HIGH-YIELD PREPARATION OF ISOLATED RAT LIVER PARENCHYMAL CELLS

A Biochemical and Fine Structural Study

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ABSTRACT

A new technique employing continuous recirculating perfusion of the rat liver *in situ*, shaking of the liver in buffer in vitro, and filtration of the tissue through nylon mesh, results in the conversion of about 50% of the liver into intact, isolated parenchymal cells. The perfusion media consist of: (a) calcium-free Hanks' solution containing 0.05% collagenase and 0.10% hyaluronidase, and (b) magnesium and calcium-free Hanks' solution containing 2 mM ethylenediaminetetraacetate. Biochemical and morphologic studies indicate that the isolated cells are viable. They respire in a medium containing calcium ions, synthesize glucose from lactate, are impermeable to inulin, do not stain with trypan blue, and retain their structural integrity. Electron microscopy of biopsies taken during and after perfusion reveals that desmosomes are quickly cleaved. Hemidesmosome-containing areas of the cell membrane invaginate and appear to pinch off and migrate centrally. Tight and gap junctions, however, persist on the intact, isolated cells, retaining small segments of cytoplasm from formerly apposing parenchymal cells. Cells which do not retain tight and gap junctions display swelling of Golgi vacuoles and vacuoles in the peripheral cytoplasm. Cytoplasmic vacuolization in a small percentage of cells and potassium loss are the only indications of cell injury detected. By other parameters measured, the isolated cells are comparable to normal hepatic parenchymal cells *in situ* in appearance and function.

In recent years a number of attempts have been made to prepare isolated parenchymal cells from rodent liver (1–5). The majority of these methods have employed mechanical force to separate the cells, inevitably resulting in damage to the cell membrane, marked changes in fine structure (6), and lack of respiration when the cells are suspended in typical extracellular media (7, 8). Recently, Howard, Christensen, Gibbs, and Pesch (9, 10) described an enzymatic technique in which isolated cells were prepared by shaking liver slices in a buffered medium containing hyaluronidase and collagenase. Cells prepared in this manner appeared to have intact cell membranes under the electron microscope, respired in the presence of calcium, and were not stained by trypan blue. A disadvantage of this technique is that the yield of isolated cells obtained from each liver represents only about 5% of the original tissue (10). Since relatively large quantities of cells are required for studies of cell permeability and metabolism, a new enzymatic technique has been developed for the rapid preparation from rat liver of isolated, intact parenchymal cells in high yield. The present paper describes this method and also describes the fine structure of the cells at several stages during the preparative procedures.

MATERIALS AND METHODS

Adult Sprague-Dawley rats weighing 200–300 g were used. Collagenase (type 1) was obtained from Worthington Biochemical Corp. (Freehold, N.J.), Nutritional Biochemical Corp. (Cleveland, Ohio), or from Schwarz BioResearch Inc. (Orangeburg, N.Y.). Hyaluronidase (type 1) and thiamine pyrophosphate (cocarboxylase) were obtained from the Sigma Chemical Co. (St. Louis, Mo.).

Preparation of Cell Suspensions

In order to achieve intimate exposure of intercellular spaces to collagenase and hyaluronidase, a buffered medium containing the enzymes was circulated continuously through the liver via the portal vein. This was accomplished by using a simplified version of the perfusion system devised by Mortimore (11). A reservoir containing the perfusion medium was held at 37°C in a thermostated cabinet. The perfusion medium was that found most suitable by Howard and Pesch (10) and comprised calcium-free Hanks' solution (12) containing 0.05% collagenase and 0.10% hyaluronidase. The enzyme medium was gassed with a mixture comprising 95% O_2 and 5% CO_2. Vigorous bubbling of gas through the solution in the reservoir maintained its pO_2 above 400 mm Hg.

The rat was anesthetized with Nembutal, the abdomen was opened, and the portal vein was cannulated with a No. 16 Rochester needle. The inferior vena cava was ligated just above the level of the renal veins. The chest was then opened and the thoracic portion of the inferior vena cava was cannulated via the right atrium with a No. 15 Rochester needle. The liver was perfused at a rate of 50–60 ml per min by the use of a Holter pump (Extracorporeal Medical Specialties, Inc., N.J.), and the perfusate was returned to the reservoir and recirculated. To prevent distention of the liver and possible cell damage, the rat was positioned about 15 cm above the reservoir, so that drainage of the liver could be assisted by a siphoning effect. The system initially contained 150 ml of medium without added enzymes. The first 50 ml of perfusate was allowed to pass through the liver directly to waste, thereby flushing all blood from the organ. The outflow was then directed back to the reservoir and the enzymes were added.

Once perfusion commenced, the pO_2 of the influent medium fell to about 200 mm Hg and that of the outflow to about 80 mm Hg. Due to carbon dioxide production by the liver, the pCO_2 rose sufficiently to lower the pH to about 7.1. At this point the gas phase was changed to 100% O_2. Control of the pH was thereafter achieved by varying the percentage of CO_2 in the gas phase, but in fact, a fall in pH of less than 0.5 did not appear to be a critical factor in determining the cell yield or percentage viability.

After 5–10 min, fluid commenced to ooze freely from the surface of the liver and accumulated in the thoracic and abdominal cavities. This fluid was collected with a syringe and needle and returned to the reservoir. Within 10–30 min, loss of fluid from the liver surface was too great for the perfusion to be maintained. At this point the consistency of the liver was so soft that it disintegrated on pressure unless handled very gently. The liver was carefully removed, transferred to a beaker containing 10–20 ml of enzyme medium at 4°C, and broken up with a blunt spatula. Scissors were used just for cutting strands of connective tissue.

Additional enzyme medium was then added to bring the volume of the suspension to 50 ml. Examination of the preparation at this point revealed clumps of intact cells, isolated intact cells, cell debris, and isolated damaged cells which had taken up trypan blue. The suspension was divided into two equal portions and transferred to 250 ml conical flasks, which were shaken at 37°C for 15 min in an atmosphere of air. The addition of sodium bicarbonate solution was usually required to maintain the pH at 7.4. Incubation of the suspension served to break up cell clumps and, more important, to digest isolated nuclei and damaged cells. Microscopic examination of the incubated suspension showed cell debris and isolated cells, of which 95–98% did not stain with trypan blue. The suspension was filtered through two layers of nylon mesh and the cells were separated from debris by centrifuging at 50 *g* for 2 min. The supernatant was removed and the cells were resuspended in fresh medium at 4°C.

In some experiments, an additional step which appeared to bring about further improvement in cell yield was included. After perfusion of the liver till it was of soft consistency, the enzyme medium was replaced with a medium comprising calcium and magnesium-free Hanks' solution containing 2 mM ethylenediaminetetraacetate (EDTA), pH 7.4. Perfusion was continued for 10 min and the remainder of the procedure was carried out as described previously. EDTA and collagenase could not be perfused simultaneously, since the enzyme was strongly inhibited by EDTA.

