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TROUT HEPATOCYTE CULTURE: ISOLATION AND PRIMARY CULTURE

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SUMMARY

Rainbow trout (*Salmo gairdneri*) hepatocytes were isolated using a two-step perfusion through the portal vein. A typical perfusion yielded 2.92×10^6 liver cells with a mean viability of 96.3%. Hepatocytes comprised 93.4% of the total cell isolate. Survival of hepatocytes in suspension culture was dependent on fetal bovine serum concentration and temperature of incubation. Serum concentrations of 5, 10, and 20% produced the highest survival during primary culture. Hepatocyte survival was in inverse proportion to the incubation temperature. Trout hepatocyte DNA synthesis and mitosis decreased during the culture period. Cytochrome p_{450} activity decreased rapidly during the first 2 d of culture and then remained low but measurable during the remaining 8 d of culture. Culture temperature also influenced the p_{450} activity with lower temperatures producing greater activity. Morphologic changes occurred in the cells during culture. Isolated hepatocytes self-aggregated, forming strands and clumps that increased in size with time in culture. Junctional complexes between cells were evident within the aggregates. Nuclear atypia, increases in size and number of autophagic vacuoles, and the appearance of bundles of intermediate filaments also were observed with increased time in culture.

Key words: trout; hepatocyte; culture; teleost; liver.

INTRODUCTION

The liver is an important site of primary tumor formation in rodents (19) and teleosts (7,16,17) after exposure to a variety of chemical carcinogens, thus making it a valuable organ for interspecies and intraspecies comparative studies of carcinogen activation, detoxification, and macromolecular interaction. Rodent hepatocytes in primary culture have been used extensively for studies of toxicity and carcinogenicity (3,18). These cells maintain many of the functional and morphologic properties of in vivo hepatocytes, therefore providing an attractive in vitro model for the investigation of carcinogen-hepatocyte interaction. Recently, liver cells have also been isolated from several teleost species and have been used for studies involving gluconeogenesis (5,20), sterol lipogenesis (6), cortisol uptake (14), aflatoxin B_1 metabolism (1), and protein synthesis (4,15) in the teleost liver. The liver cells in these reports have been maintained for short periods of time (24 h or less). Little work, however, has been directed toward the characterization of isolation and short-term primary culture of teleost hepatocytes. The purpose of the present study was to develop methods for the isolation and primary culture of rainbow trout hepatocytes and to characterize the functional and morphologic properties of the hepatocytes during the isolation and culture period.

MATERIALS AND METHODS

Animals. Male rainbow trout (*Salmo gairdneri*) weighing 150 to 200 g each were supplied by Castalia Trout Farms (Castalia, OH). Fish were maintained in living stream aquaria (Frigid Units, Inc., Toledo, OH) at 15° C for 2 wk before hepatocyte isolation. Fish were fed Purina trout chow during this holding period.

Hepatocyte isolation. Trout hepatocytes were isolated using a modification of the two-step hepatic portal perfusion method previously described for rodents (9,21) and modified for fish (12). Fish were anesthesized with tricaine methanesulfonate (MS-222) in dechlorinated water and placed ventral surface upward on a surgical board. A segment of tissue overlying the heart was removed exposing the heart and the ventral aorta. A second incision along the linea alba was made from the pectoral region to the urogenital pore exposing the abdominal viscera. A branch of the hepatic portal vein was located and cannulated with a plastic cannula secured by two ligatures of suture. Perfusion with Hanks' Ca^{++} and Mg^{++}-free salt solution containing 0.5 mm [ethyleneglycol-bis-amino ethyl ether] *N,N'*-tetraacetic acid (EGTA) and 0.01 *M* *N*-2-hydroxethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) buffer (pH 7.3) (20° C) at a perfusion rate of 10 ml/min was begun. After the liver cleared of blood and began to swell, an incision was made in the ven-

tral aorta 0.5 cm anterior to the bulbus arterious to provide an outflow for the perfusate. Perfusion with Hanks' solution continued for 10 min followed by perfusion with a collagenase solution [100 U collagenase/ml Leibowitz's L15 medium (pH 7.3); Sigma (St. Louis, MO) Type IV collagenase, 265 U/mg]. Perfusion with collagenase continued for 20 to 25 min (20° C) at a rate of 10 ml/min. On completion of the perfusion, the liver was carefully excised from the fish and placed in a 100 × 20-mm sterile dish (containing 10 ml of collagenase solution at 20° C). The gall bladder was removed and liver cells were dissociated by gentle combing of the liver with a stainless steel comb. After complete combing, the cells were further dissociated by repeated pipetting (10×), passed through sterile nylon mesh (TETKO, Nitex HC3-253) into a 50 ml centrifuge tube, and pelleted at 100 ×*g* for 5 min (10° C). The resulting pellet was resuspended in 40 ml of L15 medium containing 10% fetal bovine serum (FBS) and gentamicin (25 μg/ml). An aliquot of the cell suspension was counted on a hemocytometer. Trypan blue dye (0.4% in saline) was used to assess viability. The total number of cells and percentage of viable cells (trypan blue dye excluding) were calculated for each liver cell isolation.

