Biochem. J. (1979) **184**, 291–301
Printed in Great Britain

The Metabolism of 4-Methyl-2-oxopentanoate in Rat Pancreatic Islets

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(*Received* 4 *May* 1979)

1. Radioactively labelled 4-methyl-2-oxopentanoate was taken up by isolated pancreatic islets in a concentration- and pH-dependent manner and led to the intracellular accumulation of labelled amino acid and to a decrease in the intracellular pH. Uptake of 4-methyl-2-oxopentanoate did not appear to be either electrogenic or Na^+-dependent. The islet content of 2-oxo acid radioactivity was not affected by either 2-cyano-3-hydroxycinnamate (10mM) or pyruvate (10mM), although both these substances inhibited the oxidation of [U-^{14}C]4-methyl-2-oxopentanoate by islet tissue. 2. 4-Methyl-2-oxopentanoate markedly stimulated islet-cell respiration, ketone-body formation and biosynthetic activity. The metabolism of endogenous nutrients by islets appeared to be little affected by the compound. 3. Studies with the ^{3}H- and ^{14}C-labelled substrate revealed that 4-methyl-2-oxopentanoate was incorporated by islets into CO_2, water, acetoacetate, L-leucine and to a lesser extent into islet protein and lipid. Carbon atoms C-2, C-3 and C-4 of the acetoacetate produced were derived from the carbon skeleton of the 4-methyl-2-oxopentanoate, but the acetoacetate carboxy group was derived from the incorporation of CO_2. These results, and consideration of the relative rates of $^{14}CO_2$ and acetoacetate formation from 1-^{14}C-labelled as opposed to U-^{14}C-labelled 4-methyl-2-oxopentanoate, led to the conclusion that the pathway of catabolism of this 2-oxo acid in pancreatic islets is identical with that described in other tissues. The amination of 4-methyl-2-oxopentanoate by islets was attributed to the presence of a branched-chain amino acid aminotransferase (EC 2.6.1.42) activity in the tissue. Although glutamate dehydrogenase activity was demonstrated in islet tissue, the reductive amination of 2-oxo acids did not seem to be of importance in the formation of leucine from 4-methyl-2-oxopentanoate. 4. The results of experiments with respiratory inhibitors and uncouplers, and the finding that $^{14}CO_2$ production and islet respiration were linked in a 1:1 stoicheiometry suggested that 4-methyl-2-oxopentanoate catabolism was coupled to mitochondrial oxidative phosphorylation. The catabolism of 4-methyl-2-oxopentanoate in islet tissue appeared to be regulated at the level of the initial 2-oxo acid dehydrogenase (EC 1.2.1.25) reaction.

4-Methyl-2-oxopentanoate is a powerful stimulus for the secretion of insulin from the perfused pancreas (Fertel *et al.*, 1972; Lenzen, 1977) and from isolated pancreatic islets (Panten *et al.*, 1972). The present paper, part of which has been reported in abstract form (Hutton & Malaisse, 1977; Hutton *et al.*, 1977, 1978), attempts to define and quantify the principal metabolic fate of 4-methyl-2-oxopentanoate in islet tissue.

Experimental

Materials

Unless otherwise indicated, all biochemical reagents were obtained from Boehringer, Mannheim, West Germany. Except for L-[4,5-^{3}H]leucine (N.E.N. Corp., Boston, MA, U.S.A.) all radioisotopes mentioned were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

^{14}C- and ^{3}H-labelled 4-methyl-2-oxopentanoate were prepared by enzymic oxidation (1–2h incubation) of the correspondingly labelled leucine precursors by the method of Meister (1952). The reaction mixture was subjected to gel filtration (20cm × 1.5cm internal diam. column; Sephadex G-25; Pharmacia, Uppsala, Sweden) and ion-exchange chromatography (3cm × 0.5cm internal diam. column; Dowex 50; H^+ form; Fluka, Buchs, Switzerland), neutralized (pH7.5) and freeze-dried. The radioactive isotopes so prepared were generally used without further purification. The radioactive material could be converted, in greater than 98% yield, into a dinitrophenylhydrazone, which behaved identically with the dinitrophenylhydrazone of authentic 4-methyl-2-oxopentanoate (Sigma) with respect to its absorption spectra

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and chromatographic behaviour (solvent system: water-saturated butanol; Whatman 3MM). The ^{14}C-labelled substrates reacted completely with a 50%-satd. solution of ceric sulphate in H_2SO_4 (2M) to yield $^{14}CO_2$ and a compound that behaved as authentic isovaleric acid (Sigma) on t.l.c. [solvent system: butan-1-ol/ethanol/aq. 3M-NH_3 (4:1:5, by vol.)]. [3-^{14}C]Acetoacetate and unlabelled acetoacetate were prepared by the alkaline hydrolysis of the corresponding ethyl ester (Krebs & Eggleston, 1945) and were standardized by enzymic analysis (Mellanby & Williamson, 1974).

Islet incubations

Islets were prepared by digestion with collagenase (EC 3.4.24.3) of three to four pancreases obtained from female Wistar rats weighing about 200g (Lacy & Kostianovsky, 1967). The tissue was generally incubated under O_2/CO_2 (19:1) in Krebs–Henseleit buffer, pH7.5, containing bovine serum albumin (5mg/ml) (Malaisse *et al.*, 1970). 4-Methyl-2-oxopentanoate was added as its sodium salt and replaced an equimolar quantity of NaCl in the media. Alteration of the pH of the media was achieved by reciprocal alteration of the buffer Cl^- and HCO_3^- concentrations. HCO_3^--free media, when used, were supplemented with 10mM-sodium phosphate, pH7.5, or 25mM-Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid], pH7.5, incubations being then performed under an air atmosphere.

Uptake and distribution of 4-methyl-2-oxopentanoate radioactivity

Groups of 10 islets placed in polythene microcentrifuge tubes (Beckman Instruments, Fullerton, CA, U.S.A.) were incubated in 40μl of medium containing either 4-methyl-2-oxo[1-^{14}C]pentanoate (1–25mM; 20μCi/ml) and [6,6′-^3H]sucrose (1mM; 20μCi/ml) or 4-methyl-2-oxo[4,5-^3H]pentanoate (2.5–10mM; 20μCi/ml) and [U-^{14}C]sucrose (1mM; 10μCi/ml). At the end of the incubation period the islets were pelleted in the tip of a tube (10s centrifugation; Beckman microfuge model 152) and then isolated from the incubation media by the addition of di-n-butyl phthalate (100μl) and further centrifugation (10s). The tip of the tube was immediately removed with a scalpel and transferred to a tube in a liquid-N_2 bath. Then 1 ml of a solution of 4-methyl-2-oxopentanoate (1mM) and leucine (1mM) (pH7.0–7.5) was added to each tube, and the sample then subjected to sonification (MSE sonifier; 10s at medium setting). The sonified material was passed immediately on to a column (10cm×5mm diam.) which contained 0.5ml of Dowex 50 H^+ resin. The eluate which contained the labelled 2-oxo acid was pooled with a subsequent water wash (1ml). The column was then eluted with 1.5ml of aq. NH_3 (1M) to recover labelled amino acids. Both fractions were then mixed with 15ml of scintillation fluid (Lumagel; Lumac, Basel, Switzerland) and their ^3H and ^{14}C contents determined by dual-channel liquid-scintillation counting. The radioactivity found was expressed as the apparent intracellular contents of 4-methyl-2-oxopentanoate or leucine after correction had been performed for the extracellular contamination represented by radioactive sucrose.

