# METABOLIC PROPERTIES OF CELLS ISOLATED FROM ADULT MOUSE LIVER

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## ABSTRACT

Suspensions of isolated cells were prepared from mouse livers that had been perfused via the portal vein with a buffered medium containing sucrose. The demonstration of metabolic activities in these cells was found to be critically dependent on the composition of the suspending medium. The cells showed considerable metabolic activity in a simple medium containing 0.06 to 0.20 M sucrose, but did not respire in 0.30 M sucrose medium. Endogenous respiration was greatest when the sucrose concentration of the medium was 0.10 M or lower and was associated with the formation of acetoacetate. The cells oxidized citric acid cycle intermediates, glutamate, lactate, pyruvate, $\beta$-hydroxybutyrate, $\alpha$-glycerophosphate, and fatty acids and synthesized urea from ammonium chloride, but carbohydrate substrates did not stimulate oxygen uptake. Cells incubated in Krebs' phosphate-saline did not respire. The lack of respiration in this medium is thought to be related to increased permeability of the cell membrane with penetration of calcium ions and orthophosphate into the cells causing mitochondrial swelling and destruction. Further evidence for the loss of cellular permeability barriers is provided by the demonstration of leakage of certain soluble enzymes into the preparative media.

## INTRODUCTION

Recently methods have been devised for the preparation of isolated cells from solid organs such as liver and kidney (1–3). It has been observed that the metabolic properties of cells isolated from rat liver differ from those of liver slices (4–6). The cells lack endogenous respiration and do not respire in the presence of added substrates other than succinate. Leeson and Kalant (6) have suggested that these metabolic disturbances may be related to a loss of selective permeability of the cell membranes, with inability to retain differential intra- and extracellular concentrations of sodium and potassium ions and other substances. Evidence in support of this view is provided by the finding that several enzymes leak from the cells during their preparation (7, 8).

This report describes a biochemical study the object of which has been to obtain preparations of isolated liver cells with greater metabolic activity than those described hitherto. By incubating the cells in suitable media it has been possible to prepare suspensions which possess endogenous respiration and oxidize many substrates.

## MATERIALS AND METHODS

Adult mice of the NZY strain were generously supplied by Dr. M. Bielschowsky of the Hugh Adam Department of Cancer Research, University of Otago, New Zealand. The average weight of livers from these animals was about 1 gm.

### *Preparation of Suspensions*

Suspensions of isolated liver cells were prepared by the method of Branster and Morton (3). Livers of mice anesthetized with ether were perfused via the

portal vein for 10 to 20 seconds with about 15 ml of fluid at 37°C and gently dispersed in 7 to 8 ml of medium at 37°C. In experiments where two livers were required to provide sufficient cells, the first liver was perfused and kept at 0°C until the second liver had been perfused. Both livers were then dispersed in 14 ml of medium at 37°C. After dispersion all subsequent procedures were performed at 0–4°C. The dispersed cells were filtered through two layers of bolting nylon to remove connective tissue and clumps of cells. The cells were then freed from cell debris and erythrocytes by centrifuging for 2 minutes at 300 *g* in graduated conical centrifuge tubes. The supernatant, containing mainly isolated mitochondria and nuclei, was removed and the sedimented cells were suspended in fresh medium to give an unwashed cell suspension. Washed cells were obtained by suspending the cell pellet in twice its volume of fresh medium and again centrifuging at 300 *g*. The sedimented cells were then resuspended in the required volume of fresh medium. After preparation cell suspensions were kept at 0°C and used within 15 minutes.

The following incubation media were employed: Krebs' phosphate-saline (9); sucrose media containing various concentrations of sucrose between 0.10 M and 0.40 M; sucrose-KCl medium comprising 0.06 M sucrose, 0.08 M KCl. When cells were to be incubated in sucrose media, livers were perfused and the cells dispersed and washed with 0.30 M or 0.40 M sucrose medium. These concentrations of sucrose were chosen because in them both isolated liver mitochondria (10) and the mitochondria of dispersed liver cells (11) retain their native elongated shape. For incubations in sucrose-KCl medium, livers were perfused and the cells dispersed in 0.30 M sucrose medium. The centrifuged pellet was suspended in twice its volume of 0.12 M KCl and again centrifuged. The cell pellet was resuspended in sufficient 0.08 M KCl medium to bring the final sucrose concentration in the incubation medium to 0.06 M. All media other than Krebs' phosphate-saline were buffered at pH 7.3 with 0.02 M tris[1] HCl buffer.