Cell survival. Teleost hepatocytes failed to attach firmly to a plastic tissue culture substrate. Attempts to enhance attachment using various culture media, sources and concentrations of serum, and types of culture substrate were unsuccessful (12). Approximately one-half of plated, freshly isolated trout hepatocytes formed a weak attachment to plastic substrate but remained rounded and failed to flatten onto the culture surface with time. These weakly attached hepatocytes readily dislodged from the culture surface when the culture vessel was moved and during medium changes. Preliminary studies showed, however, that trout hepatocytes remained viable in suspension culture. Therefore, subsequent survival, functional, and morphological studies were performed on trout hepatocytes in suspension culture.

The effect of medium serum concentration and the temperature of incubation on hepatocyte survival was examined in an effort to define optimal culture conditions for these cells. For the serum experiments, five concentrations of FBS (20, 10, 5, 1, or 0%) in L15 culture medium (pH 7.3) containing 1 mg/ml glucose, dexamethasone (1 μ*M*), and gentamicin (25 μg/ml) were used. Freshly isolated trout hepatocytes (1 × 10^6 viable cells/dish) were added to 60 × 15-mm glass culture dishes in 5 ml of medium containing one of the five serum concentrations. Cell survival was determined after 1, 2, 4, 6, 8, 10, and 14 d of culture. Medium was changed 1, 2, and every 2 d thereafter. To change the medium, cells were pipetted from the culture dish into a 15-ml centrifuge tube and centrifuged for 5 min at 100 ×*g* (10° C). The supernatant fluid was discarded and the resulting pellet was resuspended in 5 ml of fresh medium (containing the same serum concentration). This suspension was replated onto the original dish. Care was taken to ensure that all cells were removed from the dish. To determine the surviving cell number, randomly selected cultures were removed and the cells pipetted into a 15-ml centrifuge tube. Each dish was washed three times with serum-free medium to ensure removal of all cells from the dish, and the washes were combined with the original medium. In the centrifuge tube, cells were treated briefly (5 min) with trypsin (0.1% in Hanks' Ca^{++}, Mg^{++}-free salt solution) at 10° C to break up clumps of cells into single cells and centrifuged at 100 ×*g* for 5 min (10° C). (This brief trypsin treatment had no apparent effect on cell viability). The cell pellet was resuspended in 10 ml of fresh, serum-free L15 medium, and five aliquots were counted on a hemocytometer using trypan blue dye exclusion as the indication for viability.

The effects of temperature on cell survival were also studied. Trout liver cells were cultured using techniques described above in L15 medium containing 5% FBS, 1 mg/ml glucose, dexamethasone (1 μ*M*), and gentamicin. Cells were cultured at either 37, 30, 25, 20, 15, 10, or 4° C. Cell survival was determined after 1, 2, 4, 6, 8, 10, and 14 d of culture. Medium was changed after 1, 2, and every 2 d thereafter as described above.

DNA synthesis. The percentage of trout hepatocytes undergoing DNA synthesis during primary culture was evaluated using autoradiography. Freshly isolated trout hepatocytes were plated (1 × 10^6 viable cells/dish) on 60 × 55-mm glass culture dishes in 5 ml of complete L15 medium (supplemented with 5% FBS) and incubated at 20° C in a humidified incubator. Medium was changed after 2 d and every 2 d thereafter. Triplicate cultures were treated with 10 μCi/ml of tritiated thymidine (50 Ci/m*M*) for 4 h before sampling at 0 time, 1, 2, 4, 6, 8, and 14 d of culture. Sampling was performed before that day's medium change. At sampling, cells were transferred to sterile glass test tubes, centrifuged at 100 ×*g* for 5 min, and washed three times (10 min each) with L15 to remove unbound thymidine. The final pellet was resuspended in 2 ml of phosphate buffered saline (PBS) and pelleted onto three clean glass slides with a cytocentrifuge. The cells were fixed by immersion in acetic acid:ethanol (1:3), air dried, and dipped in NTB-2 autoradiographic emulsion (Eastman Kodak, Rochester, NY). Autoradiographs were exposed for 2 wk at 4° C in black boxes, developed with D-19, and stained with hematoxylin and eosin. The percentage of cells undergoing DNA synthesis (as evidenced by 25 or more grains per nucleus) was determined by counting 500 cells at random per slide. Results were based on liver cell isolations from three trout.

