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Keratin immunohistochemistry in normal human liver. Cytokeratin pattern of hepatocytes, bile ducts and acinar gradient

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Summary. A panel of 2 polyclonal and 7 monoclonal antibodies directed against cytokeratins was tested on cryostat and paraffin sections of 14 normal human liver biopsies using an immunoperoxidase procedure. The staining characteristics of hepatocytes and bile ducts are reported. On cryostat sections, monoclonal antibodies directed against individual cytokeratins n°8 and n°18 stained both bile ducts and hepatocytes, whereas monoclonals anti-cytokeratin n°7 and n°19 exclusively stained bile ducts. The potential use of these 4 monoclonal antibodies in liver histopathology is briefly discussed. Monoclonal antibody anti-type II cytokeratins and the polyclonal rabbit anti-human keratin stained only bile ducts on both cryostat and paraffin sections. Using monoclonal antibody CAM 5.2 on paraffin sections, both bile ducts and parenchyma were positive. An acinar gradient was apparent in that zone 1 hepatocytes were more intensely stained. Moreover, a rim of hepatocytes around terminal hepatic venules and adjacent to subhepatic veins showed more intense staining. The same gradient could be seen in some paraffin sections stained with the monoclonals anti-cytokeratin n°18 and KL1, and the rabbit polyclonal anti-keratin "wide spectrum screening". The gradient is interpreted as reflecting quantitative differences in keratin content between hepatocytes. Polyclonal rabbit anti-human keratin is proposed as the most reliable antibody for identification of bile ducts in paraffin sections. The usefulness of reliable bile duct staining in several pathological conditions is emphasized.

Key words: Liver – Bile ducts – Hepatocytes – Keratins – Immunochemistry

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Introduction

Intermediate filaments (diameter between 7 and 11 nm) can be divided into five subclasses on the basis of chemical composition, immunoreactivity and cell type of origin (Osborn 1983). Although their function is not fully elucidated, they are believed to play a role in cell stability and intracellular organization (Lazarides 1980).

The cytokeratins are the largest group within the intermediate filament family and are characteristic of epithelial cells. Using two dimensional gel electrophoresis, Moll et al. (1982) identified 19 different cytokeratin polypeptides. These can be divided into an acidic (type I) and a (neutral to) basic (type II) subfamily (Cooper et al. 1985; Moll et al. 1982). Cytokeratins are usually expressed as polypeptide pairs consisting of a type I and a type II keratin (Cooper et al. 1985). All human epithelia can be classified according to their cytokeratin content (Moll et al. 1982).

Human hepatocytes display a relatively simple cytokeratin pattern and contain only cytokeratins n°8 and n°18 as shown by biochemical analysis (Moll et al. 1982). Two dimensional gel electrophoresis revealed the presence of cytokeratins n°7, 8, 18 and 19 in gall bladder epithelium (Moll et al. 1982).

The advent of polyclonal and monoclonal antibodies reacting with cytokeratins allows us to study the in situ distribution of cytokeratin polypeptides in human tissue. A great number of these antibodies – sometimes even directed against individual cytokeratins – have become available commercially. The original studies reporting on the reactivity of these antibodies in human tissues included only a few normal liver specimens and often cryostat sections only were used. Since immunohistochemical staining for cytokeratins has already proved useful in the study and in the differential

diagnosis of various neoplastic and non-neoplastic liver disorders (Butron Vila et al. 1984; Denk 1984; Denk and Lackinger 1984; Denk et al. 1981; Denk et al. 1982; Hazan et al. 1986; Krepler et al. 1982; Osborn et al. 1986; Sciot et al. 1986; Stosiek et al. 1985), it was felt that a detailed study of cryostat as well as paraffin sections of a fair number of normal human livers using a panel of commercially available anti-cytokeratin antibodies was indicated. Our aim was to check the reactivity of these antibodies on fresh frozen and routinely fixed and processed material as it is available to the surgical pathologist. The results obtained on these "normal livers" could then serve as a baseline for further studies of liver parenchyma and bile ducts (and ductules) in pathological cholestatic conditions.

A reliable staining method for bile duct structures would be very useful not only for research purposes but also for routine histopathological diagnosis. Hence this study was also undertaken to determine which antibody allowed the best visualization of bile ducts on paraffin sections, since for many specimens the pathologist has only paraffin embedded material available.

Materials and methods

Liver biopsies classified as "no histological abnormalities" were selected from the files of the Pathology Department, Universitair Ziekenhuis St. Rafaël, Katholieke Universiteit Leuven. Our material comprised 10 needle biopsies taken for diagnostic purposes (staging of malignant disease ($n=3$), unexplained asthenia ($n=1$), suspicion of Wilson's disease ($n=1$), exact indication unknown ($n=5$)), 2 biopsies obtained at laparoscopy and 2 post mortem specimens (routinely performed during autopsy, taken within 24 h after death). Patients ranged in age from newborn to 71 years.

