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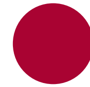

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PREPARATION AND BIOCHEMICAL CHARACTERIZATION OF PARENCHYMAL CELLS FROM RAT LIVER

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SUMMARY

1. A simple procedure for the isolation of rat liver parenchymal cells, modified from the collagenase–hyaluronidase method of Berry and Friend, is described in detail.

2. It involves a flow-through *in situ* perfusion of the rat liver at physiological flow rate and pressure, using enzymes and EDTA (2 mM) in saline medium, with or without the addition of 20 % washed bovine erythrocytes.

3. The cell preparation was studied with respect to the following biochemical characteristics: Hexokinase and glucokinase activity; respiration rate in saline medium with and without substrate; permeability to malate, glutamate, ^3H_2O and [^{14}C]inulin; glucose- and glycogen-synthesizing capacities; adenine nucleotide and K^+ contents.

4. The effects on these parameters of the omission of erythrocytes from the perfusion media, and thereby a low oxygenation of the liver during the perfusion, was examined.

5. Cells prepared with or without erythrocytes showed no differences in respiration rate, rate of gluconeogenesis or permeability to malate and glutamate. The cells were able to maintain a gradient of malate and glutamate comparable to that in the isolated perfused rat liver. The ^3H_2O/[^{14}C]inulin ratio indicated some cell destruction or penetration of inulin into the cells, independent of the isolation method used.

6. Cells prepared with erythrocytes, however, showed a four to seven times higher rate of glycogen synthesis and double the adenine nucleotide content as compared with those prepared without erythrocytes in the perfusion media.

7. Compared to the method of Berry and Friend, the method described here is simpler in perfusion procedure and uses physiological flow rate and pressure. The isolated cells recover from a loss of K^+ to return to near normal values (90 μmoles/ml packed cells) as opposed to the cells of the former method, which show a 60 % irreversible loss of the ion.

8. The results are discussed in view of earlier studies on the isolation of hepatocytes, and a comparison is drawn with the characteristics of the isolated perfused rat liver and rat liver slices.

INTRODUCTION

Studies on isolated rat liver parenchymal cells are increasingly performed, and the literature on preparation methods for their isolation offers several different procedures[1-9]. The collagenase–hyaluronidase method developed by Howard and Persch[7], and further refined and studied by Berry and Friend[8], requires a minimum of mechanical treatment and has hitherto proved to give the most convincing results.

The method used in this study for the preparation of rat liver parenchymal cells was developed from that described by Berry and Friend[8] and from the simpler one described by Capuzzi *et al.*[9]. A simple reproducible flow-through system was devised and two procedures, which were similar in all respects except for the addition or non addition of washed bovine erythrocytes to the perfusion media, were compared. The following parameters were examined: the glucokinase/hexokinase ratio, the respiration rate in modified Hanks medium with and without substrates, the concentration of adenine nucleotides, the K^+ content, the permeability to malate, glutamate, ^3H_2O and [^{14}C]inulin and the synthetic capacity for glucose and glycogen.

Cells prepared with erythrocytes in the perfusion media appeared to be superior to those prepared by Berry and Friend[8] with respect to the intracellular K^+ concentration, and superior to those obtained by the procedure of Capuzzi *et al.*[9] with respect to the rate of glycogen synthesis. The sum of the ATP, ADP and AMP contents was about twice as high in cells prepared in the presence of erythrocytes than in cells prepared without erythrocytes in the perfusion media.

MATERIALS AND METHODS

Enzymes, including collagenase (spec. act. 66 U/mg) and hyaluronidase (spec. act. 1000 U/mg), and coenzymes were purchased from Boehringer, Mannheim, Germany. Serum albumin was purchased from Armour Chemical Co., England, and treated with charcoal to remove fatty acids[10]. All chemicals were of analytical grade. Radiochemical material was from New England Nuclear Chemicals, GmbH, Germany.