Preparation of Material for Light and Electron Microscopy

Biopsies were taken before and during the perfusion and after hepatic cell isolation was complete. Small blocks of liver and the isolated liver cells were fixed at 4°C for 90 min in 0.1 M Sorenson's phosphate-buffered 1% OsO_4 (pH 7.4). In several experiments, the tissue was first fixed for 15 min in a 1% paraformaldehyde—3% glutaraldehyde mixture (13) buffered with 0.7 M Na cacodylate (pH 7.4). After

fixation, the tissue was dehydrated quickly in graded ethanols and propylene oxide and embedded in Epon 812 (14). For the localization of thiamine pyrophosphatase (TPPase), tissue was fixed in the paraformaldehyde-glutaraldehyde mixture for 3 hr and then incubated at pH 7.4 for 60 min in the medium employed by Novikoff and Goldfischer (15).

Thick sections (1 μ) and thin sections ($\sim$50 mμ) were prevpared with a Sorvall MT-2 microtome equipped with a diamond knife. Thick sections were stained lightly with 1% toluidine blue or by the periodic acid-Schiff technique and examined by bright field and phase contrast microscopy. Selected fields were photographed with a Zeiss photomicroscope at an original magnification of 400 $\times$. Thin sections were picked up on Formvar- and carbon-coated copper grids and stained in 5% aqueous uranyl acetate and alkaline lead. Electron micrographs were taken at original magnifications of 2,000 to 30,000 $\times$ with a Siemens Elmiskop 1-a operating at 80 kv with a double condenser and a 50 μ objective aperture.

Analytical Methods

Oxygen uptake was measured in a Gilson respirometer (Gilson Medical Electronics, Middleton, Wisconsin). The flasks, containing 0.15 ml of 10% KOH in their center wells, were incubated at 37° in an atmosphere of air. Glycogen was determined by the method of Good, Kramer and Somogyi (16), and the glucose formed on acid hydrolysis was measured with hexokinase and glucose-phosphate dehydrogenase (17). Lactate dehydrogenase activity was assayed by the method of Wróblewski and LaDue (18) and glutamic pyruvic transaminase by the method of Henley and Pollard (19). Inulin was measured by the method of Kulka (20), and glucose by the method of Schmidt (17). Urea was determined by conversion to ammonia with urease, and the ammonia was measured by the Berthelot reaction (21). Protein was determined according to Lowry et al. (22). K^+ and Na^+ were determined by flame photometry.

The dry weight of isolated cells was determined by mixing a measured portion of the suspension with an equal volume of 10% (w/v) trichloracetic acid (TCA). The precipitate was centrifuged, washed with 1% TCA, and weighed after drying at 100°C (TCA-insoluble dry weight) (23).

RESULTS

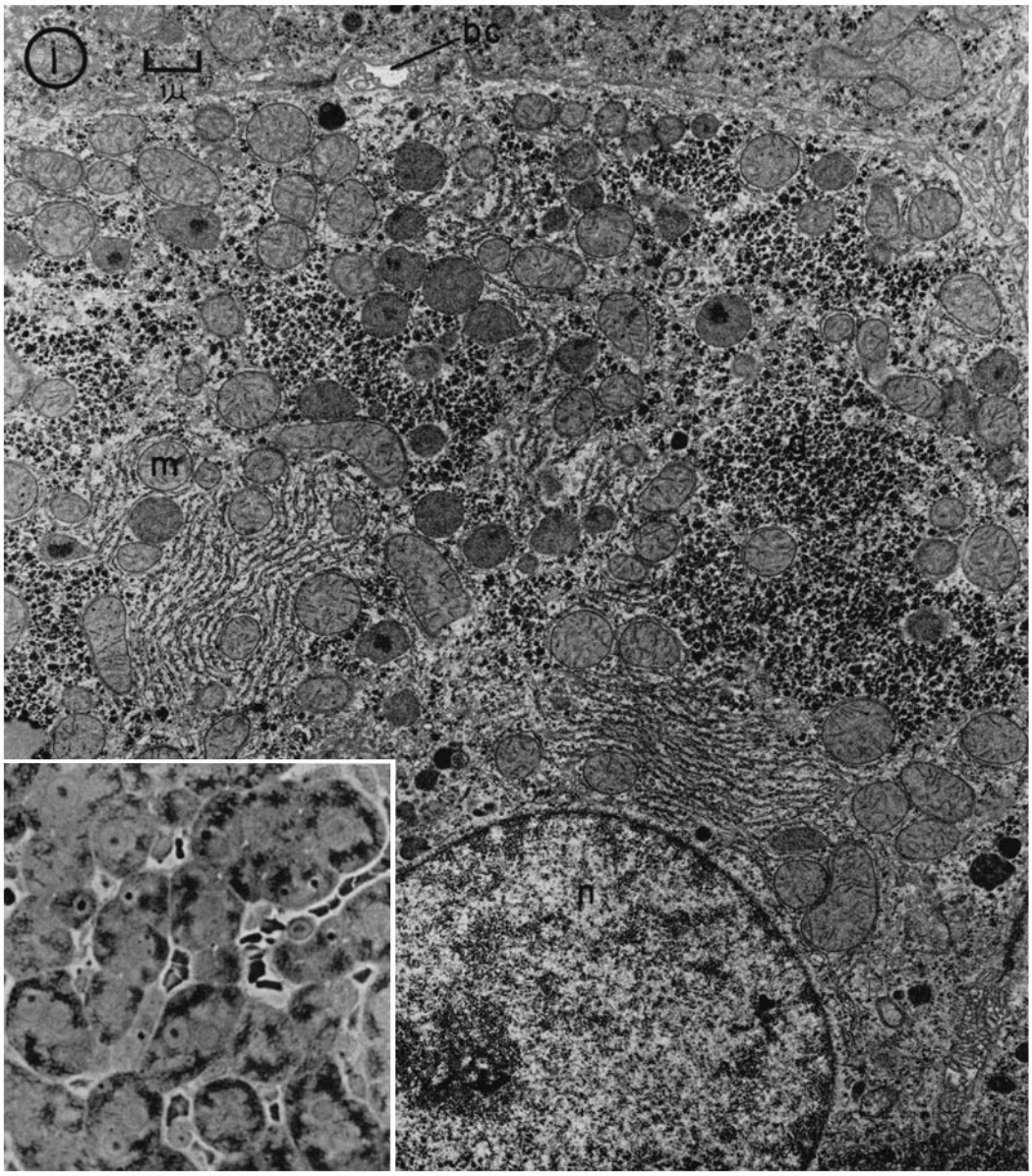
Preparation of isolated cells by continuous recirculation of the enzyme medium through the liver followed by perfusion with EDTA invariably gave yields at least 6 times as great as those obtained by Howard and Pesch (10) and sometimes represented a conversion of over 50% of the liver to isolated cells (Table I). The viability of the cells was also substantially higher than that reported by Howard and Pesch, as demonstrated by the low percentage stained by trypan blue. In order to obtain more conclusive evidence of cell integrity, other criteria were considered, namely, fine structure, permeability to substances of high molecular weight, ability to respire in a medium containing Ca^{2+} ions, and ability to synthesize glucose from lactate.