In 7 cases, only material fixed in Bouin's solution and embedded in paraffin was available. In 2 cases only frozen tissue was available. In five cases, the specimens were received fresh and a part of the biopsy was quickly frozen in nitrogen-cooled liquid isopenthane and stored at −70° until further use. The remaining part was fixed in Bouin's solution and routinely processed. Since material was collected retrospectively from our files, fixation times were not strictly standardized.

Details of the materials are listed in Table 1.

A panel of 7 monoclonal and 2 polyclonal antibodies was used for immunohistochemical staining of both paraffin and cryostat sections.

On cryostat sections an indirect immunoperoxidase procedure as described by Mason et al. (1982) was performed. The optimal dilution of the primary antibody was determined in preliminary experiments using various dilutions on 2 specimens.

Further preliminary studies included the search for optimal staining procedures for each antibody using three immunohistochemical methods on paraffin sections of 2 cases: an indirect immunoperoxidase procedure (Mason et al. 1982), an unlabeled antibody enzyme method (PAP) according to Sternberger (1974), and an avidin-biotin-peroxidase complex (ABC) technique as described by Hsu et al. (1981). All three methods were tested with and without predigestion with trypsin (obtained from Sigma and used at 0,1% in $CaCl_2$ 0,1% for 10′ at 37° C). Different dilutions of primary antisera were used. Incubation times varied from 30′ at room temperature to overnight at 4° C. For each antibody, the procedure yielding optimal staining was subsequently applied on all specimens as indicated in Table 3.

The antisera, their source, specificity and the way they were used, are listed in Tables 2 and 3.

Controls, which were always negative, consisted of omission of primary or secondary antibody or the use of chromogen alone. In cases where polyclonal rabbit antisera were used, controls also included substitution of the primary antibody by normal rabbit serum.

Table 1. Materials

Case	Sex	Age years	Specimen	Frozen tissue available	Paraffin embedded tissue available
1	M	33	N	+	+
2	M	71	L	−	+
3	M	68	N	−	+
4	F	1	N	−	+
5	F	1	N	+	+
6	M	16	N	+	+
7	M	2	N	+	+
8	F	24	N	+	+
9	F	11	N	−	+
10	M	38	L	−	+
11	M	0	x	−	+
12	M	37	N	−	+
13	M	0	x	+	−
14	M	28	N	+	−

N = needle biopsy; L = laparoscopic biopsy; x = post mortem

Results

The results are summarized in Tables 4 and 5.

In frozen sections (Table 4) using monoclonal antibodies directed against cytokeratins n°8 (II) and n°18 (III) and monoclonals KL-1 (VIII) and CAM5.2 (IX), both liver parenchymal cells and bile ducts were positive in all cases stained with these antibodies. Bile ducts were usually more intensely labeled. When monoclonal antibodies against cytokeratins n°7 (I) and n°19 (IV) were used, only the bile ducts were intensely stained in 7 and 6 cases respectively, whereas the parenchyma remained completely negative (Fig. 1). Exclusive staining of bile ducts was also seen using the 2 polyclonal antibodies (VI and VII) in 6 cases and the monoclonal anti-type II cytokeratin (V) in 5 cases.

In paraffin sections (Table 5) using the monoclonal anti-cytokeratin n°7 (I), positive staining of (mostly larger) bile ducts was only obtained in 4 cases. In the remaining cases, the nonspecific

Table 2. Monoclonal and polyclonal antibodies used in this study

	Antibody	Source	Specificity	References
I.	Monoclonal anti-cytokeratin (glandular epithelia)	Amersham International Buckinghamshire, England	Cytokeratin n°7	Moll et al. (1982) Tölle et al. (1985)
II.	Monoclonal anti-cytokeratin 8	Amersham International Buckinghamshire, England	Cytokeratin n°8	Lane (1982) Moll et al. (1982)
III.	Monoclonal anti-cytokeratin (simple epithelia)	Amersham International Buckinghamshire, England	Cytokeratin n°18	Lane (1982) Moll et al. (1982)
IV.	Monoclonal anti-cytokeratin 19	Amersham International Buckinghamshire, England	Cytokeratin n°19	Lane et al. (1985) Moll et al. (1982)
V.	Monoclonal anti-type II cytokeratins	Amersham International Buckinghamshire, England	The majority of basic type II cytokeratins, including cytokeratin 8	Lane et al. (1985) Moll et al. (1982)
VI.	Polyclonal rabbit anti human keratin	Dako Corporation Santa Barbara, USA	Keratins predominantly of 56 and 64 kD	Schlegel et al. (1980)
VII.	Rabbit polyclonal anti-keratin, wide spectrum screening	Dako Corporation Santa Barbara, USA	Bovine muzzle epidermal keratin subunits of 58, 56, 52 and also 60, 51, 48 kD	Franke et al. (1978) Steinert (1975)
VIII.	Monoclonal KL-1	Immunotech	Keratins of 55–57 kD	Viac et al. (1983)
IX.	Anti-cytokeratin CAM5.2	Becton-Dickinson	Cytokeratins n°8, 18, 19	Makin et al. (1984)

