Diabetologia 8, 93—98 (1972)
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# Oxidation of Glucose and Fatty Acids in Normal and in $A_2$-Cell Rich Pancreatic Islets Isolated from Guinea-Pigs

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Received: August 23, 1971, accepted: December 6, 1971

*Summary.* Glucose and fatty acid oxidation has been measured in normal guinea-pig islets of Langerhans, and in $A_2$-cell rich islets from streptozotocin-treated guinea-pigs. The rate of oxidation of these compounds in guinea-pig $A_2$-cells and B-cells has been estimated. In the B-cells, the oxidation of glucose and octanoic acid responded markedly to changes in the extracellular levels of these substrates. Palmitic acid did not appear to be oxidized by the B-cells. In contrast, the oxidation of octanoic acid and palmitic acid in the $A_2$-cells was very sensitive to changes in the extracellular fatty acid concentration. The sensitivity of glucose oxidation to changes in the glucose concentration was small by comparison. The high rate of oxidation of fatty acids in the $A_2$-cells supports the view that the rate of fatty acid metabolism in these cells plays an important role in the regulation of glucagon release.

*Oxydation du glucose et des acides gras dans les îlots pancréatiques normaux et dans les îlots riches en cellules $A_2$ prélevés sur le cobaye*

*Résumé.* L'oxydation du glucose et des acides gras a été mesurée dans les îlots normaux de Langerhans chez le cobaye, ainsi que dans les îlots riches en cellules $A_2$ du cobaye traité à la streptozotocine. Le taux d'oxydation de ces composés dans les cellules $A_2$ et les cellules B du cobaye a été estimé. Dans les cellules B, l'oxydation du glucose et de l'acide octanoique a fortement répondu à des changements dans les taux extra-cellulaires de ces substrats. L'acide palmitique ne semble pas avoir été oxydé par les cellules B. Au contraire, l'oxydation de l'acide octanoique et de l'acide palmitique dans les cellules $A_2$ était très sensible aux changements dans la concentration des acides gras extra-cellulaires. La sensibilité de l'oxydation du glucose envers les changements dans la concentration du glucose était comparativement faible. Le taux élevé de l'oxydation des acides gras dans les cellules $A_2$ corrobore l'hypothèse que le degré du métabolisme des acides gras dans ces cellules joue un rôle important dans la régulation de la sécrétion du glucagon.

*Die Oxidation von Glucose und Fettsäuren in normalen und isolierten, mit $A_2$-Zellen angereicherten Pankreasinseln vom Meerschweinchen*

*Zusammenfassung.* An normalen Langerhansschen Inseln vom Meerschweinchen und an mit $A_2$-Zellen angereicherten Inseln von streptozotozin-behandelten Meerschweinchen wurde die Oxidation von Glucose und Fettsäuren gemessen und die Oxidationsrate in $A_2$-Zellen und B-Zellen des Meerschweinchens bestimmt. In den B-Zellen hing die Oxidation von Glucose und Octansäure stark von den Änderungen der extracellulären Konzentrationen dieser Substanzen ab. Palmitinsäure schien in den B-Zellen nicht oxidiert zu werden. Dagegen war die Oxidation von Oktansäure und Palmitinsäure in den $A_2$-Zellen sehr von den Schwankungen der extracellulären Fettsäurekonzentration abhängig. Die Änderung der Glucoseoxidation bei Schwankungen der Glucosekonzentration war im Verhältnis dazu gering. Die hohe Oxidationsrate der Fettsäuren in den $A_2$-Zellen unterstützt die Theorie, daß der Fettsäuremetabolismus dieser Zellen eine wesentliche Rolle in der Regulierung der Glucosesekretion spielt.

*Key words*: Isolated islets of Langerhans, $A_2$-cells, B-cells, glucose oxidation, fatty acid oxidation, glucagon.

