+} (results not shown).

In good agreement with these findings, pyruvate (30.0mM) slightly but significantly ($P < 0.05$) enhanced ^{45}Ca net uptake in the absence of glucose (Table 3). Pyruvate also significantly ($P < 0.05$) enhanced ^{45}Ca uptake in the presence of 8.3mM-glucose. The value measured in the concomitant presence of 8.3mM-glucose and 30.0mM-pyruvate remained significantly lower ($P < 0.025$) than that found in the presence of 16.7mM-glucose alone.

In the presence of glucose and/or pyruvate, the relationship between insulin output and ^{45}Ca net uptake (Fig. 5*b*) displayed the same pattern as that repeatedly documented in the present system. Insulin release remained close to its basal value as long as the net uptake of ^{45}Ca did not exceed a threshold value, which itself represents approximately half of the reference value found at high glucose concentration (16.7mM).

Uptake, oxidation and reduction of pyruvate

Pyruvate rapidly accumulated in the islets (Fig. 6). The net uptake of [^{14}C]pyruvate apparently reached equilibrium between 5 and 15min of incubation. Over this period, it averaged 1.0 ± 0.2 ($n = 12$), 2.1 ± 0.3 ($n = 10$) and 7.1 ± 0.8 ($n = 28$) pmol/islet at

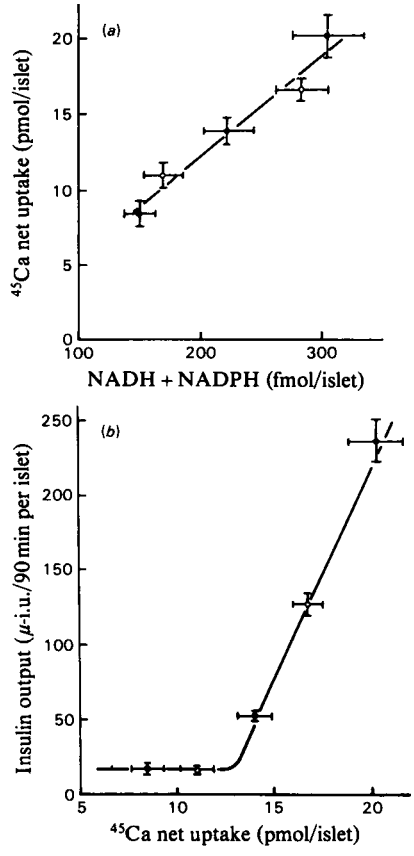

Fig. 5. *Relationship between the concentration of reduced nicotinamide nucleotides, the uptake of calcium and the release of insulin in isolated islets*

(*a*) Relationship between the mean values for net uptake of ^{45}Ca (Table 3) and the total concentration of reduced nicotinamide nucleotides (Table 7). (*b*) Relationship between the mean values for insulin release (Fig. 2) and net uptake of ^{45}Ca. Increasing values (such as illustrated from left to right) refer to data obtained respectively in the absence of nutrient, in the presence of 30.0mM-pyruvate alone, in the presence of 8.3mM-glucose alone, in the simultaneous presence of 8.3mM-glucose and 30.0mM-pyruvate, and in the presence of 16.7mM-glucose alone.

pyruvate concentrations of 1.0, 5.0 and 30.0mM respectively. For the purpose of comparison, we measured the concentration of endogenous pyruvate found in islets exposed to glucose. After 30min incubation, the concentration of endogenous pyruvate (pmol/islet) increased from a basal value of 0.54 ± 0.08 ($n = 28$) to 0.95 ± 0.08 ($n = 22$) and 1.37 ± 0.10 ($n = 28$) as the glucose concentrations were raised to 8.3 and 16.7mM respectively.

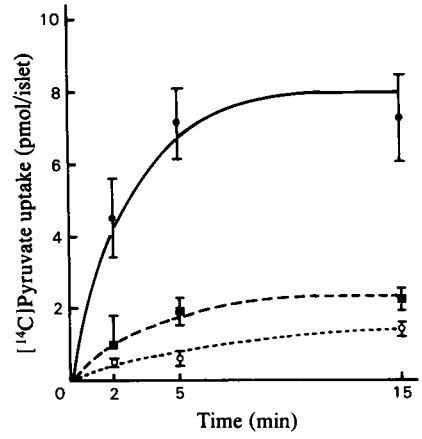

Fig. 6. *Time course for the uptake of [^{14}C]pyruvate by isolated islets*

The experiments were performed in the presence of pyruvate: 1.0 (○), 5.0 (■) or 30.0 (●) mM. Mean values (±S.E.M.) refer to 5–17 individual observations.

