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# The Application of Microcalorimetry to the Assessment of Metabolic Efficiency in Isolated Rat Hepatocytes

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1. Heat output by suspensions of isolated rat hepatocytes was determined by using a modified batch-type microcalorimeter. 2. The ratio of $O_2$ uptake (determined polarographically) to heat output was used to assess the metabolic efficiency of isolated hepatocytes. 3. Cells from starved or fed rats incubated in either bicarbonate-buffered physiological saline containing gelatin, or bicarbonate-buffered physiological saline containing amino acids, serum albumin and glucose showed no significant difference with respect to the ratio of $O_2$ uptake to heat output. 4. For liver cells from 24h-starved rats, the addition of 10mM-dihydroxyacetone and 2.5mM-fructose significantly decreased the ratio of $O_2$ uptake to heat output from $1.94 \pm 0.05$ in the controls to $1.52 \pm 0.04$ and $1.54 \pm 0.01$ $\mu$mol/J respectively. 5. Glucagon (1$\mu$M), which slightly increased both $O_2$ uptake and heat output, did not significantly alter the ratio. 6. The addition of extracellular 10mM-$NH_4Cl$ and urease to provide an energetically wasteful cycle by ensuring hydrolysis of newly synthesized urea, lowered the ratio of $O_2$ uptake to heat output from $1.81 \pm 0.08$ to $1.47 \pm 0.06$ $\mu$mol/J, indicating a reduced metabolic efficiency. 7. Metabolic efficiency in rats of different dietary regimen, age and genetically based obesity was also assessed. No differences in the ratio of $O_2$ uptake to heat output were found between liver cell suspensions prepared from rats maintained on colony diet and high-fat diet or sucrose-rich diet nor between animals ranging from 38 to 179 days of age. Comparison of the ratio of liver cell $O_2$ uptake to heat output between homozygote Zucker *fa/fa* obese rats and their lean littermates showed no significant difference. 8. It is concluded that the ratio of $O_2$ uptake to heat output for isolated hepatocytes is relatively constant unless perturbed by conditions that markedly enhance substrate cycling.

In order to maintain living processes cells obtain supplies of free energy from their environment. The mitochondria of the cell appear to be the major site where most of the useful energy derived from oxidation of fatty acids, carbohydrates and amino acids is captured in the form of the high-energy intermediate ATP. It is also recognized that ATP is a significant transducer to energy-requiring processes, such as macromolecule synthesis, muscular contraction, nerve excitation, maintenance of cellular integrity etc. In the overall processes involving the oxidation of metabolic fuel, production of ATP, hydrolysis of ATP and macromolecule synthesis, a proportion of energy is lost back to the environment in the form of heat. For systems in which a large proportion of newly formed ATP is conserved in molecular synthesis the proportion of heat loss to the environment is presumably less. Thus for a closed system the metabolic efficiency of that system could be assessed from the ratio of $O_2$ consumed to heat produced, where the more efficient system has the higher ratio.

In the present studies a modified batch microcalorimeter has been applied in conjunction with polarographic measurements of $O_2$ consumption, to assess factors that might change the ratio of $O_2$ consumed to heat produced by isolated rat hepatocytes.

## Experimental

### *Materials*

Collagenase (type II) was obtained from Worthington Biochemicals Corp., Freehold, NJ, U.S.A. Fructose, dihydroxyacetone, jack-bean urease (type VI, 7 units/mg, where 1 unit produces 1mg of ammonia nitrogen from urea in 5min at pH7.0 at 30°C), L(+)-lactic acid, sodium pyruvate and NADH were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Crystalline porcine glucagon was a gift from Dr. Wm. Bromer of Eli Lilly and Co., Indianapolis, IN, U.S.A. The mixture of essential amino acids, based on that of Eagle (1959), was obtained