## *Introduction*

It has previously been suggested that the level of circulating fatty acids is of major importance in controlling the rate of glucagon release from the $A_2$-cells of the islets of Langerhans [1, 2]. Little is known, however, about the cellular mechanisms involved in the control of glucagon release or of the metabolic properties of the $A_2$-cells in general, since it is difficult to obtain samples of these cells suitable for metabolic analyses. Recent studies have shown that the B-cytotoxin, streptozotocin, can be used to destroy the majority of B-cells in guinea-pig islets of Langerhans leaving a predominance of apparently normal $A_2$-cells. The $A_2$-cell rich islets can be isolated from the streptozotocin-treated guinea-pigs and their morphological characteristics, oxygen consumption and glucagon secretory activity have already been reported [3, 4].

In the present experiments, islets of Langerhans isolated by collagenase digestion from normal and streptozotocin-treated guinea-pigs have been used to measure the production of $^{14}CO_2$ from the metabolism of $^{14}C$-glucose, $^{14}C$-octanoic acid and $^{14}C$-palmitic acid. In this way, an indication of the rate of oxidation of these compounds within the islets has been obtained, and it has been possible to estimate the rate of oxidation of glucose and fatty acids in guinea-pig $A_2$-cells and also in B-cells. Preliminary reports of some of these observations have appeared elsewhere [5, 6].

## *Materials and Methods*

$^{14}C$-U-glucose (specific activity 3 mC/mmole), $^{14}C$-1-octanoic acid, sodium salt (specific activity 19 mC/

mmole) and $^{14}$C-U-palmitic acid (specific activity approx. 900 mC/mmole) were obtained from The Radiochemical Centre, Amersham, England. The labelled substrates were used in all experiments at specific activities of 3 mC/mmole, 0.67 mC/mmole and 4.4 mC/mmole respectively. Octanoic acid (grade 1) was obtained from Sigma Chemical Co., St. Louis, U.S.A. and palmitic acid was obtained from Fisher Scientific Co., New Jersey, U.S.A. Sodium $^{14}$C-bicarbonate (specific activity 57 mC/mmole) was also obtained from The Radiochemical Centre, Amersham.

Guinea-pigs were males weighing 200—250 g at the time of injection. The method of injection of streptozotocin (kindly supplied by Dr. W. Dulin, The Upjohn Co., Kalamazoo, U.S.A.) and of isolation of islets using collagenase was as described previously [4]. Islets were isolated from control animals (injected with buffer but without streptozotocin) in the same way. At the time of isolation of islets from the streptozotocin-treated guinea-pigs, a small piece of pancreas from each animal was fixed in Zenker-formal solution. Paraffin sections were then stained with chrome-hematoxylin ponceau fuchsin [7] and examined by light microscopy to ensure that the majority of B-cells in the islets had been destroyed.

After isolation, the islets were preincubated at 37° for 10—15 min in a bicarbonate-buffered medium [8] which was thoroughly gassed with 95% oxygen: 5% carbon dioxide before use. The islets were then distributed in groups of 25, using a calibrated braking pipette [9], into small volumes of the medium contained in incubation vessels similar to those described by Keen *et al.* [10]. The radioactive substrate was added in a further small volume of medium to give a total volume of 0.3 ml. After displacing the air in the incubation vessels with 95% oxygen: 5% carbon dioxide, the vessels were sealed and incubated in a shaking water bath at 37°. After 1 h, islet metabolism was arrested by the injection into the incubation medium of 0.1 ml of 0.05 mM antimycin A (Sigma Chemical Co., St. Louis, U.S.A.) dissolved in 70% ethanol, followed by 0.1 ml of 0.4 M phosphate buffer, pH 6. Hyamine (0.25 ml) was then injected into the outer vessels. The incubation medium was thoroughly mixed every 30 min for 2 h, and the vessels were then allowed to stand overnight to complete the absorption of $^{14}CO_2$ into the Hyamine. The radioactivity in the Hyamine was determined in a Packard Tricarb liquid scintillation spectrometer using a scintillator composed of toluene (1 l), PPO (5 g) and POPOP (0.05 g). Blank incubations without islets were carried through each experiment, and the radioactivity in the blanks was substracted from the experimental values. The radioactivity in a small volume (2 µl) of the incubation medium containing the $^{14}$C-labelled glucose or fatty acid was also determined using the same system in order to calculate the specific activity of the labelled substrate. Since it was not possible to recover all of the islets after incubation, groups of 20 non-incubated islets from every preparation were dried and weighed on platinum foils [11] so that an average dry weight of the incubated tissue could be calculated.