Mitotic index. The mitotic index of trout hepatocytes during primary culture was also studied. Freshly isolated hepatocytes (1 × 10^6 viable cells) were plated onto 60 × 15-mm culture dishes into 5 ml of L15 medium (with 5% FBS). Cells were cultured at 20° C in a 100% air incubator. Triplicate cultures were sampled after 1, 2, 4, 6, 8, 10, and 14 d of culture. Four hours before sampling, 0.25 μg/ml colchicine was added to the culture medium. At sampling, cells were collected on glass slides by cytocentrifugation, stained with hematoxylin and eosin, and the number of cells in metaphase was determined. A total of 1000 cells were counted at random for each sampling period. Hepatocytes from three trout isolations were used.

TABLE 1

TROUT LIVER CELL ISOLATION[a]

Number of Isolations	Total Number of Cells Isolated per Gram Body Weight, $\times 10^6$	Total Viability, %	Total Number of Viable Cells Isolated per Gram Body Weight, $\times 10^6$	Total Number of Hepatocytes Isolated per Gram Body Weight, $\times 10^6$
40	2.92 ± 0.42	96.3	2.81 ± 0.29	2.62 ± 0.45

[a]Values represent the mean $\pm$ 1 SD.

Cell morphology. Trout cells were examined at isolation and at 1, 2, 4, 6, 8, and 14 d of culture by phase contrast microscopy, light microscopy (LM), and transmission electron microscopy (TEM). Living cells in the culture dish were examined with an inverted phase contrast microscope. For LM and TEM, cells were fixed with 3% glutaraldehyde in 0.2 *M* cacodylate buffer (pH 7.4) for 4 h, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812. Representative semithin plastic sections (0.5-μm thick) were cut and stained with toluidine blue dye for LM. Thin sections were cut on an LKB-3 ultramicrotome and stained with uranyl acetate and lead citrate for TEM. Thin sections were examined and photographed on a Zeiss 9 electron microscope.

Cytochrome P_{450} determination. Trout hepatocytes were assayed for cytochrome p_{450} using methods previously developed for teleost liver (8). Freshly isolated trout hepatocytes were plated (1×10^6 viable cells) onto 60×15 mm-culture dishes containing 5 ml of L15 medium (5% FBS). Cells were incubated at either 25, 20, or 15° C. Medium was changed as described earlier. Triplicate dishes were sampled immediately after isolation and after 1, 2, 4, 6, and 8 d of culture, pooled, and homogenized in cold (4° C) 0.5 *M* Tris-1.15% KCl buffer (pH 7.4). Homogenates were stored at − 80° C until cytochrome determination. Hepatocytes from three isolations were used for cytochrome determinations. DNA was assayed on the homogenate using the method of Burton (2). Data were analyzed using Student's *t*-test.

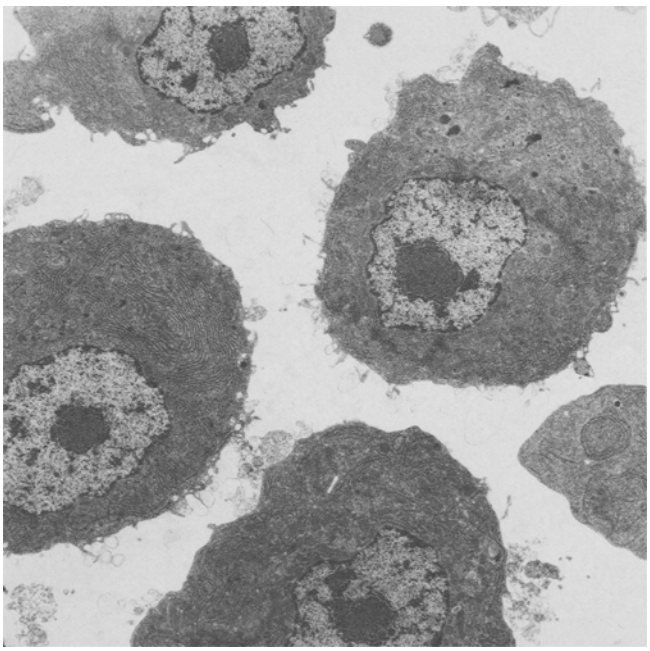

FIG. 1. Transmission electron micrograph of trout hepatocytes immediately after isolation. ×3330.

RESULTS

Forty trout liver cell isolations performed using the two-step perfusion method yielded a mean of 2.92×10^6 total liver cells/g body weight with a mean viability of 96.3% (Table 1). The percentage of hepatocytes in the isolate was determined by counting the number of hepatocytes and nonhepatocytes at random in LM preparations of the liver cell isolate. In 5000 randomly counted cells from five isolations, hepatocytes represented 93.4% of the cells isolated, with the remaining 6.6% consisting of exocrine pancreatic, endothelial, and other, unidentifiable cells.