^3H_2O and [^{14}C]inulin were counted in a Packard 2002 Tri-Carb Liquid Scintillation Spectrometer. O_2 consumption was measured by a Gilson respirometer. Incubations were carried out with atmospheric air as the gas phase, in an incubation volume of 1.5–2.5 ml (see below) and at a temperature of 37 °C. The cell concentration was 0.05–0.15 ml of tightly packed cells/ml (hepatocrit 5–15 %) corresponding to a protein concentration of 9–27 mg/ml if not otherwise stated.

Metabolites were measured enzymatically in supernatants and/or cell pellets after precipitation with half a volume of cold 2.2 M $HClO_4$ and neutralization with KOH–triethanolamine to pH 7.4. Substrate determinations were performed by the methods indicated: Glutamate[11], malate[12], lactate[13], pyruvate[14], glucose[15], glycogen[15,16], ATP[17] and ADP and AMP[18]. For the determination of ATP, ADP and AMP the total incubation mixture was precipitated without centrifugation (see Results). K^+ was determined flame-photometrically, and protein according to Groves *et al.*[19].

The hepatocrit was measured in heparinized capillary tubes in the same way as the usual haematocrit.

Solutions

Three perfusion media were used for the isolation of cells: (a) Locke's solution without glucose[20], (b) Hanks' solution[21] without Ca^{2+} and glucose *plus* 0.08 % collagenase and 0.05 % hyaluronidase and (c) Locke's solution plus 2 mM EDTA without glucose. All perfusion media contained 25 mM sodium hydrogen carbonate and were equilibrated at 37 °C with 95 % O_2 plus 5 % CO_2, to obtain a pH of 7.4. The day before the experiment, bovine erythrocytes were washed three times in the Hanks' and Locke–EDTA solutions, respectively, stored overnight at 4 °C and added to the media to a haematocrit of 20 %.

Cells were washed and incubated in Hanks' solution (without glucose) containing 1.2 mM $CaCl_2$ and 1 % serum albumin. 10 mM sodium phosphate was added and the pH adjusted to pH 7.4.

Perfusion system

The three media, kept in a thermostat at 37 °C, were successively perfused through the liver in a single pass by a peristaltic pump (LKB, Bromma, Sweden) at a constant rate of 5 ml/min through a stainless steel coil for further thermoequilibration and into an air-catching inverted "Y-piece", connected to a 20-cm vertical, open PVC tube, making registration of the perfusion pressure of the medium in cm possible. From here the media passed through a short piece of tube to the cannula. The portal vein was cannulated with a double cannula (*i.e.* a combination of a sharp inner hypodermic (Steristar rec. No. 1.05×38) and a blunt outer stainless steel cannula (Nyrop rec. No. 16). This cannulation method offers rapidity and a minimum of trauma since dissection of the ligamentum hepatoduodenale can be avoided.

Preparation procedure

Fed or fasted female Wistar rats weighing 250 g are anaesthetized with 0.5 ml of 5 % pentobarbital intraperitoneally, tracheostomized and cannulated to a rodent respirator (rate about 200 ml/min) to ensure a well oxygenated liver at the start of the perfusion. Artificial respiration does not seem to be essential for the procedure, but the respiration movements apparently facilitate the perfusion since a lowering of perfusion pressure can be observed. The abdomen is opened and 100 μl heparin injected in the inferior vena cava. Half a minute later a ligature is laid loosely around the portal vein, proximal to the splenic vein. Inclusion of the main bile duct in the ligature should be avoided. The portal vein is then cannulated with the double cannula and, after removal of the inner cannula, the liver is perfused with Locke's solution without glucose for 0.5–1 min to ensure blanching of the liver. If complete blanching is not obtained, the rat is discarded.