Radioactive palmitic acid at a concentration of 1.5 mM was maintained in solution using 20 mg/ml bovine plasma albumin as a carrier [12]. The albumin (Fraction V: Armour Pharmaceutical Co., Eastbourne, Sussex, England) was washed in heptane before use to reduce contamination by fatty acids. The low level of fatty acid which remained in the albumin was assayed using the method of Trout *et al.* [13], so that after the addition of $^{14}$C-palmitic acid to the incubation medium [14], the total concentration of fatty acid was known accurately.

In experiments using $^{14}$C-labelled fatty acids, the islets were isolated and preincubated in medium containing 5.5 mM glucose, and in experiments using $^{14}$C-glucose, medium containing 1 mM octanoic acid as the only carbon source was used. In this way it was hoped to deplete the islets of unlabelled fatty acid or glucose before incubation so that a linear rate of production of $^{14}CO_2$ should be attained as soon as possible after the addition of the labelled substrate. During incubation with radioactive fatty acids, the medium was supplemented with unlabelled glucose at a concentration of 5.5 mM since this is close to normal levels of glucose in guinea-pig blood [3]. When islets were incubated with $^{14}$C-glucose, the medium contained 1 mM unlabelled octanoic acid. It was shown in separate experiments that the addition of this concentration of octanoic acid to the incubation medium had little effect on the rate of oxidation of 11 mM $^{14}$C-glucose by the isolated islets.

Results have been expressed as the mean $\pm$ standard error of the mean. Values have been expressed as pmoles substrate completely oxidized to $CO_2$ per µg islet dry weight per hour, or in Figs. 1 and 2 as pg-atoms of C oxidized to $CO_2$ per µg islet dry weight per hour. The rate of oxidation of $^{14}$C-1-octanoic acid has been calculated assuming that the appearance of $^{14}CO_2$ reflected the complete oxidation of the octanoic acid molecule. It has previously been shown that the oxidation of fatty acids in mammalian tissues does not lead to an accumulation of intermediate oxidation products [15]. The results have been analysed statistically using Student's t-test according to Snedecor [16]. Islets of Langerhans isolated from at least 6 animals have been used for each experimental condition investigated.

*Estimation of oxidation rates by B-cells.* In these experiments, the average weight of 25 normal guinea-pig islets was found to be 34 µg. Since the percentage volume of B-cells in normal guinea-pig islets is known [17], the weight of B-cells in these islets can be estimated. The difference between the rate of oxidation of glucose or fatty acids by normal islets and by $A_2$-cell rich islets will be due to oxidation by 95% of the B-cells, subsequently destroyed by streptozoto-

cin [4]. An approximate rate of oxidation of these compounds in the B-cells can therefore be calculated. It must be stressed, however, that since the small number of non-endocrine cells in mammalian islets of Langerhans [18] have been ignored in these calculations, and since it has been assumed that both types of A-cells in the islets from streptozotocin-treated guinea-pigs behave exactly as they do in normal islets, the values for the oxidation of glucose and fatty acids in guinea-pig B-cells must be regarded only as an approximation of the true value.

## Results

In control experiments, the oxidation of 1.7 mM $^{14}$C-glucose and of 0.75 mM $^{14}$C-palmitic acid was found to be linear for at least 90 min after a short (5—10 min) delay at the start of the incubation procedure. The oxidation of 0.5 mM $^{14}$C-octanoic acid was linear for at least 60 min after the same short delay, but the oxidation of this fatty acid decreased markedly after 90 min incubation. An incubation time of 60 min was therefore used in all further experiments.