The oxidation of pyruvate progressively increased as its concentration was raised from 1.0 to 30.0mM (Fig. 7). As is the case for the effect of pyruvate on insulin release (see Fig. 1), there was a trend for the oxidation of 30.0mM-pyruvate to be somewhat higher than that expected from the extrapolation of the curve recorded at lower concentrations of pyruvate. The value noted at 30.0mM-pyruvate does not represent the maximal rate of pyruvate oxidation. Indeed, with [3-^{14}C]pyruvate as the tracer, the rate of oxidation observed at 90.0mM-pyruvate averaged $208.5 \pm 11.9\%$ ($n = 19$) of the mean reference value recorded within the same experiment at 30.0mM-pyruvate.

The rates of [2-^{14}C]- and [3-^{14}C]-pyruvate oxidation were not significantly different from one another whatever the concentration of pyruvate (Fig. 7). As judged from the output of $^{14}CO_2$, the rate of [1-^{14}C]-pyruvate decarboxylation was about 57% higher ($P < 0.001$) than that of [2-^{14}C]pyruvate oxidation (Fig. 8). As expected from these findings, the output of $^{14}CO_2$ derived from [U-^{14}C]pyruvate gave values intermediate between those seen with [2-^{14}C]- and [1-^{14}C]-pyruvate (Fig. 8). In terms of $^{14}CO_2$ output, the rate of oxidation of 30mM-[U-^{14}C]pyruvate was higher than that of 8.3mM-[U-^{14}C]glucose (Fig. 8).

The oxidation of pyruvate was inhibited by anoxia, antimycin A, KCN and iodoacetate, but unaffected by menadione (Table 4). Whereas antimycin A completely suppressed [2-^{14}C]pyruvate oxidation, it only caused a partial inhibition of [1-^{14}C]pyruvate decarboxylation.

A significant fraction of the pyruvate taken up by the islets was converted into lactate. After 120min

incubation in the presence of pyruvate (30.0mM), the total amount of lactate recovered in the islets and their surrounding incubation medium increased ($P<0.001$) from a basal value of 36.6 ± 13.8 ($n=9$) to 159.5 ± 12.0 ($n=10$) pmol/islet. When the mean rate of oxidation of 30.0mM-[U-^{14}C]pyruvate (87.3 ± 4.1pmol/120min per islet) was corrected for the pyruvate-induced increment in lactate production (i.e. 122.9 ± 18.2pmol/120min per islet), the net rate of generation of reducing equivalents (i.e. reduced nicotinamide nucleotides and reduced flavoprotein) could be estimated at 313.6 ± 27.4pmol/120min per islet.

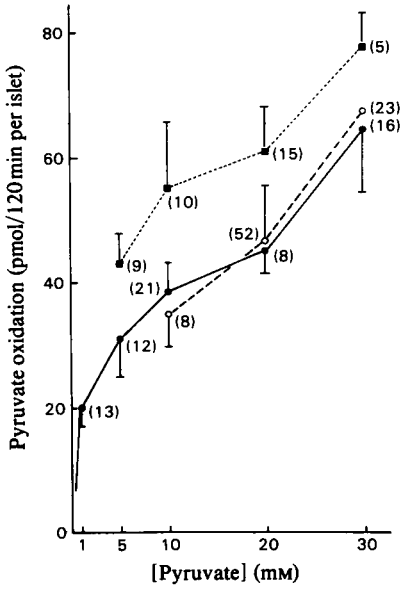

Fig. 7. *Effect of the concentration of pyruvate on its rate of oxidation*
The numbers of individual determinations are shown. [2-^{14}C]Pyruvate (●), [3-^{14}C]pyruvate (○) or [U-^{14}C]pyruvate (■) was used as the tracer molecule.

Reciprocal influence of different nutrients on their oxidation rate

The oxidation of [2-^{14}C]pyruvate (30mM) was little affected by the presence of glucose (Table 5), even at a very high concentration of the sugar (27.8mM). However, both leucine (20mM) and 4-methyl-1-oxopentanoate (10mM) were able to decrease [2-^{14}C]pyruvate oxidation.

As little as 5mM-pyruvate was sufficient to cause a significant decrease in the output of $^{14}CO_2$ from [U-^{14}C]glucose (Table 5). Pyruvate, however, failed to affect the rate of glycolysis, as judged from the conversion of [5-^{3}H]glucose into $^{3}H_2O$. At 8.3mM-glucose, the utilization of the sugar averaged 124.3 ± 8.4 and 124.9 ± 22.3pmol/120min per islet ($n=10$ in

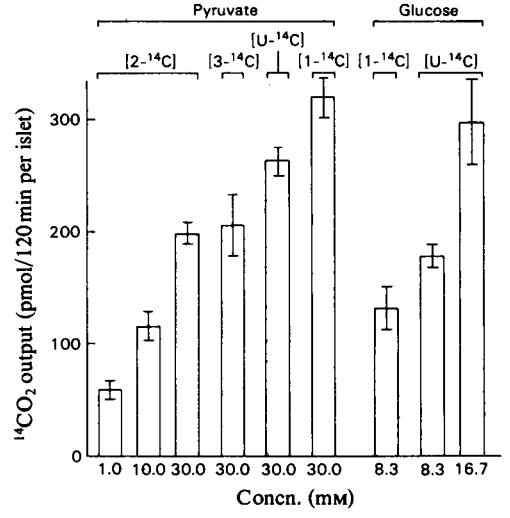

Fig. 8. *Oxidation of pyruvate and glucose by isolated islets*
The rate of oxidation (mean±S.E.M.) is shown as a function of both the concentration of the nutrient and the nature of the tracer molecule.