The dry weight of isolated cells was measured as follows: A portion of the cell suspension was precipitated with an equal volume of 10 per cent (*w/v*) TCA. The precipitate was centrifuged, washed with distilled water, and weighed after drying at 100–110°C (TCA-insoluble dry weight). The yields were between 60 and 140 mg of dry matter per liver, representing a conversion of about 40 to 80 per cent of the liver to isolated cells.

[1] The following abbreviations have been used: tris for tris(hydroxymethyl)aminomethane, ADP for adenosine diphosphate, DPN for diphosphopyridine nucleotide, TCA for trichloracetic acid.

### *Measurement of Oxygen Uptake*

Oxygen uptake was measured in conventional Warburg apparatus. The flasks, containing the complete reaction medium in the main compartment and 0.2 ml of 10 per cent (*w/v*) KOH in the centre well, were incubated at 30°C with oxygen as the gas phase. Flasks were equilibrated for 8 minutes before the first reading.

The tissue metabolism is expressed by means of the following quotients:

$Q_{O_2}$ = $\mu$l $O_2$ uptake per mg TCA-insoluble dry weight of tissue per hour.

$Q_{acac.}$ = m$\mu$-moles acetoacetic acid formed per mg TCA-insoluble dry weight of tissue per hour.

### *Analytical Methods*

Acetoacetate was estimated by the method of Walker (12). Urea was measured manometrically with urease (13). Protein was determined by the method of Lowry *et al.* (14).

For studies on enzyme distributions, livers were dispersed in 0.20 M or 0.40 M sucrose or in sucrose-KCl medium. The volume of the dispersion was measured and a sample taken for enzyme assay. The dispersion was centrifuged at 300 *g* for 2 minutes and the supernatant (first supernatant) removed and kept at 0°C. The sedimented cells were suspended in about 5 volumes of fresh ice cold medium and again centrifuged. The supernatant (second supernatant) was removed and held at 0°C. The cells were resuspended in a small quantity of fresh medium. The volumes of the fractions were measured and samples taken for enzyme assay. A portion of the cell suspension was homogenized in a ground glass homogenizer to ensure adequate access of substrates and coenzymes to intracellular enzymes, but the activities of these samples were identical with those of unbroken cells.

Aldolase was assayed by the method of Sibley and Lehninger (15). Lactic dehydrogenase was assayed spectrophotometrically by following the reduction of DPN at 340 m$\mu$. Glucose-6-phosphatase was assayed by the method of Segal and Washko (16). Spectrophotometric observations were made with a Beckman DU spectrophotometer.

## RESULTS

### *Respiratory Activity*

Cells prepared and incubated in 0.40 M sucrose medium had no endogenous respiration. Cells prepared in 0.40 M sucrose medium and resuspended in phosphate-saline also did not respire. In a medium in which the sucrose concentration was reduced to 0.20 M some endogenous oxygen uptake was observed in unwashed cells. For 21

TABLE I

*Respiration of Isolated Mouse Liver Cells in 0.20 M Sucrose Medium in the Presence of Substrates*

Warburg vessels contained cells (equivalent to 13 to 30 mg TCA-insoluble dry weight) suspended in 0.20 M sucrose solution containing 0.02 M tris HCl buffer, pH 7.3. The potassium ion concentration was brought to 20 mM with KCl. Total volume, 1 ml. Gas phase, $O_2$. Temperature, 30°C. Incubation time, 1 hour.