When isolated guinea-pig islets were incubated for 60 min at 37° in the presence of 0.01 mM antimycin, oxidation of 16.7 mM $^{14}$C-glucose and of 5 mM $^{14}$C-octanoic acid was inhibited by $97 \pm 8\%$ (6 observations). Recovery into the Hyamine of $^{14}CO_2$ from incubation medium containing 0.5 μC of sodium $^{14}$C-bicarbonate, using the method described above, was found to be $98 \pm 1\%$ (4 observations).

Table 1 shows that in normal islets the oxidation of 11 mM $^{14}$C-glucose was inhibited by 28 mM mannoheptulose (65% inhibition) and by 0.4 mM sodium iodoacetate (65% inhibition), but not by 10 mM sodium malonate. The oxidation of 2 mM $^{14}$C-octanoic acid was inhibited by sodium malonate (60% inhibition) and not by mannoheptulose or by sodium iodoacetate.

The rates of oxidation of $^{14}$C-glucose by islets from normal guinea-pigs and from streptozotocin-treated guinea-pigs are shown in Table 2. The rate of glucose oxidation by islets from the streptozotocin-treated animals was similar to that by normal islets at 1.7 mM glucose. However, when the concentration of glucose was raised to 16.7 mM, there was a larger stimulation of glucose oxidation by normal islets than by islets from the treated guinea-pigs.

The rates of oxidation of $^{14}$C-octanoic acid by isolated guinea-pig islets are shown in Table 3. The rate of octanoic acid oxidation by islets from the streptozotocin-treated guinea-pigs was found to be significantly higher than by normal islets, both at 0.5 mM and at 5 mM octanoic acid. The rates of $^{14}$C-palmitic acid oxidation are also shown in Table 3. With this fatty acid, the rate of oxidation by islets from the streptozotocin-treated guinea-pigs was over twice the rate of oxidation by normal islets at 0.75 mM and at 1.5 mM palmitic acid.

The rates of oxidation of glucose and fatty acids by the $A_2$-cell rich islets from streptozotocin-treated guinea-pigs are summarized in Fig. 1. The results have been expressed here in terms of g-atoms of C oxidized, so that a direct comparison can be made between the rates of oxidation of the different carbon sources. It can be seen that the oxidation of low levels of octanoic acid (0.5 mM) and of palmitic acid (0.75 mM) was as high as the oxidation of glucose even at a concentration of 16.7 mM. The oxidation of both octanoic acid and

Table 1. *The effects of metabolic inhibitors on the oxidation of $^{14}$C-glucose and $^{14}$C-octanoic acid by islets isolated from normal guinea-pigs. The results are expressed as pmoles substrate completely oxidized to $CO_2$ per μg islet dry weight per hour. Significant differences between incubations in the presence of the inhibitors and the control incubations are indicated* when $P < 0.001$. The number of observations is shown in parentheses*

| Substance in incubation medium | | Rate of oxidation of labelled substrate |
|---|---|---|
| 11 mM $^{14}$C-glucose | (control) | 23.9±2.1 (12) |
| ,, | + 28 mM mannoheptulose | 8.7±0.9 (7)* |
| ,, | + 0.4 mM sodium iodoacetate | 8.4±2.4 (5)* |
| ,, | + 10 mM sodium malonate | 20.2±2.9 (9) |
| 2 mM $^{14}$C-octanoic acid | (control) | 82.5±7.7 (9) |
| ,, | + 28 mM mannoheptulose | 81.0±8.1 (6) |
| ,, | + 0.4 mM sodium iodoacetate | 82.5±4.1 (4) |
| ,, | + 10 mM sodium malonate | 33.9±6.0 (7)* |