Freshly isolated hepatocytes were usually round and individual and displayed a morphology consistent with that of the in vivo trout hepatocyte (Fig. 1). The majority of cells contained a single, centrally located, round nucleus within a cytoplasm dominated by parallel stacks of rough endoplastic reticulum and oval mitochrondria. The plasma membrane formed numerous cytoplasmic projections with occasional blebbing.

Survival. The survival of trout hepatocytes in various medium serum concentrations during the first 14 d of primary culture (at 20° C) is shown in Fig. 2. Survival of hepatocytes cultured in all serum concentrations examined showed a progressive decrease during the first 14 d of culture. Cells maintained in serum-free medium displayed the greatest decrease in survival, dropping to 75.7 and 64.2% after 1 and 2 d, respectively. After 14 d of culture only 4.8% of the originally plated viable cells remained in the serum-free medium. Cells grown in 5, 10, and 20% FBS containing L15 medium displayed no

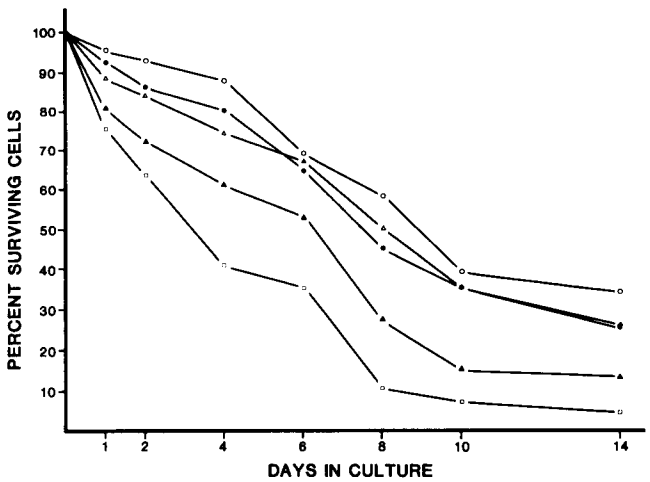

FIG. 2. Effect of serum concentration in L15 medium on trout hepatocyte survival in primary culture (— ● — 20% FBS; — ○ — 10% FBS, — △ — 5% FBS; — ▲ — 1% FBS; — □ — 0% FBS).

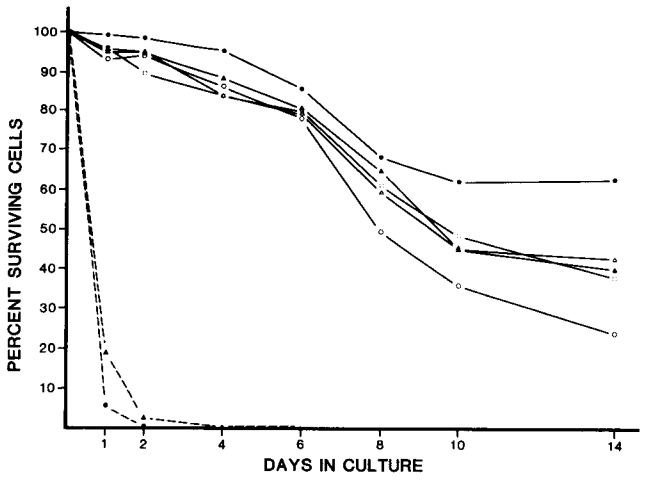

FIG. 3. Effect of temperature on trout hepatocyte survival in primary culture (--●-- 36.5° C; --▲-- 30° C; —○— 25° C; —△— 20° C; —▲— 15° C; —□— 10° C; —●— 4° C).

statistical difference ($P < 0.05$) in survival during primary culture. Survival for trout hepatocytes decreased to 75.4% in 5% FBS, 88.3% in 10% FBS, and 80.4% in 20% FBS after 4 d of culture. By 8 d of culture, hepatocytes in 10% FBS displayed a 58.6% survival rate whereas for those in 20% FBS the rate was 45.2%. Cells grown in 1% FBS showed a survival pattern intermediate to that of higher serum concentrations and that of serum-free medium during the first 8 d of culture. Based on the above results it was concluded that 5% FBS was optimal (greatest survival at the lowest serum concentration) for maintenance of trout hepatocytes in suspension culture.

The effect of temperature on trout hepatocyte survival is shown in Fig. 3. Cells maintained at 30, and 37° C displayed a rapid decrease in viability by 1 d of culture. Only 5.6% of cells maintained at 37° C, and 19.5% of cells at 30° C survived after 1 d. By Day 2 of culture the surviving fraction decreased to 2.8% at 30° C and 0.8% at 37° C. Few cells survived at these temperatures after the 2 d sampling. Cells maintained at lower incubation temperatures displayed survival patterns inversely proportional to the temperature of incubation. No significant difference ($P<0.01$) was found in survival at temperatures of 10, 15, 20, and 25° C during the first 6 d of culture. Survival of cells at 10, 15 and 20° C continued to show no significant difference at sampling on Days 8, 10, and 14. Cells maintained at 25° C however, displayed a significant ($P<0.05$) decrease in survival after Days 8, 10, and 14 in comparison to the lower incubation temperatures. At 14 d, mean cell survival at 4, 10, 15, 20, and 25° C was 63.4, 40.2, 43.2, and 23.7%, respectively.