Morphology

Figs. 1–3 illustrate features of the normal hepatic parenchymal cell pertinent to this study. Before perfusion, the polyhedral liver cells (Fig. 1) are apparently held together by tight (24) (Fig. 2) and gap (25) junctions (zonulae and maculae occludentes [26]) which border the bile canaliculi, desmosomes (maculae adherentes) (Fig. 3), and the interdigitation of cytoplasmic extensions of adjacent cells (27). Narrow zones of cytoplasm immediately adjacent to the canaliculi are free of organelles (Figs. 2 and 3). Golgi complexes (Fig. 2) are frequently seen between these zones and the nuclei, each complex consisting of stacks of four or five narrow, curved, smooth-membraned cisternae, smooth and coated vesicles, and vacuoles. Dilated ends of the Golgi cisternae and many Golgi vacuoles contain closely packed, osmiophilic, lipoprotein particles (28, 29) (Fig. 2). A few such particles are also present in the cisternae of the smooth-surfaced endoplasmic reticulum. Glycogen, closely associated with the smooth reticulum, appears as dense particles 100–300 A in diameter occurring either singly or in large aggregates (Fig. 1).

During perfusion with enzymes or with enzymes followed by EDTA, the hepatic sinusoids are distended. They contain fragmented and intact endothelial and Kupffer cells (Fig. 4). The parenchymal cells appear as naked cords, maintaining their contiguity with adjacent cells at their pericanalicular junctions. As described below, desmosomes invaginate, cellular interdigitations separate, and the free surfaces of the hepatic cells bulge (Fig. 4). Within the cells, clusters of glycogen stain poorly and lipoprotein particles are fewer and less osmiophilic than in the unperfused liver. All organelles, however, appear similar to those in the hepatic cells before perfusion.

After the isolation procedure is complete, the cell population consists nearly exclusively of

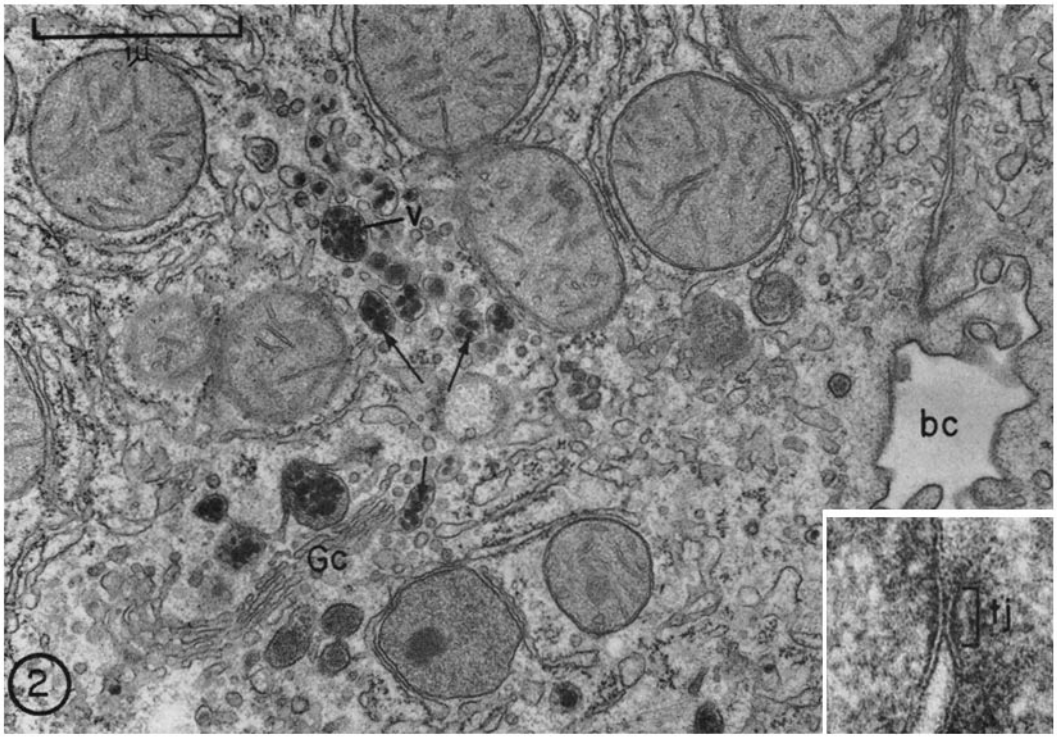


Key to Symbols

bc, bile canaliculus
cm, cell membrane
d, desmosome (macula adherens)
df, desmosomal fibers (tonofilaments)
g, glycogen
Gc, Golgi complex
l, lipid
m, mitochondrion
mvb, multivesicular body
n, nucleus
tj, tight junction (zonula occludens)
v, vacuole

FIGURES 1–3 Normal hepatic parenchymal cells before perfusion.

FIGURE 1 Phase contrast (*inset*) and electron micrographs of hepatic cells fixed in phosphate-buffered OsO_4. (*bc*), bile canaliculus; (*g*), glycogen; (*m*), mitochondrion; (*n*), nucleus. × 7500; inset × 1200.



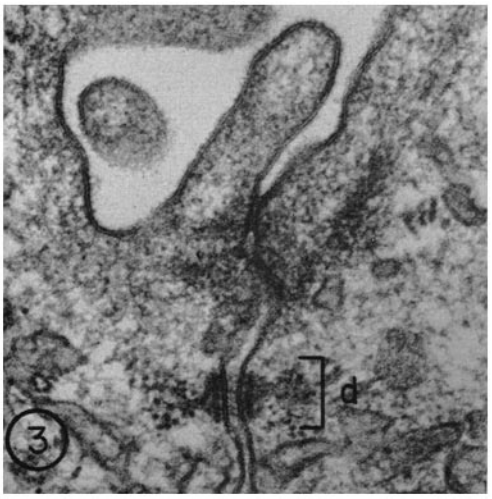


FIGURE 2 Tight junctions (*tj*, *inset*) bind adjacent hepatic cells at the margins of bile canaliculi (*bc*). Golgi complexes (*Gc*) adjacent to bile canaliculi frequently contain osmiophilic lipoprotein particles (arrows). *Inset*, tissue fixed in paraformaldehyde-glutaraldehyde mixture before OsO_4 fixation and treated with 0.5% aqueous uranyl acetate prior to dehydration. × 28,000; *inset* × 170,000.