After blanching, the inferior vena cava is cut open and the enzyme medium is passed through the liver for 20 min, followed by Locke's solution plus EDTA for 5 min. Perfusion with EDTA increases cell yield[8,22]. In experiments in which the O_2 tension of the effluent medium is measured, the inferior vena cava is cannulated and the medium passed through a Clark oxygen electrode. When the EDTA-containing medium reaches the liver the perfusion pressure rises from 8 cm to about 15 cm of water, the O_2 consumption of the liver decreases and the medium oozes from the surface of the liver. In perfusions with erythrocytes added to the perfusion fluids, subcapsular haematoma develops, indicating a collapse of the internal structure of the liver.

The liver is then rapidly excised and transferred to a Potter–Elvehjem homogenizer, where it is gently dispersed by hand (one stroke with a loose pestle (1 mm tolerance)). The cell suspension is quantitatively washed into a 1-l Erlenmeyer flask with 50 ml of enzyme medium without erythrocytes, and incubated under a carbogen (95 % O_2 and 5 % CO_2) atmosphere in the shaking bath (90 cycles/min) for 20 min at 37 °C. The cell suspension is filtered through one layer of 100 mesh nylon (pore size 160 μm) and centrifuged for 1 min at $80 \times g$. The cells are resuspended in 25 ml of washing medium (pregassed with atmospheric air) added a little at a time, and gently shaken. Sudden resuspension makes the cells aggregate immediately. The suspension is centrifuged for 1 min at $50 \times g$ and the washing procedure repeated once with centrifugation at $80 \times g$. No effect on cell clumping of the addition or non-addition of Ca^{2+} to the washing medium has been observed (*cf.* refs 3, 8 and 22). A special centrifuge with rapid acceleration and deceleration has been constructed for the washing procedure to minimize the periods of anaerobiosis. All centrifugations are done at room temperature.

The final pellet is resuspended in incubation medium to obtain the desired "hepatocrit". Until use, the cell suspension is gently bubbled with atmospheric air at 37 °C.

The importance of rapid handling of the cell pellets after each centrifugation is underlined by a 6-fold increase in the lactate/pyruvate ratio (from 29 to 181) when the cells are left packed for a period of 10 min at 25 °C.

The yield of cells is 1.5–2.5 ml of tightly packed cells from the liver of a 250 g rat.

RESULTS

O_2 consumption during cell preparation

The in- and outflow O_2 tension in the perfusion media was measured, and the O_2 consumption during perfusion with erythrocyte–enzyme medium was calculated[26] to be 2.15 μmoles O_2/g wet wt per min. The O_2 consumption declined to 0.79 μmoles O_2/g wet wt per min when the erythrocyte–EDTA medium was passed through the liver. Without erythrocytes in the perfusion medium the corresponding values for the O_2 consumption were 0.12 and 0.10 μmoles O_2/g wet wt per min, respectively.

Light microscopy

Light microscopy revealed a final suspension of isolated, rounded cells. The degree of staining with Trypan Blue (0.2 % for 5 min at 20 °C) ranged from 2–10 %. By virtue of the washing procedure, the quantity of erythrocytes, cell debris and visibly injured cells became negligible.

Activities of Hexokinase and Glucokinase

The activities of hexokinase and glucokinase were measured on cells, isolated with erythrocytes in the perfusion medium (Table I). The preparation procedure gave no loss of glucokinase activity compared with whole liver, whereas about 30 % of the hexokinase activity was lost. Results indicate an unequal distribution of the two enzyme activities in rat liver, as the ratio hexokinase/glucokinase declines from 0.31 in the whole liver to 0.028 in the isolated hepatocytes. This agrees with the data of

TABLE I

ACTIVITIES OF HEXOKINASE AND GLUCOKINASE

1. Supernatant refers to the supernatant from the first centrifugation after filtration. Filtrate residue refers to the tissue left on the nylon filter after filtration (see Materials and Methods). The hepatocytes were prepared with erythrocytes in the perfusion medium. Rats were fasted for 24 h. Results are expressed as nmoles of glucose phosphorylated per min per mg of protein (specific activity) or per g liver wet wt (total activity). Mean values ±S.E. are given with the numbers of experiments in parentheses. Hexokinase and glucokinase activities were estimated according to ref. 23.