Table 4. *Effect of metabolic inhibitors on the oxidation of pyruvate*
Mean values (±S.E.M.) are shown together with the number of individual determinations.

Substrate (mM)	Inhibitor (mM)	Oxidation rate (pmol/120min per islet)
[2-^{14}C]Pyruvate (10mM)	None	30.4 ± 3.2 (16)
	Antimycin A (0.01)	0.1 ± 0.4 (8)
	KCN (1.0)	14.8 ± 1.0 (6)
	Iodoacetate (1.0)	17.2 ± 1.6 (8)
	Menadione (0.01)	30.1 ± 10.3 (8)
[1-^{14}C]Pyruvate (20mM)	None	78.6 ± 5.1 (7)
	Anoxia	6.9 ± 2.4 (7)
	Antimycin A (0.01)	40.0 ± 1.9 (7)

Table 5. *Reciprocal influence of nutrients on their oxidation*
Mean values (±S.E.M.) are shown together with the number of individual determinations (in parentheses), and the statistical significance (*$P<0.05$ or less) of differences attributable to the unlabelled nutrient.

Nutrients (mM)		Oxidation (pmol/120min per islet)
[2-^{14}C]Pyruvate (30)	Alone	56.0±8.5 (23)
	+[U-^{12}C]glucose (8.3)	52.9±8.5 (22)
	+[U-^{12}C]glucose (27.8)	48.2±6.2 (23)
[2-^{14}C]Pyruvate (20)	Alone	49.2±6.2 (13)
	+[U-^{12}C]leucine (20)	30.7±5.8 (7)*
	+[U-^{12}C]4-methyl-2-oxopentanoate (10)	28.0±4.6 (13)*
[U-^{14}C]Glucose (8.3)	Alone	29.4±1.7 (48)
	+[U-^{12}C]pyruvate (5)	21.1±1.4 (17)*
	+[U-^{12}C]pyruvate (10)	20.9±1.2 (17)*
	+[U-^{12}C]pyruvate (30)	20.1±2.3 (17)*

Table 6. *Effect of glucose* (8.3mM) *and pyruvate* (30.0mM) *on the concentration of adenine nucleotides*
Mean values (±S.E.M.) for the concentration of adenine nucleotides were measured at 30min of incubation, and refer to 19–25 individual determinations.

	No addition	+Pyruvate	+Glucose	+Pyruvate+glucose
ATP+ADP+AMP (pmol/islet)	10.4±0.9	10.1±0.7	12.0±0.8	11.8±1.1
ATP (percentage)	58.5±2.4	60.9±1.5	69.5±1.9	67.5±3.1
ADP (percentage)	28.1±1.7	26.2±1.2	18.0±1.7	21.1±1.9
AMP (percentage)	13.3±1.6	12.8±1.1	12.6±1.2	11.4±1.7
ATP/ADP (ratio)	2.34±0.30	2.45±0.17	5.03±0.67	4.24±0.62
Adenylate charge (ratio)	0.725±0.018	0.740±0.031	0.784±0.013	0.780±0.023

each case) in the absence and presence of pyruvate (30.0mM) respectively.

From the data in Table 5, it can be calculated that, when both pyruvate (30.0mM) and glucose (8.3mM) are present in the same medium, the total output of $^{14}CO_2$ should represent 81.2±10.0% of the sum of the oxidation rates for each substrate measured separately. When [2-^{14}C]pyruvate (30mM) and [6-^{14}C]glucose (8.3mM) of identical specific radioactivity were added to the same medium, the total output of $^{14}CO_2$ indeed averaged 87.5±17.2% ($n=8$) of the sum of the oxidation rates observed within the same experiment, in islets exposed to each nutrient separately. Under these conditions, the total oxidation of exogenous nutrients in the simultaneous presence of glucose and pyruvate, which corresponded to a mean $^{14}CO_2$ output of 280±36pmol/120min per islet, was close to that observed, in the present series of experiments, when [U-^{14}C]glucose alone was used at a concentration of 16.7mM (i.e. 295±38pmol of $^{14}CO_2$/120min per islet; $n=26$). Yet, the rate of insulin secretion observed in the simultaneous presence of glucose (8.3mM) and pyruvate (30.0mM) was much lower than that recorded in the presence of 16.7mM-glucose alone (see Fig. 2).

Effect of pyruvate on the concentration of adenine nucleotides

After 30min incubation in the absence or presence of glucose (8.3mM), pyruvate (30.0mM) failed significantly to affect the concentration of ATP, ADP or AMP in the islets (Table 6). As expected from previous investigations (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1978*b*), glucose itself significantly increased the concentration of ATP, the ATP/ADP ratio and the adenylate charge ($P<0.01$ or less) in the islet cells.