Samples (1μl) of the initial incubation medium were subjected to identical processing in order to calibrate the procedure.

Intracellular pH determination

The intracellular pH was determined in a similar manner to that used to study substrate uptake. The incubation medium, however, contained 5,5-dimethyl-[2-^{14}C]oxazolidine-2,4-dione (1mM; 10μCi/ml) in the place of labelled 2-oxo acid, and the isolated islet pellet was placed directly in 10ml of Lumagel for the determination of its ^{14}C and ^3H radioactivity by liquid-scintillation counting. The intracellular pH was calculated from the observed net uptake of ^{14}C radioactivity by using the equations as described by Waddel & Butler (1959) and Hellman *et al.* (1972).

$^{14}CO_2$, ^{14}C-labelled 3-oxo acid and ^3H_2O formation

Groups of 15 islets were placed in tubes (30mm×5mm diam.) housed within a sealed 20ml liquid-scintillation vial. Incubations were performed at 37°C for 2h in 40μl of medium containing the labelled substrate (10μCi/ml), and were arrested by the addition of 20μl of a solution of antimycin A (10μM) in imidazole/HCl buffer (200mM, pH6). The $^{14}CO_2$ released was trapped in 0.5ml of Hyamine hydroxide (Packard Instruments, Downers Grove, IL, U.S.A.) (Malaisse *et al.*, 1974), and determined by liquid-scintillation counting after the addition of 10ml of scintillation fluid (Lipoluma; Lumac). Production of $^{14}CO_2$ by islets incubated for 2h in the presence of antimycin A (3μM) was not evident.

[^{14}C]Acetoacetate formation was determined in the same experiment by transferring the tube that contained the islets to another scintillation vial that contained 0.5ml of a mixture of lactic acid (85%, v/v) and hydrazine (5:2, v/v). The vial was sealed and 20μl of a mixture of aniline, citric acid (2.6M) and HCl (1M) (1:1:1, by vol.) was then injected into the tube. After the vial had been shaken for 16h at 20°C, 8ml of ethanol and 10ml of Lumagel was added, and the radioactivity was determined by liquid-scintillation counting. The recovery of [3-^{14}C]acetoacetate was determined in each experiment and used to correct the results obtained (mean recovery 40–65%).

The formation of ^3H_2O was determined simultaneously with the determination of $^{14}CO_2$ production in groups of 30 islets incubated for 2h at 37°C in 15μl of medium containing both 4,5-^{3}H- and 1-^{14}C-labelled 4-methyl-2-oxopentanoate (10–20μCi/ml each). The vial in these experiments contained from the outset 2ml of $NaHCO_3$ (100mM) equilibrated with O_2/CO_2 (19:1). The incubation was stopped by the addition of 5μl of imidazole/HCl buffer (200mM, pH7) which contained antimycin A (5μM), NADH (1mM) and 3-hydroxybutyrate dehydrogenase (2μg/ml), the last two components being included to convert labelled acetoacetate into the more stable 3-hydroxybutyrate. After a 30min incubation at 25°C, 10μl of a solution of sodium dodecyl sulphate (0.2M, pH6) was injected into the tube containing the islets, and the vials were then shaken for 17h at 37°C. The radioactivity trapped in the bicarbonate solution was determined by dual-channel liquid-scintillation counting after the addition of 20ml of Lumagel.

The efficiency of the trapping procedures used was determined under identical incubation conditions by using standard solutions of $NaH^{14}CO_3$ (recovery 70–90%) and ^3H_2O (recovery 70–90%).

Incorporation of $^{14}CO_2$ into 3-oxo acid metabolites

Groups of 10 islets placed in sealed Pyrex tubes (30mm×5mm diam.) were incubated for 2h at 37°C in 60μl of medium containing $NaH^{14}CO_3$ (24mM, initially 20μCi/ml) and various substrates. At the completion of the incubation, 10μl of HCl (1M) was added to each tube (N.B. safety precautions should be taken) and the tissue homogenized. The homogenate was subsequently gassed with O_2/CO_2 (19:1) and adjusted to pH6 by the addition of a solution containing Na_2CO_3 (250mM) and imidazole (20mM). Samples (30μl) were then either removed for the enzymic determination of acetoacetate (see below) or transferred to Pyrex tubes (30mm×5mm diam.) housed in 20ml-capacity scintillation vials containing 0.5ml of Hyamine hydroxide. Decarboxylation of the ^{14}C-labelled 3-oxo acids contained in each sample was achieved as described above by the addition of the aniline/citric acid/HCl mixture to each tube. The radioactivity trapped in the Hyamine hydroxide was determined by liquid-scintillation counting after the addition of 10ml of Lipoluma.

Incorporation of ^{14}C radioactivity into protein and lipid

Islets were incubated as described above in the $^{14}CO_2$-production experiments either in the presence of U-^{14}C]4-methyl-2-oxopentanoate (10mM; 20μCi/ml) or in media containing ^3H_2O (250mCi/ml). An extract of the tissue was then made in chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957) and treated as previously described (Sener *et al.*, 1978) to obtain a total lipid extract. Material that was not solubilized in the initial extraction was separated by centrifugation (10min at 4°C at 2000*g*) and successively extracted with 0.3M-trichloroacetic acid (15min at 90°C), 95% (v/v) ethanol and acetone. The final pellet was dissolved in 0.5ml of Hyamine hydroxide and its radioactivity determined by liquid-scintillation counting after the addition of 10ml of Lipoluma. The incorporation of [4-^{3}H]phenylalanine (1.6μM; 40μCi/ml) into islet protein was determined by the method of Malaisse *et al.* (1971) over a 90min incubation.

Incorporation of radioactivity into amino acids

Incubation conditions were identical with those described above in the $^{14}CO_2$-production experiments. After the release and capture of $^{14}CO_2$ was completed the islets and their media were homogenized in 1ml of water and passed on to an ion-exchange column (10cm×5mm diam.; 0.5ml of Dowex 50, H^+ form). The column was washed extensively (20–30ml of water), and then eluted with 1.5ml of aq. 1M-NH_3. The eluted radioactivity was determined by liquid-scintillation counting after the addition of 15ml of Lumagel. The identity of the eluted radioactivity was investigated by ascending paper chromatography in a butan-1-ol/methanol/water (2:2:1, by vol.) solvent system and analytical ion-exchange radiochromatography on a Beckman Multichrom B amino acid analyser connected to a Packard Tri-Carb flow-monitor, model 320E.