	Hexokinase activity		*Glucokinase activity*	
	Total	*Specific*	*Total*	*Specific*
Whole liver	159	1.12 ±0.11 (4)	517	3.62 ±0.23 (4)
Isolated parenchymal cells	7	0.158±0.017 (5)	255	5.67 ±1.44 (5)
1. Supernatant	23	0.778±0.120 (5)	5	0.178±0.053 (5)
Filtrate residue	80	1.35 ±0.098 (5)	234	3.96 ±0.95 (5)

TABLE II

O_2 CONSUMPTION BY ISOLATED RAT LIVER PARENCHYMAL CELLS

Endogenous respiration was measured during the first 30 min of incubation. Respiration with substrates added was measured for the first 60 min of incubation. The substrate concentration was 10 mM if not otherwise indicated. Figures are given as the mean values ±S.E. or as the range with the number of cell preparations in parentheses. Rats were fasted for 24 h.

Substrate added	*Nutritive state*	*Cells prepared without erythrocytes in perfusion media*	*Cells prepared with erythrocytes*
None	Fed	8.92± 0.49 (12)	7.35± 0.87 (9)
	Fasted	8.54± 0.55 (7)	8.41 (1)
Glucose	Fed	7.14–11.6 (2)	—
	Fasted	9.25–11.6 (2)	—
Fructose	Fed	—	15.2 (1)
	Fasted	10.6 (3) 9.62–12.3	—
G+P+G+S*	Fasted	8.72 (3) 7.37–12.3	12.3 11.7 –13.1
Lactate	Fed	10.2 –10.5 (2)	11.0 –15.6 (2)
	Fasted	9.90 (3) 6.25–12.7	10.45 (3) 8.80–12.7
L-α-Alanine	Fasted	6.22– 6.74 (2)	11.6 (1)
Glutamate	Fed	9.41 (3) 7.54–11.1	5.1 (1)
Oxaloacetate+ L-α-Alanine	Fed	8.31–12.5 (2)	5.1 (1)

* 50 mM glucose+5 mM pyruvate+5 mM glycerol+5 mM serine.

TABLE III

PERMEABILITY BARRIER FOR GLUTAMATE OF ISOLATED RAT LIVER PARENCHYMAL CELLS

The cells were prepared from fed rats with or without erythrocytes added to the perfusion media and were incubated at 37 °C in a concentration of about 0.1 ml of packed cells/ml for the times indicated. The incubation mixture was centrifuged for 30 s at 10 000 × *g* and a sample of the supernatant, and the pellet was deproteinized with $HClO_4$ for the determination of glutamate. The intracellular concentration of glutamate is uncorrected for extracellular glutamate in the cell pellet. ∞ means that concentrations of glutamate in the medium was below 4 μM. In case of three experiments the mean value and the range are given. The numbers of cell preparations are given in parentheses.

Addition to medium	*Incubation time (min)*	*Cells prepared without erythrocytes*		*Cells prepared with erythrocytes*	
		Glutamate in cells (mM)	*Glutamate in cells/ Glutamate in medium*	*Glutamate in cells (mM)*	*Glutamate in cells/ Glutamate in medium*
None	0	—	—	1.54 (1)	∞
None	60	2.29 (3) 1.68 – 3.32	13.1 7.1 – 21.3	2.03 (1)	8.5
None	90	2.12 – 2.63 (2)	9.0 – 7.9	2.10 (1)	6.4
Oxaloacetate, 7 mM + alanine, 7 mM	60	4.64 (3) 3.44 – 5.71	19.0 6.7 – 25.4	3.60 (1)	12.3
Oxaloacetate, 7 mM + alanine, 7 mM	90	5.23 – 6.40 (2)	14.1 – 17.8	2.54 (1)	5.3
Glutamate, 10 mM	60	4.08 (1)	0.44	4.81 (1)	0.59
Glutamate, 10 mM	90	—	—	4.81 (1)	0.60

Sapag-Hagar *et al.*[24] but not with the recent work of Werner *et al.*[25], who found no difference between the ratios in whole liver and in parenchymal cells. This may be due to the use of different blanks in the calculation of the hexokinase activity, as Werner *et al.*[25] find hexokinase activity about ten times higher than ours.