Table 2. *The rate of oxidation of $^{14}$C-glucose by islets isolated from normal and from streptozotocin-treated guinea-pigs. The results are expressed as pmoles substrate completely oxidized to $CO_2$ per μg islet dry weight per hour. Significant differences between the rates of oxidation by islets from normal and from streptozotocin-treated animals are indicated * when $P < 0.001$. The number of observations is shown in parentheses*

| Concentration of glucose | Rate of glucose oxidation by islets from: a) normal guinea-pigs | b) streptozotocin-treated guinea-pigs |
|---|---|---|
| 1.7 mM | 7.2±0.2 (12) | 8.5±0.7 (9) |
| 16.7 mM | 31.5±1.3 (12) | 21.4±2.0 (8) * |

palmitic acid were also much more sensitive to changes in the extracellular fatty acid concentration, than the oxidation of glucose was to large changes in extracellular glucose levels.

For comparison, the estimated rates of oxidation of glucose and fatty acids by the guinea-pig B-cells, as calculated by the method given above, are shown in Fig. 2. There was a large stimulation of glucose oxidation and of octanoic acid oxidation when the concentration of these substrates in the incubation medium was raised. Palmitic acid, however, did not appear to be oxidized by the B-cells of the guinea-pig.

Table 3. *The rates of oxidation of* $^{14}C$*-labelled fatty acids by islets isolated from normal and from streptozotocin-treated guinea-pigs. The results are expressed as pmoles substrate completely oxidized to* $CO_2$ *per* μg *islet dry weight per hour. Significant differences between the rates of oxidation by islets from normal and from streptozotocin-treated animals are indicated** when* $P < 0.01$, *and* when* $P < 0.001$. *The number of observations is shown in parentheses*

| Concentration of fatty acid | Rate of fatty acid oxidation by islets from: a) normal guinea-pigs | b) streptozotocin-treated guinea-pigs |
|---|---|---|
| 0.5 mM octanoic acid | 15.1±0.7 (12) | 21.5±1.5 (9)** |
| 5.0 mM octanoic acid | 101 ±6 (12) | 146 ±14 (9)* |
| 0.75 mM palmitic acid | 2.8±0.3 (15) | 7.7±0.6 (14)* |
| 1.5 mM palmitic acid | 4.6±0.5 (10) | 12.7±1.9 (7)* |

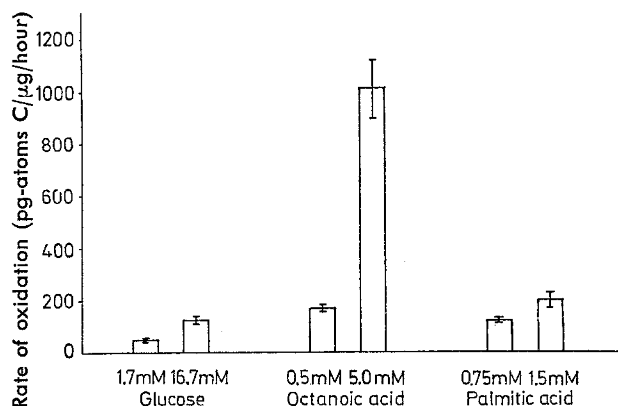


Fig. 1. The oxidation of glucose and fatty acids in $A_2$-cell rich islets isolated from guinea-pigs

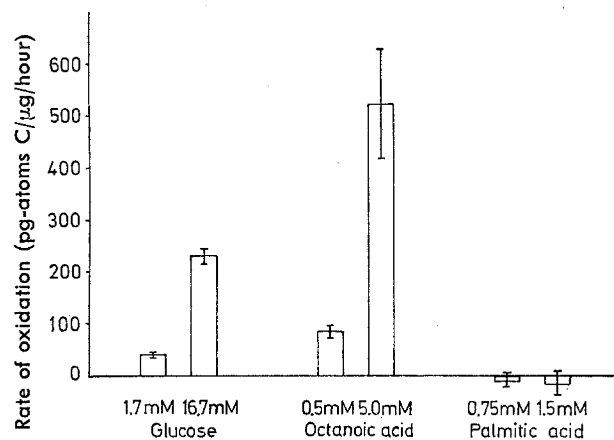


Fig. 2. The estimated rate of oxidation of glucose and fatty acids in the B-cells of guinea-pig islets

