classify them according to the patterns of infiltration proposed by Schmid and Isaacson. This proved surprisingly difficult because many of the infiltrates showed a combination of or more patterns. In particular, “dense” is not a pattern and a paratrabecular distribution may still be discerned even at an advanced stage of marrow replacement. If one considers specifically whether the infiltrate showed application to the bone trabeculae in a laminar manner, 15 of 34 trephines from cases of ML ec/cc showed a degree of paratrabecularity, compared with two of 12 ML cc. None of the infiltrates was convincingly nodular. Our experience is therefore consistent with the published findings from the University of Minnesota and contrary to those of both Bartl et al and Schmid and Isaacson.

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Glutathione S-transferase expression in primary biliary cirrhosis supports combined “ductular metaplasia” of hepatocytes

Bile duct proliferation is a feature of many cholestatic diseases. The mechanism of this phenomenon is unknown but there is some support for the concept of “ductular metaplasia”. For example, Van Eyken et al used an immunocytotoxic approach to show, in a variety of cholestatic diseases, that hepatocytes can express cytokeratins which are normally restricted to the bile duct cells. The glutathione-S-transferases (GST; EC 2.5.1.18) are a multigene enzyme family that catalyse the conjugation of reduced glutathione with a variety of electrophiles. By immunological and catalytic criteria, the major cytotoxic isoenzymes can be categorised into four classes, α, ρ, θ and π. Time and tissue specific changes in the expression of these GST classes has been described in developing human tissues and these data suggest that changes in GST expression are related to changes in cell phenotype. **

We now describe observations of glutathione-S-transferase expression in primary biliary cirrhosis which provide further evidence for the concept of “ductular metaplasia”.

Expression of GST classes in cells with intermediate phenotype

<table>
<thead>
<tr>
<th>GST Class</th>
<th>α</th>
<th>ρ</th>
<th>θ</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bile duct epithelium</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Samples of liver were obtained from 10 patients who had undergone liver transplantation at the Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham, England. A diagnosis of end stage cirrhosis as result of primary biliary cirrhosis was confirmed biochemically, serologically, and histologically. Paraffin wax sections (4 μm) were cut, dewaxed, and the expression of α, ρ, and π classes of GST demonstrated by immunocytochemical localisation as described before. **

In all cases the cirrhotic nodules of hepatocytes were positive for α, ρ, and π GST. At the periphery of these nodules, cells that might represent an intermediate phenotype undergoing transformation to bile duct type cells were negative for α GST as were bile duct epithelial cells.

The polymorphic GSTM1 gene encodes the predominant μ class isoenzyme in human liver. In those who expressed this gene, GSTM1 isoforms demonstrated strong positivity in hepatocytes but were not expressed in bile duct epithelium or those cells putatively undergoing metaplasia.

In general, μ class expression was restricted to the bile ducts and “ductular” metaplasia cells, these both strongly positive. In some cases a weak diffuse positivity was also observed in occasional regenerating nodules of hepatocytes.

As shown in the table cells with an intermediate phenotype undergoing “ductular metaplasia” from hepatocyte to bile duct-type cells expressed π class GST but not the α or μ class isoforms. Although such cells were seen only on the periphery of the cirrhotic nodules, in many instances they were clearly continuous with surrounding hepatocytes. These “metaplastic” cells, therefore, did not show the typical hepatocyte pattern of strong positivity for α and μ class isoforms but, rather, similar GST expression to bile duct epithelial cells. This observation is consistent with the concept of “ductular metaplasia” and is supported by data in patients with extrahepatic biliary atresia showing altered expression of π GST in hepatocytes. It was interesting to note diffuse positivity for π class GST in some regenerating nodules, though it is not usually expressed by adult hepatocytes. Expression of π class GST by hepatocytes is normally seen only in fetal liver and may therefore be an indication of increased cell proliferation. **

On Drs Matthews and Burt: Comments

We have read the letter of Dr Hiley et al with interest. Their observations of π class GST in cells with “an intermediate phenotype” between that of hepatocytes and bile duct epithelium in primary biliary cirrhosis are in keeping with the results of this study. ** As the authors point out it is likely that this represents a similar phenomenon to that found in our cases of neonatal cholestatic liver disease. We agree that these findings provide further evidence that under certain circumstances hepatocytes may undergo a form of transdifferentiation. It is pertinent to note, however, that this so-called ductular metaplasia may not be the only mechanism by which increased numbers of ductular structures develop in the cholestatic liver. There is strong experimental evidence that in acute biliary obstruction, proliferation of true bile duct epithelial cells has a crucial (if not exclusive) role. ** Although this may be less important in the “atypical ductular proliferation” seen in chronic biliary disease, a significant contribution cannot be discounted.

Furthermore, it is conceivable that the ductular structures may be derived from proliferation and differentiation of a putative stem cell population within portal zones. Although controversial, there is growing acceptance of the concept that the mammary liver contains a stem cell compartment at this site. **