| Substrate | Concentration | $Q_{O_2}$ |
|---|---|---|
| | *mM* | |
| None | — | 2.2 |
| K-pyruvate | 10 | 7.3 |
| K-DL-lactate | 20 | 10.1 |
| None | — | 3.4 |
| Fructose | 10 | 3.4 |
| None | — | 1.7 |
| Glucose | 10 | 1.9 |
| Glycerol | 10 | 1.8 |
| None | — | 2.0 |
| K-acetate | 10 | 4.9 |
| K-citrate | 10 | 7.1 |
| K-succinate | 10 | 10.5 |
| None | — | 3.5 |
| K-fumarate | 10 | 9.3 |
| K-L-glutamate | 10 | 10.6 |
| None | — | 1.8 |
| Na-*n*-butyrate | 2 | 2.4 |
| K-DL-*iso*-citrate | 20 | 6.3 |
| K-α-ketoglutarate | 10 | 7.6 |
| Na-DL-β-hydroxybutyrate | 20 | 4.3 |

observations the mean $Q_{O_2}$ measured over 60 minutes was 2.7 ± 0.8 (S.D.). Endogenous oxygen uptake was usually linear for 20 to 30 minutes but then declined sharply. Oxygen uptake was stimulated by citric acid cycle intermediates, lactate, pyruvate, β-hydroxybutyrate, glutamate, acetate, and butyrate, but not by carbohydrate substrates (Table I). In the presence of oxidizable substrates a linear rate of oxygen uptake was maintained for 2 hours.

The endogenous oxygen uptake of cells washed once and incubated in 0.20 M sucrose medium was less than half that of unwashed cells. For 10 observations the mean $Q_{O_2}$ measured over 60 minutes was 1.2 ± 0.2 (S.D.). One washing caused only slight diminution of the respiratory rate in the presence of substrates (Table II).

The influence of sucrose concentration on the endogenous respiration of washed cells is shown in Table III. In 0.30 M sucrose medium there was no endogenous respiration and in 0.20 M sucrose

TABLE II

*Respiration of Washed Isolated Mouse Liver Cells in 0.20 M Sucrose Medium in the Presence of Substrates*

Warburg vessels contained washed cells (equivalent to 16 to 34 mg TCA-insoluble dry weight) suspended in 0.20 M sucrose solution containing 0.02 M tris HCl buffer, pH 7.3. The potassium ion concentration was brought to 20 mM with KCl. Substrate, K salt. Total volume, 1 ml. Gas phase, $O_2$. Temperature, 30°C. Incubation time, 1 hour.

| Substrate | Concentration | $Q_{O_2}$ |
|---|---|---|
| | *mM* | |
| None | — | 1.3 |
| L-Glutamate | 10 | 7.4 |
| DL-Lactate | 20 | 7.5 |
| None | — | 1.1 |
| DL-Lactate | 20 | 5.5 |
| Pyruvate | 10 | 2.8 |
| None | — | 1.7 |
| Fumarate | 10 | 7.9 |
| L-Malate | 10 | 7.6 |

TABLE III

*Effects of Solute Concentration on Endogenous Respiration and Acetoacetate Formation in Isolated Mouse Liver Cells*

Cells were prepared in 0.30 M sucrose solution containing 0.02 M tris HCl buffer, pH 7.3. After centrifuging, the pellet was washed in twice its volume of 0.30 M sucrose medium. The cells were again centrifuged and taken up in 0.30 M sucrose medium. Samples (0.5 ml equivalent to 16 mg TCA-insoluble dry weight) were transferred to Warburg vessels which contained sufficient dissolved solute to bring the concentrations of sucrose and KCl to those indicated below. In all vessels the final concentration of tris HCl buffer was 0.02 M. Total volume, 1.5 ml. Gas phase, $O_2$. Temperature, 30°C. Incubation time, 1 hour.

| Sucrose | KCl | $Q_{O_2}$ | $Q_{acac.}$ |
|---|---|---|---|
| *M* | *M* | | |
| 0.10 | 0 | 5.6 | 66.2 |
| 0.10 | 0.05 | 6.2 | 57.6 |
| 0.20 | 0 | 0.7 | 9.9 |
| 0.30 | 0 | 0.1 | 3.6 |

medium oxygen uptake was very low. However, cells incubated in 0.10 M sucrose medium showed considerable endogenous respiration. The osmolarity of the latter medium could be raised to that of 0.20 M sucrose medium by addition of potassium chloride without inhibition of oxygen uptake. Table III also indicates that endogenous respiration was associated with the formation of acetoacetate. When oxygen uptake was depressed by incubation of the cells in 0.20 M or 0.30 M sucrose medium, acetoacetate formation was inhibited.

