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Oxygen Consumption of Isolated Pancreatic Islets of Mice Studied with the Cartesian-Diver Micro-Gasometer

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Studies of the insulin release from the endocrine pancreas and quantitative biochemical analyses of micro-dissected pancreatic islets have significantly contributed to our present knowledge of the function and biochemistry of the pancreatic islet β -cells. However, we have so far only limited information about several important metabolic characteristics of these cells, e.g. the mechanism behind the glucose stimulation of insulin release (cf. Field, 1964). The purpose of the present communication is to demonstrate a new approach to metabolic studies of mammalian β -cells, utilizing Cartesian divers in micro-respirometric analyses of isolated and surviving pancreatic islets.

Methods. Adult female mice with the obese-hyperglycaemic syndrome from a strain originating at the Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A., were used. The animals were starved for 18 hr. before decapitation. The pancreas was then excised and suspended in Krebs-Ringer phosphate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1964), maintained at 2°. The method used for the subsequent micro-dissection of fresh islets has been described in detail (Hellerström, 1964).

The oxygen consumption of the isolated islets was

measured with the aid of Cartesian divers (Linderström-Lang, 1937, 1943; Holter, 1943) with a total volume of 8–12.5 μ l. The basic equipment and the handling of the divers mainly conformed to the directions given by Holter (1943, 1961). The silicone-coated divers were either of the 'standard' type, with a bulb between the neck and the tail, or of the cylindrical type. The total gas volume of the charged divers at equilibrium pressure was about 5 μ l., i.e. a displacement in the pressure of 1 mm. of Brodie solution at 37° corresponded to a change in the gas content of about 0.0004 μ l.

When an islet had been isolated from the surrounding tissue, it was washed briefly in fresh Krebs-Ringer phosphate buffer and sucked into a braking pipette (Holter, 1943), together with 0.5 μ l. of buffer solution. The medium with the islet was placed as a bottom drop in the bulb of a standard diver, the neck of which was sealed with 0.7 μ l. of 1% (w/v) KOH, 0.5 μ l. of paraffin oil and an appropriate amount of flotation medium. After transfer to the flotation vessel the diver was allowed to equilibrate for 10 min. before the measurements started. When the islet respiration was measured at 30°, the standard divers were filled at room temperature. In experiments performed at 37° the

bottom drop with the islet was also placed as described above, but the diver was then submerged under distilled water in an aluminium thermostat maintained at 37° (Kieler, 1960). When the alkali and oil seals had been introduced, the diver mouth was temporarily closed by a water seal. The diver was transferred to the flotation vessel, where the water seal was replaced by flotation medium and the length of the mouth seal adjusted by means of a fine braking pipette, until the diver equilibrium pressure was a few cm. of Brodie solution below atmospheric pressure (cf. Kieler, 1960).

In a series of experiments designed to study the effect of various exogenous substrates on the islet oxygen uptake at 37°, cylindrical divers were used. They were charged at room temperature with 1 μ l. of KOH as bottom drop and a side drop consisting of 0.3 μ l. of phosphate buffer containing the substrate to be tested. After submersion of the diver at 37° in the aluminium thermostat, a neck seal consisting of 0.7 μ l. of buffer, including the islet, was placed 0.5 mm. above the side drop. The subsequent filling procedure was in accordance with the description above, except that the volume of the oil seal was increased to 1 μ l.

Measurements of the oxygen uptake were performed in a water thermostat with a temperature control of $\pm 0.004^\circ$. Equilibrium pressures were recorded at intervals of approx. 10 min. and a control diver without an islet was run simultaneously in each experiment. In utilizing cylindrical divers, the endogenous respiration was initially followed for about 1 hr. before the seal with the islet was made to mix with the side drop by the application of a suitable over-pressure (Anfinson & Claff, 1947). After completion of the measurements the islet was extracted by gently flushing the diver with distilled water from a hand pipette. The islet was subsequently placed on a piece of platinum foil, the weight of which had been determined previously on an ultramicro-balance (Mettler UM7) sensitive to 0.1 μ g. The weight of the islet was obtained by reweighing the dried foil.

Results. The dry weights of incubated islets were in the range 1–10 μ g., a single islet being incubated in each diver. At both 30° and 37° the respiratory rate was constant for more than 2 hr. At 30° the average ($\pm$ S.E.M.) oxygen uptake (Q_{O_2}) in 11 experiments was 3.44 ± 0.41 μ l./hr./mg. dry wt., whereas at 37° the Q_{O_2} in 34 experiments had increased to 5.41 ± 0.33 μ l./hr./mg. dry wt. A *t*-test showed the difference to be significant at the 1% level.