FIGURE 3 Junctional complex at the margin of a bile canaliculus. Tissue fixed and treated in the same manner as that in Fig. 2, *inset*. (*d*), desmosome × 35,000.

morphologically intact, free, hepatic parenchymal cells (Fig. 5). Most of the cells have the same appearance as the liver cells during perfusion (Fig. 4), but careful examination of their surfaces (Fig. 6) reveals small, membrane-bounded portions of cytoplasm of formerly apposing cells attached to persisting tight and gap junctions (Fig. 7 *a–c*). Lateral to these areas of attachment, the regions of cell membranes which contain desmosomes invaginate (Figs. 6 and 8). The invaginations subsequently appear to pinch off (Figs. 9 and 10), forming vacuoles which include hemidesmosomes on their cytoplasmic surfaces. During invagination and vacuole formation, the desmosomal fibers separate from their plaques (Fig. 9 *b*). The fibers lie free in the ground substance, while the vacuoles appear to migrate centrally. The desmosomal plaques decrease in density (Fig. 10)

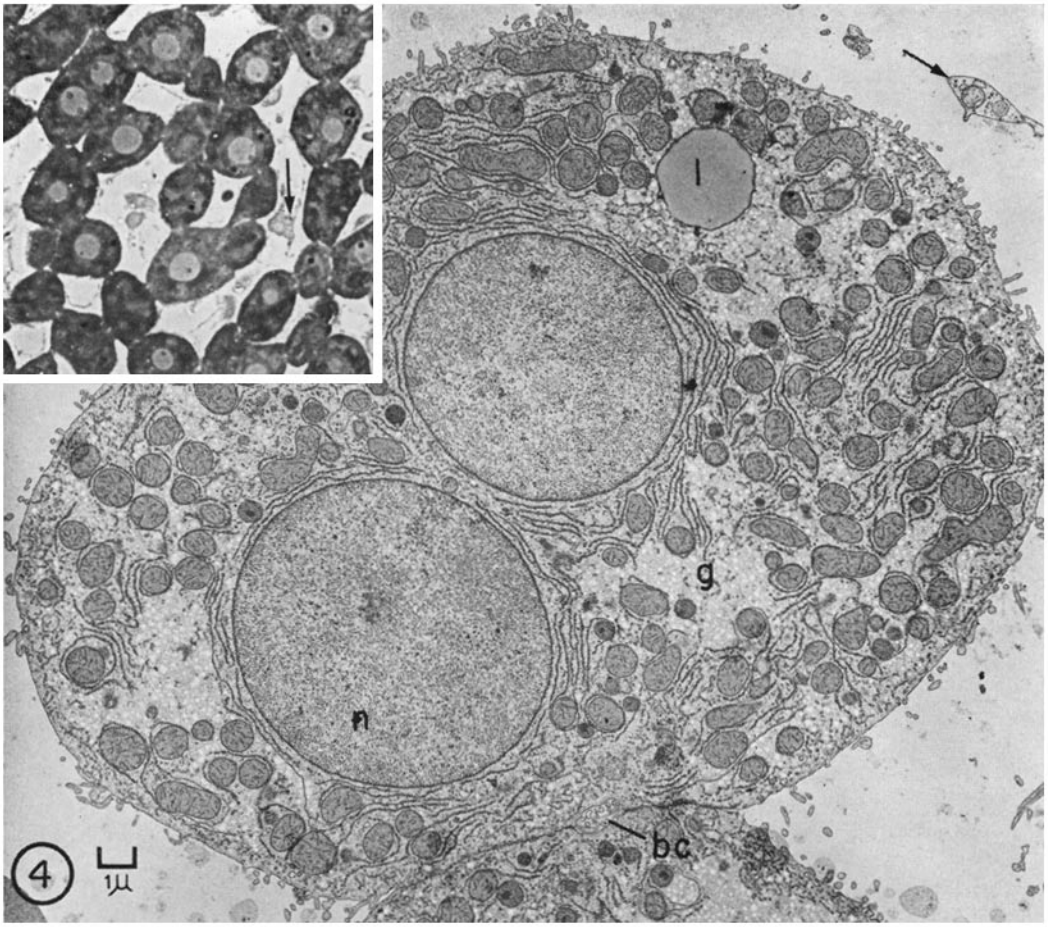
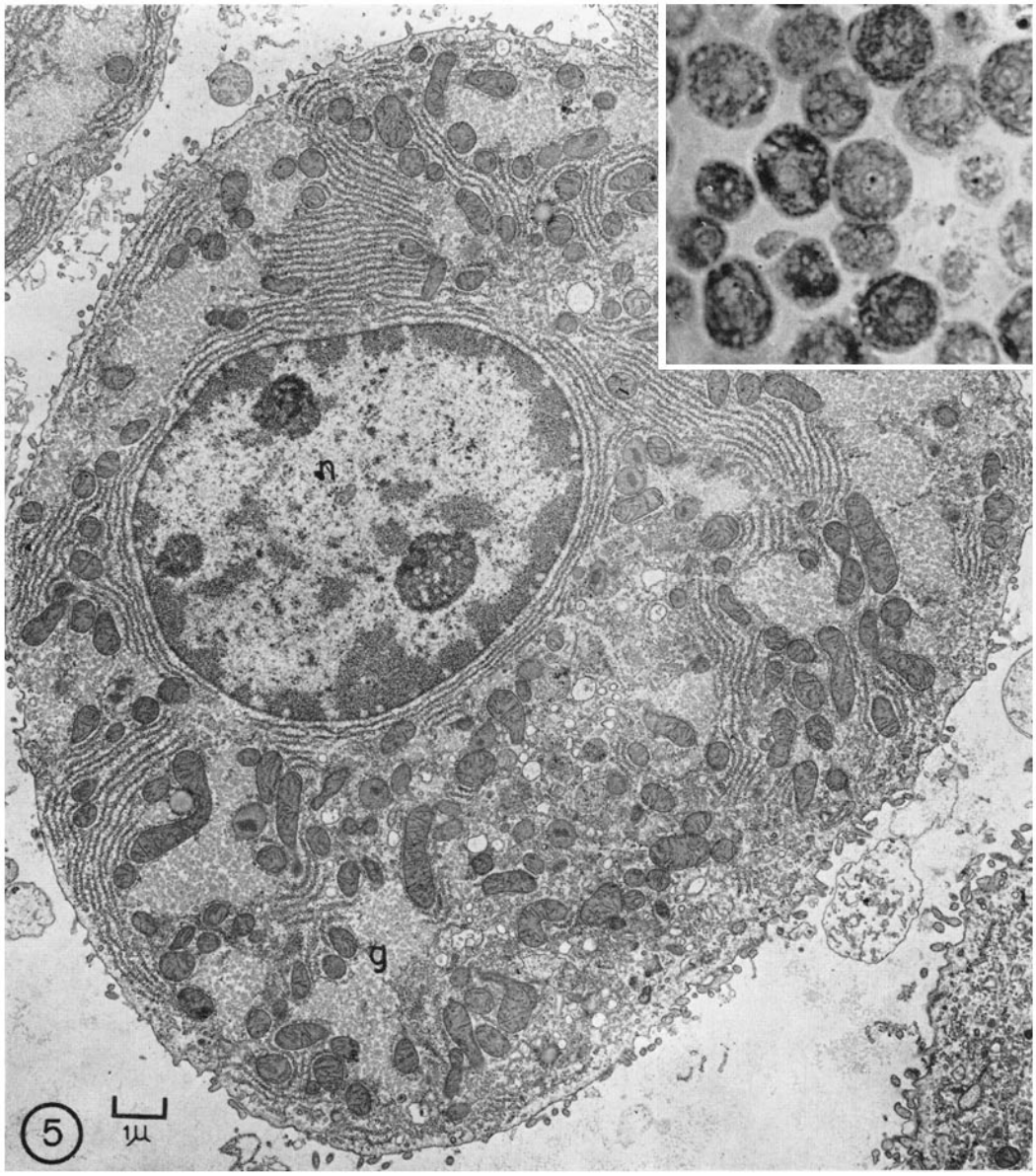


