Ultrastructure, Enzymatic, and Transport Properties of the PICM-19 Bipotent Liver Cell Line

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The pig epiblast-derived PICM-19 cell line was previously shown to spontaneously differentiate into liver-like cells and structures and to secrete serum proteins. A study was undertaken to further define the liver-like characteristics of the PICM-19 cell line. PICM-19 cells displayed in vitro ultrastructure, enzymatic, and transport characteristics similar to those of parenchymal hepatocytes and bile duct epithelium. The PICM-19 cells contained large oval nuclei, numerous oval to elongate mitochondria with flat cristae, extensive rough and smooth endoplasmic reticulum, Golgi complexes, lipid vacuoles, and glycogen granules. Biliary canaliculi with intraluminal projecting microvilli were delimited by the junctional apparatuses between adjacent PICM-19 cells. The PICM-19 cells rapidly transported fluorescein into their biliary canaliculi from the extracellular environment. PICM-19 cells that had differentiated into multicellular ductal structures had high \( \gamma \)-glutamyltranspeptidase (GGT) activity at their apical surfaces as shown by histochemical staining. PICM-19 total GGT activity was at least 19 times higher than that found in porcine hepatocytes. Metyrapone induced cytochrome P-450 content of PICM-19 cells was at least one-fourth of that found in porcine hepatocytes. PICM-19 P-450 activity induced by 7-ethoxycoumarin was nearly equivalent to that of primary cultures of pig hepatocytes. The data support the proposal that differentiated PICM-19 cells resembled hepatocytes or bile duct epithelium cells and, therefore, the PICM-19 cell line behaved like early embryonic liver progenitor cells.© 1996 Academic Press, Inc.

INTRODUCTION

The in vitro culture of the totipotent cells of the pig epiblast on STO feeder cells resulted in the isolation of specific differentiated cell types [1]. One of these epiblast-derived cell cultures, PICM-19, resembled primary cultures of fetal pig liver cells. PICM-19 was found to express liver-specific mRNA and secrete liver-specific proteins [2]. PICM-19 cells were bipotent. They spontaneously differentiated into either monolayer areas resembling in vivo cords of parenchymal hepatocytes or into multicellular ductal structures with a defined lumenal space resembling in vivo intrahepatic bile ducts [2]. Similar hepatocyte monolayers and multicellular ductal structures were spontaneously formed in hepatocyte cultures initiated from 27- to 30-day fetal pig liver tissue after colony cloning and outgrowth on STO feeder cells [3]. PICM-19 cells were in continuous culture for over 2 years and maintained their bipotent differentiation potential and secretion of liver-specific proteins over this period and after single cell donings. It is possible that the PICM-19 cell line may be an in vitro equivalent of embryonic liver progenitor cells.

To define further the bipotent and hepatic nature of the PICM-19 cell culture, an ultrastructure, transport, and enzymatic analysis of the cells was performed. Characteristics typical of both hepatocytes and bile duct epithelium were found in the cultures of differentiated PICM-19 cells.

MATERIALS AND METHODS

Cell culture. The derivation and continuous culture of the PICM-19 cell line were previously described [1, 2]. PICM-19 was grown on mitomycin C (Sigma Chemical Co., St. Louis, MO)-inactivated STO mouse fibroblast feeder cell layers (CRL 1503, American Type Culture Collection, Rockville, MD).

7-Hydroxycoumarin P-450 enzyme activity assay. Mixed oxidase function of cultured cells was studied by assaying for the formation of 7-hydroxycoumarin (7HC) glucuronide and sulfoconjugates from 7-ethoxycoumarin (7EC) substrate, according to a modification of the method of Roochvarg et al. [4]. Duplicate 10-ml cultures of cell lines were grown in phenol red-free Dulbecco's modified Eagle's medium hormonally defined as in Enat et al. [5] and exposed to 200 \( \mu \)M 7EC (Sigma). Samples (0.5 ml) of cell-free culture medium were taken in triplicate at various times as indicated, and total culture volumes
FIG. 1. Transmission electron micrograph of PICM-19 cells growing on STO feeder cells. PICM-19 cells were fixed in glutaraldehyde and osmium tetroxide solutions containing 0.05% ruthenium red in order to visualize the cell coat or glycocalyx. The ruthenium red does not penetrate past the tight junctions which delineate the biliary canaliculi (bc) between the PICM-19 cells. Note large hepatocyte-like cells (arrows) and the ductular structure composed of a confluence of six smaller biliary epithelium-like PICM-19 cells (arrowheads); original magnification, 4900×.