Effect of pyruvate on the concentration of reduced nicotinamide nucleotides

The total concentration of reduced nicotinamide nucleotides (NADH+NADPH) was measured at 30min of incubation in islets exposed to glucose and/or pyruvate (Table 7). Pyruvate (30.0mM) failed to increase significantly the concentration of reduced nicotinamide nucleotides above the basal value. As expected from previous observations (Malaisse *et al.*, 1978*b*), glucose (8.3mM) significantly increased ($P<0.005$) the concentration of NADH+NADPH. Pyruvate, in the presence of glucose, further enhanced ($P<0.05$) the concentration of reduced nicotinamide nucleotides.

There was a highly significant correlation ($r = 0.981$; $n = 5$; $P < 0.005$) between the total concentration of reduced nicotinamide nucleotides and the mean corresponding values for the net uptake of ^{45}Ca (Fig. 5*a*).

Effect of pyruvate on lipogenesis

As judged from the incorporation of ^3H_2O in islet lipids, the synthesis of lipids found in the absence of exogenous substrate averaged 8.91 ± 1.58 pmol of acetyl-CoA residues/120min per islet (Table 8). About 80% of the newly synthesized lipids corresponded to phospholipids and mono- or tri-acylglycerols. Antimycin A suppressed basal lipogenesis. Glucose (16.7mM) significantly ($P < 0.005$) augmented lipogenesis, above basal value. Pyruvate (30.0mM) mimicked, to a limited extent, the effect of glucose. By comparison with the basal data, the lipids synthesized in response to pyruvate were recovered in both the non-esterified fatty acid and triacylglycerol fractions, whereas, in response to glucose, they represented mostly triacylglycerols.

Effect of pyruvate on the utilization of endogenous nutrients

To assess the effect of glucose and pyruvate on the oxidation of endogenous nutrients, the islets were preincubated for 120min in the presence of [U-^{12}C]glucose (8.3mM) and [U-^{14}C]palmitate (0.27mM) and then washed to remove extracellular nutrients. The incorporation of radioactivity in the islets corresponded to a net uptake of 1.93 ± 0.05 pmol of palmitate/islet ($n = 121$). This radioactivity was distributed as outlined in Table 9.

After washing, the islets were incubated for 120min in the absence or presence of exogenous nutrients. Both the output of $^{14}CO_2$ and the final radioactive content of the islets were measured.

In the absence of exogenous nutrients, the output of $^{14}CO_2$ averaged $4.56 \pm 0.19\%$ ($n = 31$) of the final radioactive content of the islets (Table 10). About 30% of this control output of $^{14}CO_2$ could not be suppressed by metabolic poisons (antimycin A 10.0μM; rotenone 1.0μM; KCN 1.0mM), suggesting an extramitochondrial process of oxidation (Markwell *et al.*, 1977). Glucose (8.3 or 16.7mM) decreased the rate of $^{14}CO_2$ by about 10% to a mean value representing $4.04 \pm 0.16\%$ ($n = 30$) of the final radioactive content of the islets. Pyruvate (30.0mM) caused a much more marked inhibition in $^{14}CO_2$ output than did glucose, the data recorded in the presence of pyruvate averaging $67.9 \pm 4.2\%$ of their appropriate control values. Even in the presence of glucose (8.3mM), pyruvate still inhibited ($P < 0.005$) the output of $^{14}CO_2$, although to a somewhat lesser extent than in the absence of glucose. Thus the output of $^{14}CO_2$ recorded in the simultaneous presence of pyruvate and glucose averaged $84.4 \pm 2.6\%$ of that measured in the sole presence of glucose (8.3mM).

Assuming that the present data are representative of the overall decrease in the utilization of endogenous nutrients and by using the basal rate of O_2 consumption in the present system as a reference value, we estimated to what extent the glucose- and pyruvate-induced fall in $^{14}CO_2$ output, as documented in Table 10, may affect the net generation rate of reducing equivalents relative to the basal value found in the absence of exogenous nutrients. As illustrated in Table 11, when corrected in such a way, the net gain in reducing equivalents above basal value was much lower in the presence of pyruvate than glucose. As a matter of fact, the mean value found with 30mM-pyruvate represented 23.0% of that found

Table 7. *Concentration of reduced nicotinamide nucleotides*
Mean values (±S.E.M.) were measured at 30min of incubation, and are shown together with the number of individual determinations (in parentheses).

Nutrient (mM)	NADH+NADPH (fmol/islet)
None	150.6±13.1 (32)
Pyruvate (30.0)	170.0±16.5 (33)
Glucose (8.3)	223.8±20.4 (26)
Glucose (8.3)+pyruvate (30.0)	283.9±21.6 (28)

Table 8. *Incorporation of ^3H_2O into islet lipids*
All results are expressed in terms of acetyl-CoA residues synthesized by the islets (pmol/120min per islet). The data relative to each class of lipids refer to a duplicate determination. The mean values (±S.E.M.) for the total extract refer to eight individual observations.