FIGURE 4 Phase and electron micrographs of liver cells during perfusion with medium containing hyaluronidase and collagenase. Adjacent hepatic parenchymal cells remain contiguous around bile canaliculi (*bc*), but endothelial (arrows) and Kupffer cells are fragmented and separated from cords of parenchymal cells. Glycogen (*g*) appears as large pools of poorly stained particles. The appearance of this binucleate liver cell is otherwise comparable to that of the normal cell before perfusion. (*l*), lipid. × 5500; inset × 1000.

and closely resemble the membrane specializations (30) observed on the cytoplasmic surface of some multivesicular bodies (Fig. 11).

As in the perfused liver, particulate glycogen is difficult to detect by electron microscopy (Fig. 5), but the liver cells remain periodic acid-Schiff (PAS) positive. The cell membrane, Golgi complex, rough- and smooth-surfaced endoplasmic reticulum, mitochondria, microbodies, lysosomes, and nuclei are otherwise identical to those of the normal hepatic cell. Mitochondria show no swelling or contraction, no change in density of their inner or outer compartments, no change in configuration of their cristae, and no alteration in size or number of their matrix granules (Fig. 5).

Some isolated cells have large (~40 mμ) clear vacuoles between Golgi complexes and the cell surface (Figs. 12 and 13). Vacuoles in both Golgi and peripheral areas have the same membrane thickness, both occasionally contain lead phosphate reaction product when incubated for thiamine pyrophosphatase (*inset*, Fig. 12), and both contain occasional, lightly-staining, lipoprotein particles. Indeed, closely packed, osmiophilic

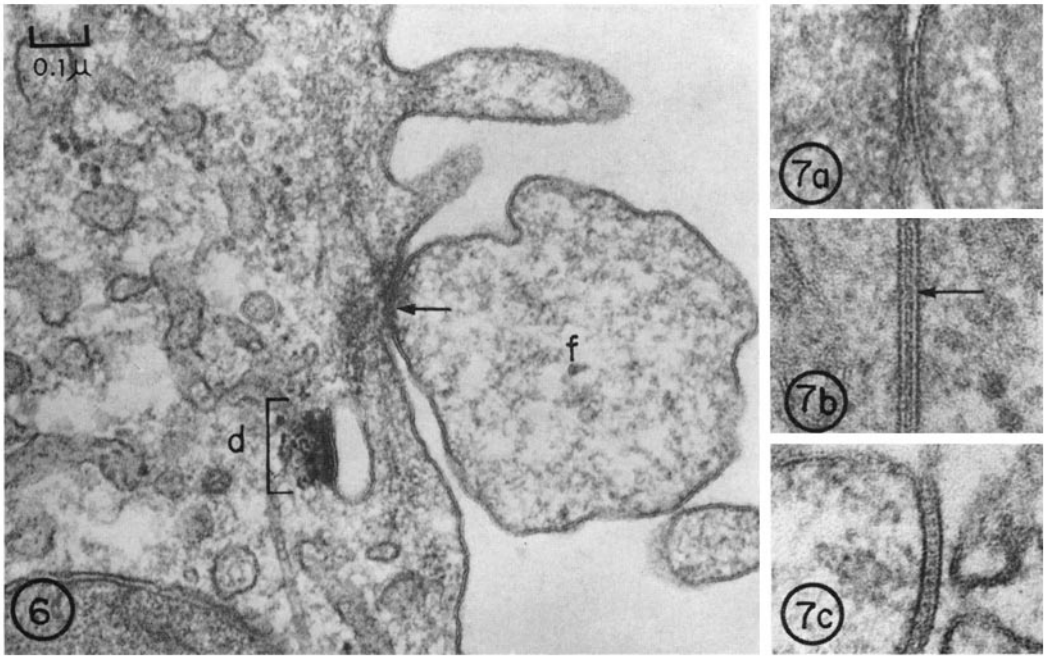


FIGURES 5–13 Isolated hepatic parenchymal cells.

FIGURE 5 Phase and electron micrographs of rounded parenchymal cells which are no longer in continuity with neighboring cells. Areas which formerly formed bile canaliculi are not discernible, but cell architecture is well preserved. At this low magnification, remnants of junctional complexes are not readily visible. Glycogen (*g*) stains poorly. × 7500; inset × 1000.

particles are seen rarely in the Golgi complex or in the smooth-surfaced endoplasmic reticulum in any of the isolated cells.

In vacuolated cells, tight and gap junctions rarely persist on the cell surface, discontinuities in cell membranes are present occasionally, and there is an increase in the width and length of the organelle-free cortical zones.



FIGURES 6–11 Tissue fixed in paraformaldehyde-glutaraldehyde mixture before OsO_4 fixation and treated with aqueous uranyl acetate before dehydration.