A striking stimulation of the oxygen uptake was noted when the phosphate buffer was supplemented with 16.7 mM-D-glucose, the Q_{O_2} in 14 experiments at 37° now being 9.06 ± 0.55 μ l./hr./mg. dry wt. This value corresponded to an increase of 67%, as

Fig. 1. Oxygen consumption of a pancreatic islet incubated in a cylindrical diver at 37°. The first part of the line (●—●) represents the endogenous respiratory rate in Krebs-Ringer phosphate buffer, and the second part (○—○) shows the respiration after the addition of D-glucose (16.7 mM). The arrow indicates mixing of the buffer seal, including the islet, with the side drop containing glucose. The oxygen uptake increased 58% after the addition of glucose.

compared with that of the glucose-free buffer at the same temperature ($P < 0.001$). The effect of glucose on islet respiration was further demonstrated in experiments with cylindrical divers. After the initial measurements of endogenous respiration, glucose was added to the Krebs-Ringer medium in a final concentration of 16.7 mM by mixing with the side drop. As shown in Fig. 1, there was immediately an increased oxygen consumption rate, which remained constant for the rest of the experiment.

Discussion. The present investigation indicates that the technique used for the preparation of fresh mammalian islets preserves the respiratory activity of the islet cells and also provides tissue samples of a size well suited for studies with the Cartesian-diver method. It is notable that the cell damage to isolated islets is kept to a minimum, since each micro-dissected islet is enclosed by an intact connective-tissue capsule (Hellerström, 1964). The increased oxygen consumption induced by a rise in the temperature or the addition of glucose suggests that the gas exchange between the islet cells and the medium was not a limiting factor. Since only small samples of fresh islet tissue can be obtained within a reasonable time, even with special pretreatment of experimental animals (Keen, Sells & Jarrett, 1965) or of the excised pancreas (Moskalewski, 1965), it seems particularly valuable that the diver method should be sufficiently sensitive to measure the respiration of individual mammalian islets.

The endogenous Q_{O_2} value of the present islet material was in the same range as that of a number of other mammalian tissues. It is assumed that this value represents the β -cells, since this cell type

composes more than 90% of the islets of obese-hyperglycaemic mice (Christophe, 1965). The striking stimulation of glucose on β -cell respiration is in line with the observations of Keen *et al.* (1965), who reported a considerably higher oxidation of [1^{14}C]glucose by isolated rat islets when the glucose concentration of the medium was increased. Friz, Lazarow & Cooperstein (1960) failed, however, to obtain a stimulating effect of glucose on the respiration of islet-tissue slices from toadfish. These results may reflect a significant metabolic difference between pancreatic β -cells in mammals and fish.

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The Enzymic Oxidation of Thiols

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Several observations have been recorded of thiols being able to reduce the coloured products formed by the action of peroxidase and hydrogen peroxide on substrates such as guaiacol and *p*-phenylenediamine, and the early observations have been reviewed by Randall (1946). Glock (1944) observed that thiourea and thiouracil inhibited the action of peroxidase and hydrogen peroxide on pyrogallol. Randall (1946) studied the action of peroxidase and hydrogen peroxide on several thiols and came to the conclusion that thiols do not inhibit peroxidase but are reducing agents acting as substrates for the peroxidase system and competing with other substrates for the available peroxide-peroxidase complex. He also found that thiols can reduce the coloured dyes formed from *p*-amino-benzoic acid and benzidine and thus they show an apparent inhibition of colour production by the peroxidase system.

Neufeld, Green, Latterell & Weintraub (1958) described an enzyme, thiol oxidase, that catalysed the oxidation of thiophenol and sodium diethyldithiocarbamate to the disulphide form, and the

reactions catalysed by this enzyme were represented thus:

(where X could be carbon, oxygen, nitrogen or sulphur). The enzyme was purified extensively by Aurbach & Jakoby (1962) and found to contain 0.01% of copper. They reported that the enzyme also catalysed the oxidation of phenols, e.g. catechol and resorcinol. These phenols have also been shown to be oxidized by laccase (*p*-diphenolase) (Benfield, Bocks, Bromley & Brown, 1964).

The oxidation of thiols by a copper oxidase is particularly interesting, since substances such as sodium diethyldithiocarbamate have been used as inhibitors of tyrosinase (*o*-diphenolase), which also contains copper as its prosthetic group (Hallaway, 1959). I have now prepared the thiol oxidase from *Piricularia oryzae* according to the method of Aurbach & Jakoby (1962) and have confirmed their