Table 3. Application of monoclonal and polyclonal antibodies (optimal staining procedure as determined in preliminary experiments)

	Antibody (Specificity)	Dilution		Incubation time		Predigestion with trypsin*	Method*
		FS	PS	FS	PS		
I	Monoclonal anti-cytokeratin (glandular epithelia) (CK 7)	1/10	1/5	30′	overnight at 4° C	–	indirect immunoperoxidase
II	Monoclonal anti-cytokeratin 8 (CK 8)	1/10	1/5	30′	overnight at 4° C	–	avidin-biotin-peroxidase complex
III	Monoclonal anti-cytokeratin (simple epithelia) (CK 18)	1/10	1/5	30′	overnight at 4° C	+	avidin-biotin-peroxidase complex
IV	Monoclonal anti-cytokeratin 19 (CK 19)	1/10	1/5	30′	overnight at 4° C	–	avidin-biotin-peroxidase complex
V	Monoclonal anti-type II cytokeratins (majority of type II CK, including CK 8)	1/10	1/10	30′	overnight at 4° C	–	indirect immunoperoxidase
VI	Polyclonal rabbit anti human keratin (CK of 56, 64 kD)	1/400	1/400	30′	overnight at 4° C	+	unlabelled antibody
VII	Rabbit polyclonal anti-keratin, wide spectrum screening (bovine muzzle CK of 58,56,52,60,51,48 kD)	1/400	1/400	30′	overnight at 4° C	+	unlabelled antibody
VIII	Monoclonal KL-1 (CK of 55–57 kD)	1/100	1/100	30′	30′	–	indirect immunoperoxidase
IX	Anti-cytokeratin CAM 5.2 (CK 8,18,19)	1/5	1/20	30′	overnight at 4° C	+	indirect immunoperoxidase

FS = frozen section; PS = paraffin section

* = on paraffin sections only

Table 4. Results: frozen sections

	Antiserum																	
	I monoclonal anti cytokeratin (CK 7)		II monoclonal anti-CK 8 (CK 8)		III monoclonal anti cytokeratin (CK 18)		IV monoclonal anti CK 19 (CK 19)		V monoclonal anti type II CK		VI polyclonal rabbit anti human keratin		VII rabbit polyclonal antikeratin, wide spectrum screening		VIII monoclonal KL 1		IX monoclonal CAM 5.2 (CK 8,18,19)	
Case	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P
1	++	−	ND	ND	ND	ND	+	−	ND	ND	+	−	+	−	+	+	ND	ND
5	++	−	+	+	+	+	++	−	++	−	+	−	+	−	+	+	+	+
6	++	−	+	+	+	+	++	−	++	−	+	−	+	−	+	+	+	+
7	++	−	+	+	+	+	+	−	++	−	−to+	−	+	−	+	+	++	++
8	++	−	+	+	+	+	++	−	++	−	+	−	+	−	++	++	++	+
13	++	−	+	+	+	+	++	−	++	−	+	−	+	−	++	+	++	++
14	++	−	+	+	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

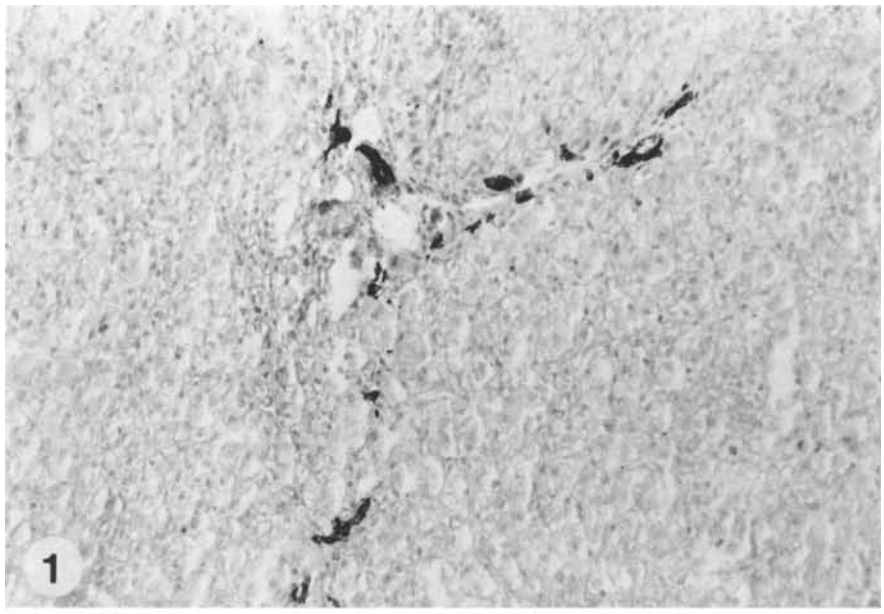
BD = bile ducts; P = parenchyma; ND = not done for lack of material; − = negative; + = positive; ++ = strongly positive