O_2 consumption of isolated parenchymal cells

In all experiments the endogenous O_2 uptake was measured. Endogenous respiration rates of cells prepared by different methods and from fed and fasted animals are shown in Table II. Neither the nutritive state of the animal nor the method of preparation alter the rate of respiration significantly. The endogenous respiration was constant with time for the first 60 min of incubation. Cells kept at 4 °C for 24 h retained an endogenous rate of respiration of about 70 %. The rate of respiration was proportional to the amount of cells up to at least 250 µl of tightly packed cells (about 40 mg of protein) per flask.

The respiration rates with various substrates during the first 60 min of incubation are shown in Table II. Most substrates stimulate respiration, although the large variation from one cell preparation to another tends to obscure the differences (see Discussion). Lactate, fructose and glucose + pyruvate + glycerol + serine stimulate the respiration rate by 10–30 % in fasted animals, when cells from the same preparation are compared.

The O_2 consumption rates of the cells in relation to Ca^{2+} and albumin concentration in the incubation medium are shown in Fig. 1. For the first 60 min of incubation a rather broad maximum of O_2 uptake was found at 1.2 mM Ca^{2+} and 1 % albumin. The values at 4 % albumin (Fig. 1) and at 0 mM Ca^{2+} + EDTA, 2.5 mM Ca^{2+} and 5 mM Ca^{2+} (Fig. 1) appear significantly different from the respective maximum values, as the S.D. in experiments with cells from the same preparation was below 4 %. In prolonged experiments the demand for a specific Ca^{2+} concentration to keep the O_2 consumption at a constant level was obvious (Fig. 1).

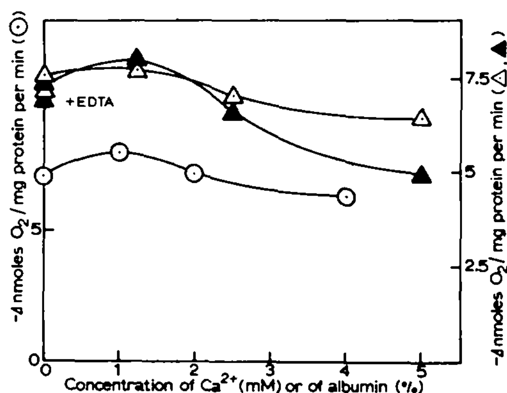

Fig. 1. O_2 consumption by isolated rat liver parenchymal cells in relation to the Ca^{2+} and albumin content of the incubation buffer. Cells prepared without erythrocytes in the perfusion media and from fasted animals were incubated as usual (see Materials and Methods) except for changes in the amount of albumin (○) (Ca^{2+} = 1.2 mM), or of Ca^{2+} (△, ▲) (albumin = 1 %), in the incubation buffer. The ordinate shows the average O_2 consumption for the first 30 min of incubation (○); for the first 60 min of incubation (△); or for the first 90 min of incubation (▲).

TABLE IV

PERMEABILITY BARRIER FOR MALATE OF ISOLATED RAT LIVER PARENCHYMAL CELLS

The cells were prepared with or without erythrocytes added to the perfusion media and were incubated at 37 °C in a concentration of about 0.1 ml of packed cells/ml for the times indicated. Experimental technique and calculations as in table III. Figures below each other belong to the same experiment. Cells were prepared from animals fasted for 24 h. Figures marked with * represent fed animals.