Ketone-body production

Groups of eight to 15 islets were incubated for 2h at 37°C in 40–60μl of medium contained in tubes (30mm×5mm diam.). The incubation was stopped by the addition of either 5μl of HCl (1M) or 5μl of $HClO_4$ (6M). The supernatant fluid obtained after centrifugation (20min at 4°C and 2000*g*) of the homogenized samples was neutralized by the addition of a solution containing imidazole (100mM) and KOH (4M), and then analysed for its content of 3-hydroxybutyrate by using a coupled enzymic/bioluminescent reaction (Hutton *et al.*, 1979*b*), or for acetoacetate. The reaction mixture used for acetoacetate determinations was an imidazole/HCl buffer (100mM, pH6.9) containing ascorbic acid (2mM), NADH (0.2mM) and 3-hydroxybutyrate dehydrogenase (2μg/ml; Boehringer grade-I enzyme). This reagent was mixed with an equivalent volume (20–50μl) of the deproteinized sample, and, after 30min at 35°C, 0.3ml of HCl (0.3M) was added to each reaction mixture to halt enzyme activity and to destroy NADH. After standing for 10min at 25°C, the NAD^+ contained in each incubation was converted into a

fluorescent product by incubation for 10min at 60°C with 1 ml of NaOH (6M) (Lowry & Passonneau, 1972). Fluorescence measurements were made at 25°C with an Aminco SPF-125 fluorimeter (American Instrument Co., Silver Springs, MD, U.S.A.).

Enzyme activities

Groups of 400 islets were homogenized with a Dounce-type homogenizer (5–20 strokes at 4°C) in 0.5ml of Hepes buffer (10mM, pH7.2) containing either KCl (80mM), NaCl (40mM) and EGTA (1mM) or sucrose (275mM) and EGTA (1mM). The suspended material was centrifuged successively for 10min at 800*g*, 10min at 15000*g* and 10min at 166000*g* in a Beckman SW 50.1 rotor. The pellet and supernatant obtained at each stage were subjected to sonification in 500μl of the same buffer at 4°C (MSE sonifier; 10s at the medium setting). The 3-hydroxybutyrate dehydrogenase reaction mixture contained imidazole/HCl buffer (50mM, pH6.9), acetoacetate (10mM) and NADH (0.2mM). The glutamate dehydrogenase reaction mixture contained triethanolamine/HCl buffer (50mM, pH8), EDTA (2.5mM), ammonium acetate (100mM), ADP (1mM), NADH (0.2mM) and 2-oxoglutarate (7mM). The reactions were started by the addition of 1, 5 or 50μl of the sonified material and were terminated by the addition of 0.3ml of HCl (0.3M). The NAD^+ produced in each reaction was determined as described in the preceding section.

Transaminase activities were determined in a Tris/acetate buffer (50mM, pH8.6) containing 4-methyl-2-oxo[4,5-^{3}H]pentanoate (2mM; 0.1μCi/ml), pyridoxal phosphate (50μM), mercaptoethanol (1mM) and an unlabelled amino acid at a 20mM final concentration. The reaction was initiated by the addition of 5–50μl of sonicated material and was stopped by diluting the reaction mixture 20-fold with water and immediately passing it through ion-exchange resin (Dowex 50; H^+ form). The dimensions of the chromatography column and the subsequent elution and radioactivity-determination steps were identical with those described above for the determination of [^{14}C]-leucine production in whole islets. All enzyme assays were conducted for 20min at 30°C in a final volume of 100μl. Control incubations were performed under identical conditions by using heat-treated samples (100°C for 2min). The enzyme activity was linear with time for at least 20min, and was proportional to the volume of homogenate.

Islet respiration

Rates of islet O_2 consumption were determined in batches of 200–300 islets perifused at the rate of 100μl/min with media equilibrated at 37°C with air/CO_2 (19:1) (Hutton & Malaisse, 1979). The pO_2 in the effluent was monitored with a Clark oxygen electrode (type E 5046 Radiometer, Copenhagen, Denmark) and recorded on a moving paper chart. The results shown are the steady-state values observed 15–20min after a change from a medium without substrate to one that contained 4-methyl-2-oxopentanoate.

Other analyses

The production of lactate from groups of 50 islets incubated for 2h at 37°C in 100μl of medium was determined by the method of Sener & Malaisse (1976). Determination of the production of $^{14}CO_2$ from islets prelabelled with [U-^{14}C]palmitate was performed with groups of 20 islets incubated for 2h at 37°C by the method of Sener *et al.* (1978).

NH_4^+ production was determined from groups of 50 islets incubated for 2h at 37°C in 60μl of albumin-free medium. The NH_4^+ in the medium was assayed by a colorimetric procedure (Chaykin, 1969), which was performed in a final volume of 300μl.

In these determinations and in all of the above-mentioned analyses, incubations that did not contain islets were carried through all incubation and analytical steps to serve as blank determinations, whose values were subtracted from those obtained in the presence of islet tissue.

Results are presented throughout as means ± S.E.M. Probability estimates were performed by Student's *t* test. Correlation coefficients were determined by least-squares regression analysis.

Results and Discussion

Uptake of radioactive 4-methyl-2-oxopentanoate

The effect of variation in the extracellular concentration of 4-methyl-2-oxo[1-^{14}C]pentanoate on the uptake of radioactivity and its distribution between the islet '2-oxo acid' and leucine pools is shown in Fig. 1. The designation '2-oxo acid' pool is used here to describe that portion of the radioactivity present in islet tissue that was not retained on a strongly acidic cation-exchange resin. The 2-oxo acid pool may be expected to contain, in addition to 4-methyl-2-oxopentanoate, the corresponding 2-hydroxy acid, but no other intermediates of the degradative pathway of 4-methyl-2-oxopentanoate metabolism, since the labelled carbon atom is removed on its conversion into isovaleryl-CoA. It is shown that 95.6±0.5% ($n=4$) of the 2-oxo acid present in islets and the contaminating extracellular fluid was converted into $^{14}CO_2$ by reaction with a 10%-satd. solution of ceric sulphate in H_2SO_4 (1M), i.e. by an agent that specifically decarboxylates 2-oxo acids. About 85% of such radioactivity, however, would be extracellular, hence it may be concluded that at least 70% of the intracellular 2-oxo acid pool was

actually 4-methyl-2-oxopentanoate. The designation '[^{14}C]leucine' pool is based on studies of the amination of 4-methyl-2-oxopentanoate (see below). The relationship of islet leucine and 2-oxo acid contents to the extracellular 4-methyl-2-oxopentanoate concentration were both hyperbolic in nature. The islet [^{14}C]leucine content, however, attained a maximal value at much lower extracellular concentrations of 4-methyl-2-oxopentanoate than did the intracellular 2-oxo acid content.