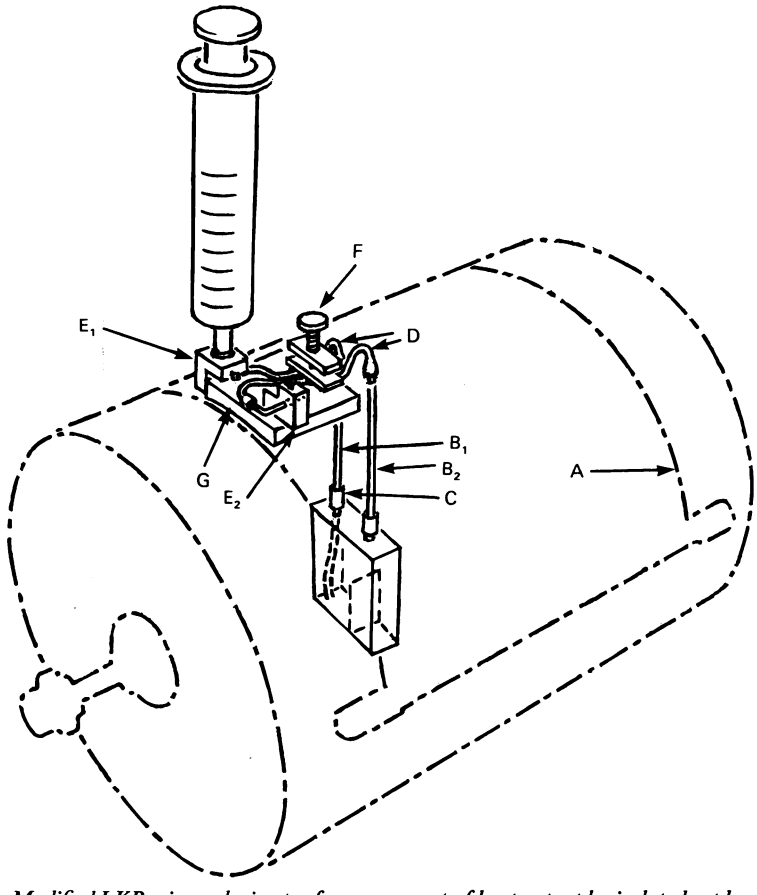


Fig. 1. *Modified LKB microcalorimeter for assessment of heat output by isolated rat hepatocytes*

The batch unit contained two gold reaction vessels of 12ml capacity each fitted internally with a baffle dividing the lower half of each vessel into two compartments. Access to these two compartments was via two gold tubes installed flush with the top surface of the vessel. The reaction vessels were contained within an aluminium block with thermopiles interposed between the vessels and block. The block was insulated from the surrounding air bath by a $1\frac{1}{2}$ inch layer of polyurethane contained in a metal jacket and could be rotated by remote control to provide initial mixing of the contents of the compartments. Originally, access for loading and emptying the vessels was through a hinged lid (A) in the jacket surrounding the aluminium block. It was because of the subsequent disturbance to the block temperature that long (approx. 90min) equilibration periods were required. In order to reduce this equilibration period a length of polythene tubing ($B_1$) (internal diam. 1.0mm; external diam. 1.4mm) was inserted through the gold tube of the larger compartment with the end reaching the floor of the compartment, a sleeve of silicone rubber tubing (C) being used to seal the polythene to the gold. Another length of polythene tubing ($B_2$) was similarly attached to the remaining gold tube, but with the end just inside the gold tube, so as to act as a vent during loading and emptying. Both tubes were passed out through the lagging and jacket via snug-fitting holes drilled directly in line with the gold tubes, the external ends being connected with silicone rubber tubing (D) to Luer-lock fittings housed in brass blocks ($E_1$ and $E_2$). A screw clamp (F) was also incorporated for closing-off the tubes at the silicone rubber connecting pieces. The brass blocks ($E_1$ and $E_2$) and screw clamp (F) were screwed to a rigid PVC block (G) which was mounted on the hinged lid (A). When loading and emptying, a temporary lid, fabricated from acrylic sheet and fitted with a pair of isolation cuffs (Isolette, Warminster, PA, U.S.A.) was used. This prevented undue disturbance to the air bath during these operations. To ensure adequate mixing of the suspension of hepatocytes the stirring mechanism was modified so that continuous clockwise/anti-clockwise movement (440 degrees of rotation each way) was maintained. The rate of rotation was approx. 3rev./min. The instrument was operated at an air-bath temperature of 37°C and located in an air-conditioned room maintained at 25°C. Thermopile output was amplified by a Keithley model 150 B Microvolt Ammeter (Keithley Instruments, Cleveland, OH, U.S.A.) coupled to a Sargent model SRG Recorder (Sargent-Welch Scientific Co., Skokie, IL, U.S.A.). The hepatocytes in suspension were pre-equilibrated for 6min in a 20ml glass syringe placed in a reciprocating water bath at 37°C (shaking at 80 oscillations/min); a 5ml gas space was left in the syringe and contained $O_2/CO_2$ (19:1). Loading of the cells into the microcalorimeter was achieved by gently discharging the syringe contents

from Grand Island Biochemical Co., Grand Island, NY, U.S.A. Foetal calf serum was obtained from the Commonwealth Serum Laboratories, Melbourne, Vic., Australia. Gelatin was obtained from Difco Laboratories, Detroit, MI, U.S.A.