Fig. 1 shows that in the presence of 10 mM orthophosphate endogenous respiration was slightly depressed, and respiration in the presence of lactate declined rapidly after about 40 minutes. The presence of calcium ions in the incubation medium abolished respiration in the presence or absence of lactate. A concentration of 1 mM was sufficient to do this.

The results of further measurements of endogenous oxygen uptake in sucrose-KCl medium are given in Table IV. The inclusion in the medium of 1 mM magnesium chloride usually caused a small stimulation of oxygen uptake and acetoacetate formation.

The influence of various substrates on the rate of respiration of cells in sucrose-KCl medium is shown in Table V. In these experiments the medium contained 1 mM magnesium chloride and 1 mM ADP. ADP was included since the rate of oxygen uptake was found to be linear for longer periods in its presence. Table V shows that the greatest rate of oxygen uptake was obtained with citric acid cycle intermediates and glutamate, the $Q_{O_2}$ ranging from about 9 to 11. β-Hydroxybutyrate, butyrate and octanoate were oxidized with the formation of acetoacetate. Citric acid cycle intermediates and glutamate consistently suppressed the accumulation of endogenous acetoacetate. Glucose or fructose did not stimulate oxygen uptake and did not affect the rate of acetoacetate production.

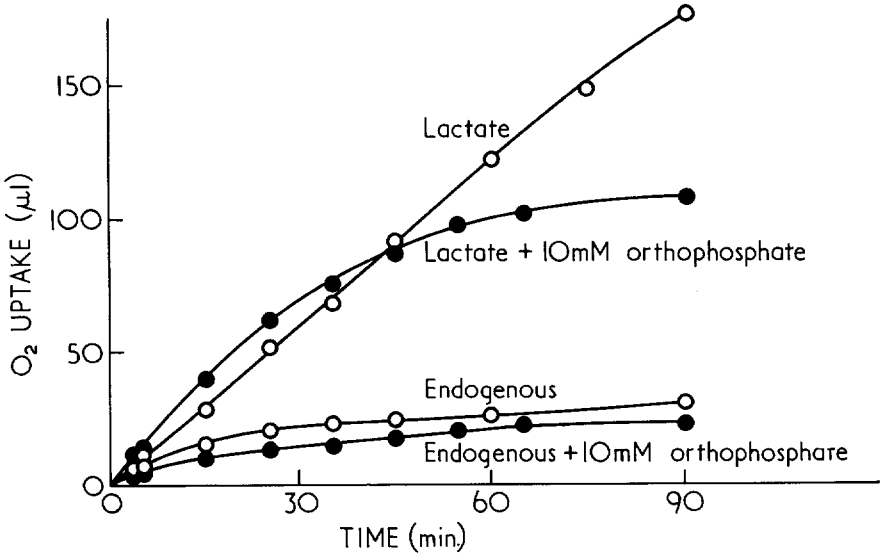


FIGURE 1

Oxygen uptake of unwashed liver cells in the presence of 10 mM orthophosphate. The medium was 0.20 M sucrose, containing 0.02 M tris HCl buffer, pH 7.3. DL-Lactate concentration, 20 mM. The TCA-insoluble dry weight of cells was 17 mg.

### *Urea Synthesis*

The results in Table VI demonstrate the capacity of unwashed cell suspensions to synthesize urea from added ammonium chloride in the presence of a bicarbonate buffer and lactate. The quantity formed was increased by addition of ornithine or ornithine plus aspartate. Urea forma-

tion from ammonium chloride could not be demonstrated with washed cells.

### *Leakage of Enzymes*

In view of the findings of previous authors (7, 8) it seemed possible that the lack of stimulation of respiration by carbohydrate substrates might be due to the loss of glycolytic enzymes from the mouse liver cells during their isolation.

