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The immunocytochemical localisation and distribution of cytochrome P-450 in normal human hepatic and extrahepatic tissues with a monoclonal antibody to human cytochrome P-450

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1 The localisation and distribution of cytochrome P-450 in human tissues has been studied by immunocytochemistry using a monoclonal antibody to a major form of human hepatic cytochrome P-450, P-450hA7, which is closely related to cytochromes P-450 HLp and P-450_{NF}.

2 Strong immunoreactivity was identified in hepatocytes, columnar absorptive epithelial cells of the small intestine, polymorphonuclear leucocytes and their precursors in the bone marrow, and in mast cells.

3 Weak immunoreactivity was present in the proximal tubules of the kidney, pancreatic acini, gall bladder epithelium, squamous epithelium and sebaceous glands of the skin, interstitial cells of the testis and luteal cells of the ovary.

4 Immunoreactivity could not be demonstrated in the adrenal gland, placenta, colonic epithelium and alveolar type II cells and Clara cells of the lung.

Keywords cytochrome P-450 hepatic extrahepatic monoclonal antibody

Introduction

The cytochromes P-450 are a superfamily of haem containing enzymes which play a central role in the metabolism of xenobiotics and many endogenous substances (Adesnik & Atchison, 1986; Black & Coon, 1986). The various forms of cytochrome P-450 have diverse substrate specificities and responses to inducing agents (Guengerich *et al.*, 1982; Le Provost *et al.*, 1983; Burke *et al.*, 1985). The balance of metabolic activation and detoxification of drugs and other chemicals by individual forms of cytochrome P-450 in different tissues is an important factor in explaining organ-specific toxicity (Gram *et al.*, 1986). Cytochrome P-450 and its associated monooxygenase activities are found predominantly in the liver, but are also present in many extrahepatic tissues although usually at lower levels than in the liver (Burke & Orrenius, 1979; Gram *et al.*, 1986).

Immunocytochemical techniques have the sensitivity and resolution to locate specific cell types containing identifiable cytochrome P-450 forms, even in tissues with a low overall cytochrome P-450 content, and in small samples of tissue, e.g. human biopsy material. The use of monoclonal rather than polyclonal antibodies greatly improves the specificity of identification of individual cytochrome P-450 forms.

We have studied the localisation and distribution of cytochrome P-450 in normal human tissues

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using a monoclonal antibody to a major hepatic form of human cytochrome P-450, P-450hA7, of subunit molecular weight 53kDa (Barnes *et al.*, 1986, 1987), which is closely related to cytochromes P-450 HLp (Watkins *et al.*, 1985) and P-450_{NF} (Beaune *et al.*, 1986).

Methods

Tissue

Normal human adult liver was obtained from renal transplant donors within 20 min of circulatory arrest, placed immediately in ice-cold 0.9% saline and, within 30 min of removal, small portions (1 × 1 × 0.5 cm) put into 10% neutral buffered formalin. Liver tissue was fixed at room temperature for 24 h then dehydrated in ethanol, cleared in xylol and embedded in paraffin wax. Examples of morphologically normal extrahepatic tissues were selected from the archive files of the Department of Pathology, University of Aberdeen. These tissue specimens, which had been submitted for diagnostic purposes, were all fixed in 10% neutral buffered formalin and processed subsequently as for the liver samples. Normal peripheral blood and bone marrow smears were obtained from the Haematology Unit of the Department of Pathology, University of Aberdeen.

Monoclonal antibody preparation

Mouse monoclonal antibodies to human hepatic cytochrome P-450 have been prepared in our laboratories (Barnes *et al.*, 1986). Briefly, human liver microsomes were used to immunise 8 week old female BALB/c mice. Three days after the final booster immunisation, mouse spleen cells were fused with mouse myeloma cells (myeloma cell line Ag 8.653). Monoclonal antibodies to cytochrome P-450 were selected by enzyme linked immunoabsorbent and immunoblot procedures, using a purified preparation of human cytochrome P-450hA7 (molecular weight on sodium dodecyl sulphate polyacrylamide gel electrophoresis = 53kDa, specific content = 15.5 nmol mg^{-1} protein), which is closely related to human cytochromes P-450 HLp (Watkins *et al.*, 1985) or P-450_{NF} (Beaune *et al.*, 1986) – see discussion. This study employed only one of the monoclonal antibodies, MAb-HL3, which recognized a single protein of the same relative molecular weight as cytochrome P-450hA7 on immunoblots of hepatic microsomes of 11 individuals (Barnes *et al.*, 1987).