Lipid Added agents (mM) ···	None	Antimycin A (0.01)	Glucose (16.7)	Pyruvate (8.3)
Non-esterified fatty acids	0.51	0.85	1.13	5.26
Triacylglycerols	1.43	0.24	10.71	4.31
Phospholipids and monoacylglycerols	4.23	0.72	4.05	2.83
Esterified cholesterol	0.84	0.14	1.74	0.68
Total extract	8.91±1.58	2.30±0.36	19.52±2.64	14.40±2.18

with 8.3mM-glucose, a finding reminiscent of the observation that the insulinotropic action of 30mM-pyruvate is comparable with that of 2mM-glucose.

Effect of dichloroacetate on glucose oxidation and insulin release

Over a wide range of concentration, dichloroacetate failed to affect insulin release evoked by 16.7mM-glucose (Table 12). The drug also failed to affect the oxidation of [U-^{14}C]glucose (16.7mM), which averaged 42.2 ± 4.6 and 41.8 ± 4.4 pmol/120min per islet in the absence and presence respectively of dichloroacetate (25mM) ($n = 11$ in both cases).

Discussion

The present findings indicate that, under suitable experimental conditions, pyruvate stimulates insulin release (Table 1, Figs. 1 and 3). The effect of pyruvate is modest relative to that of glucose (Fig. 2). In most previous studies with pancreatic tissue from fed adult animals, pyruvate (5–20mM) was reported not to stimulate insulin secretion (Coore & Randle, 1964; Montague & Taylor, 1969; Matschinsky & Ellerman, 1973; Lenzen *et al.*, 1976). It should be noted, however, that such studies were invariably carried out at either low (2.0–3.3mM) or high (16.7–27.8mM) glucose concentrations, namely under conditions that are not optimal for characterizing the insulinotropic action of pyruvate (Fig. 2). A dose-related

Table 9. *Incorporation of [U-^{14}C]palmitate into various lipid classes*

Mean values (±S.E.M.) refer to four individual determinations, and are expressed as percentage of the total ^{14}C-labelled lipids present in the islets. The islets were preincubated for 120min in the presence of [U-^{14}C]palmitate (0.27mM), washed, incubated for 120min in the absence of labelled substrate, and eventually homogenized by sonication. The sixth fraction corresponds to phosphatidylinositol (PI), phosphatidylserine (PS), and lysophosphatidylethanolamine (LPE).

Non-esterified fatty acids	20.6 ± 2.5
Monoacylglycerols	15.9 ± 1.8
Diacylglycerols	3.0 ± 1.1
Triacylglycerols	3.9 ± 0.1
Esterified cholesterol	1.8 ± 0.3
PI+PS+LPE	7.5 ± 1.1
Phosphatidylcholine	47.3 ± 4.8

Table 10. *Oxidation of endogenous nutrient*

Islets were preincubated with [U-^{14}C]palmitate, washed, incubated for 120min in the absence of radioactively labelled nutrients, and eventually examined for their radioactive content. Mean values (±S.E.M.) are shown together with the number of individual determinations (n).

Added nutrient or drug (mM)	$^{14}CO_2$ output (c.p.m./120min per islet)	Final ^{14}C content (c.p.m./islet)	$^{14}CO_2$ output / final ^{14}C content (%)	n
None	146 ± 11	3258 ± 151	4.46 ± 0.29	18
Glucose (16.7)	128 ± 7	3292 ± 142	4.02 ± 0.25	18
Pyruvate (30.0)	96 ± 10	3272 ± 231	2.98 ± 0.17	18
Antimycin A (0.01)	46 ± 5	3298 ± 168	1.32 ± 0.10	17
None	142 ± 11	3009 ± 234	4.70 ± 0.18	13
Glucose (8.3)	123 ± 9	3059 ± 224	4.06 ± 0.15	12
Glucose (8.3)+pyruvate (30.0)	105 ± 11	3024 ± 288	3.43 ± 0.11	13
Antimycin A (0.01)	41 ± 6	3031 ± 378	1.44 ± 0.14	12

Table 11. *Tentative and partial balance sheet for the generation and utilization of reducing equivalents in islets exposed to pyruvate (30mM) or glucose (8.3mM)*

The absolute value for the utilization of endogenous nutrients was derived from the basal O_2 uptake, which, in the present system, averages 176.1 ± 19.7 pmol of O_2/60min per islet ($n = 23$; J. C. Hutton & W. J. Malaisse, unpublished work).