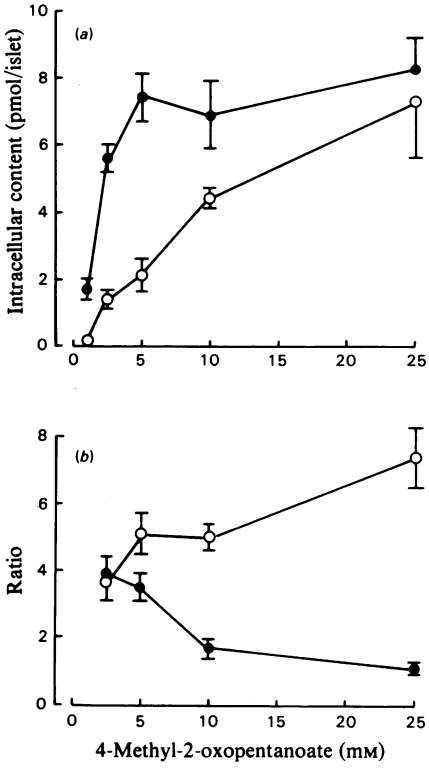

Fig. 1. *Response of the steady-state contents of 2-oxo acids and leucine in rat pancreatic islets to variations in the extracellular 4-methyl-2-oxopentanoate concentration*

Groups of 10–15 islets were incubated for 30min at 37°C in Krebs–Henseleit buffer, pH 7.5, in the presence of 4-methyl-2-oxo[1-^{14}C]penatanoate (1–25mM; 10μCi/ml) and [6,6′-^{3}H]sucrose (1mM; 20μCi/ml) as detailed in the Experimental section. The intracellular content of 2-oxo acid (○) and leucine (●) is shown in (*a*). In (*b*) the ratio of the extracellular to intracellular concentration (○) of 2-oxo acid is shown together with the intracellular [leucine]/[2-oxo acid] ratio (●). These calculations assumed uniform distribution of the tracers and an intracellular islet water space of 2.17nl/islet (Malaisse *et al.*, 1978). Each value shown is the mean ± S.E.M. of results obtained from 14–23 separate incubations.

The mean intracellular pH of islets (Fig. 2) decreased in response to increasing extracellular 4-methyl-2-oxopentanoate concentrations and, as shown in Table 1, a decrease in the extracellular pH favoured the uptake of the 2-oxo acid radioactivity by islet tissue. Islet radioactive leucine content was also increased by changing the extracellular pH from 7.5 to 7.0, but not to the same relative extent as the radioactive 2-oxo acid content. It would appear that, as in other tissues, 4-methyl-2-oxopentanoate is transported in islet tissue as the undissociated acid or either in symport with H^+ or antiport with OH^-. Elevation of the media K^+ content to a value that causes marked B-cell depolarization (Atwater *et al.*, 1978) had no significant effect on islet radioactive 2-oxo acid or radioactive leucine content (Table 1). Veratridine (0.1mM), which similarly causes cell depolarization but as the consequence of stimulated Na^+ entry (Donatsch *et al.*, 1977), also did not affect these parameters (Table 1). Such findings suggest that transport of this 2-oxo acid in islet tissue is not electrogenic. The failure of veratridine to influence the net uptake further suggests that Na^+ is not important in the transport process.

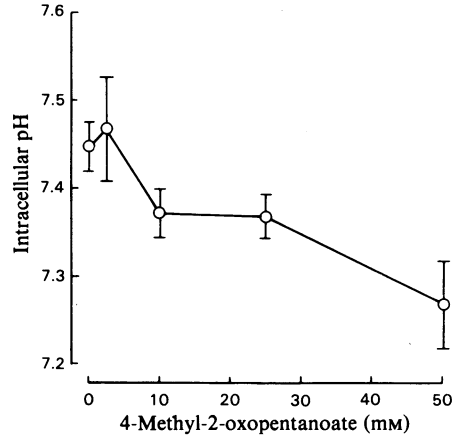

Fig. 2. *Relationship of the intracellular pH in rat pancreatic islets to extracellular 4-methyl-2-oxopentanoate concentration*

Groups of 10–15 islets were incubated for 30min at 37°C in Krebs–Henseleit buffer, pH 7.5, in the presence of 4-methyl-2-oxopentanoate (0–50mM), 5,5-dimethyl[2-^{14}C]oxazolidine-2,4-dione (1mM; 10μCi/ml) and [6,6′-^{3}H]sucrose (1mM; 20μCi/ml). The intracellular content of the ^{14}C radioactivity was determined by using the ^{3}H radioactivity determination to correct for extracellular contamination. Subsequent calculations were based upon the method of Waddel & Butler (1959) and assumed a mean intracellular water space of 2.17nl/islet (Malaisse *et al.*, 1978) and a pK_a for dimethyloxazolidinedione of 6.13. Each plotted value is the mean ± S.E.M. of results obtained in 23 separate incubations.

Table 1. *Effects of various media modifications on the uptake and distribution of radioactivity from labelled 4-methyl-2-oxopentanoate in pancreatic islets*

Groups of 15 islets were incubated for 30min at 37°C in the presence of 4-methyl-2-oxo[4,5-^{3}H]pentanoate (2.5–10mM; 10μCi/ml) and [U-^{14}C]sucrose (1mM; 20μCi/ml). Unless indicated, the pH of the media was 7.5, and was modified by changing the HCO_3^- concentration. No correction for osmolarity was made in the case of KCl addition. Each result is the mean ± S.E.M. for eight to ten separate incubations. Probability estimates refer to the determination performed at the same substrate concentration at pH7.5 in the absence of effector substances: *$P<0.005$; **$P<0.02$; ***$P<0.05$.

Extracellular concentration (mM)	Media modification	2-Oxo acid (pmol/islet)	Leucine (pmol/islet)	Leucine / 2-oxo acid
2.5	None	2.7±0.3	6.6±0.5	2.64±0.30
	pH7	5.6±0.7*	9.0±0.7**	1.78±0.21***
	pH8	2.3±0.3	7.1±0.7	3.12±0.24
	KCl (20mM)	3.5±0.3	7.5±0.3	2.27±0.21
	Veratridine (0.1mM)	3.0±0.3	5.6±0.2	2.03±0.24
10.0	None	14.5±1.5	10.1±1.2	0.71±0.05
	Pyruvate (10mM)	11.7±1.0	8.2±0.8	0.74±0.08
	2-Cyano-3-hydroxycinnamate (2.5mM)	13.5±1.3	7.9±0.8	0.60±0.05
	2-Cyano-3-hydroxycinnamate (10mM)	13.1±1.0	9.6±1.4	0.74±0.10

Table 2. *Effect of 4-methyl-2-oxopentanoate on various metabolic parameters in rat pancreatic islets*

Islets were incubated either without substrate or in the presence of 4-methyl-2-oxopentanoate (10mM). Determination of islet respiration was performed in a perifusion system; all other measurements relate to cumulative changes occurring over a 2h incubation. The formation of $^{14}CO_2$ from prelabelled islet lipids is expressed as a percentage of the radioactivity that remained in the islets at the completion of the final incubation. The initial radioactivity present was equivalent to 2pmol of palmitate/islet. Changes in islet protein synthesis were estimated from the incorporation of [4-^{3}H]phenylalanine into radioactive material that was precipitable with trichloroacetic acid. Lipid synthesis was determined from the incorporation of ^3H_2O into a total lipid extract. All values are expressed as the mean ± S.E.M. of results obtained in the number of separate incubations shown in parentheses. Abbreviation: N.S., not significant.