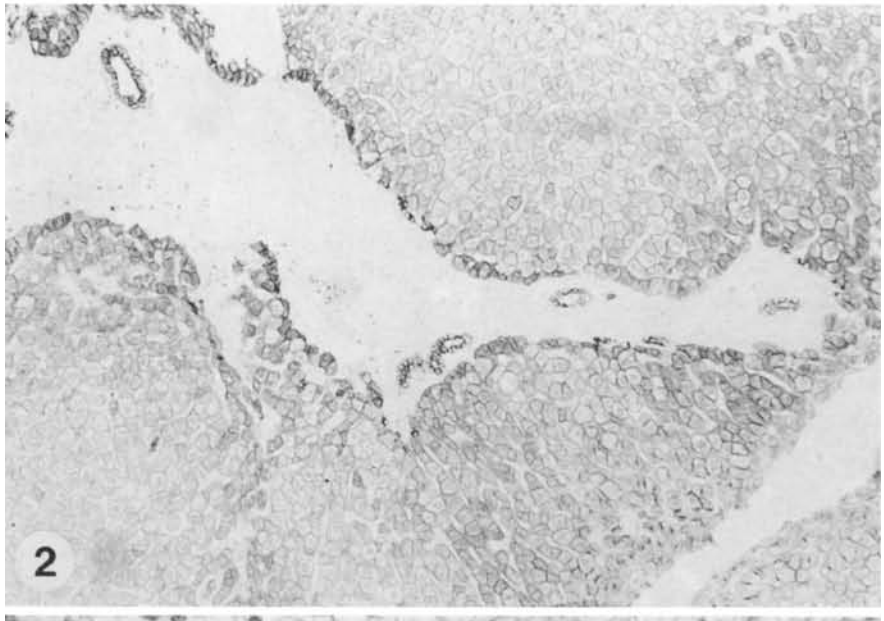
Table 5. Results: paraffin sections

	Antiserum																	
	I monoclonal anti cytokeratin (CK 7)		II monoclonal anti-CK 8 (CK 8)		III monoclonal anti cytokeratin (CK 18)		IV monoclonal anti CK 19 (CK 19)		V monoclonal anti type II CK		VI polyclonal rabbit anti human keratin		VII rabbit polyclonal antikeratin, wide spectrum screening		VIII monoclonal KL 1		IX monoclonal CAM 5.2 (CK 8,18,19)	
Case	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P	BD	P
1	BS	BS	−to+	−	−	−	+	−	+	BS	++	−	++	−to+	+	BS	+	+
2	BS	BS	−	−	−	−	−	−	+	BS	++	−	++	−to+	+	+	+	+
3	BS	BS	−to+	−	−	−	−	−	BD	BS	+	−	+	−	+	+	+	−to+
4	BS	BS	−	−	−	−	−	−	+	−	++	−	+	−	+	−to+	+	−to+
5	BS	BS	−	−	−	−	−to+	−	+	−	++	−	+	−	+	−to+	+	−to+
6	−to+	−	+	−	+	−to+	−to+	−	+	−	++	−*	++	+	+	+	+	+
7	BS	BS	+	−	−	−	−to+	−	+	−	++	−	+	−to+	+	BS	+	+
8	+	−	−to+	−	+	−to+	−to+	−	+	−	++	−	++	+	+	−to+	+	+
9	+	−	+	−	+	+	−to+	−	++	−	++	−*	+	−to+	++	−to+	+	+
10	BS	BS	−	−	−	−	−	−	BS	BS	++	−	+	−to+	BS	BS	+	+
11	BS	BS	−to+	−	−	−	−to+	−	+	BS	++	−	+	+	+	+	+	+
12	+	−	+	−	+	−to+	+	−	+	−	++	−	+	−	+	−to+	+	−to+

BD = bile ducts; P = parenchyma; ND = not done for lack of material; BS = background staining; − = negative; + = positive; ++ = strongly positive;

* Very few weakly positive hepatocytes in zone 1 or around terminal hepatic venules