for those time points were noted accordingly for normalizing cell density calculations. PICM-19 cell cultures at 7–10 days postpassage (low density; 1 × 10⁶ cells) and at 3–4 weeks postpassage (high density; 3–5 × 10⁶ cells) were grown on STO feeder cells in 25-cm² tissue culture flasks. Control STO monolayer cultures were assayed in parallel (1.5 × 10⁶ total cells per 25-cm² flask). In addition, the human hepatoblastoma cell line HepG2 (American Type Culture Collection) was cultured without STO feeder cells in parallel at (A) 6 × 10⁶ total cells or (B) 10 × 10⁶ total cells. Porcine hepatocytes were isolated as described in Morsiani et al. [6] and cultured on bovine Type 1 collagen-coated 10 × 15 mm Permanox dishes (Nunc, Inc., Naperville, IL) at 1 × 10⁶ cells in 5 ml per dish. Total cell numbers were obtained by counting cell suspensions following trypsin-EDTA harvest. To extract 7HC, 200-μl aliquots of medium were adjusted to pH 5.0 with 0.1 M acetate buffer (pH 4.0) and treated with β-glucuronidase (40 U/aliquot) and sulfatase (2.4 U/aliquot). Samples were incubated at 37°C for 15 min and extracted with 2.7 ml of a basic solution (0.01 NaOH/1.0 NaCl). Sample fluorescence was measured using a Model 450 Sequoia-Turner Digital Fluorometer (Turner Co., Mountain View, CA) with excitation and emission wavelengths of 370 and 455 nm, respectively. Serial dilutions of 7HC in the same medium and extraction protocol used for the samples generated a standard curve. Data are expressed as nanomoles of 7HC per 10⁶ cells.

P-450 enzyme content assay. Two- to four-week-old PICM-19 cultures at approximately 75% confluence or STO feeder cells alone were exposed to metyrapone (Sigma) at 0.5 mM in complete medium [2] for 48 h. Cultures not exposed to metyrapone were also assayed as noninduced controls. Three T-25 tissue culture flasks (Falcon, Becton-Dickson, Franklin Lakes, NJ) of cells were washed three times in Tris-buffered saline and scraped into 6.5 ml of buffer containing 115% KCl in 0.01 M Tris, pH 7.4. The pooled cellular material was homogenized...
for 1 min by sonication (Sonicator XL, Heat Systems, Inc., Farmingdale, NY) with a standard microtip at a setting of 3.5 and 50% pulse. The homogenates were centrifuged at 9000g (TL 100, Beckman Instruments, Palo Alto, CA) for 10 min at 4°C. The supernatant fraction was centrifuged at 125,000g for 30 min at 4°C, and the resulting “microsomal” pellet was resuspended in 1 ml of 0.1 M phosphate buffer, pH 7.0. The concentration of cytochrome P-450 was determined spectrophotometrically on microsome fractions as described by Omura and Sato [7]. A difference spectrum between 400 and 500 nm was obtained (Lambda 3B, Perkin Elmer Corp., Norwalk, CT) for samples which were reduced with solid sodium hydrosulfite (S-2156, Sigma) in the presence or absence of carbon monoxide. Protein content of microsome and homogenate fractions was determined by the method of Neururkar et al. [8] using bovine albumin as standard.

Porcine hepatocytes were isolated from crossbred pigs (40–50 kg) as previously described by Caperna et al. [9]. The microsome fractions of these freshly harvested hepatocytes were assayed for P-450 as described above for comparison.

γ-Glutamyltranspeptidase assay. Total cellular activity of γ-glutamyltranspeptidase (GTT) was determined on freshly prepared homogenate material at 37°C essentially by the method of Jacobs [10], with the following modifications. The complete reaction mix containing γ-glutamyl-p-nitroanilide and glycyl glycine (Sigma) as substrates was incubated with samples for 10 and 40 min, and the NaOH stopping solution contained Triton X-100 (0.2%). The concentration of p-nitroaniline was determined spectrophotometrically at 405 nm and enzyme activity was determined over the 30-min time interval.