Metabolic parameter	Substrate concn. (mM) ... 0	10	*P* value
O_2 consumption (pmol/h per islet)	486±46 (13)	686±64 (18)	<0.025
Lactate production (pmol/h per islet)	17.0±4.1 (8)	22.2±5.3 (9)	N.S.
Acetoacetate production (pmol/h per islet)	4.5±0.4 (34)	41.9±1.5 (96)	<0.001
β-Hydroxybutyrate production (pmol/h per islet)	0.52±0.26 (8)	3.31±0.72 (42)	N.S.
NH_4^+ production (pmol/h per islet)	9.7±2.7 (7)	≤2 (7)	<0.05
Lipid synthesis (pg-atoms of ^{3}H incorporated/h per islet)	3.4±0.8 (8)	6.8±0.6 (16)	<0.001
$^{14}CO_2$ formation from prelabelled islet lipids (%)	4.97±0.26 (14)	4.46±0.28 (15)	N.S.
$^{14}CO_2$ incorporation into 3-oxo acids (pg-atoms of ^{14}C/h per islet)	5.0±0.4 (10)	56.7±2.2 (10)	<0.001
Protein synthesis (fmol of ^{3}H incorporated/h per islet)	8.1±0.9 (10)	22.6±1.1 (20)	<0.001

4-Methyl-2-oxopentanoate together with various cinnaminic acid derivatives inhibit pyruvate transport in erythrocytes (Halestrap, 1976) and isolated mitochondria (Halestrap, 1975; Halestrap *et al.*, 1974). Neither pyruvate (10mM) nor 2-cyano-3-hydroxycinnamate (2.5 or 10mM) affected the net uptake of labelled 4-methyl-2-oxopentanoate in islet tissue or its distribution between the intracellular oxo acid and amino acid pools (Table 2). 2-Cyano-3-hydroxycinnamate nevertheless inhibited the oxidation of [U-^{14}C]4-methyl-2-oxopentanoate (Fig. 3). Such findings may be explained if the site of inhibition were a mitochondrial carrier rather than one located on the plasma membrane. It is perhaps pertinent that the pyruvate carrier in mitochondria is much more sensitive to 2-cyano derivatives and is furthermore inhibited non-competitively rather than competitively, as is the case for the plasma-membrane carrier. The finding that [U-^{14}C]glucose oxidation in islet tissue is inhibited by 10mM-2-cyano-3-hydroxycinnamate (J. C. Hutton & W. J. Malaisse, unpublished work) is consistent with such a conclusion.

Metabolic response to 4-methyl-2-oxopentanoate

Various parameters of the basal metabolic activity in rat islets are shown in Table 2 together with the effect on these parameters of the inclusion of 4-methyl-2-oxopentanoate in the incubation media.

Rat pancreatic islets, like those of *ob/ob* mice (Hellerström, 1967), exhibited a high rate of respiration for several hours, even in the absence of exo-

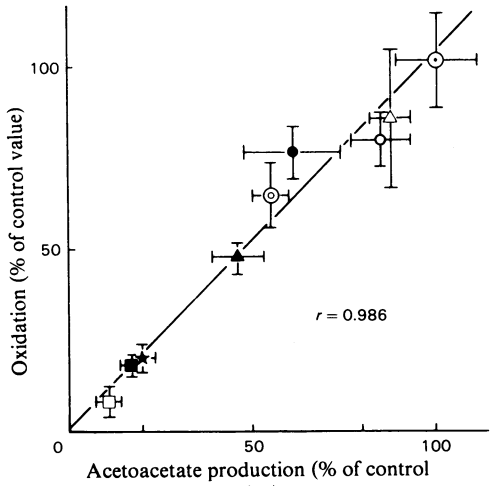

Fig. 3. *Effects of various media modifications on the oxidation of [U-^{14}C]4-methyl-2-oxopentanoate and production of acetoacetate in rat pancreatic islets incubated in the presence of 4-methyl-2-oxopentanoate*

All incubations were performed over a 2h period in the presence of 10mM-4-methyl-2-oxopentanoate. The results obtained were expressed relative to values obtained in the same experiment in incubations performed in the absence of the modifier. The reference value (⊙) and the effects of 2,4-dinitrophenol (*) (0.1mM), valinomycin (■) (0.1μM), rotenone (□) (1μM), 2-cyano-4-hydroxycinnamate (●) (10mM), 2-cyano-3-hydroxycinnamate (▲) (10mM), pyruvate (◎) (10mM), glucose (o) (20mM) or of the omission of K^+ from the media (△) are shown. Each plotted value is the mean ± S.E.M. of results obtained in eight separate incubations. *r* is the correlation coefficient.

genous substrate. The rate of islet NH_4^+ production, if it were to correspond to the complete oxidative degradation of islet protein, could account for an O_2 consumption of about 40pmol/h per islet, that is less than 10% of the endogenous rate.

The introduction of 4-methyl-2-oxopentanoate (10mM) into the media resulted in a marked increase in islet O_2 consumption, a marked decrease in NH_4^+ production, a small, but not statistically significant, decrease in the rate of $^{14}CO_2$ formation from prelabelled islet lipids and no change in islet lactate formation. 4-Methyl-2-oxopentanoate (10mM) provoked a 5–10-fold increase in the production of acetoacetate and 3-hydroxybutyrate. A similar increase in islet acetoacetate production was observed in the presence of L-leucine (40mM) (38.6 ± 7.8pmol/h per islet; $n = 16$) and a smaller increase in the presence of isovalerate (40mM) (15.7 ± 1.7pmol/h per islet; $n = 16$). Other substrates metabolized in islet tissue, such as glucose (5–20mM), pyruvate (20mM), 3-methyl-2-oxobutyrate (20mM), valine (20mM), glutamine (10mM) and octanoate (5mM), failed to cause a significant increase in islet acetoacetate production.

The formation of 3-hydroxybutyrate by islets incubated in the presence of 4-methyl-2-oxopentanoate represented less than 10% of the total ketone-body formation, a low value compared with the much higher proportion reported in rat liver mitochondria incubated with the substrate (Noda & Ichihara, 1974). The total 3-hydroxybutryate dehydrogenase activity found in a crude homogenate of islet tissue was 0.53 ± 0.05nmol/h per islet of which 62.1% was recovered in a fraction sedimenting between 400 and 15000*g* (10min centrifugation time at 3°C) and 18.2% in the postmicrosomal supernatant (60min at 4°C and 166000*g*). This distribution followed that of glutamate dehydrogenase activity (total activity 12.4 ± 1.5nmol/h per islet; $n = 16$). The 3-hydroxybutyrate dehydrogenase activity presently observed was of the same order as that determined by Berne (1976) in *ob/ob*-mouse islets; it exceeded, by two orders of magnitude, the observed rate of islet 3-hydroxybutyrate production (Table 2), and hence is unlikely to be a rate-limiting factor in the transformation of acetoacetate to 3-hydroxybutyrate.