Immunocytochemical procedures

i. *Indirect immunoperoxidase technique* Sections (5 μm thick) of fixed embedded tissue were cut and mounted on glass slides coated with poly-L-lysine (Sigma Chemical Co. Ltd), deparaffinised in xylene (two changes, 10 min each), rehydrated in ethanol and washed in 0.05 M Tris-buffered saline (TBS, pH 7.4). Endogenous peroxidase was blocked by incubating with hydrogen peroxide-methanol (1:30 v/v) for 15 min at room temperature. Sections were then further washed in TBS and labelled without prior trypsin digestion with the primary layer antibody, MAb-HL3. MAb-HL3 (hybridoma culture supernatant used undiluted) was applied for 1 h at room temperature. TBS and normal mouse immunoglobulin (Ig) applied in place of MAb-HL3 acted as negative controls. Tissue sections were then washed again in TBS and a second antibody (horse radish peroxidase-conjugated rabbit anti-mouse immunoglobulin: Dakopatts A/S, 1 in 40 dilution in 10% normal human serum) applied for 30 min. After further washing in TBS the bound peroxidase was demonstrated using 3,3 -diaminobenzidine (DAB, Sigma Chemical Co. Ltd.: 30 mg DAB and 0.5 ml hydrogen peroxide in 100 ml 0.05 M Tris buffer pH 7.6) as the substrate for 6 min. Sections were then washed in water, the nuclei counterstained with haematoxylin, dehydrated in alcohol, cleared in xylol and mounted in DPX (Raymond Lamb).

ii. *Alkaline phosphatase anti-alkaline phosphatase (APAAP) technique* Peripheral blood and bone marrow smears were fixed in acetone for 20 min at room temperature, air dried and washed in TBS. MAb-HL3 was applied to the fixed haematological samples or the rehydrated tissue sections for 1 h as above and the slides were washed in TBS. Rabbit anti-mouse immunoglobulin (Dakopatts A/S, 1 in 20 solution) was applied to the slides for 30 min and after washing again in TBS, alkaline phosphatase anti-alkaline phosphate immunoglobulin complex (Dakopatts A/S: 1 in 20 dilution) was applied for 30 min. Slides were then further washed in TBS and sites of bound alkaline phosphatase demonstrated using naphthol AS-MX phosphate (Sigma Chemical Co. Ltd, 25 mg) as the substrate with simultaneous coupling of the product to fast red TR (Raymond Lamb, 12 mg) at pH 9.2 in 0.1 M veronal acetate buffer (50 ml), containing levamisole (Sigma Chemical Co. Ltd, 12 mg) to inhibit endogenous alkaline phosphatase. The slides were then washed in water, counterstained with haematoxylin and mounted in Apathy's medium. The APAAP technique was used to avoid interference from endogenous blood cell peroxidase.

Assessment of results

All areas of each section (8–14 sections per tissue) were examined with transmitted light microscopy and the degree of immunostaining assessed on a three point scale (strong, weak, none).

Results

Light microscopic examination of all the tissues immunostained with MAb-HL3 showed that the tissues could be divided into three groups according to the intensity of immunostaining (strong, weak or no immunostaining: Table 1). The immunoreactivity of positively staining cells and tissues was abolished when MAb-HL3 was replaced by TBS or normal mouse Ig of the appropriate isotype in the immunocytochemical procedure. Prestaining absorption of MAb-HL3 with purified cytochrome P-450hA7, also abolished the positive reactions.

i. *Cells and tissue showing strong immunoreactivity*

Liver Light microscopic examination of sections of normal human adult liver immunostained for cytochrome P-450 showed that there was marked cytoplasmic (non-nuclear) staining of the hepatocytes and slight cytoplasmic staining of the epithelium of the bile ducts. There was a distinct zonal distribution of cytochrome P-450, with localisation predominantly in hepatocytes of zone 3 of the liver acinus (centrilobular region). The hepatocytes of zone 2 (midzonal) closest to zone 3 also showed some staining, but there was no staining of zone 1 (periportal) hepatocytes. There was no immunostaining of endothelium, fibrous tissue, smooth muscle, Kupffer cells or bile canaliculi (Figure 1).

Small intestine Cytochrome P-450 immunoreactivity was localised to the epithelial cells of the small intestine. There was strong cytoplasmic immunostaining of the columnar absorptive epithelial cells of the villus, whereas the goblet cells and the epithelial cells of the crypts of Lieberkuhn showed no immunostaining (Figure 2). Mast cells in the wall of the small intestine showed strong cytoplasmic staining. There was no immunostaining of the connective tissue or smooth muscle or of the blood and lymphatic vessels of the small intestinal wall.