γ-glutamyltranspeptidase histochemistry. Histochemical localization of GTT was determined by the method of Rutenberg et al. [11] on cell monolayers that were fixed for 2 min with ice-cold methanol just prior to the addition of substrate solution.

Fluorescein transport. Transcellular transport of fluorescein and fluorescein diacetate was performed as previously described [12]. PICM-19 cells cultured on STO feeder cells were exposed for 20 min to complete medium containing fluorescein (20 μg/ml) or fluorescein diacetate (25 μg/ml) that was obtained from Sigma. The monolayers were washed three times with medium and immediately viewed using a fluorescein excitation/emission filter set on an Olympus IMT-2 inverted microscope fitted with an Olympus IMT-2 reflected light fluorescence attachment (Opelco, Washington, DC). The morphology of the PICM-19 cell monolayers was evaluated by phase-contrast and Hoffman modulation photomicroscopy on an Olympus IMT-2 inverted microscope.

Transmission electron microscopy. Four- to eight-week-old cultures of PICM-19 cells were fixed and processed for electron microscopy with or without ruthenium red (Sigma, R-2751) staining as previously described [13]. Ultrathin sections (60 to 80 nm) were stained with uranyl acetate and lead citrate prior to examination with an electron microscope operating at 60 kV.

RESULTS

Ultrastructure Analysis of PICM-19 Cells

Ultrathin cross sections of PICM-19 cells in monolayer culture were inspected by transmission electron microscopy. PICM-19 cells had generally large, oval, centrally located nuclei (Fig. 1). Abundant rough endoplasmic reticulum (RER) was present in the PICM-19 cells and often occurred in extended laminar arrays and associated with mitochondria (Figs. 2 and 3A). Oval to elongate mitochondria with flat cristae extending into a dense matrix were numerous in the PICM-19 cells (Figs. 2 and 3C). PICM-19 cells contained rough and smooth endoplasmic reticulum vesicles, Golgi complexes, glycogen rosettes, and microfilaments (Fig. 2, 3C, and 3D). Lipid vacuoles were present in the PICM-19 cells, and very large lipid vacuoles were sometimes sequestered in a single pseudopod extending out from one end of a PICM-19 cell (Fig. 3B). Cilia were not observed on the PICM-19 cells.

The PICM-19 cells were closely associated with one another by extensive interdigitating microvilli along their lateral membranes (Figs. 3D and 4B). Junctional apparatus typical of epithelium was found between adjacent PICM-19 cells (Fig. 4A). These junctions established the boundaries of the biliary canaliculi that existed between adjacent PICM-19 cells. Ruthenium red staining highlighted the biliary canaliculi by staining the cell coat of the PICM-19 cells only as far as the tight junctions. The cell membranes comprising the canaliculus were consequently left unstained (Figs. 1, 2, and 3A). PICM-19’s biliary canaliculi contained numerous intraluminally projecting microvilli and variable amounts of material that took up uranyl acetate and lead citrate stain.

Three cell types were observed in the electron microscopic analysis of the PICM-19 cell culture. First, “hepatocyte-like” PICM-19 cells were found in the culture. These cells were relatively large and loosely organized (Fig. 1). Second, “bile duct-like” PICM-19 cells that were smaller were also observed (Figs. 1, 2, and 3A). These cells were sometimes sequestered in a single pseudopod extending out from one end of a PICM-19 cell (Fig. 3B). Cilia were not observed on the PICM-19 cells.