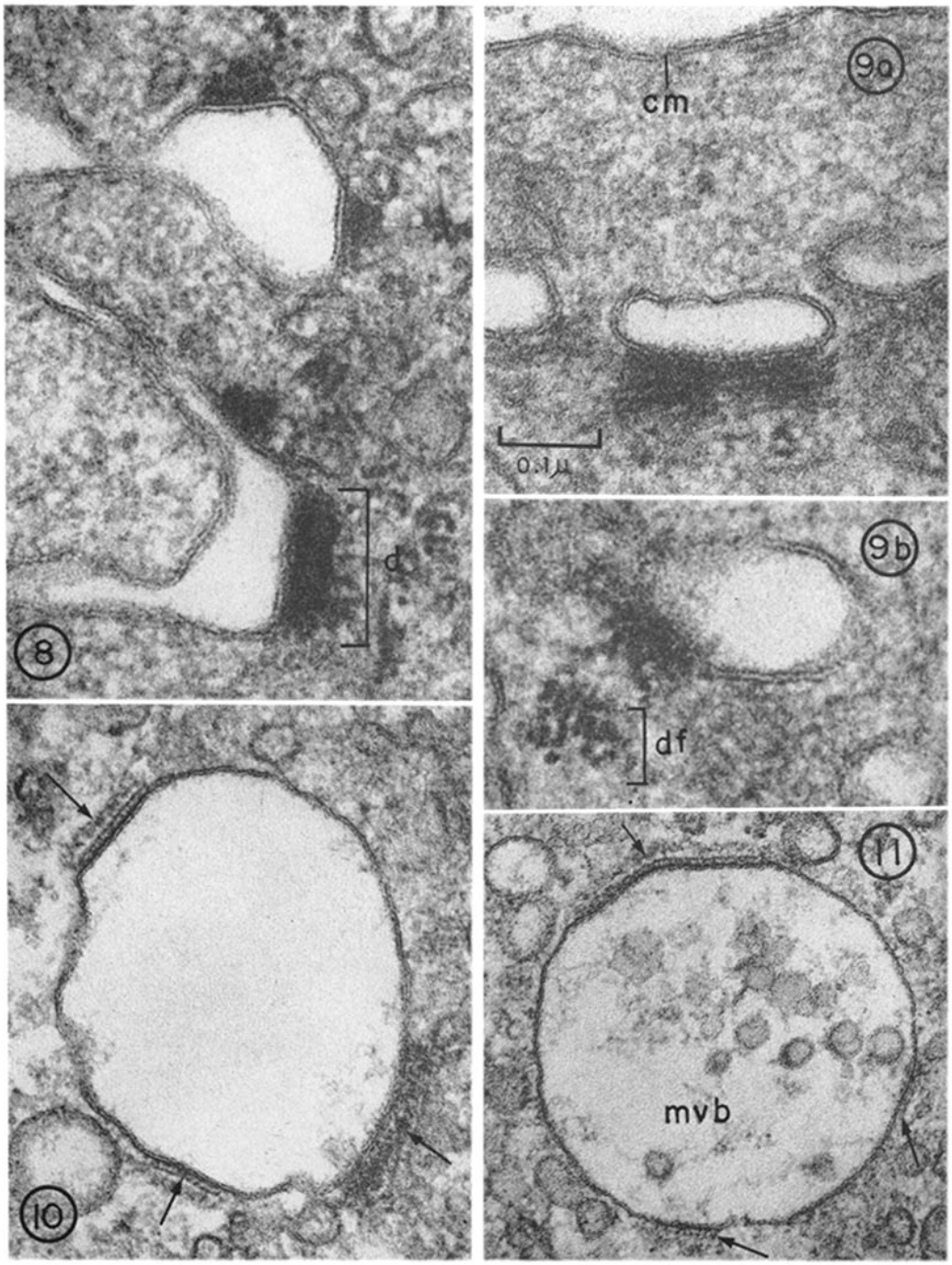
FIGURE 6 At higher magnification than Fig. 5, small segments of cytoplasm (*f*) are clearly seen attached (arrow) to the isolated cells. (*d*), desmosome. × 75,000.

FIGURE 7 Two types of junctions persist between isolated cells and cell fragments. Pentilaminar tight junctions, which have a continuous band of fusion between adjacent cell membranes (Fig. 7 *a*), and septilaminar gap junctions (Fig. 7 *b*), many of which have irregularly spaced areas of fusion (arrow) between the external leaflets of apposing cell membranes, and other gap junctions (Fig. 7 *c*), which have a periodic substructure, are shown. 7 *a*, × 160,000; 7 *b*, × 180,000; 7 *c*, × 175,000.