*Animals*

Male and female rats of the genetically obese Zucker strain were fed *ad libitum* on the colony diet. For other studies male rats of the Hooded-Wistar strain were used. The composition of the colony diet was 70% wheat-meal starch, 17.5% protein, 3.5% fat and 5.5% fibre. The fat and sucrose diets, with equal protein/energy ratios, were essentially those used by Mack *et al.* (1975) with mineral mix as described by Schwartz (1951).

*Methods*

*Preparation of hepatocytes.* Liver parenchymal cells were isolated by the technique of Berry & Friend (1969), except that hyaluronidase was omitted and the final concentration of collagenase in the perfusate (Krebs Improved Ringer I with pyruvate; Dawson *et al.*, 1969) was 0.037%. The gas phase for incubations was $O_2/CO_2$ (19:1), and the incubation buffer contained 1.5% (w/v) gelatin. The pH was 7.4 at 37°C.

*Microcalorimetry.* A modified LKB microcalorimeter of the batch-type (model 10700) was used and operated at 37°C. Fig. 1 describes in detail the modifications necessary to overcome (i) the lengthy equilibration period and (ii) insufficient mixing of the hepatocyte suspensions. Briefly the modifications involved the fitting of two plastic tubes, which allowed direct access to the reaction vessel without disturbing the block temperature, and the fabrication of an acrylic lid fitted with a pair of isolation cuffs, so that cell suspensions could be loaded manually with minimal disturbance to the air bath. Adequate mixing was achieved by continuous sequential clockwise and anti-clockwise rotation (440 degrees) of the aluminium block.

*$O_2$ uptake.* This was measured polarographically at 37°C by using a Yellow Springs Instrument model 53 oxygen electrode assembly. A portion (6ml) of the cell suspension that had been equilibrated and mixed at 37°C for microcalorimetry was transferred to the oxygen electrode assembly immediately after the loading of the microcalorimeter; this procedure permitted simultaneous measurement of heat output (microcalorimeter) and $O_2$ uptake (oxygen electrode assembly) by each cell suspension; a representative set of traces for each is shown in Fig. 2.

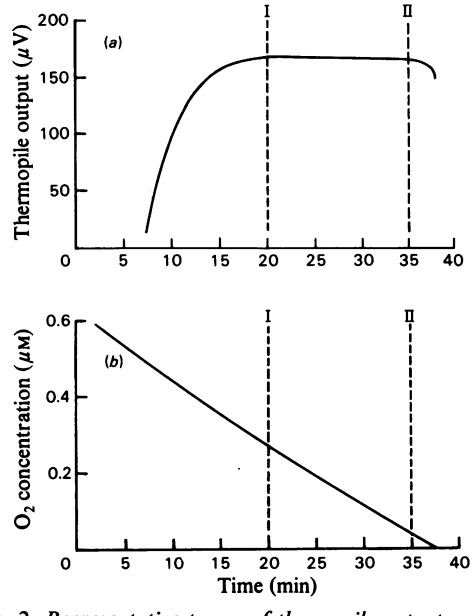


Fig. 2. *Representative traces of thermopile output and $O_2$ concentration for hepatocyte suspensions*

Isolated hepatocytes were prepared as described in the Experimental section and diluted in incubation buffer to give approx. $10^6$cells/ml or 2.7mg dry wt./ml. After pre-equilibration of the hepatocyte suspension at 37°C, with or without substrate or hormone (see under 'Methods'), for approx. 6min, 12ml was transferred to the microcalorimeter and 6ml to the oxygen electrode sample chamber. After an additional equilibration period of 12–15min, a constant thermopile output (*a*) was attained, which continued for a further 20–25min; $O_2$ concentration (*b*) was measured simultaneously. Representative traces for thermopile output and $O_2$ concentration are shown. Heat output and $O_2$ consumption of the hepatocytes were calculated over the time period I–II.

directly into the polythene tube to the reaction vessel until excess suspension overflowed from the vent tube. A suspension of approx. $10^6$cells/ml was found to give a thermopile output of approx. 150μV and such a suspension could be maintained for a least 30min by the available $O_2$. Equilibration was always obtained within 15min of loading and a stable recording of thermopile output was obtained over a subsequent 15min period for the calculation of heat output. After each day's use the instrument was calibrated electrically with the reaction vessel filled with buffer. At all times the second reaction vessel was filled with water, this arrangement providing a stable thermopile output with insignificant effects from friction within the vessels. The microcalorimeter was emptied by withdrawing the contents with a syringe. At the end of each day's use the reaction vessel was rinsed out with 70% ethanol to prevent bacterial growth.