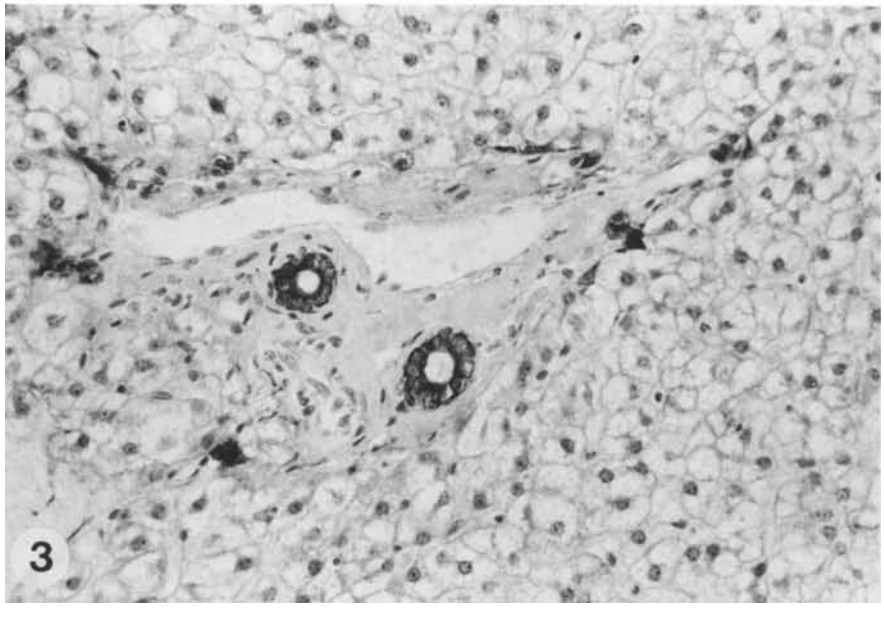
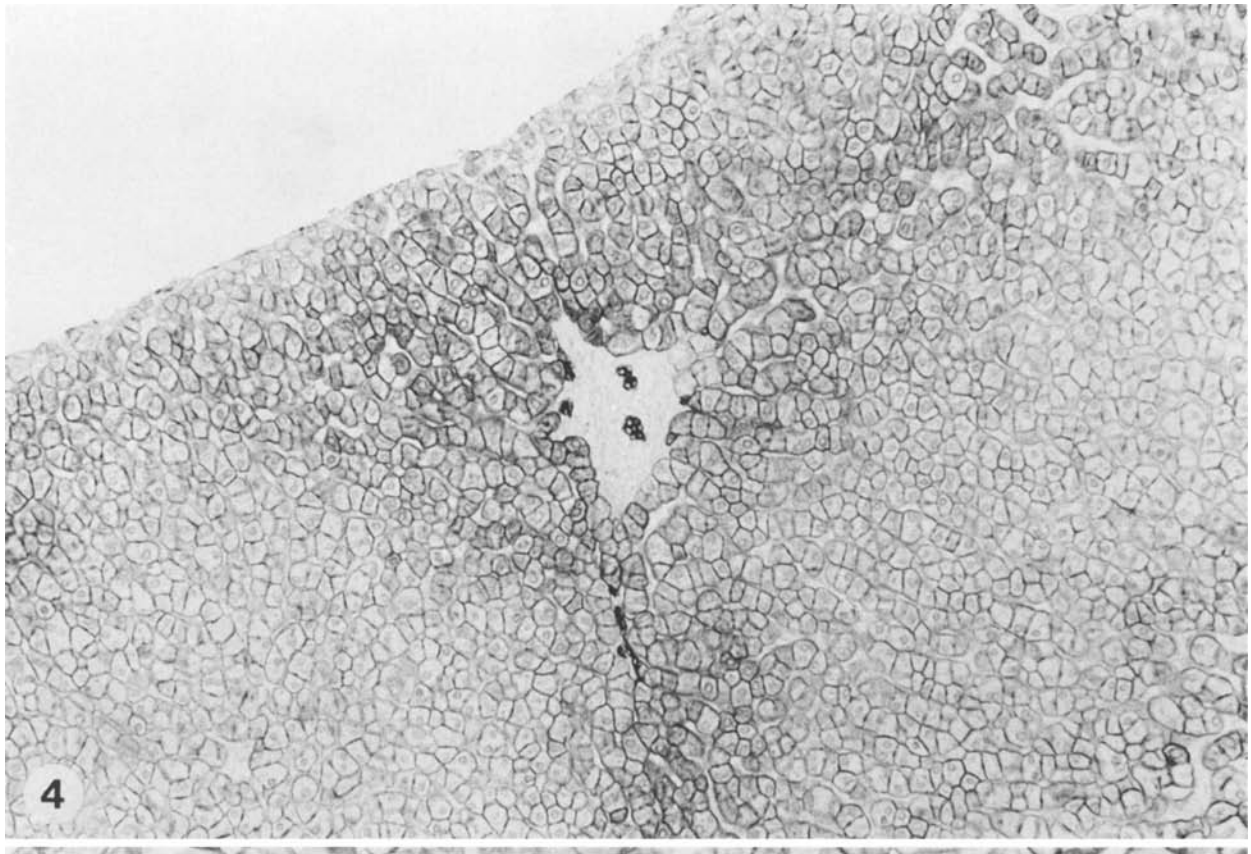