TABLE 2

DNA SYNTHESIS AND CELL DIVISION IN CULTURED TROUT HEPATOCYTES[a]

	Time in Culture						
	4 h	1 d	2 d	4 d	6 d	8 d	14 d
S-labeled cells, %	2.63	0.56	0.23	0.10	0.08	0.06	0.07
Mitotic index, %	1.07	0.13	0.07	0.05	0.05	0.01	0.01

[a]Values represent the mean of 1000 cells counted per sampling time for each trout liver isolation. A total of three isolations were performed.

The percentage of trout hepatocytes undergoing DNA synthesis and cell division are shown in Table 2. The highest percentages of both S-phase labeled cells and metaphase nuclei occurred immediately on placement in culture. Both parameters showed a decrease with increased time in culture. After 8 d of culture few S-phase labeled cells (less than 0.1%) and colchicine-arrested metaphase nuclei (0.01%) were observed.

Figure 4 illustrates cytochrome p_{450} activity in trout hepatocytes during the first 8 d of culture at incubation temperatures of 15, 20, and 25° C. At all temperatures, cytochrome p_{450} activity decreased during the first 2 d of culture from that found in the initially isolated cells. Enzyme activity remained constant during the subsequent sampling times. An effect of incubation temperature on p_{450} activity was observed, with highest activity found at 15° C and lowest at 25° C.

Phase contrast microscopy. Trout liver cells during the first 4 h of culture appeared as single round cells that settled to the bottom of the culture dish. Approximately 50% of the cells formed a weak attachment to the plastic culture dish during the first day of culture (Fig. 5). These attached cells maintained their rounded appearance and were easily dislodged when the dish was shaken gently. No difference in viability was seen in attached versus floating cells. After 2 d in culture the majority of cells were aggregated in small clumps and strands up to 100 cells in size (Fig. 6). These aggregates were usually 1 to 3 cells in thickness and 10 to 40 cells in length. With progressive time in culture the length and width of the strands increased in size until after 8 d grossly visible aggregates of cells could be observed in the culture dish. By 14 d of culture some of the clumps were 5 mm in diam-

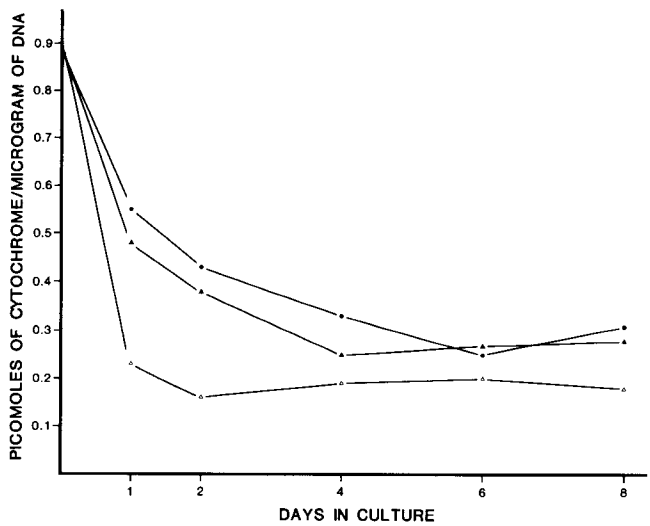

FIG. 4. Cytochrome p_{450} activity in trout hepatocytes during primary culture (— ● — 15° C; — ▲ — 20° C; — △ — 25° C).

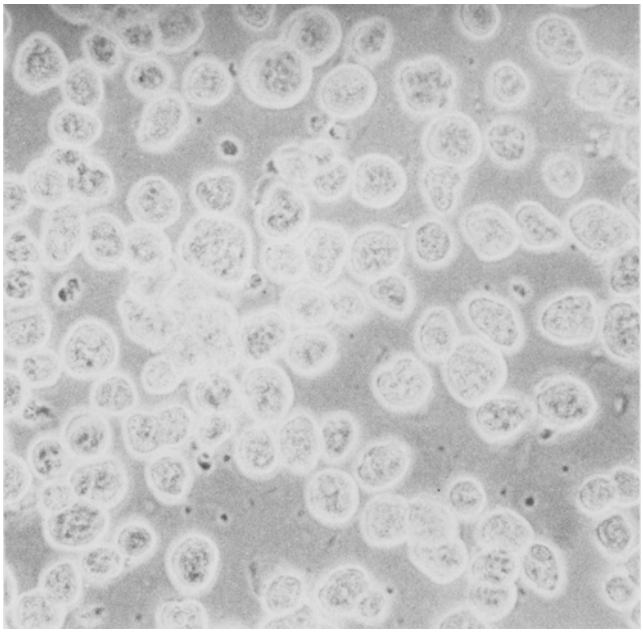

FIG. 5. Phase contrast micrograph of trout hepatocytes after 1 d of culture. ×555.