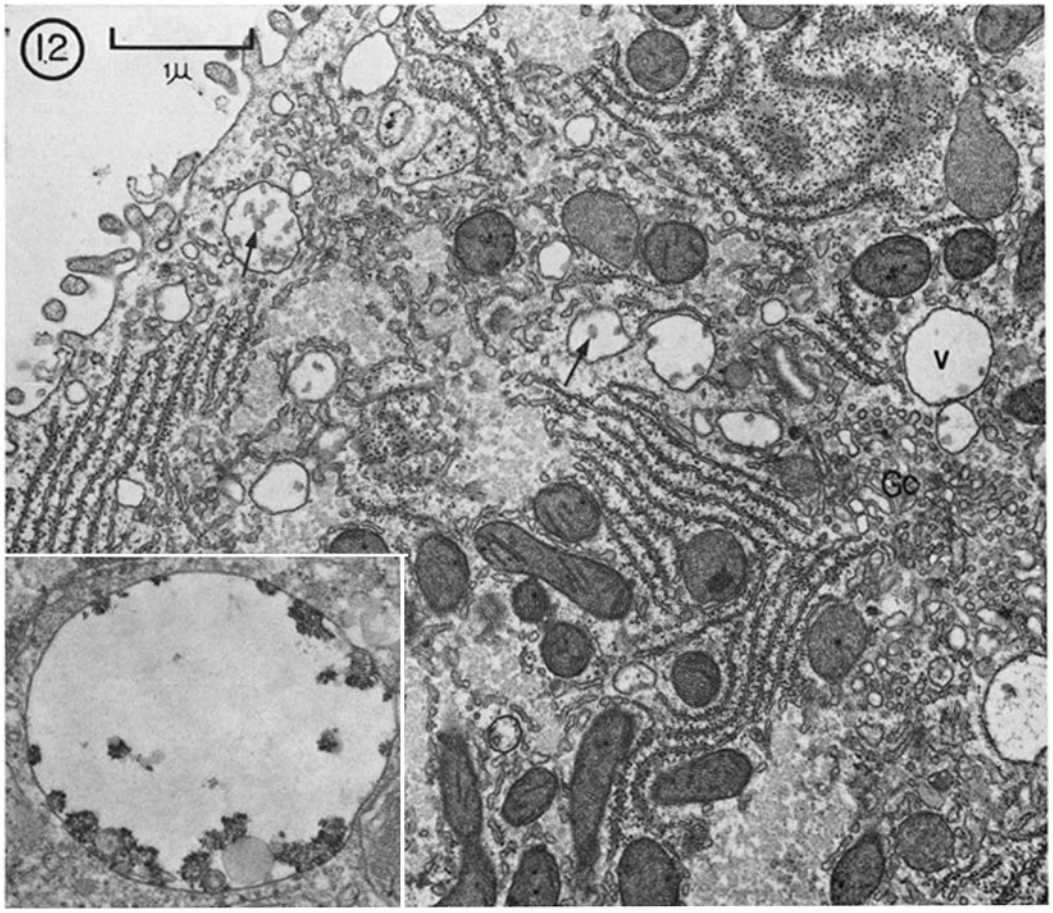
Biochemistry

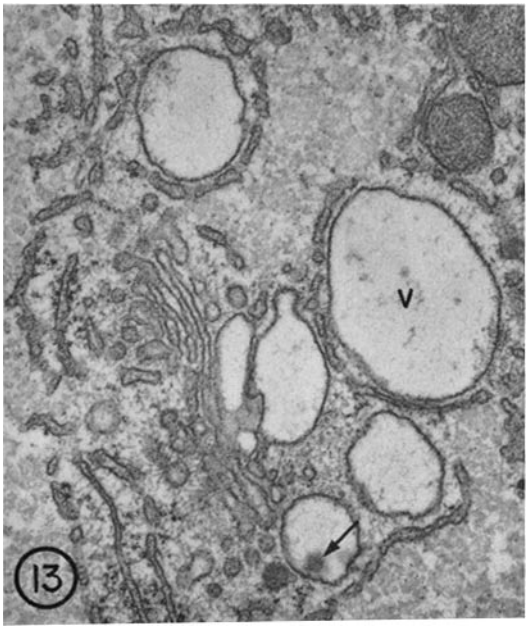
Isolated cells prepared by mechanical techniques invariably lose soluble enzymes to the suspending medium (7, 31, 32). The loss of the cytoplasmic enzyme lactate dehydrogenase (LDH) and glutamic pyruvic transaminase (GPT) was investigated by comparing the enzyme activities and specific activities in the various fractions obtained during the preparative procedure (Table I). Samples were taken from the initial dispersion after the cells were filtered through nylon mesh and from the cells after two washes with 50 ml of fresh medium. The enzyme activity of the initial supernatant obtained after centrifuging the dispersion and the amount of enzyme found in the washing media were also examined. In experiment 1, cells were prepared by the method of Howard et al. (9), in experiment 2 by perfusion with enzyme medium, and in experiment 3 by enzyme perfusion followed by EDTA perfusion. The results of the representative experiments indicate that the specific activity of LDH and GPT was as high in the cells as in the initial dispersion. If most of the soluble enzymes had been lost from the cells and only insoluble material had been retained, the specific activity of the residual cell LDH and GPT would have been substantially lower than that of the initial dispersion (7).

Cells were incubated at 37° in Hanks' medium containing 3.3% inulin and then centrifuged in tared tubes at 2° for 10 min at 18,000 *g*. In eight experiments, a mean vol representing 20% of the cell pellet was found to be penetrated by inulin. This volume, which is of the order of the theoretical estimate of the extraparticulate space for the



FIGURES 8–11 Unlike tight and gap junctions which persist, desmosomes are cleaved during enzyme perfusion. The cell membrane invaginates (Fig. 8) in the areas which contain hemidesmosomes (*d*). Some of the invaginations (Fig. 9a) appear as elongate vacuoles several microns from the cell surface (*cm*). After deep invagination or true fission of the cell membrane (Fig. 9b), fibers (*df*) appear to separate from the desmosomal plaques. Similar (Fig. 10) but less dense plaques (arrows) are seen on vacuoles deep within the cell. Such plaques (arrows) (Fig. 10) are intermediate in density and width between those of hemidesmosomes (Figs. 8 and 9) and those (arrows) (Fig. 11) occasionally seen on the surface of multivesicular bodies (*mvb*). Fig. 8, × 160,000; 9a, × 150,000; 9b, × 200,000; 10, × 105,000; 11, × 100,000.





FIGURES 12 and 13 These are "damaged," isolated cells which have tears in their cell membranes or lack tight and gap junctions. In these cells, large vacuoles are present which commonly contain faintly-staining lipoprotein particles (arrows). They appear to have their origin in the Golgi complex (Fig. 13) and then migrate towards the cell surface. Both the Golgi vacuoles and vacuoles near the cell surface occasionally contain lead phosphate reaction product (inset, Fig. 12) when the tissue is reacted for thiamine pyrophosphatase. All other organelles appear normal. (*Gc*), Golgi complex. Fig. 12, × 19,000; inset, × 39,000; 13, × 42,000.

TABLE I

Distribution of Lactate Dehydrogenase and Glutamic Pyruvic Transaminase Activities in Rat Liver Fractions

Exp. no.	Tissue fraction	Protein	% of D	Lactic dehydrogenase		Glutamic pyruvic transaminase	
				Activity*	Specific activity	Activity*	Specific activity
		mg		*units/ml*	*units/mg*	*units/ml*	*units/mg*
1	Dispersion	820	(100)	92,250	5630	9140	557
	1st supernatant	635	77	73,185	5300	8560	476
	2nd supernatant	76	9	14,700	5250	1670	595
	3rd supernatant	14	1	894	1620	186	338
	Cells	68	8	33,580	5890	2130	373
2	Dispersion	620	(100)	58,300	4410	7050	533
	1st supernatant	402	65	43,900	3880	5720	505
	2nd supernatant	82	13	11,500	4500	1020	398
	3rd supernatant	12.5	2	1,680	3360	223	446
	Cells	119	19	39,600	4350	4430	485
3	Dispersion	1500	(100)	125,810	4125	8630	283
	1st supernatant	540	36	76,585	4185	5670	310
	2nd supernatant	140	9	11,430	4970	718	312
	3rd supernatant	33	2	5,310	4825	304	276
	Cells	785	52	102,660	4775	6360	296

* One unit is the amount of enzyme required to bring about the conversion of 1 mμmole of substrate per min at 30°C. The specific activity of the fractions is defined as the number of enzyme units per mg of protein.

closest possible packing of undeformed spheres (33), is considered likely to represent only the entrained fluid of the pellet.

During the course of these experiments it was noted that the cells lost about 60% of their initial K^+ content during the isolation procedures (Table II). Measurement of the K^+ content of the liver at various stages during the preparative procedures indicated that the K^+ loss commenced after the perfusion when the digested liver was dispersed in enzyme medium. This loss took place whether the dispersion was performed at 4° or at 25°. Loss of K^+ continued during washing of the cells. Cells prepared by the technique of Howard et al. (9) also lost K^+ to the suspending medium.

Despite this loss of K^+, the cells appeared to function normally in respiratory studies (Table III). In these experiments, cells were incubated in bicarbonate-free Hanks' medium containing 2 mM $CaCl_2$. Sodium phosphate buffer (10 mM), pH 7.4, was included in the medium. Endogenous respiration was of the same order as that of liver slices. Respiration was stimulated by lactate, pyruvate, fructose, and alanine, and was not diminished in the presence of calcium. In an experiment in which 10 mM fructose was the added substrate, 22.6 μmoles of lactate were found, indicating that the glycolytic pathway from fructose to lactate was intact. In contrast, liver cells isolated by mechanical techniques do not glycolyse (7, 32).

TABLE II

Potassium Ion Loss from Isolated Liver Cells

Wet wt cell pellet	K^+ content of pellet	(K^+) of pellet	Na^+ content of pellet	(Na^+) of pellet
mg	*μeq*	*μeq/g**	*μeg*	*μeq/g*
355	4.7	13.7	42.7	150
551	6.38	11.6	63.8	116
1160	24.0	20.6		
1150	22.8	19.8		
380	10.4	27.2		
530	17.5	33.0		
141	6.2	43.5		

* No correction has been made for the K^+ content of entrained medium in the cell pellet. The mean (K^+) of the unperfused liver in five experiments was 97 μeq/g.