Peripheral blood and bone marrow Examination of peripheral blood smears demonstrated strong cytoplasmic staining of the polymorphonuclear leucocytes, but no immunostaining of the lymphocytes, monocytes, platelets or erythrocytes (Figure 3). Bone marrow smears showed strong

Table 1 Cell and tissue immunoreactivity with MAB-HL3

Tissue or cell		
i.	*Strong immunoreactivity*	*Cellular localisation*
	Liver	Hepatocytes, biliary epithelium
	Small intestine	Columnar epithelial cells
	Peripheral blood	Polymorphonuclear leucocytes
	Bone marrow	Myelocytes, metamyelocytes
	Mast cells	
ii.	*Weak immunoreactivity*	
	Pancreas	Acini
	Gall Bladder	Epithelium
	Kidney	Proximal tubules
	Skin	Epidermis, sebaceous glands
	Ovary	Luteal cells
	Testis	Leydig cells
iii.	*No immunoreactivity*	
	Adrenal, lung	
	Thyroid, colon, stomach	
	Placenta, skeletal muscle	
	Endometrium, nerve	
	Prostate, brain	
	Nasal epithelium	
	Olfactory epithelium	

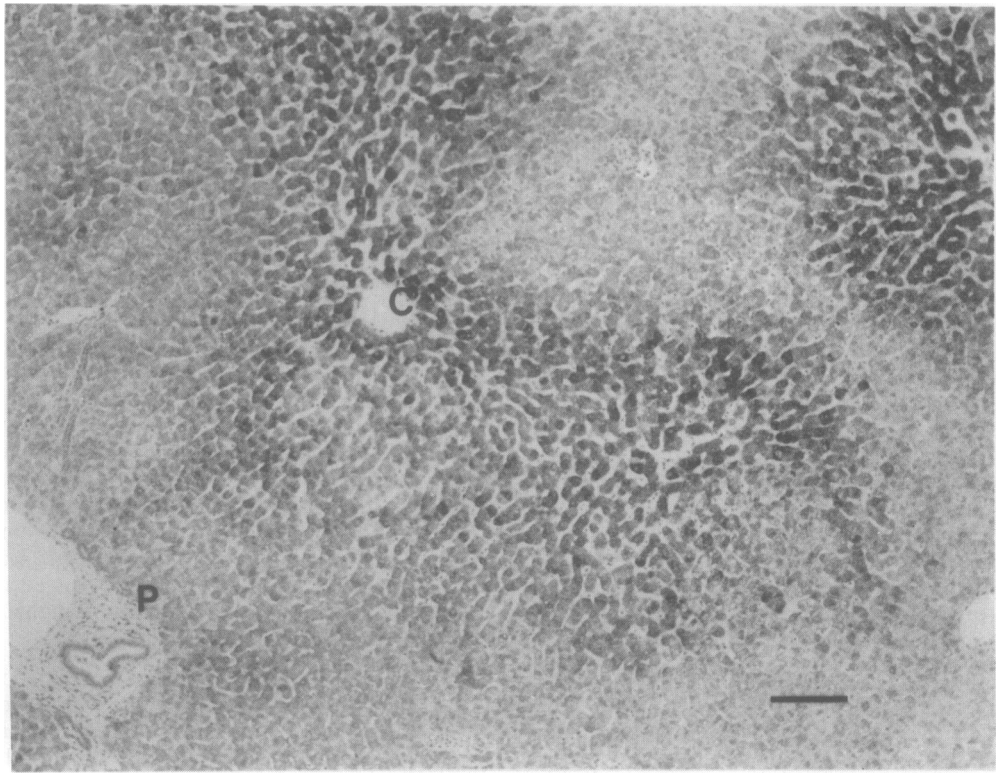


Figure 1 The immunocytochemical demonstration of cytochrome P-450 in normal human adult liver with MAB-HL3. There is a marked zonal distribution of cytochrome P-450. (P, portal area; C, central vein). (× 50, scale bar represents 160 μm.)

immunostaining of the myeloid series of cells, whereas the erythroid cell series and megakaryocytes were negative (Figure 4).

Mast cells Mast cells present in the connective tissue of the small intestine (Figure 2), dermis, colonic wall and lung connective tissue demonstrated strong cytoplasmic immunostaining (Figure 5).

ii. *Tissues demonstrating weak immunostaining*

Kidney There was slight cytochrome P-450 immunoreactivity of the cytoplasm of the proximal tubular epithelial cells, whereas the glomeruli, loops of Henle, distal tubules and collecting ducts did not show any immunostaining.