FIG. 2. Transmission electron micrograph of PICM-19 cells growing on STO feeder cells. PICM-19 cells were fixed in glutaraldehyde and osmium tetroxide solutions containing 0.05% ruthenium red in order to visualize the cell coat or glycocalyx. The ruthenium red does not penetrate past the tight junctions which delineate the biliary canaliculi between the PICM-19 cells. Note the biliary canaliculi (bc) at the confluence of six PICM-19 cells and the presence of material in the canaliculus lumen. Other features include round or oval nuclei (nu). Original magnification, 12,800×.
FIG. 3. Transmission electron micrograph of PICM-19 cells in STO coculture. (A) Biliary canaliculus (bc) between three juxtaposed PICM-19 cells highlighted by ruthenium red staining. Note numerous microvilli projecting into the canaliculus lumen and laminar array of rough endoplasmic reticulum (small arrowheads); magnification, 18,900×. (B) PICM-19 pseudopod containing large lipid vacuoles; magnification, 9500×. (C) PICM-19 cell with extensive Golgi complex (gc), oval mitochondria (m), and large oval nucleus (nu); magnification, 21,200×. (D) PICM-19 cells containing glycogen rosettes (g); note microvilli between cells; magnification, 15,500×.
FIG. 4. Transmission electron micrograph of PICM-19 cells grown in STO coculture. (A) Union of PICM-19 cells at biliary canaliculus showing tight junctions between adjacent cells (arrowheads); note microvilli within biliary canaliculus (bc); magnification, 33,500×. (B) Multilayer of PICM-19 cells with biliary canaliculus and microvilli between adjacent cells. Note the STO feeder cell beneath the PICM-19 cells in the lower righthand corner (asterisks denote the large, elongated, indented STO nucleus); magnification, 5700×.
diacetate for 20 min. The PICM-19 cells rapidly took up fluorescein and concentrated it into their biliary canaliculi, where it could be visualized (Figs. 6A and 6B). The nonfluorescent substrate, fluorescein diacetate, was processed by PICM-19 cell esterase activity to free fluorescein, and the fluorescein was similarly concentrated into the biliary canaliculi (Figs. 6C and 6D). No background fluorescence was seen within the PICM-19 cells or with STO feeder cells after exposure to fluorescein. After exposure to fluorescein diacetate, background fluorescence was not observed in the PICM-19 cells, but was observed in the STO feeder layer cells. This indicated that the STO feeder cells were able to process the fluorescein diacetate to free fluorescein, but were unable to transport it as efficiently as the PICM-19 cells.

GGT Activity in PICM-19 Cells

Histochemical staining showed intense localized expression of GGT activity on the interior aspect of the multicellular ductal structures, i.e., the apical portion of the small columnar cells forming the luminal space (Figs. 7D, 7E and 7F). GGT activity was also present on the canicular surfaces of recently passaged PICM-19 cells that had started to organize into small groups (Figs. 7A and 7B). PICM-19 cells that were not associated with one another were negative for GGT histochemical stain. GGT activity appeared to be more diffuse or lacking in "hepatocyte-like" monolayer areas of the PICM-19 cells (Figs. 7E and 7F). STO feeder cells were negative when histochemically stained for GGT activity in the presence (Fig. 7) or absence of PICM-19 cells (not shown).

GGT activity was present in homogenates of PICM-19 cells cultured for 2 to 4 weeks (Table 1). Total GGT activity was approximately 19 times that found in the homogenates of freshly harvested porcine hepatocytes (Table 1). The STO feeder cell homogenates showed very low or no GGT activity when grown alone under the same conditions as the PICM-19 cells (see legend, Table 1). The reported specific activity of GGT in PICM-19 was therefore underestimated because approximately two-fifths of the cell culture homogenate protein was derived from the STO feeder cells. Calculation after subtracting out the STO microsomal component indicated that PICM-19 cells actually had 29 times as much GGT activity as the freshly harvested pig hepatocytes preparation.

Cytochrome P-450 Content and Activity in PICM-19 Cells

Cytochrome P-450 was present in the microsomal fraction of PICM-19 cells that had been exposed to metyrapone for 48 h prior to assay (Table 1). In contrast, P-450 was undetectable in PICM-19 cell culture homogenates that had not been exposed to metyrapone (data not shown). The crude homogenate and microsomal fraction of STO feeder cells grown under identical conditions and treated with metyrapone had no detectable P-450 content. Because the microsomal protein associated with STO cells represented more than one-third of the total harvested microsomal protein, the actual activity...
specific content of P-450 (nmol/mg/protein) in the PICM-19 cells would be markedly greater than reported and would actually be approximately 40–50% of that found in the freshly harvested porcine hepatocytes (Table 1).

