Lamprey biliary atresia: First model system for the human condition?

J.H. Youson and E.W. Sidon[1]

Department of Zoology, Scarborough College, University of Toronto, West Hill (Ontario, Canada M1C 1A4), 30 January 1978

Summary. Degeneration of the bile ducts and gallbladder occurs during metamorphosis of the lamprey. Morphological aspects of this process suggest a similarity to human biliary atresia.

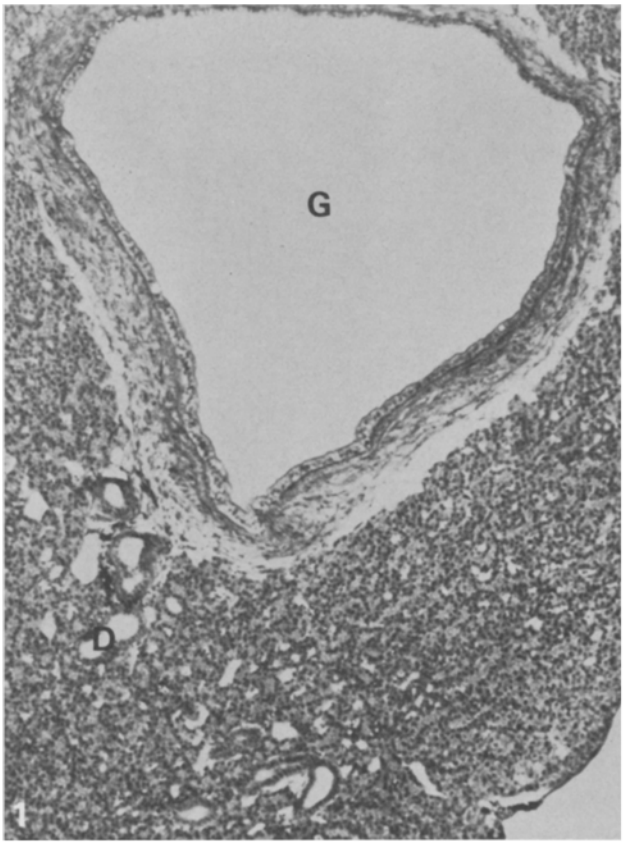
Biliary atresia is a fatal disease of unknown etiology which afflicts human infants[2-6]. Investigations into this disease have been handicapped by the absence of a method for experimental production of bile duct atresia. Morphological studies on the liver of the 'primitive' cyclostome, the lamprey[7-9], have suggested that the entire bile-transport apparatus of the larvae must disappear during its period of metamorphosis for it is absent in the adult. This results in cholestasis and accompanied bile pigment accumulation in the liver of the adult[7]. The adults feed and grow rapidly on the fluids of bony fish over the next 2 to 3 years after which time they spawn and die. It was proposed that the loss of this exocrine function in lamprey may be related to human biliary atresia[8]. Despite the potential interest of lamprey in this regard, to date there is no definitive information on the process of biliary degeneration in lamprey and whether the atretic process is similar in these 2 diverse groups of vertebrates. The present report describes the morphological events of metamorphic transformation of the liver and gallbladder in the sea lamprey, *Petromyzon marinus,* and discusses the validity of using the liver of this organism as a model system for studying human biliary atresia.

