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THE EFFECTS OF DEXAMETHASONE ON METABOLIC ACTIVITY OF HEPATOCYTES IN PRIMARY MONOLAYER CULTURE

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SUMMARY

The effects of dexamethasone on multiple metabolic functions of adult rat hepatocytes in monolayer culture were studied. Adult rat liver parenchymal cells were isolated by collagenase perfusion and cultured as a primary monolayer in HI/WO/BA, a serum free, completely defined, synthetic culture medium. Cells inoculated into the culture medium formed a monolayer within 24 hr. Electron microscopy showed that the cells in primary culture had a fine structure identical to liver parenchymal cells in vivo, including the observation of desmosomes and bile canaliculi in intercellular space. There was significant gluconeogenesis by the cells 24 hr postinoculation but it had decreased markedly by 48 hr. There was a marked induction of tyrosine aminotransferase (TAT) by dexamethasone, which was maintained for up to 72 hr postinoculation of cells. The transport of α-aminoisobutyric acid into the cells in monolayer culture was stimulated by dexamethasone and was dependent on the concentration of dexamethasone. Albumin synthesis and secretion by the cells was measured by a quantitative electroimmunoassay. Albumin production was shown to increase linearly over an incubation period of 24 to 48 hr postinoculation. Dexamethasone depressed the albumin synthesis. The effects of dexamethasone are slow, and at times require more than 6 hr to show variation from the control, indicating that dexamethasone is not a single controlling hormone. Possibly it functions in a cooperative and coordinating role in the regulation of cell metabolism.

Key words: dexamethasone; hepatocytes; cell culture; liver metabolism.

INTRODUCTION

In order to study the function of adult rat liver parenchymal cells in vitro, multiple techniques such as isolated liver perfusion (1-4), incubated liver slices (5), and isolated liver cells (6,7) have been used. However, these methods have several limitations, and, more important, there is a marked decrease in metabolic activity after 3 or 4 hr in these systems. In contrast to these techniques, adult rat liver parenchymal cells in non-proliferating monolayer culture offer several advantages. These include: homogeneity of parenchymal liver cells, preservation of specific liver cell functions, and stability of various metabolic activities for variable periods up to several days as reported by Bissell et al. (8). Also, this system permits the liver cells from a single rat to be used under various experimental conditions. Some investigators have used partially resected and regenerating liver to promote monolayer formation of liver cells (8,9). However, it can be questioned whether or not there is normal adult liver parenchymal cell metabolic activity in regenerating liver. To avoid this possibility, normal adult rat liver cells can be successfully cultured on collagen-coated dishes as a monolayer without previous partial hepatectomy (10). We have used

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this system to study the effects of dexamethasone on multiple metabolic functions of adult rat liver parenchymal cells.

MATERIALS AND METHODS

Isolation and culture of rat liver parenchymal cells. Male Sprague-Dawley rats weighing 250 to 300 g were fasted for 24 hr prior to isolation of liver cells. The method of Berry and Friend (6) for isolating liver cells with collagenase was used with some modifications (7). Rats were anesthetized with an i.p. injection of sodium pentobarbital (Nembutal Sodium, Abbott Laboratories, North Chicago, IL, 5 mg/100 g body wt). All procedures for isolation and culture of liver cells were carried out under strict aseptic conditions. The abdomen was opened, and, after the portal vein was cannulated, both the hepatic artery and the splenic vein were tied. The thorax was then opened aseptically and the inferior vena cava was cannulated via the right atrium. The inferior vena cava was then tied above the renal vein. The liver was perfused via the portal vein with Krebs-Henseleit bicarbonate buffer (equilibrated with 95% O_2:5% CO_2, 37° C, pH 7.4) at a flow rate of 40 ml/min, using a Manostat Varistaltic Pump (VWR Scientific, Denver, CO). After flushing all blood from the liver, collagenase solution (Type II, Worthington Biochemical Corp., Freehold, NJ), which was sterilized previously by passage through a Nalgene filter unit (pore size 0.22μ, Nalge Sybron Corp., Rochester, NY), was added to the perfusion system in a final concentration of 50 mg/100 ml.

Five minutes after adding collagenase, calcium chloride was added to give a final concentration of 2.5 m*M*. Within 20 min the liver became markedly soft and fluid began to ooze from the surface. At this point the liver was removed aseptically and transferred to a beaker containing 20 to 30 ml of HI/WO/BA culture medium (International Scientific Industries, Cary, IL). The liver was minced gently with scissors and filtered through silk cloth of approximately 90 mesh. The cells were centrifuged at 400 rpm for 2 min at 4° C. The supernatant containing Kupffer cells and cell debris, was removed by aspiration and discarded. The pellet, containing parenchymal cells was resuspended in HI/WO/BA culture medium. After recentrifugation and removal of the supernatant, the cells were resuspended in a volume of 60 to 70 ml, which gave approximately 10×10^6 cells/ml. The number of cells was determined with a hemocytometer and viability was estimated by Trypan Blue dye exclusion. The exclusion of dye observed was greater than 95%. Suspensions of 2.5×10^6 cells in a final volume of 2.5 ml of HI/WO/BA containing gentamicin, 50 μg/ml (Geramycin, Schering Corp., Kenilworth, NJ) were inoculated into 60-mm tissue culture dishes (Falcon Plastics, Oxnard, CA), which had been coated previously with calf skin collagen (acid soluble, Type III, Sigma Chemical Co., St. Louis, MO) to promote monolayer formation according to the method of Lin and Snodgrass (10). It should be noted that cell population and the depth of medium influence plating efficiency. Also, the antibiotic gentamicin has several advantages: pH and heat stability, broad spectrum bactericidal activity, and lack of any notable influence on cell metabolic activity (11). After inoculation, the culture dishes were gently swirled to disperse the cells and were placed in a humidified incubator (Bellco Glass, Inc., Vineland, NJ) at 37° C containing 5% CO_2:95% air. Within 4 hr, the inoculated cells adhered to the bottom of the culture dishes. The first change of medium was carried out at this time. Twenty hours later, and subsequently, every 24 hr, the medium was changed in order to remove loosely attached cells, cell debris, and potentially toxic metabolic products.