eter (Fig. 7). The presence of serum in the culture medium seemed to affect the ability of the cells to aggregate. Cells grown in serum containing medium formed larger aggregates than those in serumless medium. Cells cultured at 10, 15, 20, and 25° C displayed no apparent differences in cell morphology. However, hepatocytes cultured at 4° C failed to form the large cellular aggregates characteristic of higher culture temperatures, instead forming small clumps, 10 to 20 cells in size. Cells cultured at 30 and 36.5° C failed to aggregate, and displayed extensive plasma membrane blebbing.

Light and electron microscopy. Light and electron microscope examination of trout hepatocytes after 1 d of culture revealed individual cells with a centrally located nucleus containing a single nucleolus (Fig. 1). Figure 8 illustrates a group of six hepatocytes from a 2-d-old culture. Tight junctions were evident on the plasma membranes between adjacent hepatocytes. Intercellular spaces frequently contained microvilli-like, cytoplasmic projections of the plasma membrane. Nuclei appeared more elongated than in earlier sampling times and frequently had cytoplasmic indentations of the nuclear envelope. The cytoplasm contained multiple, parallel stacks of rough endoplasmic reticulum and numerous mitochondria. Secondary lysosomes appeared to be increased in size and number from earlier sampling times. Hepatocytes sampled after 4 and 6 d of culture showed a further progression of the changes seen after 2 d, specifically, elongation of nuclei, cytoplasmic invaginations of the nuclear envelope, and increases in secondary lysosomes. After 8 d of culture (Fig. 9) the majority of hepatocytes were found in large aggregates. These cells were oval shaped with a single elongated nucleus. The most striking cytoplasmic feature was the presence of large secondary lysosomes. These organelles frequently were larger than nuclei. Rough endoplasmic reticulum and mitochondria (in elongated form) remained major components of the cell. Bundles of intermediate (10 NM) filaments and clear intracellular vacuoles were also found in the majority of the cells sampled. The intercellular space between hepatocytes appeared more extensive than seen in early sampling times. Dead cells were frequently observed in the aggregates and individually in the medium. The nuclear and cytoplasmic changes seen at 14 d of culture reflected a continuation of those observed after earlier sampling times. An increase in the number of dead cells within hepatocyte aggregates from earlier sampling times was also evident. Cells grown at 15, 20, and 25° C displayed similar patterns of morphologic changes.

DISCUSSION

The trout hepatocyte isolation method reported in this paper is a modification of the well-established rodent, two-step isolation procedure. Mean isolated cell viability in the present study was over 95%. Bailey et al. (1) and Walton and Cowey (20) also achieved viabilities of 95% in trout hepatocyte isolations. Hayashi and Osshiro (5) noted mean viabilities of 85 to 90% in freshly isolated eel liver cells. Total liver cell yield and hepatocyte yield have not been well documented in previous studies. Walton and Cowey (20) reported a total cell yield of 20 to 30% of trout liver weight. Hayashi and Osshiro (5) noted an average of 6.8×10^6 liver cells isolated per total liver for a 150 to 200-g fish. In the present study the yield was expressed in terms of total number of liver cells isolated and number of hepatocytes isolated per gram body weight. The mean number of viable liver cells isolated from the trout in the present study (2.81×10^6/g

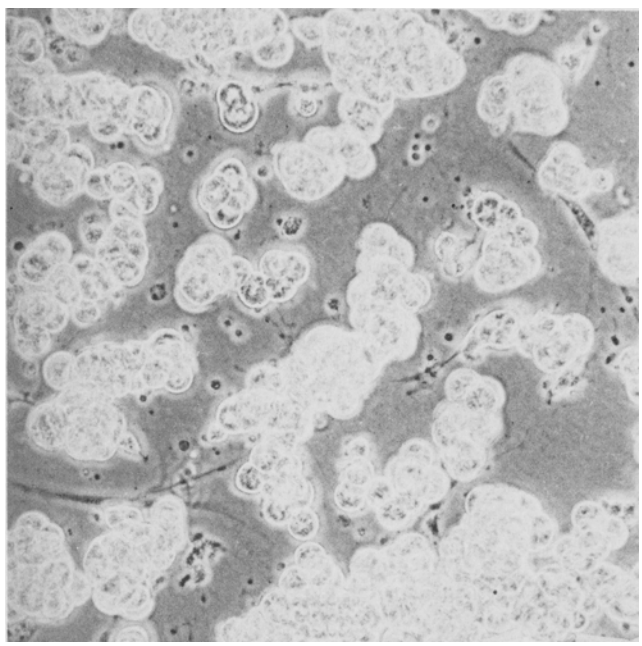

FIG. 6. Phase contrast micrograph of trout hepatocytes after 2 d of culture. ×389.