TABLE IV

*Endogenous Respiration and Acetoacetate Formation of Isolated Mouse Liver Cells in Sucrose-KCl Medium*

Warburg vessels contained cells (equivalent to 23 to 35 mg TCA-insoluble dry weight) suspended in sucrose-KCl medium comprising 0.06 M sucrose, 0.08 M KCl, 0.02 M tris HCl buffer, pH 7.3. In the experiments in group B the medium contained in addition 1 mM $MgCl_2$. Total volume, 1.5 ml. Gas phase, $O_2$. Temperature, 30°C.

| Incubation time | $Q_{O_2}$ | $Q_{acac.}$ |
|---|---|---|
| *min.* | | |
| Group A | | |
| 45 | 3.9 | 33.5 |
| 45 | 4.5 | 36.2 |
| 45 | 3.3 | 38.6 |
| 45 | 4.6 | 45.2 |
| 60 | 4.7 | 31.3 |
| 60 | 7.7 | 43.4 |
| Group B | | |
| 30 | 6.7 | 58.2 |
| 30 | 8.7 | 47.8 |
| 40 | 4.1 | 55.0 |
| 40 | 6.1 | 60.8 |
| 45 | 7.9 | 34.9 |
| 45 | 7.2 | 60.8 |
| 45 | 8.2 | 67.6 |
| 60 | 5.6 | 97.4 |

To test this possibility the leakage of aldolase and lactic dehydrogenase from these cells was examined. The possibility of leakage of a particulate enzyme, glucose-6-phosphatase, was also investigated. The results are shown in Tables VII and VIII. Over 90 per cent of the aldolase activity was consistently lost from the cells during their preparation. The pattern of distribution of lactic dehydrogenase activity was similar to that of aldolase, but glucose-6-phosphatase activity was greatest in the cell pellet, indicating that the latter enzyme was retained in its known situation in the endoplasmic reticulum of the cells.

TABLE V

*Respiration and Acetoacetate Formation of Isolated Mouse Liver Cells in Sucrose-KCl Medium in the Presence of Substrates*

Warburg vessels contained cells (equivalent to 27 to 40 mg TCA-insoluble dry weight) suspended in sucrose-KCl medium comprising 0.06 M sucrose, 0.08 M KCl, 1 mM $MgCl_2$, 1 mM ADP, 0.02 M tris HCl buffer, pH 7.3. Total volume, 1.5 ml. Gas phase, $O_2$. Temperature, 30°C. Incubation time, 45 minutes.

| Substrate | Concentration | $Q_{O_2}$ | $Q_{acac.}$ |
|---|---|---|---|
| | *mM* | | |
| None | — | 8.1 | 23.7 |
| K-citrate | 6.7 | 11.1 | 3.7 |
| K-DL-*iso*-citrate | 13.3 | 10.0 | 6.7 |
| K-succinate | 6.7 | 10.2 | 0 |
| K-fumarate | 6.7 | 10.5 | 0 |
| None | — | 6.9 | 33.2 |
| Na-$\alpha$-glycerophosphate | 6.7 | 7.9 | 21.9 |
| K-L-glutamate | 6.7 | 10.0 | 8.9 |
| K-$\alpha$-ketoglutarate | 6.7 | 8.9 | 1.8 |
| K-L-malate | 6.7 | 9.7 | 0.4 |
| None | — | 5.9 | 16.1 |
| K-acetate | 6.7 | 7.6 | 22.4 |
| Na-*n*-butyrate | 3.3 | 6.6 | 82.2 |
| K-*n*-octanoate | 1.0 | 7.5 | 56.8 |
| Na-DL-$\beta$-hydroxybutyrate | 13.3 | 8.0 | 269 |

TABLE VI

*Urea Synthesis by Unwashed Isolated Mouse Liver Cells in a Sucrose Medium*

Warburg vessels contained cells (equivalent to 51 mg TCA-insoluble dry weight) in a suspending medium comprising 0.20 M sucrose, 0.01 M tris HCl buffer, pH 7.3, 0.025 M $KHCO_3$, 20 mM potassium DL-lactate. Total volume, 2 ml. Gas phase, 95 per cent $O_2$/5 per cent $CO_2$. Temperature, 30°C. Incubation time, 2 hours.