Morphological examination. Monolayer cells were examined by light and electron microscopy and photographs were taken at 4, 24, 48, and 72 hr postinoculation. For electron microscopy, monolayer cells in culture dishes were fixed with 4% glutaraldehyde in phosphate buffered 0.154 *M* NaCl, pH 7.4, at room temperature for 30 min and postfixed at 4° C for 30 min in 1.0% osmium tetraoxide. After fixation, cells were dehydrated in graded ethanol and propylene oxide. Cells were scraped and embedded in a capsule with resin. Ultrathin sections were prepared with a Porter-Blum Ultramicrotome MTI (Ivan Sorvall, Newton, CT) and stained with uranyl acetate and Karnovsky's lead hydroxide. These samples were examined in a Hitachi HU-11B electron microscope (Hitachi Ltd., Tokyo, Japan).

Gluconeogenesis. Gluconeogenesis of cultured hepatocytes was studied in rat liver cells at 24 and 48 hr postinoculation. After the cells were rinsed with Krebs-Henseleit bicarbonate buffer (pH 7.4) three times, they were incubated for 2 hr in 3.0 ml of Krebs-Henseleit bicarbonate buffer con-

taining 2.5 m*M* calcium chloride with or without 10 m*M* L-lactate (lithium salt, Calbiochem, San Diego, CA) and 1 m*M* pyruvate (Sigma), with or without 2.6 μM glucagon (crystalline porcine glucagon, Lilly Research Laboratories, Indianapolis, IN). The culture dishes were gently shaken on a rocker platform (Bellco Glass, Inc.) under 5% CO_2:95% air at 37° C. At 0, 1, and 2 hr a 300-μl aliquot of medium was removed from each dish and measured for glucose by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim Biochemicals, New York, NY) (12). Cells were removed from the dishes by scraping with a rubber policeman in 1.5 ml of Krebs-Henseleit solution, 4° C, and cell protein was measured by the method of Lowry et al. (13). Gluconeogenesis was calculated as nanomoles of glucose per milligram cell protein.

Induction of tyrosine aminotransferase (TAT) by dexamethasone. Monolayer liver cells at variable periods after inoculation (24, 48, and 72 hr) were washed two times with fresh HI/WO/BA medium to remove cell debris and loosely attached cells. The cells were then incubated in HI/WO/BA medium with or without 10^{-5} *M* dexamethasone (Merck Sharp & Dohme Research Laboratories, West Point, PA) for 6 hr at 37° C. After incubation, the cells were washed two times with 0.125 *M* phosphate buffer (pH 7.6, 4° C) containing 10^{-3} *M* sodium ethylenediaminetetraacetate (EDTA) (Sigma) and 10^{-3} *M* dithiothreitol (Sigma). The cells were then scraped with a rubber policeman and suspended in 1.5 ml of the phosphate buffer. Scraped cells were sonicated by a Biosonik microprobe (Bronwill Scientific, Rochester, NY) at a probe intensity of 50, for 15 s. This sonicated sample was directly assayed for TAT activity by the method of Spencer and Gelehrter (14) and for cellular protein by the method of Lowry et al. (13). Enzyme specific activity was expressed as milliunits per milligram cell protein. One milliunit of enzyme activity is that quantity of enzyme that catalyzes the formation of 1 nmole *p*-hydroxyphenylpyruvate/min at 37° C.

Effect of dexamethasone on ^{14}C-aminoisobutyric acid (AIB) uptake. After rinsing two times with fresh HI/WO/BA medium, the monolayer cells were incubated in 2.5 ml of HI/WO/BA medium containing 0.15 μCi α-[1-^{14}C]-AIB (New England Nuclear Corp., Boston, MA) and 1.5 m*M* native AIB (Sigma). After incubation, the medium was removed and cells were rinsed immediately with 0.125 m*M* phosphate buffer, 4° C. Cells were digested by adding 2.0 ml of 0.2 *N* NaOH, scraped with a rubber policeman, transferred to a counting vial and mixed with a universal liquid scintillation fluid, Instagel (Packard Instrument Co., Downers Grove, IL). Radioactivity in both cells and medium was counted on a liquid scintillation counter (Packard). Cell protein was measured by the method of Lowry et al. (13). To determine the AIB distribution ratio, the intracellular water space was determined by the method of Kletzien et al. (15). The cells were incubated with 0.1 μCi of 3-*O*-methyl [^{14}C]-D-glucose (New England Nuclear Corp.) and 10 m*M* unlabeled methylglucose (Aldrich Chemical Co., Milwaukee, WI) for 2 hr in 2.5 ml of Krebs-Henseleit bicarbonate buffer. An equilibrium between the cells and the medium was achieved after 30 min. Intracellular water space, calculated from the average of values at 30, 60, and 120 min, was 2.455 μl/mg cell protein. The AIB uptake was expressed as the distribution ratio of disintegrations per min per milliliter intracellular fluid to disintegrations per minute per milliliter extracellular fluid.