Line no.	Reducing equivalents (pmol/120min per islet)	Pyruvate (30.0mM)	Glucose (8.3mM)
1	Produced through the oxidation of exogenous nutrient	436.5 ± 20.5	352.8 ± 20.4
2	Lost through the conversion of exogenous nutrient into lactate	122.9 ± 18.2	—
3	Generated by the metabolism of exogenous nutrient (1–2)	313.6 ± 27.4	352.8 ± 20.4
4	Utilized for lipogenesis in excess of basal value	11.0 ± 5.4	≤ 21.2 ± 6.2
5	Utilized in substitution to endogenous nutrients	244.1 ± 27.3	77.2 ± 8.7
6	Net gain relative to basal value (3−4−5)	58.5 ± 39.1	254.4 ± 23.1

Table 12. *Effect of dichloroacetate on insulin release*
Mean values (±S.E.M.) for the rate of insulin release evoked by glucose (16.7mM) are shown together with the number of individual observations (in parentheses).

Dichloroacetate (mM)	Insulin output (μ-i.u./90min per islet)
0	270.4±14.2 (20)
1.0	282.2±17.8 (20)
10.0	305.8±21.9 (10)
50.0	307.7±18.7 (10)
100.0	267.1±27.3 (10)

effect of pyruvate on insulin release, comparable with that illustrated in Fig. 1, was already observed in cultured explants of foetal rat pancreas incubated in the presence of 10mM-caffeine (Lambert, 1970).

The present study is aimed at re-evaluating the effect of pyruvate on islet function within the framework of recent investigations that suggest that the insulinotropic action of different nutrients is related to their readiness to be metabolized and to increase the concentration of reduced nicotinamide nucleotides in islet cells (Malaisse *et al.*, 1978*b*,*c*).

Insulinotropic action of pyruvate

At first glance the present data seemed incompatible with the above-mentioned hypothesis. Indeed, up to 30.0mM, pyruvate, although being readily oxidized by the islets (Figs. 7 and 8), failed to stimulate insulin release in the absence of glucose (Table 1, Fig. 3). The failure of 30mM-pyruvate to provoke insulin release in the absence of glucose was confirmed by the fact that, even in the presence of theophylline and cytochalasin B, compounds that invariably augment stimulated insulin output, the rate of secretion remained close to its basal value (Table 2). In the absence of glucose, pyruvate also fails to stimulate proinsulin biosynthesis (Lin & Haist, 1969).

Whereas glucose slightly decreases the steady-state values for net uptake of $^{22}Na^+$ by the islets (Kawazu *et al.*, 1978), pyruvate tends to exert an opposite effect. Thus after 90min incubation in the presence of pyruvate (30.0mM), the net uptake of Na^+ averaged 171.6±17.0pmol/islet ($n=17$) as distinct from a basal value of 143.0±1.6pmol/islet ($n=14$). We considered, therefore, that the transport of pyruvate in islet cells might somehow be associated with an increased inflow of Na^+ and hence with a higher activity of the membrane-associated ($Na^+ + K^+$)-activated adenosine triphosphatase. However, even in the presence of ouabain, pyruvate failed to stimulate insulin release (Table 2).

A significant effect of pyruvate (30.0mM or less) on insulin secretion was only detected in the presence of glucose or other insulinotropic nutrients, such as fructose, glyceraldehyde, leucine and 4-methyl-2-oxopentanoate (Table 2, Fig. 1). The relationship between the glucose concentration of the incubation medium and the magnitude of the proper insulinotropic action of pyruvate clearly indicates that pyruvate should be considered as a glucose-simulating agent, as defined elsewhere (Malaisse, 1973). Thus pyruvate shifts to the left the sigmoidal curve relating insulin output to the ambient glucose concentration (Fig. 2). The magnitude of such a shift is related to the concentration of pyruvate (Fig. 1). When the latter concentration is sufficiently high, a significant but modest increase in insulin output is evoked by pyruvate in the absence of glucose (Table 1). Incidentally, the view that pyruvate mimics the effect of glucose does not imply that the modality by which pyruvate and glucose respectively stimulate insulin release cannot be distinguished from one another. And indeed, the data collected after a preincubation of 90min in the absence of glucose suggest that such a prior nutritional manipulation decreases more markedly the insulinotropic capacity of glucose than that of pyruvate. Likewise, when the pancreas is removed from starved or hyperthyroidic rats, the secretory response to glucose (16.7mM) is impaired, so that the enhancing action of exogenous pyruvate can now be documented at this high glucose concentration (Lenzen *et al.*, 1975).

The pyruvate-induced shift to the left of the sigmoidal curve relating insulin output to glucose concentration indicates that the effect of 30.0mM-pyruvate is comparable with that of 2.0mM-glucose. The major aim of the present study was to discover whether such a difference could be explained by the different metabolic situations found in the islets exposed to glucose and pyruvate. The experimental findings are compatible with the view that pyruvate, like glucose (Malaisse *et al.*, 1978*b*,*c*), stimulates insulin release through a sequence of events involving (i) the metabolism of the nutrient, (ii) a resulting increase in the concentration of reduced nicotinamide nucleotides, (iii) a subsequent remodelling of calcium fluxes in the islet cells, and (iv) the eventual activation by intracellular calcium of the insulin-releasing effector system.

Metabolism of pyruvate in the islets

In considering the metabolism of pyruvate in the islets, it should be first remembered that the islets represent a heterogeneous cell population including not only insulin-producing cells.