PICM-19 P-450 activity was measured by O-deethylation of 7EC to the highly fluorescent product 7HC. Over the first 48 h of assay the PICM-19 cells converted 7EC to 7HC at rates approximately one-half of that of primary cultures of pig hepatocytes (Fig. 8). However, by 72 h (Fig. 8; PICM lo B and C) or 120 h (Fig. 8; PICM hi A and B) the conversion of 7EC to 7HC was roughly comparable between the two cultures. PICM-19 cells that had been recently passaged 7–10 days prior to assay (PICM lo B and C) showed higher activity than cultures that had been maintained over 3–4 weeks postpassage (PICM hi A and B). STO feeder cells and HepG2 cells showed no detectable cytochrome P-450 activity (Fig. 8).

**DISCUSSION**

Ultrastructure, transport, and enzymatic analysis demonstrated that the PICM-19 cell line possessed characteristics of parenchymal hepatocytes and bile duct epithelium cells. These findings were consistent with PICM-19's liver-specific gene expression, liver-specific protein secretion, and bimorphic phenotype previously described [2]. The data were also consistent with the proposed stem cell nature of the PICM-19 cell line since both hepatocytes and bile duct epithelium cells are thought to come from a common precursor cell derived from the embryonic endoderm of the primitive gut [14]. Embryological data based on morphological characteristics [15] and histochemical analysis of cytokeratins [16] indicated that intrahepatic biliary epithelial cells arise from hepatoblasts or parenchymal cells. It has been concluded that early embryonic parenchymal liver cells possess characteristics of both hepatocytes and biliary epithelium cells [17]. In addition, some evidence exists for liver stem cells in adult animals [18]. The proposed liver stem cells were postulated to be able to give rise to both hepatocytes and bile duct epithelial cells and to be of fetal phenotype, e.g., express α-fetoprotein [18]. As previously shown, the PICM-19 cells are of fetal phenotype since they express α-fetoprotein [2]. Thus, the present study supports the proposal that the PICM-19 cell culture is an in vitro equivalent of the embryonic liver's progenitor cells or the hypothesized adult liver stem cell compartment.

The ultrastructural feature perhaps most defining of hepatocytes was the specialized cell-to-cell junctions that combine to form the biliary canaliculi between adjacent hepatocytes [13]. PICM-19 cells cocultured with STO feeder cells had biliary canaliculi similar to those found in thin sections of human embryonic, piglet, and rodent liver [15, 19]. Multicellular tubular formations of PICM-19 cells were strikingly similar to in vivo human embryonic intrahepatic biliary epithelium and piglet interlobular bile duct [15, 19]. The ultrastructural analysis of the STO-cocultured PICM-19 cells supports and extends the previous conclusion drawn from light microscopic analysis that the PICM-19 cells displayed an in vivo-like hepatic or biliary epithelium morphology [2].

Other PICM-19 ultrastructural features were typical of hepatocytes. Extensive RER arranged in long lamellar cisternae was found in both the hepatocyte-like and bile duct epithelium-like cells. Hepatoblasts in human fetal liver were reported to have extensive multizonal collections of RER with long cisternae, whereas fetal and adult bile duct epithelium cells were found to have very little and short tubular RER [15, 20, 21]. This distinction was not apparent in the PICM-19 cells, which may indicate either that the PICM-19 cells are fundamentally different from in vivo fetal liver cells or that the PICM-19 cells displayed a transitional morphology that could “mature” given the proper environment.

PICM-19 mitochondria were typical of hepatocytes and bile duct epithelium cells in having lamellar cristae [13, 19]. This is in contrast to the tubular and vesicular cristae found in adrenal cortex cell mitochondria, another cell type known to have high levels of P-450 [22]. Adult hepatocyte mitochondria were generally reported as numerous and round to oval in shape [15, 23]. However, fetal hepatocytes and piglet hepatocytes were described as varied in size and shape [19]. Enzan et al. [15] reported that fetal bile duct epithelium cells had fewer mitochondria per cell than hepatoblasts and that the mitochondria were long oval forms with incomplete traversing cristae. In contrast, Phillips et al. [21] described bile duct cell mitochondria as having cristae traversing their entire width. The PICM-19 mitochondria were elongated oval forms and some appeared to have cristae that traversed their width. No obvious differences were noted in the character or number of mitochondria in comparing “hepatocyte-like” to “bile duct-like” PICM-19 cells.

