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Histochemical localization of heart-type fatty-acid binding protein in human and murine tissues

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Abstract Cellular fatty acid-binding proteins (FABP) are a highly conserved family of proteins consisting of several subtypes, among them the mammary-derived growth inhibitor (MDGI) which is quite homologous to or even identical with the heart-type FABP (H-FABP). The FABPs and MDGI have been suggested to be involved in intracellular fatty acid metabolism and trafficking. Recently, evidence for growth and differentiation regulating properties of MDGI and H-FABP was provided. Using four affinity-purified polyclonal antibodies against bovine and human antigen preparations, the cellular localization of MDGI/H-FABP in human and mouse tissues and organs was studied. The antibodies were weakly cross-reactive with adipose tissue extracts known to lack H-FABP, but failed to react by Western blot analysis with liver-type FABP (L-FABP) and intestinal-type FABP (I-FABP). MDGI/H-FABP protein was mainly detected in myocardium, skeletal and smooth muscle fibres, lipid and/or steroid synthesising cells (adrenals, Leydig cells, sebaceous glands, lactating mammary gland) and terminally differentiated epithelia of the respiratory, intestinal and urogenital tracts. The results provide evidence that expression of H-FABP is associated with an irreversibly postmitotic and terminally differentiated status of cells. Since all the antisera employed showed spatially identical and qualitatively equal immunostaining, it is suggested that human, bovine and mouse MDGI/H-FABP proteins share highly homologous epitopes.

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Introduction

A number of intracellular proteins, which are characterised by the capability to bind hydrophobic ligands, have been isolated from a variety of tissues from various species of animals as well as from humans. With regard to their physicochemical, chemical and structural characteristics and to their ligand spectrum, they have been divided into three groups: (I) fatty acid-binding proteins (FABPs), (II) cellular retinol-binding proteins (CRBPs), and (III) cellular retinoic acid-binding protein (CRABP; for reviews see Bernier and Jollès 1987; Clarke and Armstrong 1989; Glatz and Van der Vusse 1989; Veerkamp et al. 1993). Data from both nucleic acid and amino acid sequence analyses revealed varying degrees of homology among members of the family of fatty acid-binding proteins, but with a highly conservative secondary and tertiary structure (Ockner 1990; Veerkamp et al. 1991). Furthermore, the interspecies homology among family members appears more pronounced than the intraspecies homology of this protein family (Bass 1993).

Although the physiological functions and biological significance of the fatty acid-binding proteins are not yet fully determined, there is experimental evidence that FABPs are involved in the cellular uptake and intracellular trafficking of long-chain fatty acids (Bass 1990; Kaikaus et al. 1990; Ockner 1990; Sweetser et al. 1987). Thus FABPs may regulate cytoplasmic fatty acid concentrations and oxidative capacity (Glatz and Van der Vusse 1989; Kaikaus et al. 1990; Matarese et al. 1989; Veerkamp and Van Moerkerk 1993). The capability of FABPs to bind retinoic acid and metabolites of the eicosanoid pathway (Boylan and Gudas 1991; Dutta-Roy et al. 1987; Raza et al. 1989) suggests, moreover, their possible participation in cellular growth regulation and differentiation processes (Glatz et al. 1993). This suggestion was corroborated by the isolation of a growth inhibiting and differentiation promoting protein from bovine mammary gland, which was originally referred to as mammary derived growth inhibitor (MDGI; Böhmer et al. 1987a). MDGI was later found to be highly homolo-

gous to, if not identical with, heart-type fatty acid-binding protein (H-FABP; Spener et al. 1990; Treuner et al. 1994). The expression of MDGI in murine mammary gland was shown to be under strong hormonal control (Binas et al. 1992). Recently, direct evidence was found for both antiproliferative and differentiation promoting capabilities of MDGI/H-FABP in mammary gland organ cultures (Yang et al. 1994).