Exogenous pyruvate rapidly accumulated in the islet cells (Fig. 6). An ionophoretic rather than diffusional process probably mediates the entry of pyruvate in islet cells, as in other tissues (Spencer & Lehninger, 1976; Halestrap, 1976). Taking into

account the intracellular water space of the islets (2–3 nl/islets; see Malaisse *et al.*, 1976), the steady-state concentration of pyruvate (and its metabolites) in the islets was much lower than its extracellular concentration. The uptake of pyruvate as a function of its extracellular concentration appeared as a saturable phenomenon with a K_m close to 8–9 mM.

In the islets exposed to exogenous pyruvate, the true intracellular concentration of pyruvate was not measured. Assuming rapid interconversion of pyruvate and lactate, the latter concentration may not be vastly different from that of endogenous pyruvate derived from the metabolism of glucose. Differences in either pool size or fractional turnover of these nutrient pools may explain why pyruvate (30.0 mM), although not altering the rate of glycolysis, lowered the rate of oxidation of [U-^{14}C]glucose, whereas glucose failed to affect [2-^{14}C]pyruvate oxidation.

The rate of pyruvate oxidation by the islets was relatively high, when compared with that of glucose (Fig. 8). Several findings indicate that this situation is not attributable to an experimental artifact. The limited and occasional contamination of our islets preparation by acinar tissue cannot be considered a significant factor of error (see the Experimental section). The rate of pyruvate oxidation, as a function of its extracellular concentration, displayed a pattern compatible with the existence of a saturation process (Fig. 7). The oxidation of pyruvate was decreased in the presence of either leucine or 4-methyl-2-oxopentanoate (Table 5). The oxidation of pyruvate was partially or totally suppressed by suitable metabolic poisons (Table 4). Almost identical data were obtained with [2-^{14}C]- and [3-^{14}C]-pyruvate (Fig. 7). At a concentration of 30.0 mM, the rate of decarboxylation of [1-^{14}C]pyruvate exceeded that of [2-^{14}C]- or [3-^{14}C]-pyruvate oxidation by 39.0 ± 9.1 pmol/120 min per islet. The latter value happens to be close to the rate of [1-^{14}C]pyruvate decarboxylation in the presence of antimycin A, which suppressed [2-^{14}C]pyruvate oxidation. Some of the acetyl-CoA residues, which are derived from pyruvate but are not oxidized, could conceivably be used for the purpose of lipogenesis, which was indeed modestly stimulated by pyruvate (Table 8).

Our metabolic data are in good agreement with other published values. In rat islets, Lin & Haist (1971) noted that [U-^{14}C]pyruvate (33.3 mM) was oxidized at a rate of 90 ± 27 pmol/120 min per islet, a value almost identical with the 87 ± 4 pmol/120 min per islet here observed with [U-^{14}C]pyruvate (30.0 mM). In islets from normal mice, [1-^{14}C]pyruvate (10.0 mM) is decarboxylated at a rate of 186 ± 40 pmol/120 min per μg dry wt. (Hedeskov *et al.*, 1972). In islets removed from *ob/ob* mice, 33.3 mM-[U-^{14}C]pyruvate was oxidized at a lower rate, averaging 41.8 ± 5.2 pmol/120 min per μg dry wt. (Gunnarsson & Hellerström, 1973).

A fraction of the exogenous pyruvate taken up by the islets was converted into lactate. The latter phenomenon was taken into account when calculating the rate of generation of reducing equivalents in the islets exposed to pyruvate (Table 11). Even so, there remained an apparent discrepancy between the aptitude of pyruvate to generate reducing equivalents and to stimulate insulin release.

Role of reduced nicotinamide nucleotides as a coupler between metabolic and cationic events

Several findings in the present study support the view that, in the process of pyruvate-induced insulin release, the generation of reduced nicotinamide nucleotides plays an essential role, possibly by coupling the metabolism of pyruvate to the subsequent accumulation of calcium in the islet cells. First, the aptitude of pyruvate to stimulate insulin release, as a function of the presence or absence of glucose, coincided with its aptitude to increase the concentration of reduced nicotinamide nucleotides in the islets (Table 7). Secondly, and as already observed under other experimental conditions (Malaisse *et al.*, 1978*b*), there was a tight proportionality (Fig. 5*a*) between the steady-state concentration of NADH+NADPH and the size of the exchangeable pool(s) of calcium, as judged from the net uptake of ^{45}Ca. And thirdly, menadione inhibited the effect of pyruvate on insulin release (Table 2). We have previously indicated that the inhibitory effect of 0.01 mM-menadione on glucose-induced insulin release is indeed attributable to a fall in the concentration of reduced nicotinamide nucleotides rather than to any abnormality in glucose metabolism (Malaisse *et al.*, 1978*b*). Likewise, at the same concentration (0.01 mM), menadione impaired the insulinotropic action of pyruvate, but failed to affect its rate of oxidation (Table 4). The modality by which reducing equivalents may affect cation transport by native ionophoretic systems in the islet cells was previously considered (Malaisse *et al.*, 1978*a*).