## *Discussion*

In previous experiments to measure the oxidation of radioactively-labelled compounds by incubated islet tissue, an excess of a strong acid has been used to stop cellular metabolism and to release the labelled carbon dioxide from the incubation medium [10, 19]. In experiments reported here, antimycin, a potent respiratory poison [20], has been used to arrest islet metabolism, and the labelled carbon dioxide has been absorbed into Hyamine after the addition of phosphate buffer, pH 6, to the incubation medium. It has been shown that the production of carbon dioxide by the islets is completely stopped by antimycin, and that the radioactive carbon dioxide is recovered quantitatively from the incubation medium. The advantages of using this system are that small amounts of acid-labile impurities in the $^{14}C$-glucose [21] are not decomposed to give $^{14}CO_2$, and also that absorption of $^{14}C$-octanoic acid by the Hyamine is not so high when the incubation medium is maintained at pH 6, as when lower pH values are used. Radioactivity in the blanks is therefore reduced and better reproducibility is achieved.

The oxidation of glucose and fatty acids by the isolated islets appeared to be linear for at least 60 min after an initial small delay at the start of the incubation procedure, which may have been due to the presence of pools of unlabelled metabolites in the islets or to temperature equilibration. The rate of oxidation expressed for 60 min incubation may therefore be slightly lower than a true hourly rate of oxidation. Values obtained for the oxidation of 1.7 mM $^{14}C$-glucose by normal guinea-pig islets were very similar to those reported by Ashcroft *et al.* [19] for glucose oxidation by isolated mouse islets. Oxidation of 16.7 mM glucose by guinea-pig islets, however, was

lower than reported for mouse islets, a fact which may be due to the smaller proportion of B-cells in guinea-pig islets [17, 22].

Experiments with metabolic inhibitors showed that the production of carbon dioxide from glucose was inhibited by mannoheptulose, iodoacetate and antimycin, whereas oxidation of octanoic acid was inhibited by malonate and antimycin but not by mannoheptulose or iodoacetate. These results showed that the metabolism of glucose and fatty acids by the islets appeared to follow the established pathways of oxidation of these compounds, and that the small amounts of $^{14}CO_2$ produced by the islets (less than 0.3% of the total radioactivity in the incubation medium) did not appear to be from the oxidation of impurities in the radioactive substrates. Although malonate inhibited carbon dioxide production from octanoic acid by the guinea-pig islets, there was no significant effect of this agent on the production of carbon dioxide from the oxidation of glucose. It has been noted before that there is little inhibitory effect of malonate on glucose-stimulated insulin release from the B-cells of rabbit pancreas [23], and that there is no effect of malonate on the oxygen consumption by obese-hyperglycaemic mouse islets in response to glucose [24]. Such observations suggest that there is no inhibitory effect of malonate on glucose metabolism in the B-cell. The marked inhibition of octanoic acid oxidation and the lack of effect on glucose oxidation by malonate in guinea-pig islets is explicable if it is assumed that malonate has an inhibitory effect in the A-cells only. It was calculated that at high concentrations of glucose and octanoic acid, about 75% of the total glucose oxidation by guinea-pig islets takes place in the B-cells, whereas 60% of the octanoic acid oxidation occurs in the A-cells.

Islets from the streptozotocin-treated guinea-pigs have been shown to contain about 70% $A_2$-cells [4] and the metabolism of these islets must therefore reflect the metabolism of the $A_2$-cells. There does not appear to be any effect of the streptozotocin treatment on the ultrastructure, oxygen consumption of glucagon-releasing activity of these $A_2$-cells [3, 4]. Furthermore, although streptozotocin destroys the majority of B-cells in the treated guinea-pigs, blood glucose levels remain close to normal [3]. The surviving cells in the islets of Langerhans are therefore not subjected to high levels of blood glucose before isolation, and it has been assumed that the surviving $A_2$-cells behave normally in every respect.