Fig. 1. Frozen section of case 5 showing bile ducts and ductules strongly positive for cytokeratin n°19 (IV). The liver parenchyma is negative. Indirect immunoperoxidase method for cytokeratin n°19, counterstained with Mayer's haemalum, ×100

Fig. 2. Paraffin section of case 9, showing intense staining of bile ducts for cytokeratin n°18 (III). Liver parenchymal cells are also positive but positivity is more marked in acinar zone 1. ABC complex method for cytokeratin n°18, predigestion with trypsin, counterstained with Harris' haematoxylin, ×100

Fig. 3. Paraffin section of case 5. Bile ducts and ductules are intensely stained by the polyclonal rabbit anti human keratin antiserum (VI). Liver parenchymal cells are negative. Unlabeled antibody method, predigestion with trypsin, counterstained with Harris' haematoxylin, ×250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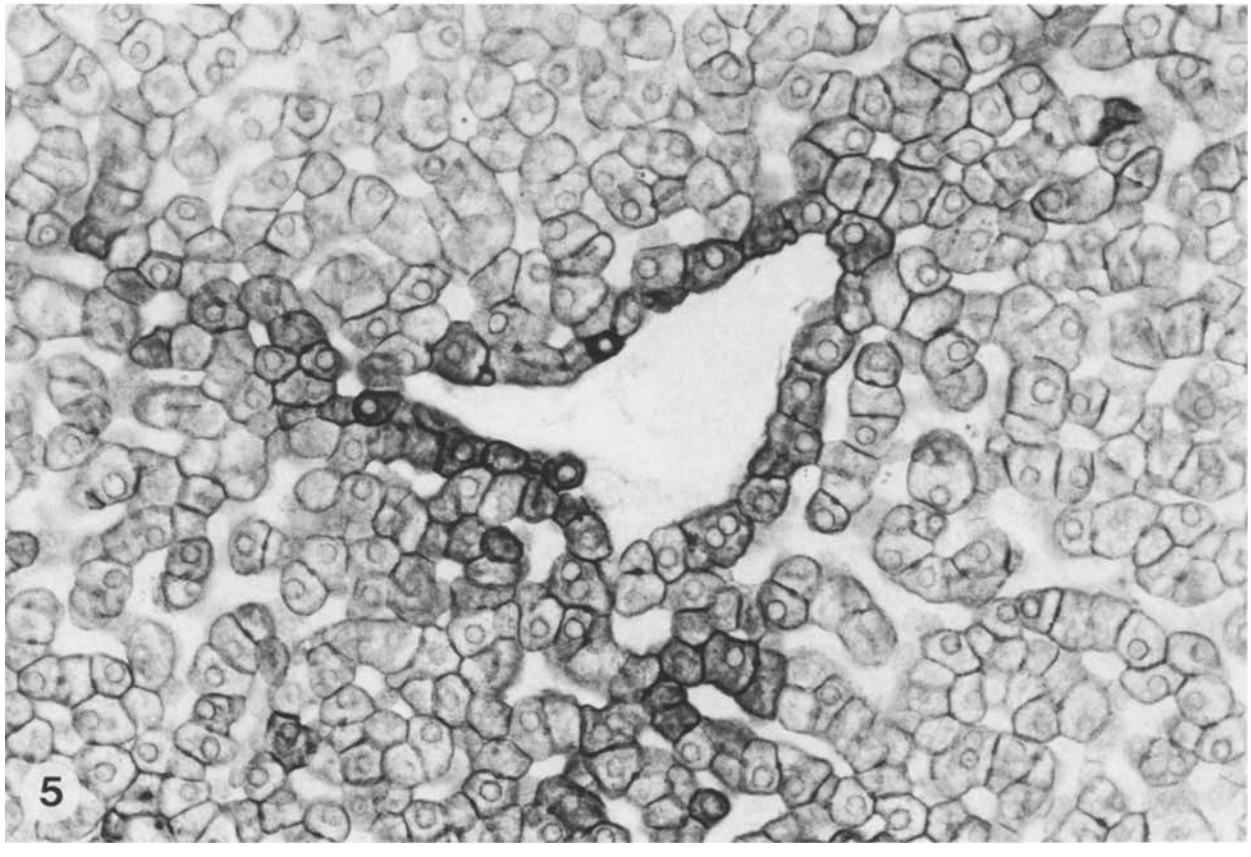
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background staining was too intense for true positivity to be assessed.

With monoclonal anti-cytokeratin n°8 (II) most bile ducts were stained in eight cases. The parenchyma always remained negative.

With the monoclonal anti-cytokeratin n°18 (III), a positive result was only obtained in 4 cases. In all these cases, bile ducts and a number of zone 1 hepatocytes and of hepatocytes around the terminal hepatic venules or adjacent to a larger subhepatic vein were clearly positive, often with more intense staining at the canalicular membrane (Fig. 2).