Addition to medium	*Incubation time (min)*	*Cells prepared without erythrocytes*		*Cells prepared with erythrocytes*	
		Malate in cells (mM)	*Malate in cells/ Malate in medium*	*Malate in cells (mM)*	*Malate in cells/ Malate in medium*
None	0	0.118–0.890*	1.13– 1.65*	0.161–0.167	2.0–4.4
None	60	—	—	0.055–0.060	∞ –6.7
None	120	0.090–0.734*	23.7 –33.3*	—	—
L-Lactate, 10 mM	30	—	—	0.384	6.0
L-Lactate, 10 mM	60	—	—	0.254	4.0

Permeability of the cell membrane

In isolated tissue (perfused liver or liver slices), permeability barriers for intermediates of the tricarboxylic acid cycle exist[27,28]. Similarly, cells prepared by the methods described in the present paper maintain a gradient of glutamate and of malate across the plasma membrane at least for several hours of incubation (Tables III and IV). Glutamate generated inside the cell membrane from oxaloacetate plus alanine remains inside the cell membrane, whereas glutamate added to the incubation buffer in a concentration of 10 mM is excluded from the intracellular space (Table III). These results are quite similar to those obtained in liver perfusion experiments[27,28] and in isolated rat liver cells[4], and indicate that the integrity of the plasma membrane is maintained throughout the preparation procedure and the time of incubation.

A further indication of the integrity of the plasma membrane was obtained by comparison of the water space and inulin space in the pellet obtained by centrifugation of a cell suspension: the cell suspension was equilibrated with ^3H_2O and [^{14}C]carboxylinulin for 10 min and centrifuged at 10 000 × *g* for 3 or 30 s. The ^{3}H and ^{14}C label were measured in the supernatant and in the pellet, and the spaces were calculated. In five experiments a mean ratio of 2.8 (range 1.8–3.2) between water space and inulin space was obtained in the pellet of freshly prepared cells. Differences in centrifugation time did not affect the ratio. Incubation of the cells for 60 min at 37 °C lowered the ratio between the water space and inulin space to 2.2 (range 1.7–2.6, three experiments). The decrease of the ratio during incubation might indicate some cell destruction, but could also be due to a lowering of the water content of the cells during incubation. Indeed, the dry weight of cell pellets from cell suspensions increased from 18 to 27 % during incubation. The relatively low ratios between water space and inulin space may indicate that inulin slowly penetrates the cell membrane[29].

Gluconeogenesis and glycogen synthesis

Glucose and glycogen synthesis from various substrates were measured with cells prepared from livers of 24 h fasted rats (Table V). Gluconeogenesis from lactate is linear with time for at least 2 h when cells are prepared without the addition of erythrocytes to the perfusion media. Cells prepared in the presence of erythrocytes show a slightly higher rate of gluconeogenesis from lactate than cells prepared in the absence of erythrocytes (Table V). Glucose synthesis from L-α-alanine is as high as glucose synthesis from lactate within the first hour of incubation, but declines to almost zero during the second hour of incubation (Table V). The rate of glucose synthesis from β-alanine is low and constant with time for 2 h (Table V). In isolated rat liver parenchymal cells, Berry and Friend[8] measured a synthesis of glucose from lactate of 1.8–2.3 μmoles/g dry wt per min.

In experiments with lactate or α-alanine as the gluconeogenic substrates no net glycogen synthesis was detectable, in agreement with liver perfusion experiments[30,31]. However, a high concentration of glucose (50 mM) in combination with a mixture of gluconeogenic substrates (pyruvate + glycerol + serine) gives rise to a net glycogen synthesis by the hepatocytes (Table V). The same effect has been observed in liver perfusion experiments[31,32] and is in agreement with the stimulation *in vivo* of mouse liver glycogen synthetase by glucose[33]. The average rate of glycogen synthesis (μmoles/min per mg of protein), is four to seven times higher in cells pre-

TABLE V

SYNTHESIS OF GLUCOSE AND OF GLYCOGEN BY ISOLATED RAT LIVER PARENCHYMAL CELLS

The cells were prepared from 24-h-fasted rats with or without erythrocytes in the perfusion media and incubated as described in Materials and Methods. The figures represent average rates of synthesis in the time intervals indicated as nmoles/mg of protein per min ±S.E. or as the range with the number of experiments in parentheses.