Lipid vacuoles were typically found in liver cells of a variety of species [24]. The cultured PICM-19 cells accumulated large amounts of lipid under certain culture conditions [2]. As demonstrated in Fig. 3B, lipid vacuoles could be pronounced in the PICM-19 cells. The lipid was mainly composed of triglyceride as indicated by oil red O staining [2] and lipid analysis by thin-layer and high-performance liquid chromatography (unpublished data).

Cilia were not observed by electron microscopy in the PICM-19 thin sections. The presence of cilia was shown to be a characteristic feature of fetal human, neonatal
**FIG. 6.** Uptake and transport of fluorescein and fluorescein diacetate by PICM-19 cells. (A) PICM-19 cells exposed to fluorescein containing medium (20 min) transported and concentrated the fluorescein in their biliary canaliculi (arrowheads); FITC excitation; bar, $= 25 \mu m$. (B) Phase-contrast microscopy of corresponding area to that shown in (A); note coincident biliary canaliculi between PICM-19 cells (arrowheads); bar, $= 28 \mu m$. (C) PICM-19 cells exposed to fluorescein diacetate containing medium (20 min). Freed fluorescein was transported and concentrated in the PICM-19 biliary canaliculi as in (A) (arrowheads), but accumulated in the STO feeder cells; FITC excitation; bar, $= 25 \mu m$. (D) Hoffman modulation microscopy of corresponding area shown in (C); note coincident biliary canaliculi (arrowheads); bar, $= 25 \mu m$.

**FIG. 7.** $\gamma$-glutamyltranspeptidase (GGT) activity in PICM-19 cells. (A and B) Four days postpassage, subconfluent PICM-19 cells; note GGT staining at interior junction of cells (canaliculi) and lack of signal in PICM-19 cells that have not associated with each other (A, scale bar, $= 26 \mu m$; B, scale bar, $= 78 \mu m$; phase-contrast). (C and D) Four-week postpassage, differentiated PICM-19 cells; note GGT staining at apical surfaces on lumens of multicellular ductal formations (C, scale bar, $= 39 \mu m$; D, scale bar, $= 78 \mu m$; Hoffman modulation). (E and F) Four-week postpassage, differentiated PICM-19 cells; note low GGT activity in monolayer portions of the culture (E, scale bar, $= 39 \mu m$; F, scale bar, $= 78 \mu m$; phase-contrast).
TABLE 1

| Cell type                      | P-450
(nmol/flask) | P-450
(nmol/mg microsomal protein) | GGT
(munit/flask) | GGT
(munit/mg total cell protein) |
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<tr>
<td>PICM-19 (11)</td>
<td>91.7 ± 7.5</td>
<td>154 ± 21</td>
<td>219 ± 21</td>
<td>70.3 ± 5.45</td>
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<tr>
<td>Porcine hepatocytes (4)(^b)</td>
<td>—</td>
<td>617 ± 47</td>
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<td>3.7 ± 0.4</td>
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^a^ PICM-19 cells were assayed between passage 26 and passage 39. PICM-19 cells were harvested between 12 and 34 days postplating. Values represent means ± standard error of the means of 11 independent determinations. Total protein of cocultures was determined to be 3.17 ± 0.22 mg per flask, while protein content of independent control cultures of STO feeder cells alone was 1.13 ± 0.12 mg per flask (n = 10). Microsomal protein of cocultures was 0.593 ± 0.035 mg per flask, while microsomal protein of STO feeder cells alone was 0.267 ± 0.020 mg per flask. P-450 was undetectable in metyrapone-treated STO feeder cells, and background GGT activity associated with STO feeder cells was less than 0.4% of the activity associated with PICM-19 cells.