Preparation of antiserum to rat albumin. Rat serum albumin (Fraction V, fatty acid free, Sigma) was mixed with Freund's complete adjuvant (Difco Laboratories, Detroit, MI). Final concentration of albumin as antigen was 5 mg/ml. One milliliter of this mixture was injected i.p. and s.c. into white male New Zealand rabbits once a week for 3 or 4 weeks. Blood was withdrawn directly from the heart with the rabbit under ketamine anesthesia (40 mg/kg, Ketaset, Bristol Co., Syracuse, NY). The purity of rabbit antirat albumin serum was examined by immunodiffusion and immunoelectrophoresis. Immunoprecipitin lines between bovine albumin and rat albumin to rabbit antirat albumin serum were observed. In order to avoid the cross-reaction with bovine albumin in HI/WO/BA, bovine albumin (Pentex, Fraction V, fatty acid free, Miles Laboratories, Elkart, IN) was mixed with the antiserum and centrifuged. The supernatant was used as a monospecific antiserum against rat albumin for the quantitative albumin assay.

Quantitative immunoelectrophoresis assay for albumin. The methods of Laurell (16) and Curry et al. (17) were employed. Agarose HAA, 2.5% (Accurate Chemical & Scientific Corp., Hicksville, NY), was dissolved in 0.05 *M* barbital buffer (pH 8.4, ionic strength 0.07) in a boiling water bath and then cooled to 62° C. Diluted antiserum

was mixed with the agarose solution (final concentration of antiserum was 1:40 or 1:60). The agarose antiserum mixture was poured into a mold, which was formed by two glass plates, U-Frame, and strip of Cronar Graphic Arts Film (Dupont Instruments, Wilmington, DE). Ten microliters of the appropriate rat albumin standard solutions (0.5 μg/ml to 25 μg/ml) or samples was applied to holes (4-mm diameter) in the agarose gel with a microdispenser (Scientific Manufacturing Industries, Emeryville, CA). The agarose plate was used within 1 week after preparation. The electrophoresis was run with 10 V/cm width for 5 hr with the electrophoresis tray and buffer maintained at 15° C. After electrophoresis, the agarose plate was placed in 0.154 *M* NaCl for 3 hr, dried, and stained with Coomassie Brilliant Blue solution for 20 to 30 min followed by destaining of the background with 95% ethanol. The rocket height (the distance between the center of the well and the peak of the immunoprecipitate) was measured. There was a linear relationship between the amount of albumin and the height of the rocket in which the regression line was $y = 1.040X + 3.599$; $r = 0.999$. By this immunochemical assay, a minimum of 5 ng of albumin was measurable in the sample.

Effect of cycloheximide and dexamethasone on albumin synthesis by liver parenchymal cells in monolayer culture. Monolayer cells were incubated in HI/WO/BA medium with or without 10^{-5} *M* dexamethasone or with cycloheximide (20 μg/ml). After 0, 1, 3, 6, 12, and 24 hr, the medium from each dish was removed and centrifuged at 1500 rpm for 15 min to remove cell debris. The supernatants were used for measurement of albumin synthesis. Cells were removed with a rubber policeman and suspended in 1.5 ml Krebs-Henseleit bicarbonate buffer. The suspension was sonicated and analyzed for protein. Albumin synthesis was measured following incubation of the cultured cells in the presence of various concentrations of dexamethasone (10^{-7} - 10^{-3} *M*). Units of albumin synthesis were expressed as micrograms albumin per milligram cell protein per 24 hr or micrograms albumin per milligram cell protein at the indicated time.

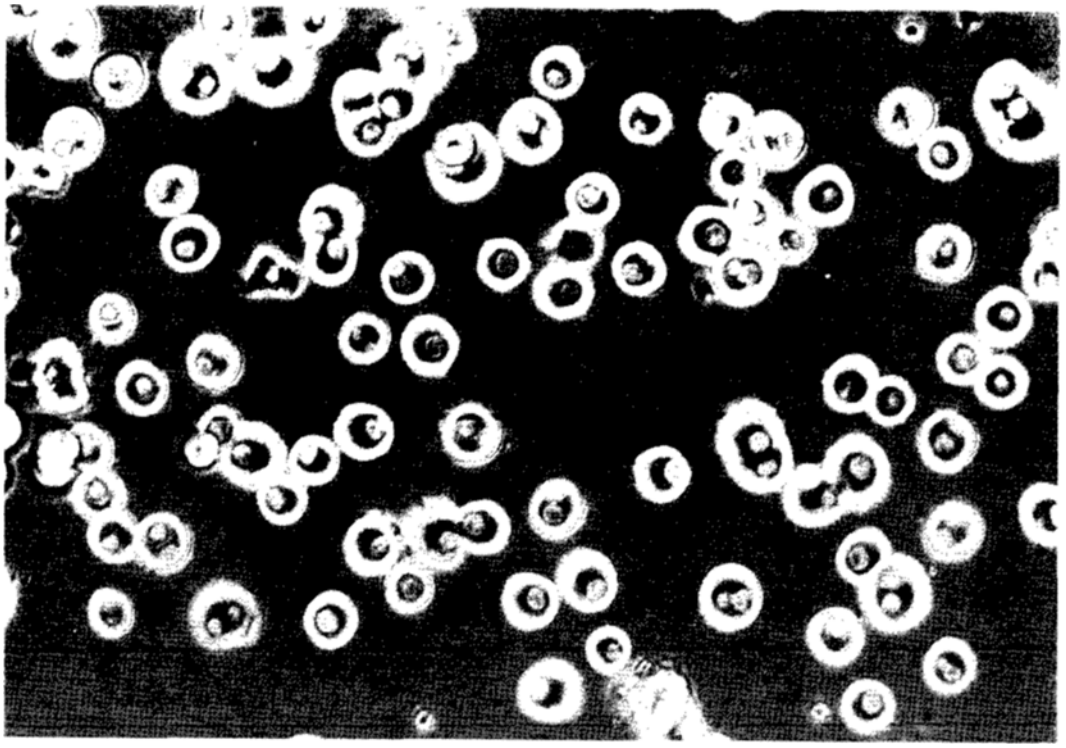

FIG. 1. Primary rat liver cell culture 4 hr postinoculation. The cells were round and had bright halos. Phase contrast. ×170.