Pancreas The acinar epithelial cells and the epithelium of the ducts of the exocrine pancreas showed slight immunostaining. The islets of Langerhans and the fibrous tissue septa were negative.

Ovary The luteal cells of the ovary displayed slight immunoreactivity with MAb-HL3, whereas the ova, *corpora albicantia* and stromal cells were negative.

Testis The Leydig or interstitial cells demonstrated slight cytoplasmic immunoreactivity and the seminiferous tubules, epididymis and connective tissue all displayed no immunoreactivity.

Gall bladder The cytoplasm of the columnar epithelial cells lining the gall bladder showed slight immunoreactivity with MAb-HL3. The connective tissue and muscularis of the gall bladder wall were negative.

Skin The non-keratinised layers of the epidermis and the epithelium of the sebaceous glands both showed weak immunoreactivity. Mast cells within the dermis showed strong immunostaining. Immunostaining was absent in sweat glands, hair follicles, dermal connective tissue and blood vessels.

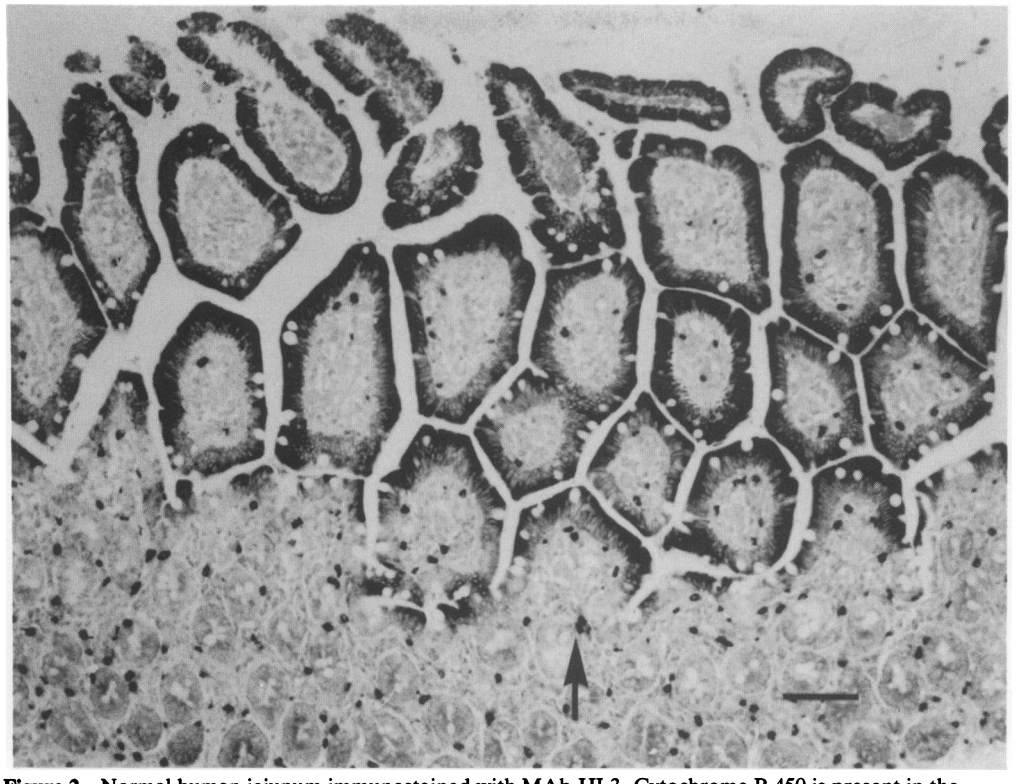


Figure 2 Normal human jejunum immunostained with MAb-HL3. Cytochrome P-450 is present in the columnar epithelial cells of the villi and not the crypts. There is strong immunostaining of the mast cells (arrow identifies a mast cell) within the lamina propria. (× 90, scale bar represents 90 μm.)

iii. *Tissues demonstrating no immunostaining with MAb-HL3* Immunoreactivity with MAb-HL3 was absent from a variety of tissues (Table 1), including the adrenal gland, lung, colon and placenta. There was no immunostaining of the alveolar type II cells and Clara cells of the lung, although mast cells within the connective tissue of the lung displayed strong immunoreactivity. The colonic epithelium displayed no immunostaining, although here also the mast cells in the colonic wall displayed strong immunoreactivity.