It was found that the rates of glucose oxidation by normal guinea-pig islets and by the $A_2$-cell rich islets from the streptozotocin-treated guinea-pigs were similar at a glucose concentration of 1.7 mM. When the glucose concentration was raised to 16.7 mM, however, there was a smaller increase in glucose oxidation by the $A_2$-cell rich islets than by the normal islets. Similar increases in oxygen consumption in response to glucose by normal islets and by $A_2$-cell rich islets have previously been reported [3]. In comparison with the decreased response to changes in glucose concentration, it was found that the oxidation of both octanoic acid and palmitic acid in the $A_2$-cell rich islets was higher than in normal islets.

In experiments in which glucagon release from isolated guinea-pig islets was measured [1], it was found that the release of glucagon was more sensitive to changes in fatty acid levels than to changes in the concentration of glucose. It has also been suggested that the oxidation of fatty acids inhibits glucagon release from the $A_2$-cells [25]. The present results on the rate of oxidation of glucose and fatty acids in the $A_2$-cells correlate with the observed effects of these compounds on glucagon release. It was found that fatty acids are oxidized at a faster rate than glucose in the $A_2$-cells. The oxidation of octanoic acid was also very sensitive to changes in the extracellular octanoic acid concentration, and the oxidation of palmitic acid rose markedly in response to a small physiological increase in the concentration of this fatty acid in the incubation medium. The increase in glucose oxidation in response to a much larger rise in the glucose concentration was small by comparison. It was also noted that when the concentration of fatty acid in the incubation medium was increased, a condition which would inhibit glucagon release from the $A_2$-cell [1], fatty acid oxidation was increased.

In the guinea-pig B-cell, the oxidation of glucose and octanoic acid was found to be sensitive to changes in the extracellular levels of these substrates, but palmitic acid did not appear to be oxidized by the guinea-pig B-cells. The effects of these compounds on insulin release in the guinea-pig have not so far been reported, but in other rodents high levels of glucose and octanoic acid stimulate insulin release from the B-cell [26, 27] whereas palmitic acid does not affect insulin release at least in the rabbit [28]. In contrast to the $A_2$-cells therefore, high rates of glucose and octanoic acid oxidation appear to take place when hormone release from the B-cell is stimulated.

In conclusion, the results of this paper indicate that there are fundamental differences in the oxidation of various substrates in the $A_2$-cells and B-cells of guinea-pig pancreatic islets. The observations also lend support to previous suggestions that the control of the hormone release mechanisms in these two cell types is substantially different.

*Acknowledgements.* Financial support from the Medical Research Councils of Britain and Sweden are gratefully acknowledged. J.C.E. is in receipt of a Wellcome-Swedish Travelling Research Fellowship.

*References*

1. Edwards, J.C., Howell, S.L., Taylor, K.W.: Fatty acids as regulators of glucagon release. Nature **224**, 808–809 (1969).
2. Luyckx, A.S., Lefebvre, P.J.: Arguments for a regulation of pancreatic glucagon secretion by circulating