RESULTS

Culture medium. Before beginning the present study on effects of dexamethasone, we compared the morphological changes of cells in three different media, widely used for primary liver cell culture: Leibovitz-15 (18), Ham's F-12 (19), and HI/WO/BA (20). The Leibovitz-15 and Ham's F-12 media were supplemented with fetal calf serum for the initial 24 hr postinoculation according to the method of Lin and Snodgrass (10). In Ham's F-12 medium, low plating efficiency, poor monolayer formation, and poor maintenance of cell structure were noted. Cells maintained in HI/WO/BA medium were superior to those in Leibovitz-15 as measured by the following metabolic functions: TAT induction by dexamethasone, AIB transport, and albumin synthesis (*data not shown*). Morphological appearance was also better in HI/WO/BA than in Leibovitz-15. Accordingly, HI/WO/BA was the medium selected for our studies.

Morphology. Phase contrast photographs of cells 4 hr after inoculation revealed absence of significant monolayer formation. The cells were adherent to the culture dishes but the shape of the cells was still round with bright halos, as in freshly isolated cells (Fig. 1). After 24 hr in culture, most cells were flattened and cuboidal and had formed a monolayer (Fig. 2). Binucleate cells were also observed, as is associated with normal liver parenchymal cells. Straight borders between cells were clearly recognized. Cell morphology showed no appreciable change until after 72 hr or more, when cell borders were becoming poorly defined and vacuoles and granules were increasing in the cytoplasm. Even with these morphological changes, most cells (approximately 95%) still excluded Trypan Blue dye, indicating minimal cell membrane damage.

Electron microscopy (Fig. 3). At 24 hr postinoculation intact parenchymal cells were approximately 90 to 95% of total cells. Kupffer cells were 1.0% or less. Desmosomes and bile canaliculi were observed in the intercellular spaces. The cell membranes, cytoplasmic organelles, Golgi apparatus, and rough and smooth endoplasmic reticulum appeared identical to those of the normal adult rat liver cells. A noticeable difference

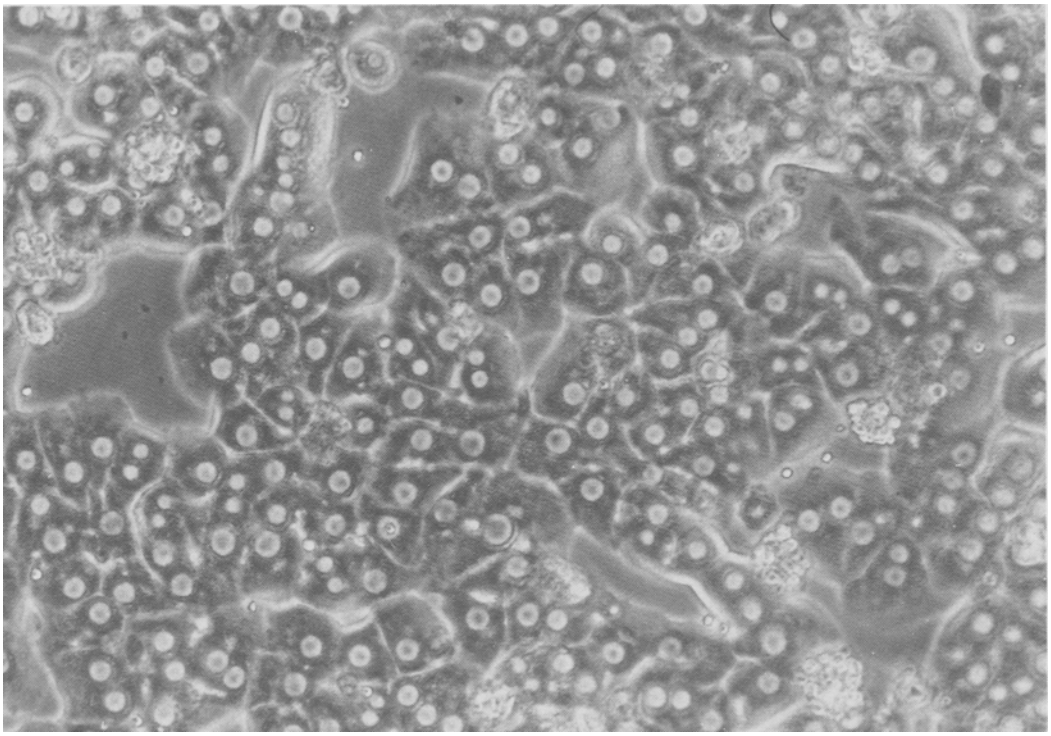

FIG. 2. Twenty-four hours postinoculation, the polygonal epithelial-like cells had formed a monolayer. Phase contrast. ×170.

between freshly isolated rat liver cells and the cultured cells was that the mitochondria were larger in the cultured cells. Most mitochondria were surrounded by rough endoplasmic reticulum. The matrix and the granules of mitochondria also appeared normal. Vacuoles were observed in 30% of cells 24 hr postinoculation. These vacuoles increased in number and size during the subsequent 48 hr in culture.