Participation of calcium in pyruvate-induced insulin release

The involvement of calcium in the insulinotropic action of pyruvate is supported by the finding that the usual relationship between the net uptake of ^{45}Ca and the rate of insulin release, as previously documented under a variety of experimental conditions (Malaisse-Lagae & Malaisse, 1971), was also applicable to the process of pyruvate-induced insulin release (Fig. 5*b*). Moreover, pyruvate mimicked the ability of glucose to lower the efflux of ^{45}Ca from islets exposed to a Ca^{2+}-free medium (Fig. 4). The latter effect was sufficiently rapid to account for the dynamics of pyruvate-induced insulin release, as documented in

Fig. 3. It is reasonable, therefore, to assume that the insulinotropic action of pyruvate, like that of glucose, is indeed mediated by the accumulation of calcium in the β-cell and the eventual activation by calcium of the appropriate effector system controlling the exocytosis of secretory granules.

Incidentally, in the absence of glucose, the aptitude of pyruvate (30mM) to affect ^{45}Ca efflux and uptake, although unable to stimulate insulin release, is understandable in view of the existence of a threshold phenomenon for the stimulant action of calcium on insulin release (see Fig. 5*b*). In this respect, the effect of pyruvate (30.0mM) in the absence of glucose resembles that of low glucose concentrations (up to 4.5mM), which also affect calcium handling without causing insulin release (Herchuelz & Mahy, 1974).

Interference of pyruvate with the utilization of endogenous nutrients

It remained to be understood why pyruvate (30.0mM), although oxidized at a higher rate (Fig. 8) than glucose (8.3mM), was much less efficient than the sugar in raising the concentration of reduced nicotinamide nucleotides, as already observed by Panten *et al.* (1973). The rate of pyruvate conversion into lactate was not sufficiently high to account fully for this discrepancy (Table 11). The effect of pyruvate and glucose on the rate of lipogenesis (Table 8) also failed to account for the discrepancy. The latter was apparently attributable to the fact that pyruvate exerts a more pronounced inhibitory effect than does glucose on the utilization of endogenous nutrients (Table 10). Indeed, when corrected for such a phenomenon, the net generation of reducing equivalents in the presence of glucose and pyruvate became strictly proportional to their insulinotropic capacity (Table 11).

The findings illustrated in Table 11 are in good agreement with previous reports indicating that the rate of O_2 consumption by isolated islets is increased much more (above its basal value) by glucose than by pyruvate (Hedeskov *et al.*, 1972; Hellerström *et al.*, 1970). For glucose, it was already underlined that the sugar-induced increase in O_2 consumption coincides with the amount of O_2 required for the oxidation of exogenous glucose (Hellerström & Gunnarsson, 1970). Our data support this observation, since they suggest that glucose only exerts a marginal effect on the utilization of endogenous nutrients.

The calculations outlined in Table 11, which suggest that the overall gain in reducing equivalents is relatively modest in the presence of exogenous pyruvate, could also explain why pyruvate, in contrast with glucose, fails to prevent the fall in ATP concentration and adenylate charge (Table 6) normally seen when islets are incubated in the absence of exogenous nutrients (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1976).

At this point it should be mentioned that the metabolic situation found in the concomitant presence of glucose and pyruvate may not strictly coincide with that expected from the summation of the individual effects of each nutrient. First, and as already discussed, allowance should be made for the reciprocal influence of glucose and pyruvate on their respective oxidation rates (Table 5). Secondly, the data outlined in Table 10 indicate that, in decreasing the utilization of endogenous nutrients, the effects of pyruvate and glucose were not additive.

Possible role of endogeneous pyruvate in glucose-induced insulin release

In the last part of the present study we have attempted to elucidate to what extent the situation seen with exogenous pyruvate might be extrapolated to endogenous pyruvate derived from the metabolism of glucose. The finding that glucose causes a dose-related increase in the endogenous concentration of pyruvate (see the Results section) is compatible with the view that the metabolism of pyruvate somehow participates in the process of glucose-induced insulin release. To document further the role of endogenous pyruvate, we have used dichloroacetate with the hope that this drug might affect the activity of the islet pyruvate dehydrogenase system (Randle, 1976). However, dichloroacetate failed to affect both glucose oxidation and glucose-stimulated insulin release (Table 12). These negative data obviously do not rule out a possible role for the oxidation of endogenous pyruvate in the normal secretory response evoked by glucose in the islets.

Taken as a whole, the findings outlined in the present paper could even raise the question as to whether, in the stimulus-secretion coupling of glucose-induced insulin release, glycolysis plays any role other than being the major pathway for the conversion of glucose into endogenous pyruvate and contributing modestly to the overall generation rate of reduced nicotinamide nucleotides, which would be mostly derived from the mitochondrial oxidation of pyruvate.

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