Studies of the pathway of leucine degradation in other tissues have revealed that the carboxy group of the metabolic product acetoacetate is derived from the fixation of CO_2 in the 3-methylcrotonoyl-CoA carboxylase (EC 6.4.1.4) reaction (Coon, 1950). The incorporation of radioactivity from $NaH^{14}CO_3$ into the carboxy group of islet 3-oxo acids was investigated as a means of establishing the presence of a similar metabolic pathway in pancreatic islet tissue. The introduction of 4-methyl-2-oxopentanoate into the incubation medium caused a 10-fold increase in the amount of radioactivity incorporated compared with incubations performed in the absence of substrate or in the presence of glucose (16.7mM) or octanoate (5mM). The rate of ^{14}C incorporation was close to the rate of acetoacetate formation determined in the same incubations.

Amination of radioactively labelled 4-methyl-2-oxopentanoate

The metabolic fate of radioactively labelled 4-methyl-2-oxopentanoate in islet tissue is shown in Table 3. The incorporation of 4-methyl-2-oxopentanoate into amino acids constituted quantitatively the most significant pathway of substrate utilization in islet tissue, In experiments in which islets were incubated with 4-methyl-2-oxo[1-^{14}C]-pentanoate (10mM), more than 95% of the radioactive material recovered on a cation-exchange resin at the end of incubation was identifiable as leucine by analytical ion-exchange radiochromatography. Analyses performed on control incubations did not reveal the presence of any labelled amino acids. In

experiments with islets incubated with [U-^{14}C]-4-methyl-2-oxopentanoate, incorporation of radioactivity into leucine was observed together with another compound which behaved like glutamate on ion-exchange and paper chromatography. This second compound constituted 7–10% of the total radioactivity recovered and could account for the slightly higher rate of amination observed with U-^{14}C-labelled as opposed to 1-^{14}C-labelled 4-methyl-2-oxopentanoate (Table 3). Above 90% of the radioactive material incorporated from [U-^{14}C]-4-methyl-2-oxopentanoate into amino acids was recovered from the medium surrounding the islets. Treatment of the incubation media with L-amino oxidase and catalase (Meister, 1952) resulted in an 87% decrease in the amount of radioactive material that could subsequently be retained on a Dowex 50 H^+ ion-exchange resin. It was concluded that 4-methyl-2-oxopentanoate was aminated principally to the L-stereoisomer of leucine, which was then transported into the incubation medium. Such findings agree with previous investigations performed with non-radioactive substrates (Panten, 1975).

The relationship between the rates of 4-methyl-2-oxopentanoate oxidation and amination in islet tissue was investigated further in an experimental series (Table 4) in which the effect of various metabolic inhibitors or agents that modify insulin release (Hutton *et al.*, 1979*a*) were tested in the presence of [U-^{14}C]4-methyl-2-oxopentanoate (10mM). A clear dissociation between the rates of amination and oxidation were observed in several cases. The incubation of islets in the presence of 4-methyl-2-oxopentanoate led to a decrease in islet NH_4^+ production (Table 2), but the basal rate of islet NH_4^+ production was not of sufficient magnitude to account for the rate of amination observed. The addition of NH_4^+ (0.1–5mM) to the incubation media did not

Table 3. *Metabolic fate of labelled 4-methyl-2-oxopentanoate in rat pancreatic islets*

All incubations were conducted for 2h in the presence of 4-methyl-2-oxopentanoate (10mM) radioactively labelled in the positions indicated. The incorporation of radioactivity into the various metabolic products is expressed as pmol of oxo acid residues/h per islet. Results are also expressed as a percentage of the sum of the rates of amination and decarboxylation. Each value is the mean ± S.E.M. of results obtained from the number of separate incubations shown in parentheses.

Metabolic product	Radioisotopic precursor	Rate of incorporation	Incorporation (%)
$^{14}CO_2$	[1-^{14}C]-	42.1 ± 8.9 (89)	54.3 ± 11.5
$^{14}CO_2$	[U-^{14}C]-	25.7 ± 1.2 (108)	33.1 ± 1.5
^{14}C-labelled amino acids	[1-^{14}C]-	35.4 ± 3.5 (45)	45.7 ± 4.5
^{14}C-labelled amino acids	[U-^{14}C]-	39.2 ± 2.6 (83)	50.6 ± 3.6
[^{14}C]Acetoacetate	[U-^{14}C]-	29.5 ± 3.5 (30)	38.1 ± 4.5
^{14}C-labelled protein	[U-^{14}C]-	2.6 ± 1.3 (10)	3.4 ± 1.7
^{14}C-labelled total lipid	[U-^{14}C]-	1.6 ± 0.3 (8)	2.1 ± 0.4
^3H_2O	[4,5-^{3}H]-	38.9 ± 5.8 (13)	50.2 ± 7.5

Table 4. *Effects of various media modifications on the amination of 4-methyl-2-oxopentanoate*

Rates of amination and oxidation were determined in the presence of [U-^{14}C]4-methyl-2-oxopentanoate (10mM; 5μCi/ml) over 2h incubations with the same batches of islets, except for 2,4-dinitrophenol addition when experiments were performed as described in the legend of Table 5. Oligomycin, 2,4-dinitrophenol, cytochalasin B and suloctidil were added to incubations in 10^3-fold concentrated solutions in dimethyl sulphoxide. The solvent, however, did not affect either amination or oxidation rates. Each value is the mean ± S.E.M. of results obtained in the number of separate incubations shown in parentheses. The statistical significance of differences refers to the same determination performed without the media modification (first line): *$P<0.001$; **$P<0.025$; ***$P<0.05$.