Gluconeogenesis by rat liver parenchymal cells at 24 and 48 hr postinoculation (Fig. 4). Glucose in the incubation medium was measured at 0, 1, and 2 hr of incubation. Cells that were incubated with 10 m*M* lactate, 1 m*M* pyruvate, and 2.6 μM glucagon gave the highest level of glucose production, which was 767 nmoles per mg cell protein per 2 hr. Glucose production from glycogen breakdown was 416 and 353 nmoles per mg cell protein per 2 hr in cells incubated with glucagon alone and with no substrate, respectively. After 48 hr in culture, the cells failed to show glucose production above the levels of glycogenolysis. The glucose level in these cells was approximately 250 nmoles per mg cell protein per 2 hr of incubation

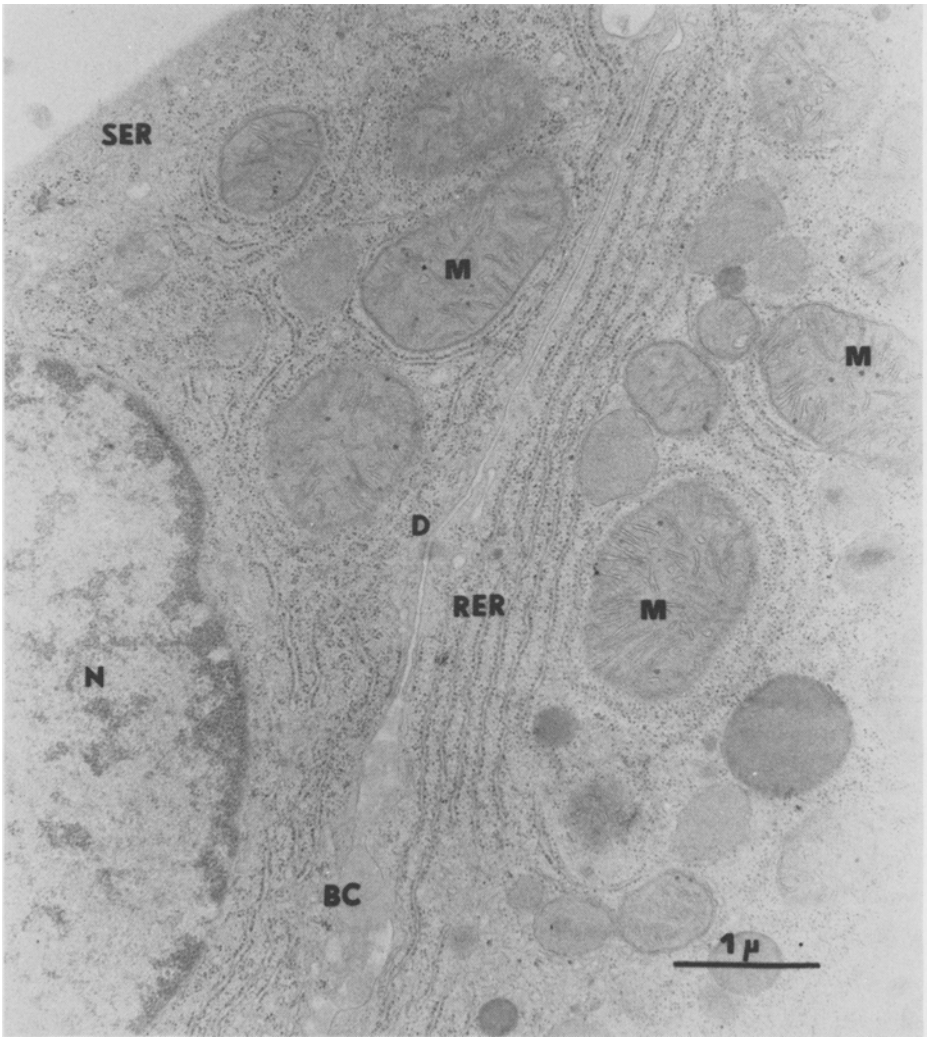

FIG. 3. Electron micrograph of liver cells in monolayer culture, 24 hr postinoculation. The fine structure and organelles of the monolayer cells were similar to those of normal rat liver cells. The enlarged mitochondria (M) were surrounded by the rough endoplasmic reticulum (RER), completely or partially. The desmosomes (D) and bile canaliculi (BC) were observed at intercellular junctions. N = nucleus; SER = smooth endoplasmic reticulum. ×25,410.

and was approximately 70% of the level in control cells 24 hr postinoculation. After incubation, cytosomal glucose was negligible, as determined by the method of Nakai et al. (7).

Induction of TAT by dexamethasone (Fig. 5). TAT activity levels in the resting state at 24, 48, and 72 hr postinoculation appeared relatively constant: 12.4, 15.6, and 14.2 mU/mg cell protein, respectively. After 6 hr of incubation with 10^{-5} *M* dexamethasone, TAT activity was increased to 43.8 mU/mg cell protein in cells 24 hr postinoculation. This level was approximately 4 times higher than in the resting state. This TAT induction by dexamethasone was also significant at 48 hr postinoculation. However, after 72 hr in culture, TAT induction by dexamethasone had decreased noticeably.

Effect of dexamethasone on AIB uptake. Figure 6 shows the time course of AIB uptake by liver cells in monolayer culture. The cells were incubated with or without 10^{-5} *M* dexamethasone over a 24-hr period. After 3 hr of incubation with AIB (starting 24 hr post-cell inoculation), the uptake of ^{14}C-AIB in both groups was linear. There were no remarkable differences between them at 3 hr. On the other hand, the AIB distribution ratio in cells treated with dexamethasone was 6.1 and 11.2 at 12 and 24 hr of incubation, respectively. Each value was 24% and 18% higher than

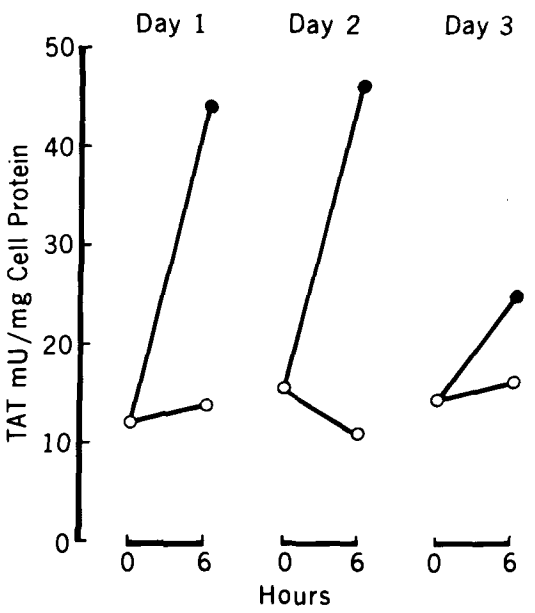

FIG. 5. Induction of tyrosine aminotransferase activity by dexamethasone in liver cells in monolayer culture is shown. The monolayer cells, 24, 48, and 72 hr postinoculation, were incubated for 6 hr in HI/WO/BA with 10^{-5} *M* dexamethasone (●——●) and without dexamethasone (○——○). Each value is the average of duplicate samples.