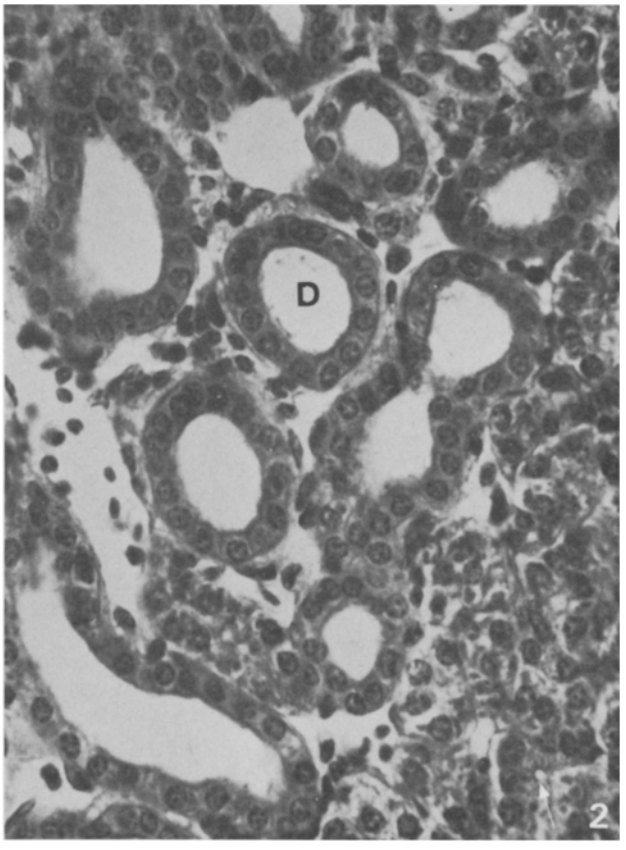
Metamorphosing and larval stages of *P. marinus* were captured and maintained as previously described[10]. Metamorphosing animals in the stages 1 to 7 of Potter et al.[11] were sacrificed along with larvae in various age classes. The entire liver and gallbladder were removed from each animal and were fixed in Bouin's fluid, dehydrated, and embedded in tissue-prep. Serial sections of 8–10 µm were stained with periodic acid-Schiff (PAS) and haemalum. In larvae, biliary channels follow a curvilinear course through the liver parenchyma (figure 1). Diminutive ducts collect bile from canaliculi which are tubular lumina surrounded by 3–6 hepatocytes (figure 2). These ducts lead to larger ducts situated in the central part of the organ which fuse to produce the common bile duct. The latter is joined by a cystic duct originating at the gallbladder. Bile ducts are composed of a simple cuboidal to columnar epithelium with apical brush border and the largest ducts are surrounded by fibrous connective tissue and blood vessels. The gallbladder is contained within the anterior segment of the liver (figure 1) and is lined with a low columnar epithelium with a brush border. Irregular dense connective tissue, smooth muscle fibres, and blood vessels separate the gallbladder from the liver parenchyma.

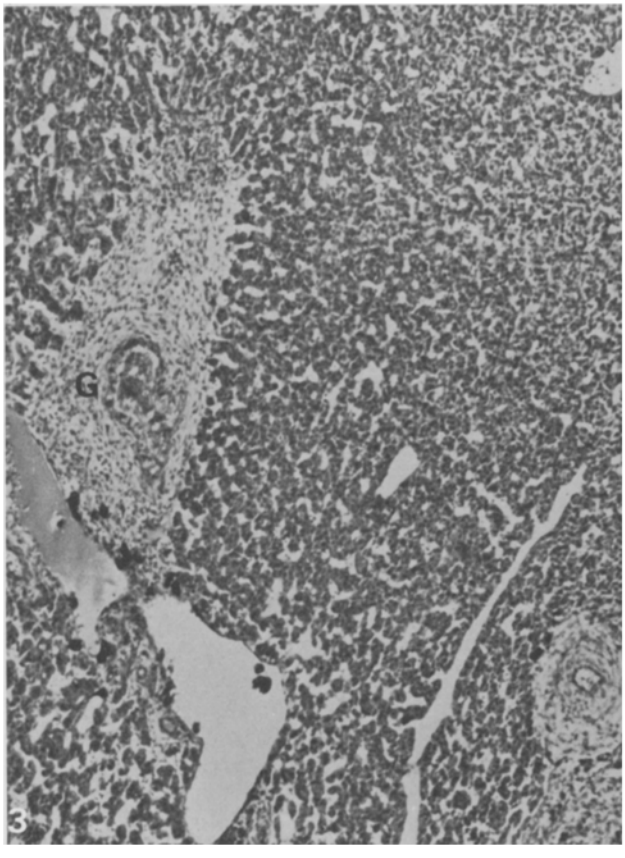
Although a few subtle changes in liver morphology (e.g. more pronounced PAS-staining of the basement mebrane) occur in the earliest phase (stages 1 and 2) of metamorphosis, alterations are most dramatic by stages 3 and 4 (figure 3). There is a significant asynchrony of degeneration within each class of ducts but in general, small biliary components degenerate faster than large ducts and the process seems to progress from peripheral locations to central sites. Occasionally, 1 or 2 bile ducts persist into stages 5 and 6 but by stage 7 the liver has lost its exocrine function. Branches of the intrahepatic common bile duct are also observed in stages 5 and 6 while extrahepatic segments are never present beyond stage 4. The cystic duct is not present later than stage 3 and the regression of the gallbladder is essentially complete by stage 4. The process of degeneration appears to be identical in the ducts of all sizes and in the gallbladder. The thickening of the basement mebrane is accompanied by a reduction in the height of the epithelium and a partial occlusion of the lumina with a PAS-positive residue (figure 4). Further degeneration is marked by a loss of organization of the ductular cells, a loss of the lumina, and ultimately, a complete loss of the biliary structures. This regression is accompanied by a fibrosis in the juxtapositional areas with the production of 'scar tissue' and sometimes, by an infiltration of leukocytes. Eventually, hepatocytes arranged into cords, replace the connective tissue.