With the monoclonal anti-cytokeratin n°19 (IV), bile ducts were also positive in 8 cases. The larger interlobular bile ducts were often more intensely stained. The parenchyma remained negative.

With the monoclonal anti-type II cytokeratin (V), positive staining of bile ducts was observed in all cases. The parenchyma often showed some non-specific background staining.

With the polyclonal rabbit anti-human keratin (VI), very intense staining of all bile ducts was obtained in all cases (Fig. 3). Parenchymal cells were completely negative except in cases 6 and 9 where a few positive hepatocytes were seen in zone 1 or around the terminal hepatic venules.

With antibody CAM 5.2 (IX), bile ducts were stained in all cases. Liver parenchymal cells were also positive in most cases and exhibited a striking gradient. Hepatocytes in acinar zone 1 were intensely positive (Fig. 4). Positivity decreased towards zone 3 but around the terminal hepatic venules a rim of more positive hepatocytes could be seen (Fig. 5). Liver cells adjacent to subhepatic veins were also more positive. In all cases, the positivity was more pronounced at the canalicular pole of the hepatocytes (Fig. 5).

With monoclonal KL-1 (VIII), bile ducts were positive in 12 cases. Parenchymal cells were mostly positive and in 6 cases again a gradient was apparent: zone 1 hepatocytes as well as a rim of hepatocytes around terminal hepatic venules were more intensely stained. Staining was more prominent at the canalicular pole.

With the polyclonal rabbit anti-keratin "wide spectrum screening" (VII), positive staining of all bile duct structures was seen in all cases. In 8 cases, a variable number of hepatocytes were clearly positive, often at the canalicular membrane. Positive hepatocytes were mainly found in zone 1 and in cases 7, 8 and 9 also in a rim around the terminal hepatic venules.

Discussion

Cryostat and/or paraffin sections of fourteen human liver specimens classifed as "no histological abnormalities" were stained with a panel of monoclonal and polyclonal antibodies directed against cytokeratins. For each antibody, an "optimal" immunoperoxidase procedure, as determined in preliminary experiments, was used.

On frozen sections, liver parenchymal cells were stained by monoclonal antibodies anti-cytokeratin n°8 and n°18 but remained negative when the monoclonals anti-cytokeratin n°7 and n°19 were used. Bile duct cells, however, were positive with monoclonals anti-cytokeratin n°8, n°18, n°7 and n°19. Biochemical analysis using two dimensional gel electrophoresis has revealed the presence of cytokeratins n°8 and n°18 in liver parenchymal cells and cytokeratins n°7,8,18 and 19 in gall bladder epithelium (Moll et al. 1982). Using monoclonal antibodies directed against these individual cytokeratin polypeptides in an indirect immunofluorescence technique on cryostat sections of 2 normal human liver specimens, Osborn et al. (1986) confirmed the reactivity of hepatocytes with monoclonals anti-cytokeratin n°8 and n°18 and reported the staining of bile ducts with monoclonals anti-cytokeratin n°7, 8, 18 and 19. These data are confirmed in the present study, which (except for the monoclonal anti-cytokeratin n°7) used other monoclonal antibodies and an immunoperoxidase instead of a fluorescence procedure.

On paraffin sections, staining of bile ducts with the monoclonal anti-cytokeratin n°7 was only obtained in 4 cases and with monoclonal anti-cytokeratin n°19 in 8 cases. Bile ducts were stained with monoclonal anti-cytokeratin n°8 in 8 cases but

Fig. 4. Paraffin section of case 9. Bile ducts and ductules are strongly labeled by the monoclonal antibody CAM5.2 (IX). Liver parenchymal cells are positive. Positivity is more pronounced in acinar zone 1. Note also more intense staining of hepatocytes at the canalicular membrane. Indirect immunoperoxidase procedure, predigestion with trypsin, counterstained with Harris' haematoxylin, ×140

Fig. 5. Paraffin section of case 6 showing a rim of hepatocytes around a terminal hepatic venule (THV) strongly positive with antibody CAM5.2 (IX). Staining of hepatocytes is more marked in the canalicular area. Indirect immunoperoxidase procedure, predigestion with trypsin, counterstained with Harris' haematoxylin, ×140

liver parenchyma remained negative. Fixation has apparently destroyed part of the immunoreactivity of cytokeratin n°8. Bile ducts were still positive in 8 cases and this probably reflects a higher content of cytokeratin n°8 in bile duct cells than in hepatocytes. Electron microscopy has indeed shown bile duct cells to have a higher intermediate filament investment than hepatocytes (Biava 1964; Chedid et al. 1974; Sternlieb 1965). Bile ducts and a variable number of hepatocytes were stained using the monoclonal anti-cytokeratin n°18 in 4 cases. Thus, the four monoclonal antibodies directed against individual cytokeratin polypeptides clearly give better results when used on cryostat sections.