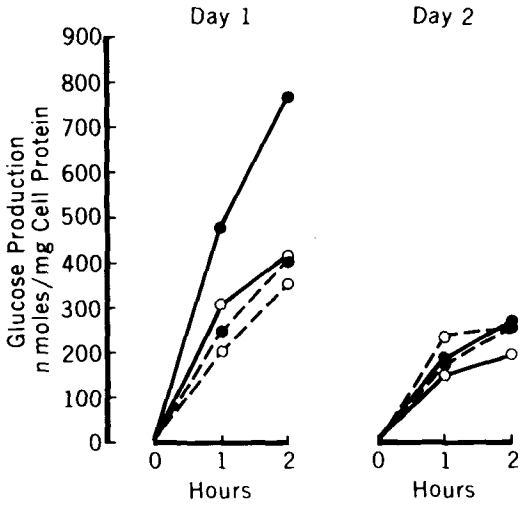

FIG. 4. Glucose production in cultured hepatocytes 24 and 48 hr postinoculation. Cells were incubated for 2 hr with lactate and pyruvate (●), without lactate and pyruvate (○), or with glucagon (——) and without glucagon (--). Glucose concentration in the medium was measured. Each point represents the average of duplicate samples.

the control. After 24 hr of incubation with increasing concentrations of dexamethasone (10^{-7} to 10^{-3} *M*), the distribution ratio increased markedly (Fig. 7). Dexamethasone accelerated AIB transport across the cell membrane. At the highest concentration of dexamethasone, 10^{-3} *M*, the distribution ratio was 13.0, which represented a 33% increase over the control.

Albumin synthesis. Albumin synthesis and secretion are shown in Fig. 8. Albumin synthesis and secretion by cells 24 to 48 hr postinoculation increased linearly with incubation time. The synthesis rate was 1.56 μg per mg cell protein per hr. Albumin synthesis was completely inhibited by the addition of cycloheximide (20 μg/ml). Addition of 10^{-5} *M* dexamethasone depressed albumin synthesis by approximately 10% as compared to control cells. Increasing concentrations of dexamethasone (10^{-7} *M* – 10^{-3} *M*) depressed the rate of albumin synthesis as shown in Fig. 9. In the presence of 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} *M* dexamethasone, albumin synthesis was 42.6 ± 3.1, 39.6 ± 3.8, 39.0 ± 1.9, and 37.0 ± 1.8 μg/mg cell protein (mean±SD), respectively. At 10^{-3} *M* dexamethasone, the highest concentration used,

the rate of albumin synthesis fell to 71% of the control.

DISCUSSION

Monolayer cultures of rat liver parenchymal cells provide a useful experimental model for the study of hepatic function. There are several advantages of cell culture that include: cell homogeneity, prolonged cell viability, and stable metabolic function for several days. Since Bissell et al. (8) and Bonney et al. (9,21) successfully studied adult rat liver cells in monolayer culture, several studies of rat liver cells in monolayer culture have been reported (22-24). There are still numerous questions on the exact metabolic activity of the liver parenchymal cells under the various culture conditions. We have studied further some of the metabolic functions of rat liver parenchymal cells and the effects of dexamethasone on these functions, in monolayer culture using a completely synthetic culture medium, HI/WO/BA.

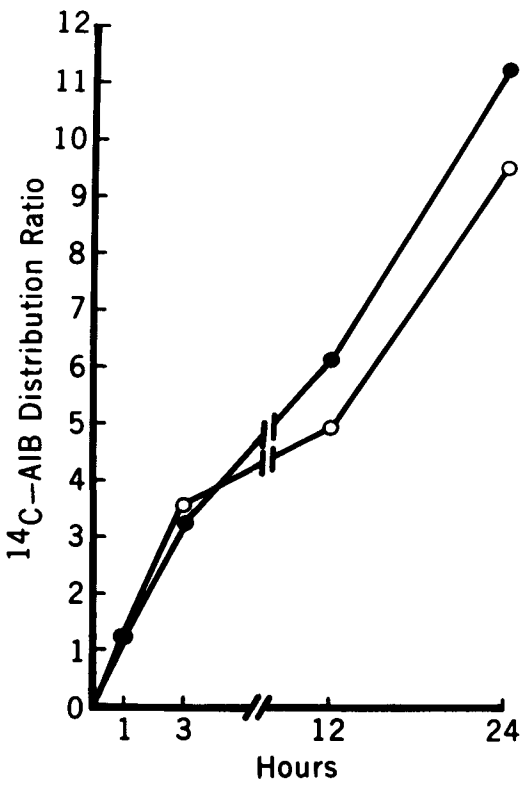

FIG. 6. Effect of dexamethasone on aminoisobutyrate (AIB) transport by rat liver monolayer cells in culture is shown. Cells, 24 hr postinoculation, were incubated in HI/WO/BA medium containing ^{14}C-AIB in the presence of (●——●) and in the absence of (○——○) of 10^{-5} *M* dexamethasone. The radioactivity in the cells and in the medium was counted at the times indicated. Results are expressed as a distribution ratio. Intracellular water space was calculated to be 2.455 μl/mg cell protein by using ^{14}C-methylglucose. Each point is the average of duplicate samples.