*Measurement of cellular integrity.* Lactate dehydrogenase (EC 1.1.1.27) activity released into the medium was assayed directly (Kornberg, 1955). Total activity in cells plus medium was measured in a cell suspension diluted 10–20-fold in the presence of 0.5% Triton X-100.

The proportion of non-viable cells was estimated by mixing the cell suspension with 2vol. of freshly prepared Trypan Blue (0.4% in incubation buffer), after which a minimum of 100 cells were examined for Trypan Blue uptake.

*Analytical determinations.* The dry weight of the liver-cell suspension was determined gravimetrically. Cell suspensions were counted in an improved Neubauer haemocytometer. Urea was determined by the colorimetric method of Oginsky (1957).

## Results and Discussion

### *Heat output by suspensions of isolated hepatocytes*

*Measurement of heat output.* Previous applications of microcalorimetry (Wadsö, 1968, 1975) to the measurement of heat evolved by isolated mammalian cells have been restricted to cell types that either do not respire [e.g. erythrocytes (Levin, 1973*a*; Monti & Wadsö, 1976)] or respire at relatively low rates [e.g. leucocytes (Levin ,1973*b*), pancreatic $\beta$-cells (Gylfe & Hellman, 1975), fat-cells (Nedergaard *et al.*, 1977)] and therefore do not drastically lower buffer $O_2$ concentration during the extensive equilibration period.

Although the equilibration period associated with a flow microcalorimeter would be suitable for isolated hepatocyte suspensions, preliminary studies indicated that the cells sedimented in the heat exchangers and associated pump lines. For this reason the batch-type microcalorimeter was employed, and modifications were implemented that reduced equilibration time and enhanced mixing.

As the incubation conditions used in the microcalorimeter differ from those commonly employed, an assessment of cell viability was performed (e.g. Hopgood *et al.*, 1977). Representative values for the uptake of Trypan Blue were 4.5 and 10.8% for samples taken at the commencement and end of a microcalorimetry experiment respectively; values for loss of lactate dehydrogenase were 9.7 and 16.5% respectively.

*Factors affecting heat output by isolated hepatocytes.* Substrate or futile cycles must be considered as pre-eminent among those reactions occurring in the cell that can contribute to heat loss and therefore to a lowering of the overall metabolic efficiency of that system. Such energetically wasteful cycles have been proposed to provide regulatory advantage (Newsholme & Gevers, 1967; Newsholme & Start, 1973), and even thermogenesis (Newsholme & Crabtree, 1970; Newsholme *et al.*, 1972; Clark *et al.*, 1973). Several workers have demonstrated the occurrence of substrate cycles in liver. The glucokinase/glucose 6-phosphatase cycle (Clark *et al.*, 1973), the phosphofructokinase/fructose 1,6-bis-phosphatase cycle (Clark *et al.*, 1973; Clark *et al.*, 1974*a*,*b*) and the pyruvate kinase/pyruvate carboxylase/phosphoenolpyruvate carboxykinase cycle (Friedman *et al.*, 1971; Rognstad, 1975) have each been demonstrated. Further, approximations of the rates of all three have been made (Katz & Rognstad, 1976): these are 0.4–1.7 (Katz & Rognstad, 1976), 0.12 (Clark *et al.*, 1974*b*) and 0.6 $\mu$mol/min per g wet wt. (Rognstad, 1975) respectively for the glucokinase/glucose 6-phosphatase, phosphofructokinase/fructose 1,6-bisphosphatase and pyruvate kinase/pyruvate carboxylase/phosphoenolpyruvate carboxykinase substrate cycles of fed rat liver. Thus a range of substrate-cycling rates of 1.1–2.4 $\mu$mol/min per g wet wt. appears possible; this would be equivalent to 11–24% of the rate of $O_2$ consumption by fed rat liver.