Discussion

We report the first detailed immunocytochemical study of the localisation and distribution of cytochrome P-450 in normal human tissues using a monoclonal antibody raised against human cytochrome P-450. This is a major form of human hepatic cytochrome P-450, P-450hA7 (Barnes *et al.*, 1987), which, on the basis of its subunit molecular weight (53 kDa, Barnes *et al.*, 1986, 1987), its *N*-terminal amino acid sequence over 18 amino acids and its high activity for nifedipine oxidation (unpublished data), is probably closely related to human cytochrome P-450 HLp (Watkins *et al.*, 1985) and human cytochrome $P\text{-}450_{NF}$ (Beaune *et al.*, 1986). Baron *et al.* (1983) demonstrated cytochrome P-450 in the epithelium of human breast, skin and prostate gland using immunocytochemistry with polyclonal antibodies to an unidentified form of human cytochrome P-450.

We have reported elsewhere the immunocytochemical characterisation of the anti-cytochrome P-450 monoclonal antibody (MAb-HL3) used in this study (Murray *et al.*, 1988). It was shown that the epitope on cytochrome P-450 recognised by MAb-HL3 is not destroyed by standard histological processing, ie. formalin fixation, dehydration and clearing in organic solvents and wax embedding, thus enabling the study of a wide range of normal human tissues.

Five cell types were identified which demonstrated strong immunoreactivity with MAb-HL3. These were hepatocytes, small intestinal columnar

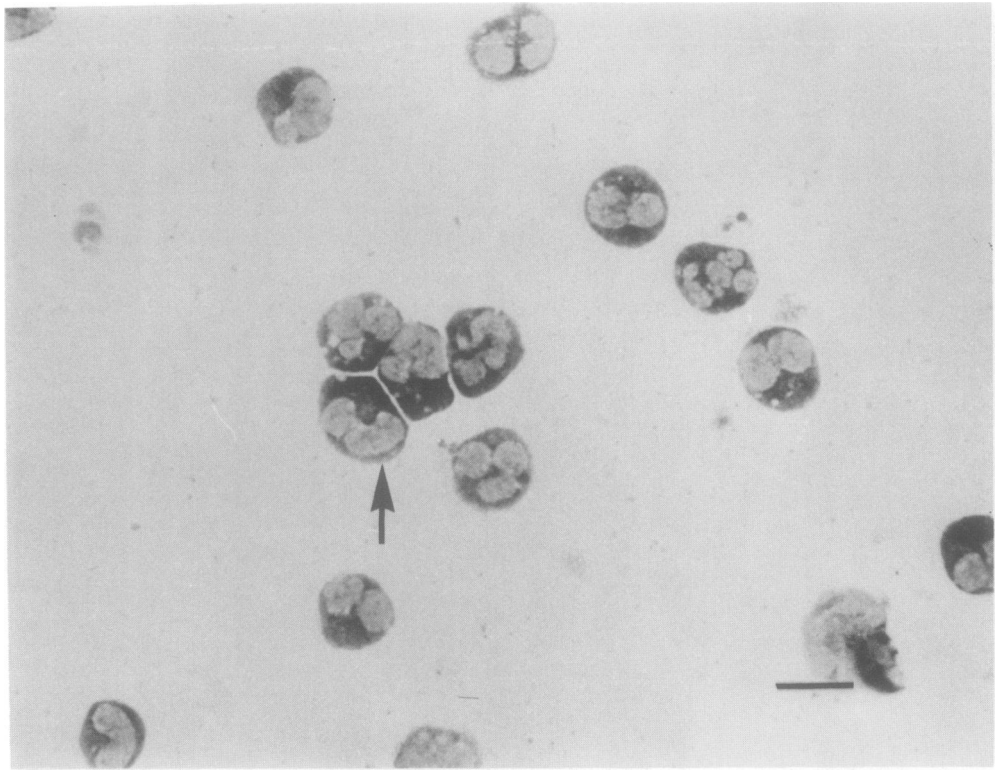


Figure 3 Cytochrome P-450 immunolocalisation in polymorphonuclear leucocytes (arrow identifies a polymorph). There is strong cytoplasmic staining of the polymorphs with MAb-HL3. (× 540, scale bar represents 15 μm.)

absorptive cells, polymorphonuclear leucocytes and their precursors in the bone marrow, and mast cells. Although strongly immunoreactive mast cells were identified in a variety of tissues, the immunostaining classification of these tissues was made according to the degree of staining shown by the epithelium.

In the liver cytochrome P-450 was predominantly localised to the zone 3 (centrilobular) hepatocytes, with a slight amount of immunostaining of the biliary epithelium. A detailed account of cytochrome P-450 immunolocalisation and distribution in normal adult human liver with MAb-HL3 has been reported elsewhere (Murray *et al.*, 1988).