Media modification	Amination (pmol/h per islet)	Oxidation (pmol/h per islet)	Amination / oxidation
None	38.3 ± 2.9 (50)	22.8 ± 1.7	1.68 ± 0.14
Oligomycin (0.4μM)	23.4 ± 2.7 (8)**	1.2 ± 0.5*	⩾20*
Dinitrophenol (50μM)	34.5 ± 3.8 (8)	31.3 ± 3.2***	1.10 ± 0.13
Amino-oxyacetate (5mM)	8.1 ± 1.3 (8)*	18.6 ± 2.4	0.43 ± 0.08*
Glutamate (10mM)	91.2 ± 9.8 (16)*	23.8 ± 2.0	2.70 ± 0.30*
Adrenaline (1μM)	42.0 ± 3.7 (8)	23.2 ± 3.2	1.98 ± 0.22
Theophylline (1.4mM)	30.1 ± 4.0 (9)	19.3 ± 3.6	1.36 ± 0.16
Ca^{2+}-free + EGTA (1mM)	38.6 ± 4.5 (10)	25.0 ± 2.6	1.52 ± 0.18
Suloctidil (5μM)	31.5 ± 2.9 (8)	20.9 ± 1.5	1.50 ± 0.22
Cytochalasin B (21μM)	38.3 ± 3.0 (8)	24.7 ± 4.8	1.83 ± 0.25
HCO_3-free Hepes (25mM)	32.0 ± 2.5 (6)	8.1 ± 2.5*	4.05 ± 0.40*

promote the amination of 4-methyl-2-oxopentanoate (Hutton *et al.*, 1979*c*), yet the addition of glutamate more than doubled the amination rate (Table 4). The finding that 2,4-dinitrophenol and oligomycin did not markedly inhibit the amination of 4-methyl-2-oxopentanoate in intact islets further suggested that the provision of reducing equivalents or metabolic energy was not essential for the reaction to occur. All these findings were consistent with the hypothesis that the amino-group donors involved in the amination reaction were free amino acids, but that these were not regenerated by reductive amination of the 2-oxo acid reaction product either directly or in transaminase reactions coupled to glutamate dehydrogenase. The participation of a transaminase in the production of leucine from 4-methyl-2-oxopentanoate by intact islets was suggested from the finding that amino-oxyacetate inhibited the reaction (Table 4).

Transaminase activity in islet tissue was investigated by the determination of the incorporation of radioactivity from 4-methyl-2-oxo[4,5-^{3}H]pentanoate into cationic products in the presence of various amino acids. No incorportaion was observed in the presence of the 2-oxo acid substrate alone or with alanine or aspartate. The rates of transamination with L-glutamate, L-valine and L-isoleucine, L-norleucine and L-norvaline were respectively 0.36 ± 0.03, 0.50 ± 0.04, 1.26 ± 0.07, 0.15 ± 0.02 and 0.15 ± 0.01 nmol/h per islet ($n=4$). In the reaction of 4-methyl-2-oxopentanoate and glutamate, the $K_{m_{app.}}$ was 2.5mM for the 2-oxo acid and 12.0mM for the amino acid. Lineweaver–Burk plots were linear. A radioactive-isotope exchange reaction between 4-methyl-2-oxopentanoate and leucine was also catalysed. The substrate specificity and relative rates of transamination presently observed with different amino acids were similar to those reported for the pig heart branched-chain amino acid aminotransferase enzyme in an assay with 2-oxoglutarate as the 2-oxo acid substrate (Ichihara & Koyama, 1966). Rat pancreatic islets contain an appreciable pool of free amino acids (Gylfe, 1974), many of which, on the basis of the present enzymic evidence, would be suitable transamination partners for 4-methyl-2-oxopentanoate.

Incorporation of labelled 4-methyl-2-oxopentanoate into protein and lipid

The incorporation of radioactivity from [U-^{14}C]-4-methyl-2-oxopentanoate into protein represented only a small proportion of the utilization of this substrate by islet tissue. Such incorporation seemed to result from the stimulation of protein synthesis, since the addition of unlabelled 4-methyl-2-oxopentanoate to the medium of islets incubated in the presence of tracer concentrations of [4-^{3}H]phenylalanine (Table 2) caused an almost 3-fold increase in the amount of radioactivity subsequently recovered in trichloroacetic acid-precipitable material isolated from the islets.

The rate of islet lipid synthesis, determined from the incorporation of radioactivity from ^3H_2O into islet total lipids, was stimulated by the addition of 4-methyl-2-oxopentanoate (10mM) to the incubation media (Table 2). The increase provoked by 4-methyl-2-oxopentanoate was equivalent to about 0.7pmol of 2-oxo acid residues/h per islet if it were assumed that each g-atom of ^{3}H incorporated represented the equivalent of 0.58 acetyl-CoA moieties (Jungas, 1968) or 0.19 4-methyl-2-oxopentanoate residues. Such a rate of lipid synthesis was of the same order as the observed incorporation of radioactivity from [U-^{14}C]4-methyl-2-oxopentanoate and represented only a small proportion of the rate of utilization of this substrate by this tissue.

Incorporation of labelled 4-methyl-2-oxopentanoate into acetoacetate and CO_2

The incorporation of [U-^{14}C]4-methyl-2-oxopentanoate residues into the methyl, carbonyl and methylene carbon atoms of acetoacetate was equivalent to the rate of incorporation of this precursor into $^{14}CO_2$. The ratio $^{14}CO_2$ formation/[^{14}C]acetoacetate formation expressed in pmol of 2-oxo acid residue was 1.03 ± 0.13 ($n=10$) when determined in the same experiment. The incorporation of radioactivity from [U-^{14}C]4-methyl-2-oxopentanoate into the carboxy group of acetoacetate by islets (1.78 ± 1.0pmol of 2-oxo acid residues/h per islet; $n=8$), however, was negligible in comparison with the incorporation of this precursor into the other three carbon atoms of acetoacetate. Such findings, along with the H$^{14}CO_3^-$-incorporation experiments, are consistent with the pathway of 4-methyl-2-oxopentanoate degradation described in other tissues (Meister, 1965).

As generally depicted, the pathway of degradation of 4-methyl-2-oxopentanoate is initiated by the irreversible oxidative decarboxylation of the 2-oxo acid to isovaleryl-CoA and proceeds via further dehydrogenation, carboxylation and hydration reactions leading to formation of 3-hydroxymethylglutaryl-CoA, which is subsequently cleaved into acetoacetate and acetyl-CoA. As expected, the rate of 4-methyl-2-oxo[1-^{14}C]pentanoate decarboxylation was equivalent to the rate of acetoacetate production determined enzymically, but was much higher than the rate of [U-^{14}C]4-methyl-2-oxopentanoate oxidation expressed in terms of oxo acid residues. The ratio [U-^{14}C]4-methyl-2-oxopentanoate oxidation/4-methyl-2-oxo[1-^{14}C]pentanoate decarboxylation, determined in the same experiment, was 0.63. The rate of ^3H_2O production from 4-methyl-2-oxo-[4,5-^{3}H]pentanoate was also equivalent to the rate of decarboxylation of the 1-^{14}C-labelled substrate, the

ratio 1-^{14}C-labelled/4,5-^{3}H-labelled 4-methyl-2-oxopentanoate metabolism being 1.13 ± 0.06 ($n = 13$) when compared within the same experiment. Further indirect evidence for the existence of the pathway in islet tissue was the finding that the production by islets of $^{14}CO_2$ from [U-^{14}C]4-methyl-2-oxopentanoate was markedly decreased by the addition of unlabelled isovalerate to the incubation media (J. C. Hutton & W. J. Malaisse, unpublished work).