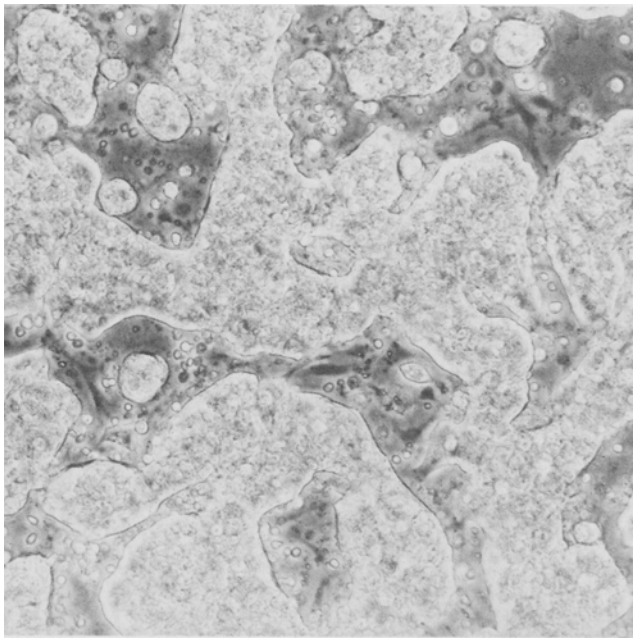

FIG. 7. Phase contrast micrograph of trout hepatocytes after 14 d of culture. ×111.

body weight) was in the range for that reported in rodent liver (2.3 to 2.9 × 10^6 viable cells/g body weight) (9,21). The percentage of hepatocytes in the isolate was also similar; 93-96% for the rodent liver (9,21) and 93.4% for the trout liver (this study).

Few attempts at culture of teleost liver cells have been made (4,15). Haschemeyer and Matthews (4) have recently reported on the primary culture of hepatocytes isolated from two antarctic fish. In that study, hepatocytes attached to either collagen-coated or uncoated culture dishes and synthesized and secreted protein over 6 d of culture when maintained at 0 or 5° C. Saez.et al. (15) described morphologic and functional properties of carp hepatocytes in primary culture for over 2 wk.

The inability of trout (this study), catfish (12), and carp (15) hepatocytes to attach firmly to culture substrate is interesting. Haschemeyer and Matthews (4) noted attachment of antarctic fish hepatocytes at low temperature (0 to 5° C) on either collagen-coated or uncoated culture dishes after 2 d. However, the morphology of the cellular attachment was not described for those fish. Trout and catfish hepatocytes do attach to plastic and collagen but fail to flatten out and securely attach to the culture substrate (12). The discrepancy in the attachment behavior of the cells in the present study and that of Haschemeyer and Matthews may be related to the species of fish, temperature of isolation, or method of cellular isolation. Further investigation into the attachment behavior of teleost hepatoctyes is warranted.

Survival of trout hepatocytes in suspension-type primary culture displayed patterns similar to that reported in mouse and rat hepatocyte culture (9,21). The trout hepatocytes exhibited a gradual decrease in survival over time. The percentage of surviving hepatocytes was dependent on both serum concentration and incubation temperature. Rodent hepatocytes also require serum in the medium for optimal survival (9). The influence of temperature on survival is not surprising. Nonhepatocyte cell lines derived from cold water teleosts such as salmon and trout have been reported to grow best at 2 to 20° C and do not tolerate temperatures above 27° C whereas cell lines from warm water fish such as catfish and carp do well at 15 to 30° C and can tolerate temperatures up to 37° C (22).

The maintenance of trout hepatocyte cells at 4 to 25° C enables these cells to be used for metabolic and physiologic studies at various temperatures. Studies using rainbow trout hepatoctyes from both cold and warm water-acclimated fish for fatty acid oxidation and lipogenesis have been reported by Hazel and Prosser (6).

Based on the DNA synthesis and mitotic index data, it appears that trout hepatocytes in suspension culture rarely divide or undergo DNA replication. A similar effect occurs in rodent hepatocyte cultures (9). The percentage of S-phase labeled hepatocytes and cells in mitosis peaked in the trout after 4 h of culture. This pattern has also been observed in rodent hepatocytes immediately after isolation (10). Liver cell isolation procedures might promote certain hepatocytes to divide or undergo DNA synthesis.

The activity of cytochrome p_{450} decreased steadily during the first 4 d of trout hepatoctye culture before leveling to low but detectable concentrations during the remaining culture period. A similar pattern has been observed in rodent hepatocytes (11,13,18).