Since nutritional-state changes, in particular starvation, have been shown to decrease the activity of glycolytic enzymes and to enhance those of gluconeogenesis, i.e. the activity of glucokinase (Vinuela *et al.*, 1963) and L-type pyruvate kinase (Krebs & Eggleston, 1965) decrease whereas those of phosphoenolpyruvate carboxykinase (Shrago *et al.*, 1963) and glucose 6-phosphatase (Ashmore & Weber, 1959) increase, initial experiments were conducted to assess the effect of food deprivation on the rates of $O_2$ uptake and heat output, as well as the ratio of $O_2$ uptake to heat output (Table 1). In addition, two incubation buffers of differing composition were tested. In comparing cells from fed and starved animals incubated in Krebs–Henseleit bicarbonate buffer containing gelatin, both the rates of $O_2$ uptake and heat output were slightly higher in the starved animal when expressed as a function of the dry wt. of cells. In general, cells incubated in Eagle's minimal essential medium showed lower rates of $O_2$ uptake and heat output, but each rate was significantly increased when the animal had been starved before cell isolation. However, no significant difference in the ratio of $O_2$ uptake to heat output was noted with either of the incubation buffers. These results are consistent with a view that starving the animal did not alter the metabolic efficiency of the isolated liver cells when expressed as a function of the ratio of $O_2$ uptake to heat output. This suggests that the rates of substrate cycling between glucose/glucose 6-phosphate, fructose 6-phosphate/fructose 1,6-bisphosphate and pyruvate/phosphoenolpyruvate in addition to the cycles between triacylglycerol/fatty acids, protein/amino acids and glycogen/glucose are not appreciably altered by starvation.

Substrates represent a second factor that may alter the rate of substrate cycling in liver. Substrates,

such as dihydroxyacetone, ethanol, fructose etc., which increase the so-called 'extra oxygen consumption' (Newsholme & Gevers, 1967; Berry, 1974), may do so by enhancing the rate of energy dissipation. Table 2 compares the effects of dihydroxyacetone, lactate and fructose on the rates of $O_2$ uptake and heat output. Dihydroxyacetone (10mM) significantly increased the rate of heat output of cells from starved animals when compared with controls. Since the rate of $O_2$ uptake was not greatly increased, the ratio of $O_2$ uptake/heat output was significantly lower than that for the control experiments (no additions). Fructose (2.5mM) produced similar effects to those of 10mM-dihydroxyacetone. With 10mM-lactate there was no change in the rate of heat output, but the increase in $O_2$ consumption, although not statistically significant, led to a significant increase in the ratio of $O_2$ uptake to heat output. Increased glycolytic rate produced by either dihydroxyacetone or fructose when rates of gluconeogenesis are high (Clark *et al.*, 1974*b*) may account for the observed increase in heat output produced by these substrates. It is of interest that Katz *et al.* (1975) have shown that 10mM-dihydroxyacetone increases the rate of futile cycling at the glucose/glucose 6-phosphate and the fructose 6-phosphate/fructose 1,6-bisphosphate futile cycles in isolated hepatocytes from either fed or starved rats.

Hormones such as glucagon and adrenaline, which have been shown to inhibit metabolic flux through phosphofructokinase (Clark *et al.*, 1974*b*; Kneer *et al.*, 1974) and pyruvate kinase (Rognstad, 1975; Feliu *et al.*, 1976) while enhancing flux through fructose 1,6-bisphosphatase (Clark *et al.*, 1974*b*; Kneer *et al.*, 1974), might be expected to increase the ratio of $O_2$ uptake/heat output by decreasing the rate of energy dissipation through substrate cycling at these sites. In Table 3 the effect of glucagon on the rates of $O_2$ uptake and heat output are shown. Whereas there were slight increases in each rate and the ratio of $O_2$ uptake/heat output, none of these changes was statistically significant. On this basis it appeared likely that glucagon's action in altering metabolic flux rates in the hepatocyte resulted from, or coincided with, an appreciable decrease in substrate cycling, and hence an increase in metabolic efficiency.