\(^b\) Porcine hepatocytes were isolated from four crossbred pigs (40–50 kg) and microsomes were prepared from freshly isolated cells [9].

pig, and adult rat bile duct epithelium cells [15, 20, 19]. In contrast, cilia were not found in human hepatoblasts or piglet hepatocytes [15, 19]. The lack of cilia in the PICM-19 cells comprising duct-like structures may again indicate that the cells have a transitory phenotype and require further developmental cues. Alternatively, a larger sampling of PICM-19 thin sections may be required to find ciliated cells since they were a rare feature even in in vivo bile duct cells [15, 19]. STO coculture supported the survival of adult pig bile duct epithelial cells because ciliated cells were observed in cocultures of primary adult pig hepatocytes (manuscript in preparation).

The PICM-19 cells that were found to stain relatively darkly and that had very large, distinct Golgi complexes were only rarely found (Fig. 5). These PICM-19 cells may be similar to an undefined cell observed by Enzan et al. [15] which they described as an “unusual”

**FIG. 8.** 7-Hydroxycoumarin P-450 activity assay of STO-cocultured PICM-19 cells in comparison to HepG2 [36] and primary pig hepatocyte cell cultures. Values represent the means of three independent measurements ± SD and were normalized to cell counts made at the end of the assay period.
intermediate type of cell bearing features of both hepatoblasts and bile duct epithelium cells. Their conclusion was that the "unusual" cell was probably a hepatoblast that coincidentally displayed a biliary epithelial cell size and shape. The large and extensive Golgi complexes exhibited by the PICM-19 variant cell is a distinguishing feature. Based on this, the cell is most similar to secretory cells of the glands of the extrahepatic bile duct [25].

Fluorescin and the nonfluorescent compound fluorescein diacetate were used to demonstrate the polarized nature and biliary transport function of hepatocytes in primary culture [12]. The transport of fluorescein into the biliary canaliculi formations between the PICM-19 cells confirmed their similarity and functional equivalence to cultures of primary hepatocytes.

GGT is highly expressed in bile duct epithelium and thought to be a good marker for this cell type [20, 26]. GGT activity was expressed at or near the apical portion of the PICM-19 cell membrane directly adjacent to the canaliculi or the lumen of the ductal structures. No staining was observed on the outer aspect of the ductal structures indicating a well-polarized condition within the PICM-19 cells adopting this differentiated phenotype. A similar polarized and apical distribution of GGT was demonstrated in acinar cells of the pancreas [11], in the canicular regions of hepatocytes, and in bile duct epithelium [27]. This polar distribution of GGT was indicative of the transport function of the enzyme in the specialized apical areas of these cells [27]. Finally, in direct comparison with PICM-19 cells, a similar distribution of GGT histochemical staining was found in STO-cocultured adult pig hepatocytes that had differentiated into similar ductal formations (manuscript in preparation). It seems probable, therefore, that the in vitro formation of the multicellular ductal structures by the PICM-19 cells represents differentiation into bile duct epithelium [26, 28, 29].

PICM-19 cells displayed inducible cytochrome P-450 content or activity when exposed to metyrapone [30] or 7EC, respectively. The ability to induce relatively high amounts of P-450 in the PICM-19 cells was consistent with a hepatic phenotype [18, 31] and occurred despite the apparent fetal character of the PICM-19 cells [2]. P-450 activity was not present in embryonic or fetal rat hepatocytes, although P-450 content was found in human fetal liver cells by immunocytochemical analysis [31, 32]. However, P-450 was not found in pig fetal liver microsomes (unpublished observations). The STO coculture conditions may allow the induction of P-450 in the PICM-19 cells, or fetal pig liver cells may have the ability to synthesize P-450 when exposed to an inducing agent. Clearly, the levels of inducible P-450 can be manipulated depending on the culture environment [33], and in the PICM-19 cell culture, P-450 levels probably also depend upon the relative proportions and extent of the alternative differentiated phenotypes.

This further characterization of the PICM-19 cell line confirms their hepatic nature. The findings also confirm, in part, the pluripotency of the pig epiblast mass as defined by the reported anatomy of the pig blastocyst and our isolation procedure [1]. It is possible that other biomedically important cell types could be isolated and grown from the culture of epiblast cells given the appropriate conditions [1, 34]. Furthermore, the culture of epiblasts from humans and other species might also yield hepatocyte cell lines of interest and utility [35].

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