Substrate	*Parenchymal cells prepared by perfusion media without erythrocytes*		*Parenchymal cells prepared by perfusion media with erythrocytes*	
	0–60 min	*0–120 min*	*0–60 min*	*0–120 min*
Glucose synthesis				
L-Lactate, 10 mM	1.31±0.26 (6)	1.26±0.29 (5)	1.78 (3) 1.21–2.68	2.02 (3) 1.96–2.10
L-α-Alanine, 10 mM	1.27–1.41 (2)	0.67 –0.74 (2)	1.28 (1)	—
β-Alanine, 10 mM	—	—	0.48–0.59 (2)	0.48–0.40 (2)
Glycogen synthesis				
Glucose, 50 mM+pyruvate, 5 mM+ glycerol, 5 mM+serine, 5 mM	0.09 (3) 0.02–0.14	0.20 (3) 0.11–0.37	0.66 (3) 0.43–1.03	0.83 (3) 0.34–1.68

pared in the presence of erythrocytes than in cells prepared without the addition of erythrocytes to the perfusion media (Table V).

K^+ and adenylates

Cells prepared by the present method and kept as a pellet for 10 min showed a loss of about 50 % of the K^+ content (to 50 μmoles/ml of tightly packed cells). This K^+ loss was reversible, and could be restored to values of about 90 μmoles/ml of packed cells by incubation in the usual incubation buffer (see Solutions) for 30 min at 37 °C. The initial loss of K^+ from the cells could be prevented by rapid suspension and gentle shaking of the cell pellet at a cell concentration of about 0.1 ml of packed cells/ml. Omission of erythrocytes from the perfusion media did not affect the K^+ content of the cells.

The loss of potassium ions from cell pellets was paralleled by a loss of adenylates. Even a short centrifugation (30 s) of the cell suspension at $10\,000 \times g$ caused a 30–40 % fall in the sum of the adenylates. The supernatant after centrifugation showed a strong 260-nm absorption, but contained less than 10 % of the total adenylates, indicating a deamination of AMP during the centrifugation procedure. For this reason, all cell suspensions were acid-precipitated without separation of the cells from the incubation medium.

The adenylate content of cells incubated for various time intervals is shown

TABLE VI

ADENYLATE CONTENT OF ISOLATED RAT LIVER PARENCHYMAL CELLS

Cells were prepared from 24-h-fasted rats with or without erythrocytes in the perfusion media and incubated as described in Materials and Methods. For further details see text. Figures are nmoles/mg of protein and are the means and ranges of experiments with three cell preparations. Cells prepared without the addition of erythrocytes to the perfusion media were incubated for 60 min; those prepared in the presence of erythrocytes were incubated for 20 min.

	Incubation time (min)	*Parenchymal cells prepared by perfusion media without erythrocytes*	*Parenchymal cells prepared by perfusion media with erythrocytes*
ATP	0	6.11	13.50
		4.35 – 7.23	9.88 – 15.78
	20 or 60	6.74	13.17
		4.27 – 9.14	11.63 – 14.06
ADP	0	1.84	2.64
		0.70 – 3.22	2.08 – 3.23
	20 or 60	1.80	1.86
		1.27 – 2.72	1.40 – 2.18
AMP	0	0.31	0.56
		0.13 – 0.65	0.19 – 0.76
	20 or 60	0.14	0.87
		0.005 – 0.31	0.19 – 1.47
ΣAXP	0	8.27	16.70
		6.12 – 11.16	12.68 – 18.79
	20 or 60	8.68	15.75
		5.98 – 11.86	14.00 – 17.09

in Table VI. It is seen that the addition of erythrocytes to the perfusion media results in a higher content of adenylates, especially ATP. No difference was observed in adenylate content of cells prepared from fed or fasted rats.

DISCUSSION

Compared with the method of Berry and Friend[8], the present method for the preparation of liver parenchymal cells has the advantage of simplicity and of the exposure of the liver to only physiological perfusion pressure and flow rate, as determined by Brauer *et al.*[43], at an average of 13 cm of water and of 1 ml/g liver per min, respectively.