Table 1. *Effect of food deprivation and composition of incubation buffer on $O_2$ uptake and heat output by suspension of isolated hepatocytes*

Isolated hepatocytes were prepared from either fed or 24h-starved colony rats as described in the Experimental section and diluted in incubation buffer to give approx. $10^6$ cells/ml. Two incubation buffers were used: Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969) with 1.5% (w/v) gelatin, Eagle's minimum essential medium (MEM) (Eagle, 1959) with 5% (w/v) foetal calf serum. After pre-equilibration at 37°C (see under 'Methods'), 12ml was transferred to the microcalorimeter and 6ml to the oxygen electrode assembly. For each cell preparation the simultaneous rates of $O_2$ uptake and heat output have been calculated. Values are means ± S.E.M. with the numbers of cell preparations shown in parentheses.

| Nutritional state | Body wt. (g) | Incubation medium | $O_2$ uptake (μmol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake/heat output (μmol/J) |
|---|---|---|---|---|---|
| Fed (6) | 291 ± 11 | Krebs–Henseleit + 1.5% (w/v) gelatin | 13.5 ± 0.7 | 7.43 ± 0.51 | 1.84 ± 0.10 |
| Fed (4) | 302 ± 8 | Eagle's MEM | 10.8 ± 0.7 | 5.88 ± 0.38 | 1.83 ± 0.03 |
| Starved (7) | 233 ± 12 | Krebs–Henseleit + 1.5% (w/v) gelatin | 15.4 ± 0.7 | 8.83 ± 0.58 | 1.76 ± 0.05 |
| Starved (5) | 287 ± 16 | Eagle's MEM | 14.8 ± 0.6 | 7.87 ± 0.49 | 1.89 ± 0.04 |

Table 2. *Effect of substrates on $O_2$ uptake and heat output by suspensions of isolated hepatocytes*

Isolated hepatocytes were prepared from 24h-starved colony rats (209 ± 14g body wt.) as described in the Experimental section. Cells were diluted in Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969) containing 1.5% (w/v) gelatin to give approx. $10^6$ cells/ml. The substrates were added to the incubations before equilibration. The rates of $O_2$ uptake and heat output were determined. Values are the means ± S.E.M. with the numbers of cell preparations shown in parentheses. Student's *t* test was applied to assess significant differences when compared with the corresponding control (no additions) value; n.s., not significant.

| Substrate | $O_2$ uptake (μmol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake/heat output (μmol/J) |
|---|---|---|---|
| No additions (6) | 14.1 ± 0.29 | 7.50 ± 0.39 | 1.88 ± 0.05 |
| 10mM-Dihydroxyacetone (3) | 16.2 ± 0.66 (n.s.) | 10.7 ± 0.39 ($P<0.01$) | 1.52 ± 0.04 ($P<0.01$) |
| 10mM-Lactate (3) | 15.9 ± 1.39 (n.s.) | 7.67 ± 0.62 (n.s.) | 2.07 ± 0.03 ($P<0.05$) |
| 2.5mM-Fructose (3) | 15.3 ± 1.0 (n.s.) | 9.94 ± 0.67 ($P<0.05$) | 1.54 ± 0.01 ($P<0.01$) |

Table 3. *Effects of glucagon and artificially stimulated futile cycling on the rates of $O_2$ uptake and heat output by suspensions of isolated hepatocytes*

Isolated hepatocytes were prepared from fed rats (285±27g body wt.) as described in the Experimental section and diluted in Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969) containing 1.5% (w/v) gelatin to give approx. $10^6$cells/ml. Incubations were conducted in the above buffer. Values are means ± S.E.M. for three cell preparations. The order of experimental conditions was altered for each of the three cell preparations to a latin square arrangement. Student's *t* test was applied to assess significant differences when compared with the control value; n.s., not significant.

| Additions | $O_2$ uptake (μmol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake/heat output (μmol/J) |
|---|---|---|---|
| None | 9.30±0.82 | 5.12±0.33 | 1.81±0.08 |
| 1μM-Glucagon | 10.2 ±0.66 (n.s.) | 5.39±0.22 (n.s.) | 1.90±0.13 (n.s.) |
| 10mM-$NH_4Cl$+7μg of urease/ml | 8.03±0.06 (n.s.) | 5.47±0.24 (n.s.) | 1.47±0.06 ($P<0.05$) |

Comparisons of the rates of $O_2$ uptake and heat output obtained in these experiments (Table 3) with those of similar experiments (Table 1) demonstrate a highly significant decrease in the rate of $O_2$ uptake ($P< 0.001$), a significant decrease in heat output ($P< 0.05$) and no change in the ratio of $O_2$ uptake/heat output. The hepatocytes used in these experiments (Table 3) were prepared from a different strain of Hooded-Wistar rats. Very similar low results for $O_2$ uptake and heat output were obtained with an albino strain of Wistar rats (results not presented).