| Additions | Urea formed |
|---|---|
| | *μmoles* |
| None | 1.0 |
| 5 mM ammonium chloride | 2.9 |
| 5 mM ammonium chloride + 10 mM L-ornithine | 4.4 |
| 5 mM ammonium chloride + 10 mM DL-aspartate | 3.5 |
| 5 mM ammonium chloride + 10 mM L-ornithine + 10 mM DL-aspartate | 7.8 |

TABLE VII

*Distribution of Aldolase Activity in Mouse Liver Fractions*

Livers were perfused with 0.40 M sucrose medium and dispersed in the following media: experiment 1, 0.40 M sucrose; experiment 2, 0.125 M KCl; experiment 3, 0.20 M sucrose; all media contained 0.02 M tris HCl buffer, pH 7.3. For other experimental details see "Methods." Aldolase activity is defined arbitrarily on the basis of extinction at 550 mμ, one unit of enzyme being the amount of enzyme required to bring about the formation of sufficient triose chromogen in 15 minutes under the conditions of Sibley and Lehninger (15) to give an optical density change of 1.0 measured with the Beckman DU spectrophotometer in cuvettes with a light path of 1 cm. In the column headed "Percentage of dispersion" the activity in each fraction is given as a percentage of the total activity in the dispersion.

| Experiment no. | Tissue fraction | Protein | Activity (enzyme units) | Percentage of dispersion |
|---|---|---|---|---|
| | | *mg* | | |
| 1 | Dispersion | 157 | 63.8 | (100) |
| | 1st supernatant | 83 | 46.4 | 73 |
| | 2nd supernatant | 6.3 | 7.5 | 12 |
| | Cells | 61 | 4.5 | 7 |
| 2 | Dispersion | 190 | 137 | (100) |
| | 1st supernatant | 98 | 103 | 75 |
| | 2nd supernatant | 15 | 24 | 17 |
| | Cells | 69 | 4.1 | 3 |
| 3 | Dispersion | 150 | 131 | (100) |
| | Cells | 43 | 7.2 | 5 |

## DISCUSSION

The present work shows that the demonstration of respiratory activity in isolated mouse liver cells is critically dependent on the composition of the suspending medium. The influence of sucrose concentration on mitochondrial structure and the possible mode of action of high concentrations of sucrose in inhibiting respiration are discussed in the accompanying paper (11). Similar inhibitory effects of high concentrations of sucrose on respiratory activity have previously been observed with preparations of isolated mitochondria (17, 18). It seems likely that the inhibition of respiration by 1 mM calcium ions and the decline of respiration in the presence of added orthophosphate are due to mitochondrial damage following the penetration of these ions into the cells. Calcium ions and orthophosphate are known to cause swelling of isolated mitochondria (19), and observations by phase contrast microscopy (20) and by electron microscopy (21) have shown gross swelling and destruction of the mitochondria of dispersed liver cells in the presence of these agents. Changes in the fine structure of the cell membrane (11) and the findings on enzyme leakage indicate that isolated liver cells probably lose permeability barriers during their preparation. Loss of these barriers would facilitate the penetration of calcium ions and orthophosphate into the cells. This would explain the lack of metabolic activity in cells incubated in Krebs' phosphate-saline and would account for the negative findings of previous authors (4, 22).

TABLE VIII

*Distribution of Lactic Dehydrogenase and Glucose-6-phosphatase Activities in Mouse Liver Fractions*

The fractions were prepared in 0.40 M sucrose medium. For other experimental details see "Methods." Enzyme activities are defined as follows: For lactic dehydrogenase one unit of enzyme is the amount required to bring about the reduction of 0.1 μmole of DPN per minute at pH 9.6 and 20°C with a concentration of 120 μmoles of potassium DL-lactate and 1.2 μmoles of DPN in 2.4 ml. For glucose-6-phosphatase one unit of enzyme is the amount required to liberate 1 μmole of orthophosphate in 10 minutes at pH 6.4 and 30°C with a substrate concentration of 20 mmoles in a total volume of 2 ml. The specific activity (sp. act.) of the fractions is defined as the number of enzyme units per mg of protein.