Morphologic changes during culture of trout hepatocytes are interesting. Separated trout hepatocytes seemed

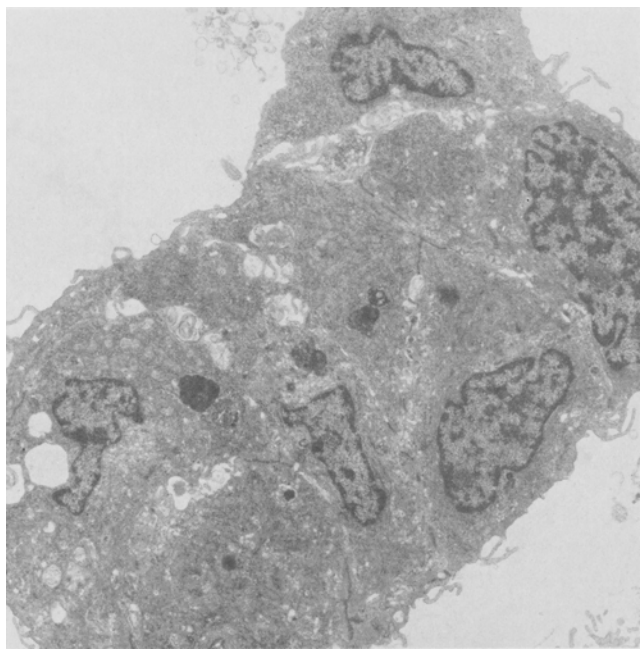

FIG. 8. Electron micrograph of a group of aggregated trout hepatocytes after 2 d of culture. ×3420.

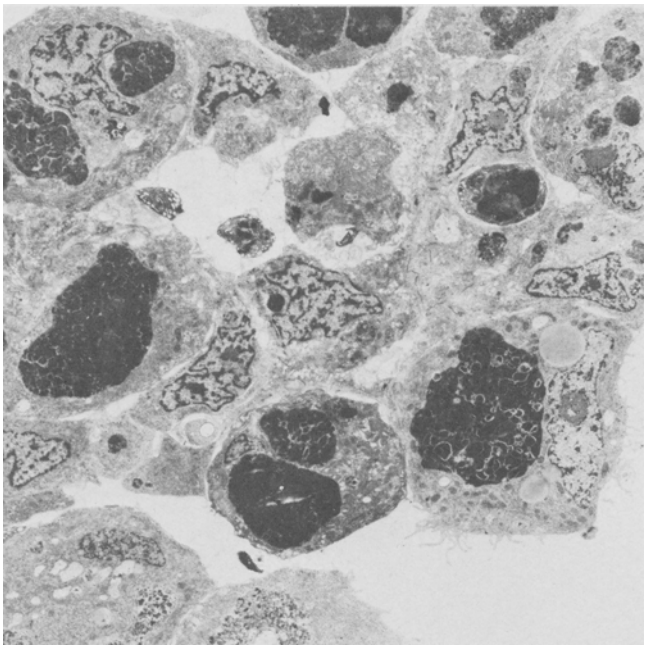

FIG. 9. Electron micrograph of a group of aggregated trout hepatocytes after 8 d of culture. ×2775.

to have a strong tendency to complex back together and, once joined, to form cell to cell junctional complexes. A similar self-aggregation was found in carp hepatocytes in primary culture (15). The three prominent morphologic changes that occurred in trout hepatocytes during culture also have been observed in rodent primary hepatoctye cultures (10). These include (a) nucleus elongation and cytoplasmic indentation of the nuclear envelope, (b) an increase in the number and size of autophagic vacuoles, and (c) the occurrence of bundles of 10 NM intermediate filaments within the cytoplasm (10). These changes seem to be related to the aging and death of the cultured hepatic cells. A delay in the onset of these changes can be accomplished in mouse liver cultures with the use of dexamethasone or insulin supplementation in the medium (11). Further studies with the trout hepatoctyes are required to assess the influence of medium additives such as insulin, dexamethasone, vitamins, etc., on cell survival and cell morphology.

Isolated hepatocytes from trout and other teleosts are potentially valuable for the in vitro study of normal liver metabolism and physiology as well as for use in toxicology and carcinogenesis research. Trout hepatocytes have already been used in the study of aflatoxin B_1 metabolism and DNA interaction (1). Interspecies studies between teleosts and higher vertebrates and between teleosts are possible using cultured hepatoyctes. Inasmuch as the liver is the primary detoxification organ and the major site of tumor formation after carcinogen exposure in most vertebrates, studies of the similarities and differences in carcinogen activation and deactivation, DNA repair, and carcinogen-macromolecular binding in various species may help elucidate poorly understood hepatic functions and give further insight into the mechanisms of experimental liver tumorigenesis.

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