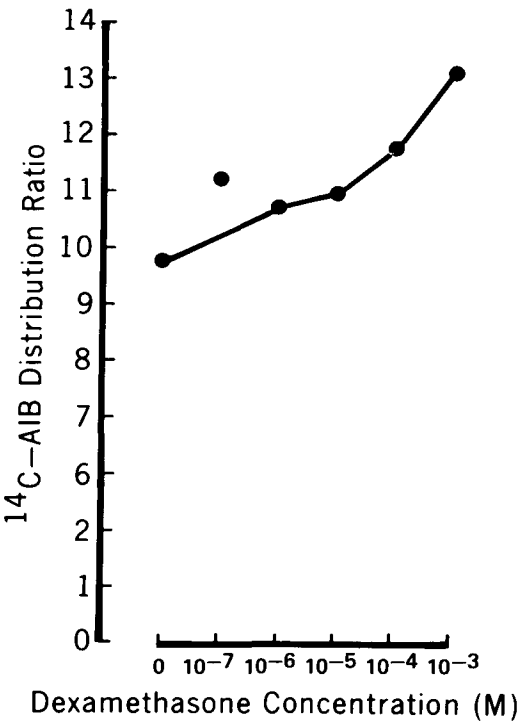

FIG. 7. Effect of various concentrations of dexamethasone on AIB transport by liver cells is shown. After 24 hr incubation in HI/WO/BA medium, cells were incubated with different concentrations of dexamethasone (10^{-7} *M* to 10^{-3} *M*) for 24 hr. As the dexamethasone increased in concentration, the distribution ratio of AIB also increased. Each ratio is the average of duplicate samples.

Morphologic study of cells 24 hr postinoculation. Cells, 24 hr postinoculation, formed excellent monolayers. On examination by electron microscopy, the desmosomes and bile canaliculi were observed in the intercellular space between adherent cells. These findings are the same as is found in normal rat liver cells in vivo. Also, the subcellular organelles were the same as those found in normal adult rat hepatocytes. In contrast, our liver cells in monolayer culture had enlarged mitochondria, although the fine structure and density of these mitochondria were like those of in vivo liver cells. This enlargement of the mitochondria became more pronounced with increasing time in culture, which is similar to that reported by Chapman et al. (25). The cause of enlarged mitochondria is considered to be the result

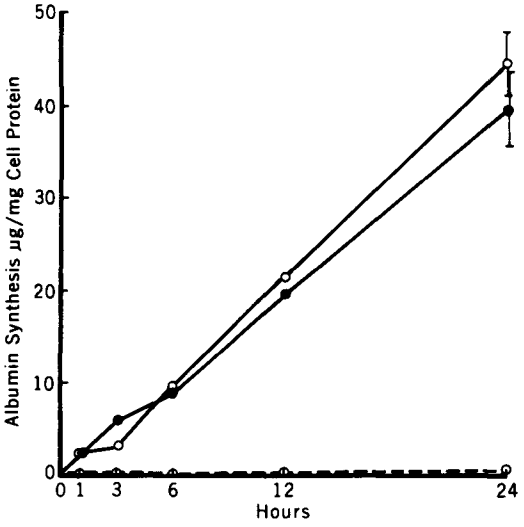

FIG. 8. Effect of dexamethasone and cycloheximide on synthesis and secretion of rat albumin in liver cells in monolayer culture is shown. Cells were incubated in HI/WO/BA medium with (● —— ●) or without 10^{-5} *M* dexamethasone (O—O) and with cycloheximide (20 µg/ml) (O---O). The synthesized and secreted albumin increased linearly with incubation time and was depressed by dexamethasone and inhibited by cycloheximide completely. Each point is the average of duplicate samples except for the 24-hr value which is the mean of six culture dishes ± SD.

of some nutritional deficiency in the culture system (26) or hypoxia (27). Another interesting observation was the presence of vacuoles in the cells in culture. Vacuole formation has been shown to be increased by hypoxia (27) and glycogenolysis (28). The high concentrations of glucose in HI/WO/BA medium may be responsible for maintaining the ultrastructure of the cells by inhibiting glycogenolysis.

Glucose production by cells 24 hr postinoculation. Glucose production is one of the principle functions of hepatocytes. Numerous publications have documented gluconeogenesis by hepatocytes (6,7,29). In our system, the hepatocytes were cultured in a high concentration of glucose (2.7×10^{-2} *M*) contained in HI/WO/BA medium. These cells can therefore be considered to be in a fed state. Glycogen synthesis has been reported to be increased in the presence of glucose in the culture medium (8). After incubation of cells with glucagon, pyruvate, and lactate, the greatest amount of glucose was observed. This was the result of gluconeogenesis from pyruvate and lactate, and from breakdown of glycogen (30). However, in cultured cells of 48 hr postinoculation, there were no remarkable differences in the presence or absence of glucagon and/or pyruvate and lactate. Gluconeogenesis from the substrate was not observed at this time period and glucose in the medium apparently was the result of glycogenolysis.