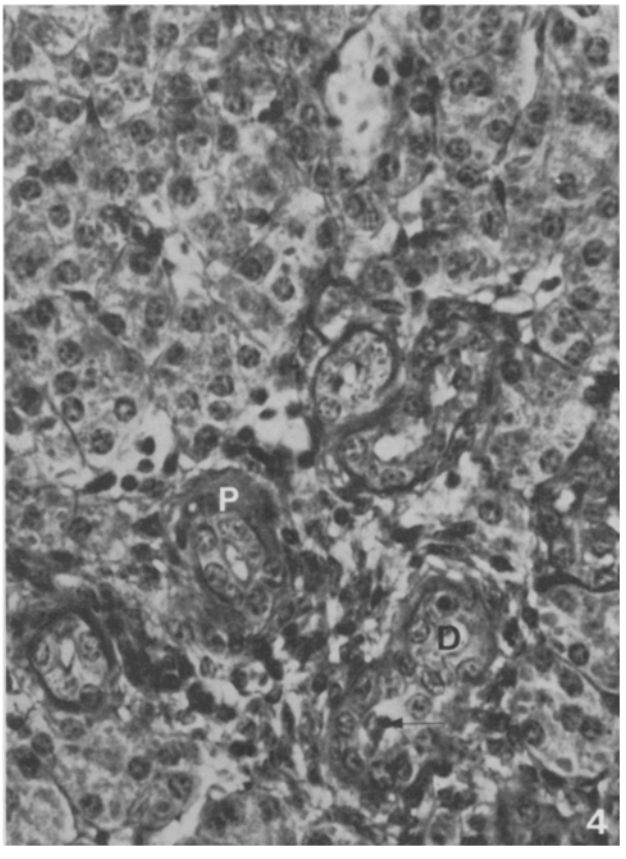
The events of degeneration of the bile duct system during lamprey metamorphosis seem to resemble previous descriptions of the events of human biliary atresia. This is particularly true with respect to the inflammatory response[12], the accumulation of a stainable debris in the lumina[3], the thickening of the basement mebrane[4], disorganization of the hepatic architecture[3], extrahepatic bile duct atresia[13], and shrinkage or loss of the gallbladder[14,15]. We therefore propose that further studies now being undertaken may underscore the value of this animal as the 1st experimental model for studying human hepatic biliary atresia. Metamorphosis in lamprey represents a genetically-programmed event with a high degree of synchrony[11] so that large numbers of animals at the various stages of biliary degeneration and liver repair are available. Although the majority of degenerative changes occur in the 1st month of metamorphosis, the entire process of biliary atresia extends over a 3-month period. There is also a similarity in the general form of the livers of the developing human embryo and the larval lamprey. Transformation of the liver in lampreys involves a change in organization of the hepatocytes from tubules to cords which also occurs in the human liver from early embryological development to early infancy[16]. Furthermore, a characterization of the physiological differences in the liver of lampreys during larval life, metamorphosis, and the parasitic adult stage will be useful for correlating the ability of an organism with biliary atresia to survive for an extended period of time. That lampreys establish an alternate route for the elimination of bile components is not known, but the mechanism that has developed at metamorphosis to prevent excessive cholestasis seems to breakdown during their spawning migration. Lampreys at this stage are characterized by orange pigmentation of their skin apparently as a result of