The immunostaining of small intestine showed a distinct crypt to villus gradient, with cytochrome P-450 localised to the cytoplasm of the columnar epithelial (absorptive) cells of the villus. Cytochrome P-450 associated monooxygenase activity has previously been identified in human jejunal microsomes (Finnen *et al.*, 1983), although the preferential localisation of cytochrome P-450 in absorptive cells of the small intestinal villus has not previously been documented in human small intestine. However, the distribution of cytochrome P-450 in human small intestine is similar to the distribution of cytochrome P-450 determined either immunochemically (Bonkovsky *et al.*, 1985) or spectrophotometrically (Hoensch *et al.*, 1975) in cells scraped from rat small intestine.

In the blood cytochrome P-450 was localised to the polymorphonuclear leucocytes and their precursors in the bone marrow. Cytochrome P-450 has previously been identified and partially purified in human polymorphonuclear leucocytes (Mungikar & Gothoskar, 1986; Shak & Goldstein, 1984, 1986). Cytochrome P-450 has not previously been identified in human bone marrow, although cytochrome P-450 associated monooxygenase activity has been demonstrated in cultured human sternal marrow cells (Fujino *et al.*, 1982). Cytochrome P-450 recognised by MAb-HL3 was not present in platelets, monocytes or lymphocytes, although cytochrome P-450 or cytochrome P-450 associated activity has been described in these human cell types

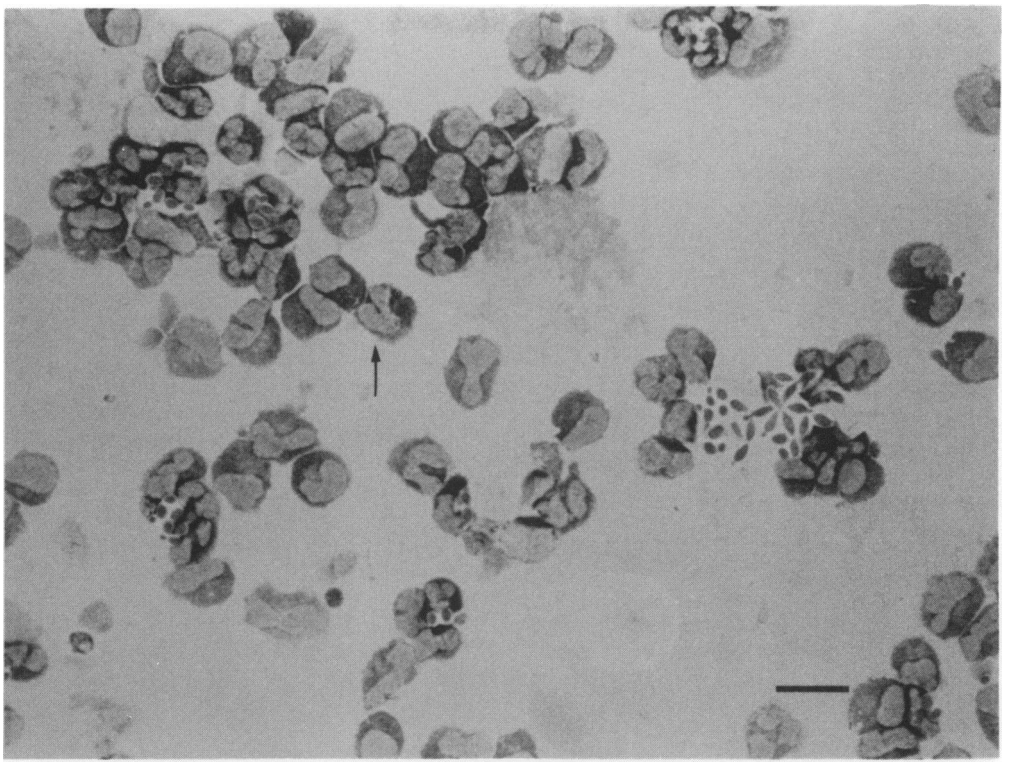


Figure 4 Immunostaining of a group of myeloid cells with MAb-HL3 showing strong cytoplasmic staining (arrow identifies a myeloid cell). (× 340, scale bar represents 25 μm.)