Direct stimulation of substrate cycling in liver by exogenous agents has not previously been demonstrated. Thus in the present experiments extracellular urease was added in conjunction with ammonia in an attempt to stimulate energy dissipation by providing an immediate urea-hydrolysing system. Table 3 shows that the addition of both urease and 10mM-$NH_4Cl$ produced a significant increase in the rate of heat output and a lowering of the ratio of $O_2$ uptake/heat output from 1.81±0.08 to 1.47±0.06. Since the amount of urease added was sufficient to prevent urea accumulation and to keep $NH_4^+$ concentration at approx. 10mM, any extra heat produced could be concluded to arise from the sum of the energy required to synthesize urea and the heat output from the hydrolysis of the urea formed.

Rates of urea synthesis by hepatocytes (from fed rats) in the calorimeter gave values between 5.0 and 5.3μmol of urea/min per g dry wt. These values are within the range reported by Krebs *et al.* (1974). The enzymic hydrolysis of urea in a Krebs–Henseleit bicarbonate buffer plus gelatin demonstrated that 25.7–27.9mJ of heat was released/μmol of urea. This agreed with the published value of 25.9mJ/μmol of urea (Wall & Laidler, 1953). The extracellular hydrolysis of 5.2μmol of urea would therefore result in the release of 0.14J. From these calculations it would appear that the presence of urease would only lead to an increase of about 3% in heat production over the control.

Measurements of heat production and $O_2$ consumption with 10mM-$NH_4Cl$ gave virtually the same results, both in the presence and absence of urease (results with $NH_4Cl$ alone not shown). This is not surprising, since a 3% increase in heat output is only just detectable by the calorimeter.

In these experiments (Table 3), although the decrease in $O_2$ consumption was not statistically significant, the ratio of $O_2$ uptake/heat output was significantly decreased. This observation would be consistent with lower metabolic efficiency in the presence of $NH_4Cl$ and urease. This could be explained by an increase in substrate cycling.

### *Metabolic efficiency of isolated hepatocytes and obesity*

James and co-workers (James & Trayhurn, 1976; Trayhurn *et al.*, 1977) have extended the original hypothesis of Miller (1974) that obesity or the accumulation of triacylglycerol in cells reflects a change in metabolic efficiency, i.e. that excessive net synthesis of free fatty acids etc. arises because less energy is dissipated in cycling and more is conserved in chemical-bond formation. Support for this hypothesis has come from studies with genetically obese (*ob/ob*) mice where (i) these have been shown to develop obesity even when pair-fed to their lean littermates (Alonso & Maren, 1955; Hollifield & Parson, 1958) and (ii) these strains of mice, and other strains of genetically obese rodent, are unable to maintain their body temperature when exposed to cold (Davis & Mayer, 1954; Trayhurn *et al.*, 1977). The latter effect has been concluded to result from a defect in thermogenesis rather than heat conservation (Davis & Mayer, 1954; Trayhurn *et al.*, 1977). On this basis a comparison of hepatocyte suspensions from normal and obese littermates might be expected to reveal a difference in the ratio of $O_2$ uptake/heat produced. For this reason an assessment of the rates of $O_2$ uptake and heat output were made for liver cells from normal, dietary obese and genetic obese

rats. In Table 4, rats that were clearly obese as a result of being fed on lard-rich or sucrose-rich diets showed no significant change in $O_2$ uptake, heat output or the ratio of $O_2$ uptake/heat output. In Table 4 a comparison was also made between hepatocyte suspensions from homozygote obese (*fa/fa*) rats and their lean littermates. For the obese animals the rate of $O_2$ uptake was significantly less than their lean counterparts. This contrasted with the rate of heat output, which was only slightly less, giving rise to a slight decrease in the ratio of $O_2$ uptake/heat output. The decrease in the ratio, although not statistically significant, was contrary to the predictions made above and suggested that obesity does not alter the metabolic efficiency of the liver as assessed by the ratio of $O_2$ uptake/heat output for the isolated hepatocytes.