Since neoplastic cells usually retain the intermediate filament type of the cell of origin, antibodies against intermediate filaments can be used in the differential diagnosis of human neoplasms (Corson 1986; Osborn et al. 1984). Moreover, tumour cells show a tendency to express the same cytokeratin pattern as their non-tumour counterparts (Moll et al. 1982). In this respect immunohistochemical analysis of cytokeratin expression has proven useful in the differential diagnosis between hepatocellular carcinoma and cholangiocellular carcinoma (Krepler et al. 1982; Osborn et al. 1986). The four commercially available antibodies used in this study might also serve this purpose. However, it should be borne in mind that the best results with these antibodies are obtained on cryostat sections. Indeed, our results clearly indicate that reactivity with these 4 monoclonals is reduced or even completely lost in paraffin embedded material. Therefore, on paraffin sections, only positive results can be used in differential diagnosis.

The monoclonals anti-cytokeratin 7, 8, 18 and 19 might also be used as "differentiation markers" to study ductular proliferation (and its origin) and ductular metaplasia of hepatocytes. These phenomena have so far only been studied using a polyclonal rabbit anti-keratin antiserum (Butron Vila et al. 1984; Sciot et al. 1986; Vanstapel et al. 1984) and a monoclonal anti-cytokeratin n°19 (Stosiek et al. 1985).

Using monoclonal CAM5.2 on paraffin sections, a striking finding was the gradient in staining of the liver parenchyma. When Makin et al. (1984) originally described this antibody, they reported that (on paraffin sections) only a few periportal hepatocytes were positive. In our material, on paraffin sections, almost all hepatocytes were positive, those of zone 1 of the liver acinus being clearly more positive. An unexpected finding was also the presence of a rim of more intensely stained hepatocytes around the terminal hepatic venules and adjacent to subhepatic veins. Using CAM5.2 on cryostat sections the parenchyma was uniformly positive. Apparently part of the immunoreactivitiy of the cytokeratin filaments is destroyed by fixation so that quantitative differences in cytokeratin content become apparent when paraffin embedded material is used. Zone 1 hepatocytes and those around terminal hepatic venules or close to subhepatic veins are apparently richer in cytokeratin filaments.

Positive hepatocytes were detected using anti-cytokeratin (simple epithelia) (anti CK 18), monoclonal KL1 and rabbit polyclonal anti-keratin "wide spectrum screening". In those cases positivity was nearly always detected in zone 1, around terminal hepatic venules or adjacent to subhepatic veins, indicating that the observed gradient is real. In cases where hepatocytes were positive on paraffin sections, the positive immunostaining was always more pronounced at the canalicular membrane. This is in keeping with the observations of others using immunofluorescence on frozen sections (Denk et al. 1981; Franke et al. 1979; Franke et al. 1981).

An unexpected finding was the absence of reactivity of hepatocytes with the monoclonal anti-type II cytokeratin. Although according to the data sheet provided by the firm monoclonal anti-type II cytokeratin reacts on Western blotting with the majority of basic type II cytokeratins including cytokeratin n°8, only bile ducts were stained positively on both cryostat and paraffin sections. This might be due to conformational masking of the epitope recognized by the antibody on cytokeratin n°8 in the keratin filaments present in hepatocytes in vivo. Hazan et al. (1986) have reported the first case of conformational masking of an epitope in an intermediate filament system. Alternatively, the absence of reactivity of hepatocytes with this antibody might also indicate that an epitope present on cytokeratin n°8 from other tissues is absent from hepatocytic cytokeratin n°8. The staining of bile duct cells might be due to the presence of the basic cytokeratin n°7 present in bile duct cells.

The two polyclonal antibodies (polyclonal rabbit anti human keratin and rabbit polyclonal anti-keratin, wide spectrum screening) used in this study clearly gave better results on paraffin than on cryostat sections. Rabbit polyclonal anti-keratin "wide spectrum screening" also stained hepatocytes on paraffin sections in a fair number of cases.

The testing of a panel of 9 antibodies against cytokeratins enabled us to select an antiserum that

produces a very intense staining of bile ducts on paraffin sections: the polyclonal rabbit anti-human keratin. Recently, the usefulness of keratin staining to identify bile ducts and assess their changes in orthotopic liver transplantation was stressed (De Lellis et al. 1986). It may indeed on occasion be difficult to identify and evaluate bile ducts and ductules on routinely stained sections, especially in neonates (Alagille and Odièvre 1978; Dahms et al. 1982). The rabbit polyclonal anti-human keratin appears most useful to identify bile duct structures in pathological conditions in which their presence and appearance is crucial, such as atresia or paucity of bile ducts, primary biliary cirrhosis and sclerosing cholangitis.

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