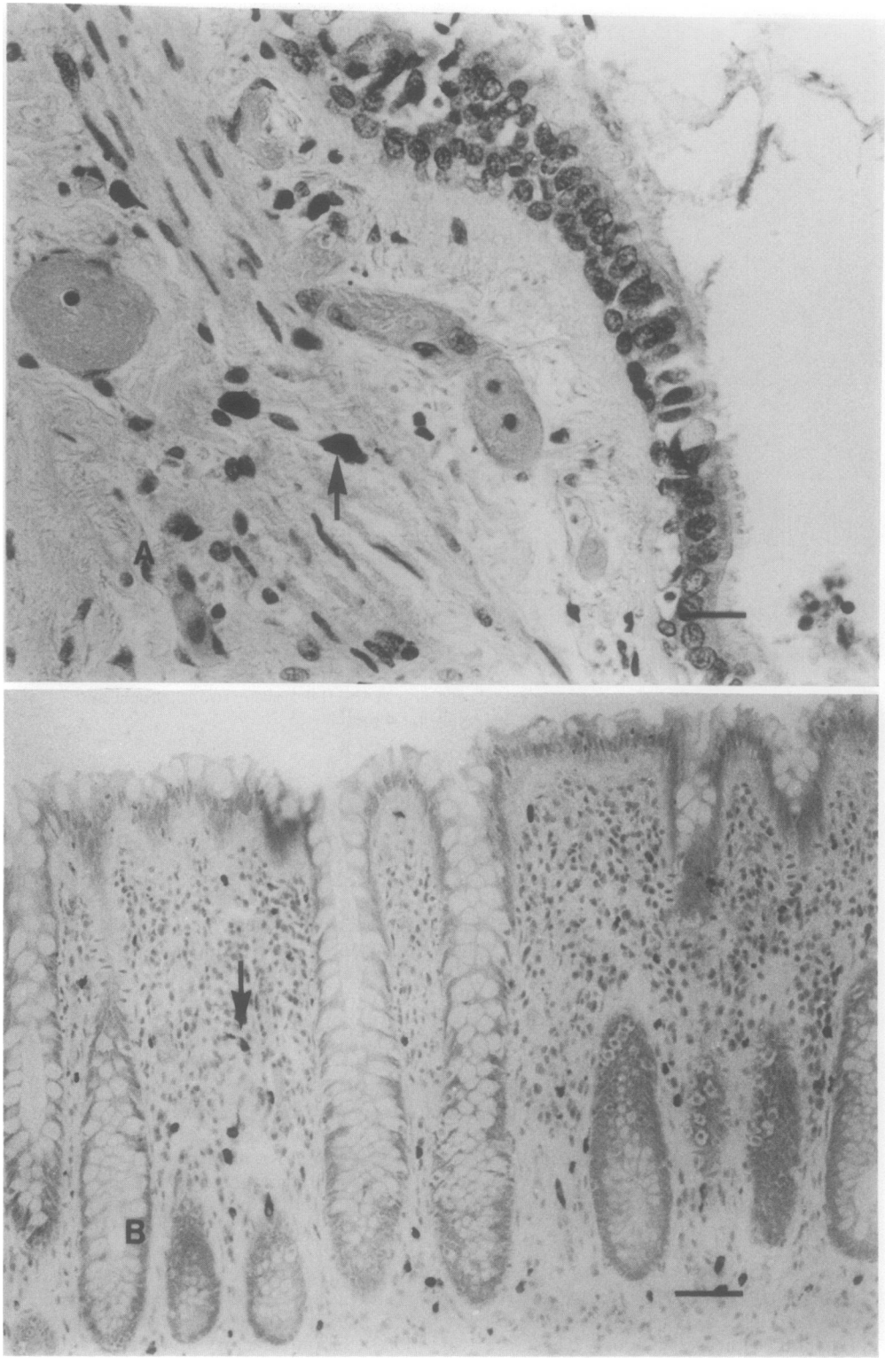
(Cinti & Feinstein, 1976; Haurand & Ullrich, 1985; McLemore *et al.*, 1978; Burke *et al.*, 1977; Fujino *et al.*, 1982).

The presence of cytochrome P-450 in mast cells has not been previously reported in either humans or animals.

Weak immunostaining was identified in the proximal tubular cells of the kidney, the epidermis of the skin, the luteal cells of the ovary and the Leydig cells of the testis. We do not know at this stage, however, whether weak immunostaining reflected a small amount of cytochrome P-450hA7 or binding with low affinity to another cross-reacting protein. Cytochrome P-450 has nevertheless been demonstrated in all these human tissues, using immunocytochemistry (Baron *et al.*, 1983) and non-immunological methods (Okita *et al.*, 1979; Finnen *et al.*, 1983; Chung *et al.*, 1987; Voutilainen *et al.*, 1986).