Effect of dexamethasone on TAT activity. Dexamethasone is known to induce specific hepatic enzymes such as TAT (31,32) and tryptophan pyrrolase (33,34). These liver enzymes are specifically induced by glucocorticoids while many other enzymes are not affected. The induction of these enzymes is apparently associated with newly synthesized protein and is inhibited by cycloheximide, puromycin, and actinomycin-D (35). TAT induction by dexamethasone was observed in

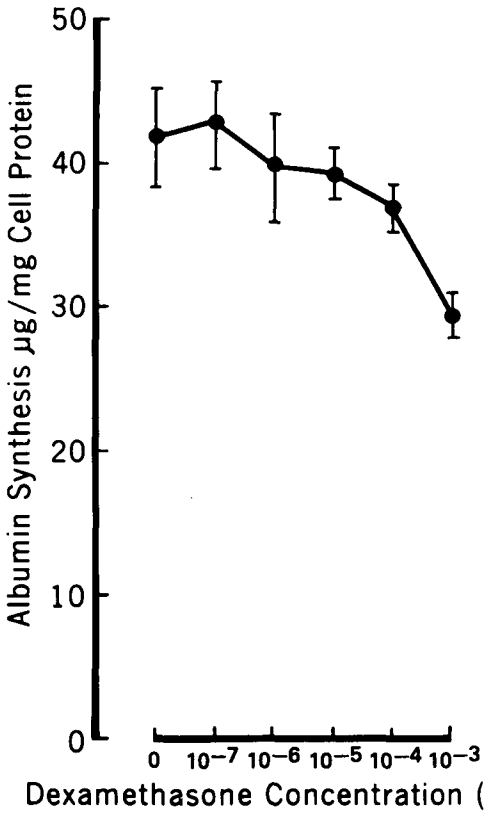

FIG. 9. Albumin synthesis by liver cells in monolayer culture at various concentrations of dexamethasone is shown. After 24 hr of culture in HI/WO/BA medium, the cells were incubated with various concentrations of dexamethasone (10^{-7} to 10^{-3} *M*). The entire amount of rat albumin in the culture medium was divided by total cell protein. Each point is the mean of five culture dishes ± SD. As the concentration of dexamethasone in the culture medium increased, the synthesis rate of albumin was depressed.

monolayer cells in culture in the completely synthetic HI/WO/BA medium even though fetal calf serum was not used. The level of TAT induction in our cells at 24 and 48 hr postinoculation was similar to other published data (40 to 50 mU/mg cell protein) (9). However, after 72 hr of culture, the enzyme level fell 50%. Therefore, using TAT as a marker of cell function and viability, it appears that, up to 72 hr postinoculation, cell functions associated with the production of this enzyme are functioning normally.

Effect of dexamethasone on AIB transport. In general, glucocorticoids are known to increase the uptake of amino acids and increase protein synthesis in liver. However, these steroids play a catabolic role in the metabolism of protein, glucose, and lipids in extrahepatic tissues. Much has been written on the effect of glucocorticoids on amino acid transport in liver in vivo (36) and in vitro (1,37). The administration of glucocorticoids in vivo appears to stimulate amino acid transport across cell membranes. The AIB uptake by the perfused liver has been shown to be increased in the presence of hydrocortisone (1). However, Kletzien et al. (38,39) could not find any remarkable differences in ^{14}C-AIB uptake by either dexamethasone treated or nontreated liver cells. Conversely, in HTC line cells, AIB uptake was depressed by dexamethasone (40). The distribution ratio of ^{14}C-AIB was 10 in our system after incubation for 24 hr. The HI/WO/BA medium was used as the incubation medium for the study of ^{14}C-AIB transport in cells to maintain cell viability for 24 hr. However, ^{14}C-AIB transport in cells was influenced by other amino acids contained in HI/WO/BA medium and this may account for the determination of a low ratio in our system as compared to other investigators (38). We also found no remarkable difference between dexamethasone-treated cells and nontreated cells for the initial 3 to 6 hr starting 24 hr postinoculation. However, after 12 to 24 hr of incubation, dexamethasone stimulated AIB transport in liver cells, and the magnitude of transport was dependent on the concentration of dexamethasone (Fig. 7).

Albumin synthesis. Albumin synthesis represents a major liver cell function (41). It is well known that albumin synthesis is stimulated by insulin (42,43,44), growth hormone (45), and testosterone (46,47). The effect of corticosteroids on albumin synthesis has been frequently reported (44). Administration of a single dose of cortisol has been shown to depress albumin synthesis, whereas repeated injections of this hormone have increased albumin synthesis and accelerated albumin catabolism (48). Gordon has demonstrated that albumin synthesis is depressed by cortisol in vivo (49) and Crane and Miller have shown albumin synthesis is depressed by cortisol in suspended rat liver cells (42). In clinical investigation studies, Lewis et al. (50) have reported that hypoalbuminemia is observed as a side effect in patients treated with prednisone. In Cushing's syndrome, low levels of serum albumin have been associated with high blood glucocorticoid levels (51). In monolayer cells treated with dexamethasone, synthesized and secreted albumin was depressed with dexamethasone. The depressed synthesis and secretion became apparent after 12 to 24 hr or more of incubation. Even if albumin is degraded by our cells, the major fraction of it is probably bovine albumin in the HI/WO/BA medium, since catabolism of newly synthesized albumin by liver cells is thought to be minimal. Therefore, we considered the effect of albumin degradation to be negligible. Glucocorticoids are well known to accelerate the uptake of amino acids, which are utilized to synthesize albumin and proteins or as substrates for gluconeogenesis. At 10^{-3} *M*, a high concentration of dexamethasone, AIB transport in cells is markedly stimulated. In contrast, at the same concentration of dexamethasone, albumin synthesis was depressed. A possible explanation for this may be that amino acids taken up may be deaminated to form urea or utilized for gluconeogenesis rather than being incorporated to albumin or other proteins (36).

Conclusions. We have shown that dexamethasone has several effects on the metabolism of liver cells in culture including induction of TAT activity, stimulation of AIB transport, and depression of albumin synthesis. There appears to be no added direct effect on cell viability. However, it has been reported that dexamethasone has some effect on maintaining cell structure (23). The effect of dexamethasone is slow and it does not appear to play a role as a single controlling hormone. Possibly it functions in a cooperative and coordinating role in the regulation of cell metabolism.

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