Further investigation of the relationship of acetoacetate production to 4-methyl-2-oxopentanoate oxidation by islet tissue was undertaken by using a series of experimental conditions previously found to affect the insulinotropic action of 4-methyl-2-oxopentanoate (Hutton *et al.*, 1979*a*). The results are summarized in Fig. 3. Omission of K^+ from the incubation media or the addition of glucose (20mM) tended to decrease, though not significantly, the production of acetoacetate and $^{14}CO_2$ by islets incubated in the presence of [U-^{14}C]4-methyl-2-oxopentanoate (10mM). The addition of pyruvate to the media caused a parallel inhibition in 4-methyl-2-oxopentanoate oxidation and acetoacetate production, an effect that was attributable to decreased utilization of 4-methyl-2-oxopentanoate, since islet $^{14}CO_2$ production from 4-methyl-2-oxo[1-^{14}C]pentanoate was also inhibited to a similar extent (J. C. Hutton & W. J. Malaisse, unpublished work). The oxo acid-transport inhibitors 2-cyano-3-hydroxycinnamate (10mM) and 2-cyano-4-hydroxycinnamate also caused parallel inhibitions of islet acetoacetate and $^{14}CO_2$ production, as did the respiratory poison rotenone, the uncoupler of oxidative phosphorylation 2,4-dinitrophenol, and the K^+-ionophore valinomycin. The effect of valinomycin (0.1μM) on 4-methyl-2-oxopentanoate oxidation contrasts with its effects on [U-^{14}C]glucose oxidation in islet tissue (Boschero *et al.*, 1978) and may be attributed to the inability of 4-methyl-2-oxopentanoate, unlike glucose, to generate ATP by substrate-level phosphorylation. As ATP is an obligatory substrate for the 3-methylcrotonoyl-CoA carboxylase reaction it is expected that when oxidative phosphorylation is uncoupled, a dual effect on 4-methyl-2-oxopentanoate metabolism may occur, depending on the degree of uncoupling achieved. When islets were preincubated for 10min in the presence of 4-methyl-2-oxopentanoate to restore their ATP content and then exposed for 60min to 2,4-dinitrophenol indeed both stimulatory and inhibitory effects could be produced. As shown in Table 5, under such conditions the presence of 50μM-dinitrophenol in the incubation media resulted in a stimulation of the incorporation of 1-^{14}C- and U-^{14}C-labelled 4-methyl-2-oxopentanoate into $^{14}CO_2$, but these effects were reversed in the presence of 200μM-dinitrophenol. In every case changes in the rate of acetoacetate production were proportional to the rate of $^{14}CO_2$ formation.

It would appear that islet tissue when metabolizing 4-methyl-2-oxopentanoate does not utilize further the ketone bodies produced from its metabolism. This occurs in spite of its having the capacity to oxidize these metabolic products (Berne, 1976). In hepatic tissue, ketone bodies formed from 4-methyl-2-oxopentanoate are similarly not further degraded (Krebs & Lund, 1977).

Studies on rat liver 4-methyl-2-oxopentanoate dehydrogenase have indicated that the intracellular concentration of ATP, Ca^{2+} and P_i may regulate its activity (Johnson & Connelly, 1972). The present finding that 2,4-dinitrophenol, under suitable experimental conditions, induced a marked increase in the rate of decarboxylation of 4-methyl-2-oxopentanoate in islet tissue was consistent with the concept that ATP or some other component of the respiratory chain regulated the rate of 4-methyl-2-oxopentanoate metabolism in intact islet tissue.

Relationship of respiratory changes to 4-methyl-2-oxopentanoate oxidation

The production of $^{14}CO_2$ by islets incubated in the presence of [U-^{14}C]4-methyl-2-oxopentanoate (Table 3) occurred at a rate that was 0.8 times the increase in the respiratory rate induced by this substrate (Table 2). Calculation from the reaction:

$$3\ O_2 + [U\text{-}^{14}C]\text{oxo acid} + {}^{12}CO_2 \rightarrow [{}^{14}C]\text{acetoacetate} + 3\ {}^{14}CO_2 + 2H_2O$$

Table 5. *Effect of* 2,4-*dinitrophenol on the metabolism of* 4-*methyl-2-oxopentanoate by pancreatic islets*

Incubations were performed as described in the Experimental section, except that 2,4-dinitrophenol was not added to the incubation media until 10min after the addition of the labelled substrate and that the incubation was then performed only for a further 60min. Each value is the mean ± S.E.M. of results obtained from the number of separate incubations shown in parentheses. The statistical significance of differences refers to a control incubation (column 1): *$P<0.001$; **$P<0.005$; ***$P<0.01$.

Metabolic parameter	[2,4-Dinitrophenol] (μM)	Rate (pmol/h per islet)			
		0	20	50	200
1-^{14}C Decarboxylation		41.8 ± 3.2 (15)	47.7 ± 5.3 (8)	70.5 ± 11.4 (7)**	55.5 ± 9.6 (8)
U-^{14}C Oxidation		26.0 ± 2.5 (16)	29.0 ± 2.9 (8)	39.1 ± 3.9 (16)***	30.9 ± 4.4 (18)
Acetoacetate formation		42.2 ± 5.4 (8)	51.7 ± 4.3 (8)	89.8 ± 15.4 (8)*	58.5 ± 11.7 (8)

would give a value of 1 for such a parameter, thereby suggesting that little change in the oxidation of endogenous substrates occurred in islet tissue exposed to 4-methyl-2-oxopentanoate. Such a conclusion is consistent with the observation that the addition of 4-methyl-2-oxopentanoate to the incubation media caused little change in the oxidation of prelabelled lipids in islet tissue (Table 2). It also implies that the production of reducing equivalents by 4-methyl-2-oxopentanoate was to a large extent linked to O_2 consumption by a stoicheiometry similar to that of the respiratory chain in other tissues. Such a conclusion is consistent with the observed effect of 2,4-dinitrophenol (Table 5) and oligomycin (Table 4) on 4-methyl-2-oxopentanoate metabolism and suggests that the increased respiratory activity induced by 4-methyl-2-oxopentanoate was associated with increased turnover of ATP. The nature of the reactions consuming metabolic energy that are stimulated in the presence of secretagogues remains obscure, but could be related to the observed changes in islet biosynthetic activity (Table 2) or to changes in ionic fluxes (Hutton *et al.*, 1979*a*). The latter postulate is consistent with the changes in 4-methyl-2-oxopentanoate oxidation induced by the omission of HCO_3^-, K^+ or Ca^{2+} from the media (Fig. 3 and Table 4). The relationship that the observed changes in metabolic flux bear to changes in the secretory capacity in islet tissue is considered in the following paper (Hutton *et al.*, 1979*c*).

This work was supported in part by grants from the Belgian Foundation for Scientific Medical Research (3.4527-75, 3.4537-76, 3.4528-79 and 3.0067-74). J.C.H. received a Pfizer Travel Award from the European Association for the Study of Diabetes. A. Tinant, S. Procureur, B. Cocks and R. Kutzner are thanked for their technical assistance, C. Demesmaeker for secretarial help, and Professor J. Christophe for his collaboration in the performance of amino acid analyses.

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