| Tissue fraction* | Protein | Lactic dehydrogenase | | | Glucose-6-phosphatase | | |
|---|---|---|---|---|---|---|---|
| | | Activity | Sp. act. | % of D | Activity | Sp. act. | % of D |
| | *mg* | *units* | *units/mg* | | *units* | *units/mg* | |
| D | 147 | 213 | 1.45 | (100) | 35.4 | 0.24 | (100) |
| S | 70 | 152 | 2.17 | 72 | 12.7 | 0.18 | 36 |
| W | 18 | 35 | 1.94 | 17 | 6.9 | 0.38 | 19 |
| C | 41 | 10 | 0.24 | 5 | 16.7 | 0.41 | 47 |

* D, dispersion; S, first supernatant; W, second supernatant; C, cells.

The rat liver fractionation studies of LePage and Schneider (23) have shown that the enzymes of the Embden-Myerhof pathway are located in the soluble fraction. Glucose-6-phosphatase activity, however, appears to be predominantly associated with the microsomal fraction (24). Tables VII and VIII show that the specific activity of aldolase and lactic dehydrogenase is greatest in the first supernatant but the specific activity of glucose-6-phosphatase is greatest in the cell pellet. These findings provide evidence that some enzymes found mainly in the soluble phase of the cytoplasm leak from the cells during their preparation, whereas glucose-6-phosphatase is retained in the endoplasmic reticulum.

Zimmerman *et al.* (8) considered that the loss of enzymes from isolated liver cells might explain the lack of endogenous respiration found by them and by previous workers. The considerable respiratory activity of isolated mouse liver cells found in the present study indicates that they retain a complement of enzymes sufficient to permit oxidative pathways to function. However, the inability of isolated liver cells to metabolize glucose, glycerol, or fructose can be explained on the basis of loss of glycolytic enzymes.

In all cases examined, the cells have been found to carry out oxidations which isolated mitochondria are known to perform. These are the oxidation of citric acid cycle intermediates, pyruvate, fatty acids, glutamate, $\beta$-hydroxybutyrate, and $\alpha$-glycerophosphate. These activities can be demonstrated in media which have not been supplemented with magnesium ions, orthophosphate, ADP, or adenosine triphosphate. In fact considerable endogenous respiration and acetoacetate formation occurs in a medium comprising 0.10 M sucrose and 0.02 M tris HCl buffer, pH 7.3. The rate of acetoacetate formation from endogenous substrates is similar to that from slices from the livers of fasted rats (*cf.* 25). This rate seems abnormally high since all experiments in the present study were conducted with cells derived from fed mice. It seems possible that factors responsible for the suppression of acetoacetate formation in the fed animal may be washed from the cells during their preparation.

Washing brings about some diminution in respiratory activity and completely abolishes the ability of the cells to synthesize urea from ammonium chloride. It seems probable that these changes in activity are due to loss of soluble substrates, cofactors, and enzymes during the washing procedure.

The preparation of isolated liver cell suspensions is relatively easy and rapid. Measurements of oxygen uptake can be commenced within 30 minutes of anesthetizing the experimental animal. The survival times of metabolic activities are similar to those found in liver slices.

From the point of view of structural damage the cells may be said to stand between the slice and the homogenate. Their mitochondria remain in morphological relation to the endoplasmic reticulum (11) and may carry out activities in concert with the endoplasmic reticulum. The loss of soluble enzymes is an obvious disadvantage which however might be turned into an experimental advantage. Since many enzymes are now available in purified form, the experimenter is at liberty to replace missing enzymes and cofactors under controlled conditions. Thus, in spite of the loss of soluble enzymes, liver cell suspensions may prove suitable for the study of reactions mediated in part by enzymes of the soluble phase.

Although the abnormal permeability of the isolated cells makes them more sensitive than slices to the composition of the incubation medium, and therefore more liable to damage, the lack of permeability barriers may in some respects prove advantageous. There are many substances of metabolic interest whose study *in vitro* is limited by their apparent failure to penetrate liver slices (see, for example, the discussion by Ratner (26) on urea synthesis in relation to cell permeability). Isolated liver cells may provide a more suitable *in vitro* system for studying the action of these substances.

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