Fig. 1. Portion of the liver of a stage 1-2 metamorphosing lamprey demonstrating the gallbladder (G) and bile ducts (D). × 100. Fig. 2. Bile ducts (D) and hepatocytes, with their canaliculi (arrow), in a stage 1-2 metamorphosing lamprey. × 560. Fig. 3. The liver of a stage 4 metamorphosing lamprey indicating the cord-like arrangement of hepatocytes, the regressing gallbladder (G), and the absence of ducts. × 100. Fig. 4. Portion of the liver of a stage 3 metamorphosing lamprey showing the thickened basement membrane (P) and the luminal contents of regressing ducts (arrow). × 560.


G
D
1


D
2


G
3


P
D
4

an increase in concentration of bilirubin[17]. It is noteworthy that jaundice which also arises from elevated bilirubin levels occurs consistently in human biliary atresia[18].

1 Acknowledgments. This investigation was supported by grant No. A5945 from the National Research Council of Canada. Dr M.M. Fisher, Department of Pathology, University of Toronto, kindly reviewed the manuscript.
2 R. Cameron and G.L. Bunton, Br. Med. J. *2*, 1253 (1960).
3 S. Stowens, Ann. N. Y. Acad. Sci. *111*, 337 (1963).
4 M. Hollander and F. Schaffner, Am. J. Dis. Child. *116*, 49 (1968).
5 J.N. Levin, J. Pediat. *79*, 130 (1971).
6 D.M. Hays, Surg. Clins. N. Am. *53*, 1257 (1973).
7 B. Bertolini, Z. Zellforsch. *67*, 297 (1965).
8 J.A. Sterling, D.R. Meranze and S. Winsten, Int. Surg. *49*, 157 (1968).
9 R. De Vos, C. De Wolf-Peeters and V. Desmet, Z. Zellforsch. *136*, 85 (1973).
10 J.H. Youson, G.M. Wright and E.C. Ooi, Can. J. Zool. *55*, 469 (1977).
11 I.C. Potter, G.M. Wright and J.H. Youson, Can. J. Zool. *56*, 561 (1978).
12 P.M. Hatfield, E.J. Scholz and R.E. Wise, Semin. Roentgenol. *11*, 235 (1976).
13 J.A. Sterling, Int. Surg. *46*, 228 (1966).
14 J.A. Sterling, Am. J. Gastroent. *45*, 261 (1966).
15 K.J. Foster and S.J. Karran, Br. Med. J. *26*, 815 (1977).
16 A. Koga, Z. Anat. Entw. Gesch. *135*, 156 (1971).
17 E. Kott, Can. J. Zool. *51*, 101 (1973).
18 A.A. DeLorimier, Surg. Clins N. Am. *57*, 429 (1977).

Relationship between A-type and C-type particles in Ehrlich ascites tumor cells

E.C. Chew[1]

Department of Anatomy, University of Hong Kong, Hong Kong (Hong Kong), 7 February 1978

Summary. Intracisternal and intracytoplasmic A-type particles were discovered in Ehrlich ascites tumor cells. In addition, 'mature' and 'immature' C-type particles were also seen in the intercellular space. It is believed that A particles may represent a precursor or a formative stage of the C particles.