free fatty acids. Proc. Soc. exp. Biol. Med. **133**, 524—529 (1970).
3. Petersson, B., Hellerström, C., Gunnarsson, R.: Structure and metabolism of the pancreatic islets in streptozotocin treated guinea-pigs. Horm. Metab. Res. **2**, 313—317 (1970).
4. Howell, S.L., Edwards, J.C., Whitfield, M.: Preparation of B-cell deficient guinea-pig islets of Langerhans. Horm. Metab. Res. **3**, 37—43 (1971).
5. Edwards, J.C., Hellerström, C., Petersson, B.: Oxidation of glucose and fatty acids in normal islets and in $A_2$-cell rich islets from guinea-pigs. Proceedings of the 7th meeting of the Scandinavian Society for the Study of Diabetes. Abstract. Diabetologia **7**, 401 (1971).
6. — — — Taylor, K.W.: Oxidation of glucose and fatty acids in the $A_2$-cells of isolated guinea-pig islets of Langerhans. Proceedings of the 7th meeting of the European Association for the Study of Diabetes (1971). In press.
7. Bencosme, S.A.: Studies on the methods of staining the islet cells of the pancreas. Arch. Path. **53**, 87—97 (1952).
8. Gey, G.O., Gey, M.K.: The maintainance of human normal cells and tumor cells in continuous culture. Amer. J. Cancer **27**, 45—49 (1936).
9. Holter, H.: Technique of the Cartesian diver. C. R. Lab. Carlsberg, Ser. Chim. **24**, 399—478 (1943).
10. Keen, H., Field, J.B., Pastan, I.: A simple method for in vitro metabolic studies using small volumes of tissue and medium. Metabolism **12**, 143—147 (1963).
11. Hellerström, C.: Effects of carbohydrates on the oxygen consumption of isolated pancreatic islets of mice. Endocrinology **81**, 105—112 (1967).
12. Goodman, D.S.: The interaction of human serum albumin with long chain fatty acid anions. J. Amer. chem. Soc. **80**, 3892—3898 (1958).
13. Trout, D.L., Estes, E.H., Friedberg, S.J.: Titration of free fatty acids of plasma: A study of current methods and a new modification. J. Lipid Res. **1**, 199—202 (1960).
14. Garland, P.B., Newsholme, E.A., Randle, P.J.: Regulation of glucose uptake by muscle. Biochem. J. **93**, 665—678 (1964).
15. Greville, G.D., Tubbs, P.K.: The catabolism of long chain fatty acids in mammalian tissues. In: Essays in Biochemistry (P.N. Campbell and G.D. Greville, eds.), Vol. 4, p. 155. London: Academic Press 1968.
16. Snedecor, G.W.: Statistical methods, 5th Ed., p. 35. Ames: The Iowa State University Press 1956.
17. Petersson, B.: The two types of alpha cells during the development of the guinea-pig pancreas. Z. Zellforsch. **75**, 371—380 (1966).
18. Lacy, P.E.: Quantitative histochemistry of the islets of Langerhans. Diabetes **11**, 96—100 (1962).
19. Ashcroft, S.J.H., Hedeskov, C.J., Randle, P.J.: Glucose metabolism in mouse pancreatic islets. Biochem. J. **118**, 143—154 (1970).
20. Webb, J.L.: Enzyme and Metabolic Inhibitors, Vol. 1, p. 518. New York: Academic Press 1963.
21. Snyder, P.J., Kashket, S., O'Sullivan, J.B.: Pentose cycle in isolated islets during glucose stimulated insulin release. Amer. J. Physiol. **219**, 876—882 (1970).
22. Ferner, H., Kern, H.: Die Vergleichende Morphologie der Langerhansschen Inseln. In: Handbuch des Diabetes Mellitus (E. Pfeiffer ed.), Vol. 1, p. 39—62. München: J.F. Lehmanns Verlag 1969.
23. Coore, H.G., Randle, P.J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. **93**, 66—78 (1964).
24. Hellerström, C., Westman, S., Marsden, N., Turner, D.: Oxygen consumption of the B-cells in relation to insulin release. In: The Structure and Metabolism of the Pancreatic Islets (S. Falkmer, B. Hellman, I.-B. Täljedal, eds.), p. 315. Oxford: Pergamon Press 1970.
25. Edwards, J.C., Taylor, K.W.: Fatty acids and the release of glucagon from isolated guinea-pig islets of Langerhans incubated in vitro. Biochim. Biophys. Acta **215**, 310—315 (1970).
26. Sanbar, S.S., Martin, J.M.: Stimulation by octanoate of insulin release from isolated rat pancreas. Metabolism **16**, 482—484 (1967).
27. Montague, W., Taylor, K.W.: Regulation of insulin secretion by short chain fatty acids. Nature **217**, 853 (1968).
28. Howell, S.L.: Ph. D. Thesis, University of London (1967).

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