Cytochrome P-450 was also demonstrated in the acinar cells of the pancreas and the columnar epithelium of the gall bladder. These are two human tissues in which cytochrome P-450 has not previously been reported. The distribution of cytochrome P-450 in human pancreas was similar to that reported for hamster pancreas using polyclonal antibodies to rat cytochrome P-450 (Kawabata *et al.*, 1984).

Cytochrome P-450 could not be demonstrated in a variety of tissues that have been reported to contain cytochrome P-450 or associated monooxygenase activity. The Clara cells of several animal species contain cytochrome P-450 and are an important site of the pulmonary metabolism of xenobiotics (Boyd, 1977; Serabjit-Singh *et al.*, 1980; Foster *et al.*, 1986). Cytochrome P-450 associated monooxygenase activity has been demonstrated in human alveolar type II cells (Devereux *et al.*, 1986) and colonic epithelium (Autrup *et al.*, 1982; Cohen *et al.*, 1983). Cytochrome P-450 has been shown in human adrenal gland, placenta, breast and prostate gland (Chung *et al.*, 1987; Mendelson *et al.*,1985; Baron *et al.*, 1983), in rat olfactory epithelium and rat and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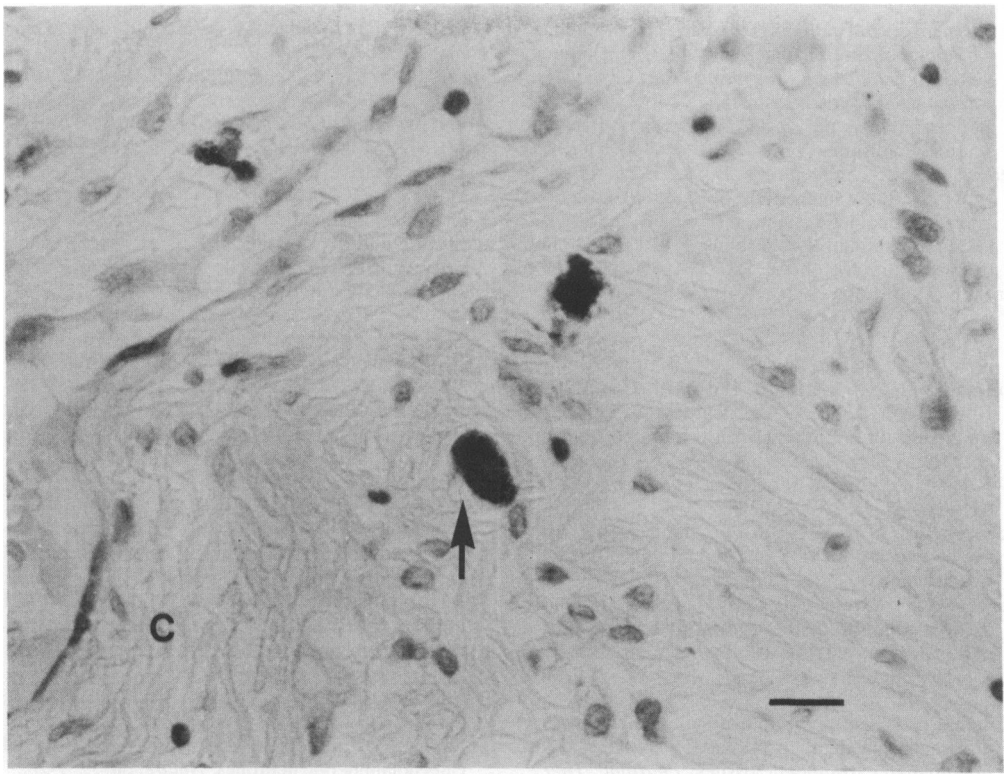

Figure 5 The immunolocalisation of cytochrome P-450 in mast cells (arrow identifies a mast cell) of the
(a) wall of a bronchus (× 340, scale bar represents 25 μm),
(b) colon (× 140, scale bar represents 40 μm),
(c) dermis (× 340, scale bar represents 25 μm).
In (a) there is no immunostaining of the ciliated epithelium.

rabbit nasal epithelium (Reed *et al.*, 1986; Foster *et al.*, 1986; Ding *et al.*, 1986; Voigt *et al.*, 1985) and in rat brain (Haglund *et al.*, 1984; Walker *et al.*, 1986). The absence of immunostaining by MAb-HL3 in tissues which have been reported to contain cytochrome P-450 suggests that these tissues contain forms of cytochrome P-450 different from that (or those) recognised by MAb-HL3.

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