In the Ehrlich ascites (EA) tumor cells, a virus with morphological characters of A-type particles was first described by Yasuzumi and Higashizawa[2] and Friedländer and Moore[3]. Since then these particles have been reported in several mouse tumors[4-7]. The particles are usually intracytoplasmic and/or intracisternally located. Recently Myking and Åbro[8] were able to demonstrate C-type particles in a transplant of EA tumor cells. The present communication reports the presence of A- and C-type particles in EA tumor cells obtained from peritoneal fluid containing tumor cells and i.m. transplants.

Materials and methods. The Ehrlich ascites tumor cells in suspension were kindly provided by Dr Y.C. Kong of the Department of Biochemistry, The Chinese University of Hong Kong. The tumor is carried in this laboratory by i.m. and i.p. transplantations in WHT/HT (Swiss) mice.

Small pieces of solid tumor pellets made from peritoneal fluid containing tumor cells were fixed in 2.5% glutaraldehyde in phosphate buffer and post-fixed in 1% osmium tetroxide. Routine procedures were followed for dehydration and embedding in Epon 812. Sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 300 at 60 kV.

Results. The details of cell organelles of EA tumor cells have been described by many investigators[2,8-11]. Intracisternal A-type 'virus-like' particles were found in the cytoplasm of most of the tumor cells. On rare occassions, intracytoplasmic A particles were also detected (figure 1). Most of the A particles were in the juxta-nuclear position. The particle consisted of 2 concentric shells and a rather electron-lucent center. The outer and inner shells have diameters of 70 and 40 nm respectively. In addition, 'mature' and 'immature' C-type particles were observed in some tumor cells obtained from solid i.m. transplants and from peritoneal fluid containing tumor cells (figure 2). The C-particle has an envelope of 90 nm in diameter. Intracisternal and intracytoplasmic A-type particles were sometimes located near the cell membrane and were released as 'doughnut-shaped' enveloped nucleoids called 'immature' C-type particles (figure 5). Many 'mature' C-type particles were located only in the intercellular space (figures 2-5).

Discussion. Previous investigations have revelaed the presence of intracisternal and intracytoplasmic A particles in EA tumor cells[9-11]. Myking and Åbro[8] also reported the presence of C-type particles in EA tumor cells. However, the relationship of the A- and C-type particles in this tumor line was not discussed. The present study has revealed a possible gradual 'shift' of the intracisternal and intracytoplasmic A-particles from the juxta-nuclear position to the cell periphery and eventually to produce the 'immature' C-type particles (figure 5). 'Mature' C-type viron is later formed by condensation of the nucleoid.

Guili et al.[12] suggested a relationship between cytoplasmic A particles and the C-type Rous sarcoma virus in chicken cells after revealing that the A particles contain components immunologically related to the protein of C-type virus. However, Dalton believes that no true intracellular A-type particle is ever involved in C particles formation[13]. Our results seem to indicate a gradual transformation of A particles to C particles. This view is also shared by Bibby and Smith[14] in the study of neoplastic transformation of epidermal cell of BalB/c Mice. Intracisternal A particles have never been shown to possess biological activity, whereas C-type particles have been demonstrated as causative agents in avian, murine and feline leukemia and sarcomas[15]. The presence of C-type particles in Ehrlich ascites tumor cells may also imply that it is likely to be one of the causative oncogenic agents. Perhaps it is also reason-

Fig. 1. An EA tumor cell (T) showing intracisternal A particles. × 12,300. Fig. 2. 'Mature' and 'immature' C particles in the intercellular space. × 19,400. Fig. 3. Higher power view of an EA tumor cell showing many A particles at the cell periphery. Intracytoplasmic A particles are also seen (arrow). × 37,600. Fig. 4. Higher power view of EA tumor cells showing an intracytoplasmic A particle (arrow) adjacent to the cell membrane. × 23,900. Fig. 5. C-type particles budding from cell membrane (arrow). × 23,900.