*Effect of development on hepatocyte metabolic efficiency*

Growth curves for the laboratory rat indicate that he maximum rate of growth occurs shortly after weaning (see Table 5, where the highest growth rate is 5.0g body wt./10 days between 38 and 48 days of age). Thus experiments were conducted to assess whether changes in the rate of growth reflected changes in the metabolic efficiency of the hepatocyte. Despite large increases in the rates of $O_2$ uptake and heat output (per $10^8$ cells) with increase in age (due in the main to concomitant increases in individual cell mass), there was no significant change in the ratio of $O_2$ uptake/heat output.

In summary, it is apparent that the metabolic efficiency of the liver, as assessed by the ratio of $O_2$ uptake/heat output of the isolated hepatocyte suspension, remains relatively constant at 1.8 $\mu$mol/J unless perturbed by conditions that markedly enhance substrate cycling. There is no evidence from this study that obesity or growth in the rat is associated with a change in the metabolic efficiency of liver.

This work was supported in part by a grant to M. G. C. from the National Health and Medical Research Council of Australia.

Table 4. *Effect of dietary regimen (a) and genetically inherited obesity (b) on $O_2$ uptake and heat output by suspensions of isolated hepatocytes*

Colony rats were fed on diets rich in either sucrose or lard from weaning (*a*). Obese and lean littermates of the genetically obese Zucker strain of rats were fed on a colony diet (*b*). Hepatocytes were prepared as described in the Experimental section and diluted in Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969), containing 1.5% (w/v) gelatin, to give approx. $10^6$ cells/ml. Incubations were conducted in the above buffer and the rates of $O_2$ uptake and heat output determined. Values are the means ± S.E.M. for the numbers of cell preparations shown in parentheses. Student's *t* test was applied to assess significant differences when compared with the colony diet group (*a*) and between lean and obese (*b*); n.s., not significant.

| | Body wt. (g) | $O_2$ uptake ($\mu$mol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake/heat output ($\mu$mol/J) |
|---|---|---|---|---|
| (*a*) Diet | | | | |
| Colony (6) | 291±11 | 13.5 ±0.66 | 7.43±0.51 | 1.84±0.10 |
| High-lard (4) | 521±46 | 11.4 ±1.48 (n.s.) | 6.21±0.61 (n.s.) | 1.85±0.15 (n.s.) |
| High-sucrose (4) | 365±6 | 11.9 ±0.69 (n.s.) | 6.98±0.37 (n.s.) | 1.71±0.07 (n.s.) |
| (*b*) Phenotype | | | | |
| Lean (5) | 292±33 | 11.3 ±0.18 | 6.12±0.47 | 1.88±0.13 |
| Obese (5) | 414±20 | 8.46±0.78 ($P<0.01$) | 5.50±0.80 (n.s.) | 1.58±0.08 (n.s.) |

Table 5. *Effect of age on $O_2$ uptake and heat output by suspensions of isolated hepatocytes*

Isolated hepatocytes were prepared from fed (colony) rats, at the ages shown, as described in the Experimental section. Cells were diluted in Krebs–Henseleit bicarbonate-buffered saline (Dawson *et al.*, 1969), containing 1.5% (w/v) gelatin, to give approx. $10^6$ cells/ml. The rates of $O_2$ uptake and heat output were determined. Values are means ± S.E.M. for three cell preparations from each age group. Weight at weaning (25 days) was 71±2g.

| Age (days) | Body wt. (g) | $O_2$ uptake ($\mu$mol/min per g dry wt.) | Heat output (J/min per g dry wt.) | $O_2$ uptake/ heat output ($\mu$mol/J) | $O_2$ uptake ($\mu$mol/min per $10^8$ cells) | Heat output (J/min per $10^8$ cells) | Wt. of $10^8$ cells (g dry wt.) |
|---|---|---|---|---|---|---|---|
| 38 | 112±14 | 11.6±0.8 | 6.39±0.37 | 1.82±0.06 | 2.21±0.18 | 1.22±0.12 | 0.19±0.01 |
| 48 | 162± 8 | 12.0±0.6 | 6.60±0.21 | 1.79±0.13 | 2.82±0.15 | 1.57±0.16 | 0.24±0.02 |
| 91 | 348±14 | 14.5±0.8 | 7.50±0.26 | 1.93±0.06 | 4.46±0.57 | 2.32±0.23 | 0.31±0.04 |
| 179 | 479±27 | 13.1±1.3 | 7.14±0.36 | 1.82±0.09 | 4.26±0.26 | 2.35±0.20 | 0.33±0.04 |

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