Furthermore, the K^+ content of cells prepared by the present method is very similar to that of the intact liver. By the procedure of Berry and Friend[8] an irreversible loss of up to 60 % of the total K^+ content occurs[8]. This loss may be caused by the low temperature during cell wash[44] or to the high perfusion flow rate and pressure applied in that method.

The operationally simple method of Capuzzi *et al.*[9] makes control of perfusion flow rate and pressure difficult, and the liver is exposed to relative anaerobiosis for at least 15 min. The effect of insufficient O_2 supply to the liver during perfusion is relatively small (see Results and below) in agreement with the observation that dog liver may be exposed to 40 min of anaerobiosis without apparent irreversible damage[34]. The only striking metabolic differences between cells prepared in the presence of erythrocytes and cells prepared in the absence of erythrocytes appear to be in the rate of glycogen synthesis in response to a high concentration of glucose (Table V) and the higher content of adenylates in cells prepared with erythrocytes in the perfusion media (Table VI). These differences indicate that the mechanisms responsible for metabolic regulation are more intact in cells prepared with erythrocytes in the perfusion media. The addition of erythrocytes to the perfusion media may, besides increasing O_2 supply, have some effect upon substrate supply, osmoregulation and pH regulation.

Generally, the results obtained in the present study are similar to those of other authors. Direct comparison of results from the literature is, however, complicated by the fact that data from different authors are related to a variety of measures for the amounts of cells (*e.g.* cell number, mg of protein, mg dry wt, ml cell suspension, ml of tightly packed cells). A comparison of the present results (expressed per mg of protein) with results obtained in perfusion experiments or in experiments with liver slices, has been attempted either by measurement of the protein content of intact liver tissue (mean $\pm$ S.E. $= 142 \pm 5$ mg/g wet wt ($n = 7$)) and supposing an equal relative protein content of all cell populations of the liver, or by measurement of the protein content per ml of tightly packed cells (mean $\pm$S.E. $= 179 \pm 6$ mg/ml of tightly packed cells ($n = 23$)). Thus, an average factor for the recalculation of nmoles/mg of protein to μmoles/g wet wt is 0.14–0.18. The recalculated data presented in Table VII together with values for perfused rat liver and rat liver slices were obtained by applying the factor of 0.14 and must, therefore, be considered as minimum values.

Results obtained with different cell preparations show relatively large variations (Tables II and V, *e.g. cf.* refs 8, 35 and 36). These variations, however, present no serious problems in work with isolated liver parenchymal cells, as the excellent

TABLE VII

COMPARISON OF DATA OBTAINED WITH ISOLATED RAT LIVER PARENCHYMAL CELLS, PERFUSED RAT LIVER AND RAT LIVER SLICES

Values are μmoles/g wet wt per min except for the adenylate sum, which is μmoles/g wet wt. Data for perfused liver and liver slices were taken from the papers indicated.

	Parenchymal cells (present paper)	*Perfused liver*	*Liver slices*
O_2 consumption	0.7 – 2.2	1.8 – 2.8 (30, 32, 37)	0.8 (38)
Gluconeogenesis (from lactate)	0.2 – 0.4	0.7 – 1.0 (30, 32)	0.3 – 0.6 (27, 39)
Glycogen synthesis	0.03 – 0.24	0.2 (32)	—
Adenylate sum	0.8 – 2.4	3.3 – 4.2 (40, 41)	0.9 (42)

reproducibility within the same cell preparation (S.D. below 4 %) and the possibility of performing a large number of experiments with cells prepared from the same liver, allow the use of the paired-data *t*-test in the statistical analyses.

In conclusion, cells prepared by the present method in the presence of erythrocytes are quantitatively comparable to perfused liver tissue with respect to membrane permeability (Tables III and IV), gluconeogenesis and glycogenesis (Table V), K^+ content (see Results) and ATP/ADP and adenylate content (Table VI).

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