TABLE III

Respiration of Isolated Liver Cells in a Saline Medium

Cells were incubated at 37°C in bicarbonate- and glucose-free Hanks' medium containing 10 mM phosphate buffer, pH 7.4. Substrate was added at a final concentration of 10 mM. Gas phase air Total vol 4 ml

Nutritional state of rat	Incubation time	Substrate	Oxygen uptake	Dry weight of cells	$Q O_2$
	min		*μl*	*mg*	*μl/mg dry wt/hr*
Fed	65	None	236	26.1	8.4
		Lactate	326		11.5
		Pyruvate	316		11.2
		Fructose	263		9.3
Fed	60	None	738	61.0	12.1
		Pyruvate	836		13.7
		Alanine	876		14.4
Fed	40	None	113	15.0	11.3
		Lactate	169		16.9
		Pyruvate	180		18.0
		Fructose	164		16.4
Fasted	40	None	325	62.4	7.8
		Lactate	625		15.0
		Fructose	493		11.6
Fasted	35	None	325	59.8	9.3
		Lactate	565		16.2
		Pyruvate	547		15.7

As pointed out by Krebs et al. (34), a stringent test of the metabolic integrity of the liver cell is its ability to synthesize glucose from lactate, since this anabolic process involves both the mitochondrial and cytoplasmic fractions of the cell working in unison. Accordingly, the synthetic ability of isolated liver cells was examined. For the study of glucose synthesis, cells were prepared from the livers of rats fasted for 24 hr. The experiments recorded in Table IV demonstrate that isolated cells were able to convert lactate and pyruvate to glucose. As in the case with perfused liver (34), no glycogen was synthesized by the isolated cells from fasted rats. However, cells obtained from the livers of fed animals lost only about 10–20% of their glycogen during the preparative procedures. The mean glycogen content of the cells after isolation in six experiments was 2.4%, and it is likely that this glycogen was an important source of endogenous substrate.

DISCUSSION

The major advantage of the technique described in this paper is that yields of parenchymal cells 6 times those achieved by Howard et al. can be obtained within 45 min. The perfusion technique employed in this work was a modification of the Mortimore system (11). However, a technique based on the apparatus described by Miller et al. (35) in which the liver is removed from the rat before perfusion should prove equally satisfactory.

As determined by electron microscopy, more than half of the hepatic cells isolated by the procedure reported here, with or without EDTA, completely retained their structural integrity and most of the remainder showed only swelling of Golgi and cytoplasmic vacuoles. Few breaks in plasma membranes, virtually no transformation of endoplasmic reticulum into isolated vesicles, and no mitochondrial damage were seen in any hepatic parenchymal cells.

TABLE IV

Synthesis of Glucose by Isolated Liver Cells
Incubation conditions as for Table III

Incubation time	Dry weight	Additions	Glucose formed	Rate of glucose formation
min	*mg*		*μmoles*	*μmoles/g dry weight/min*
40	29.2	None	0.24	0.08
		Pyruvate	4.37	1.5
		Glycerol	2.78	1.0
40	62.4	None	0.39	0.15
		Fructose	23.8	9.5
		Lactate	4.78	1.9
		Glycerol	3.96	1.6
40	40.4	None	0.25	0.15
		Pyruvate	3.76	2.3
		Lactate	3.23	2.0
		Glycerol	2.36	1.5
55	45.7	None	0.63	0.25
		Pyruvate	7.03	2.8
		Lactate	6.38	2.5
		Alanine	5.06	2.0

Perfusion rapidly separated interdigitations and desmosomes. The hemidesmosomes of hepatic cells appeared to have the same fate as those in trypsinized embryonic epithelial tissue (36), and some may be the precursors of plaques occasionally seen on the cytoplasmic surfaces of multivesicular bodies.

Previous studies (9, 37, 38) have overlooked the persistence of tight and gap junctions on the surfaces of isolated cells, but searching the surfaces of isolated hepatic parenchymal cells at high magnification consistently revealed these portions of junctional complexes binding segments of formerly adjacent cells. The findings suggest that neither enzymes nor EDTA, both of which appeared to have the same effect on desmosomes, cleaves tight or gap junctions. Tight and gap junctions appear to be only mechanically torn from cells after the disruption of other adhesive elements. Cells of the perfused liver and isolated cells in which tight junctions persisted did not show widening of the cortical area of organelle-free cytoplasm, swelling of Golgi vacuoles, nor did they show the emergence of vacuoles between the Golgi complex and the cell surface. The findings suggest that, on dispersion of the cells, tearing of tight and gap junctions results in defects in the cell membrane, possibly accompanied by loss of potassium and the entrance of fluid. The influent medium is apparently sequestered within Golgi vacuoles and then transported to the cell surface along with the remaining lipoprotein particles. That lipoprotein-containing Golgi vacuoles do migrate to the cell surface has been amply documented by other investigators (29, 38, 39) and is further supported here by the presence of TPPase reaction product in these vacuoles. Whether or not the Golgi vesiculation and potassium loss are reversible remains to be determined. The defects in cell membrane apparently repair quickly.

Although many workers have attempted to prepare isolated parenchymal cells from rodent liver (1–5), Howard et al. (9) have been the only group up to now to present convincing evidence that the cells produced were relatively intact. Berry and Simpson (6) showed that cells prepared by perfusion of the liver under pressure followed by mechanical dispersion had disrupted cell membranes and grossly-distorted fine structure. In spite of this, several groups of workers have used such cells to perform studies including those on the effects of hormones on cell permeability (40, 41) and the uptake of chylomicrons (42). When in the present study trypan blue was used to stain cells prepared by high-pressure perfusion, 100% of the isolated cells took up the stain. It was noted consistently that the more severe the mechanical treatment, the greater was the percentage of damaged cells, and it was for this reason that mechanical treatment was reduced to a minimum.

Nevertheless, even with the most gentle handling, cells prepared with this technique show some morphological and biochemical abnormalities, namely, the vesiculation of the Golgi apparatus and the loss of K^+. Moreover, the rate of glucose synthesis from lactate, although rather higher than that observed with liver slices, is only about 50% of that obtained with the perfused liver (34).

In spite of these limitations, it is evident that the cells should prove a useful preparation for metabolic studies. For respiratory experiments, cells can be prepared from one liver which are sufficient for at least 40 incubation vessels. They are easier to handle than surviving slices, and, since the suspension is drawn evenly from all regions of the liver, a greater experimental uniformity can be achieved with cells than with slices. Perhaps one

of their greatest advantages is that metabolic studies can be combined with rapid examination of cell morphology. Moreover, all the problems of oxygen diffusion associated with surviving slice techniques are overcome by the use of isolated cell preparations.

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