Using immunochemical methods, H-FABP has been detected in a variety of organs and tissues, e.g. heart and skeletal muscle, lung, kidney, stomach, placenta and testes (Bass and Manning 1986; Kleine et al. 1993; Paulussen et al. 1989; Veerkamp et al. 1990). To our knowledge, however, only a few studies describe the cellular and tissue localisation of H-FABP (Watanabe et al. 1991, 1993). In the present study using affinity purified antibodies, the spatial distribution of MDGI/H-FABP was investigated, not only in a larger variety of tissues than was investigated by Watanabe et al. (1991, 1993), but also in two different species, namely man and mouse. This systematic investigation of the spatial distribution of MDGI/H-FABP was expected to reveal possible regularities of the expression patterns of MDGI/H-FABP in various tissues and organs and, furthermore, to give evidence for presumed relations between the presence of MDGI/H-FABP and specific cellular organ functions.

Materials and methods

Animals

BALB/c inbred strain mice of both sexes and about 6-week-old as well as pregnant female mice at day 17 of gestation were employed. After the animals were killed by cervical dislocation, organ specimens were excised and fixed in phosphate-buffered formalin (4%) at 4° C and routinely embedded in paraffin.

Human tissues

Human tissue specimens were obtained from autopsies (Department of Pathology, Academic Hospital Maastricht, The Netherlands) up to 24 h after death. The material, which showed no detectable pathological alterations, was fixed in phosphate-buffered formalin (4%) at 4° C and was routinely embedded in paraffin.

Preparation of antigens and antibodies

The purification procedure for bovine H-FABP used for immunisation and the preparation of polyclonal antisera was described elsewhere (Jagschies et al. 1985). Purification of bovine MDGI was as described earlier (Böhmer et al. 1987a). Polyclonal antisera were affinity purified on Sepharose columns covalently derivatised with bovine MDGI (Böhmer et al. 1987b). For this purpose, 1 mg of bovine MDGI was coupled to CNBr-activated Sepharose (Pharmacia/LKB, Uppsala, Sweden), incubated overnight with 5 ml anti-MDGI rabbit antiserum and subsequently washed extensively with phosphate-buffered saline (PBS). The bound specific immunoglobins were eluted with 100 m*M* glycine buffer, pH 2.8.

The preparation of recombinant human H-FABP from skeletal muscle was described by Peeters et al. (1991) and polyclonal antisera against it were prepared according to Paulussen et al. (1990). Human H-FABP from heart, needed for the generation of affinity-purified monospecific polyclonal rabbit IgG, was isolated and purified by gel permeation and anion-exchange chromatography as previously described (Van Nieuwenhoven et al. 1991; Kleine et al. 1992). Briefly, homogenates of human heart tissue (25%, w/v) were prepared in buffer, consisting of 10 m*M* TRIS-HCl (pH 8.0), 150 m*M* KCl and 1 m*M* dithiothreitol. After centrifugation of the homogenate at 2600 *g* for 10 min and the supernatant at 105000 *g* for 90 min, the final supernatant was obtained containing cytosolic proteins. The final supernatant was concentrated by ultra-filtration using a Diaflo YM5 membrane (Amicon, Danvers, Mass., USA), and applied to a Sephacryl S200-SF column (Pharmacia/LKB) equilibrated with homogenisation buffer. Elution of proteins was monitored spectrophotometrically at 280 nm. FABP containing fractions, identified by the Lipidex 1000 assay (Glatz and Veerkamp 1983), were pooled, concentrated, dialysed overnight at 4° C against 5 m*M* TRIS-HCl (pH 8.0), and applied to a Sepharose-Q fast flow column (Pharmacia/LKB) equilibrated with dialysis buffer.

H-FABP was collected after stepwise gradient-elution (0–30 m*M* NaCl in 5 m*M* TRIS-HCl, pH 8.0). The purity of H-FABP was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing. Finally, the protein was dialysed against 10 m*M* potassium phosphate (pH 7.4), containing 150 m*M* NaCl, and stored in aliquots at –20° C. To prepare antisera against human H-FABP, rabbits (Flemish giant) were immunised with 200 µg of pure human H-FABP each in a mixture of 2 ml PBS and Freund's Complete Adjuvant (FCA) (1:1, v/v), and were boosted at weeks 4 and 8 with antigen in a mixture of 2 ml PBS and Freund's Incomplete Adjuvant (FIA, 1:1, v/v). Two weeks after the last booster injection, blood was collected in glass tubes and spun for 10 min at 1500 *g*. From the resulting polyclonal antiserum the IgG fraction was isolated by means of protein-A chromatography (Pharmacia/LKB). Monospecific rabbit IgG against human H-FABP (αP-IgG) was subsequently isolated from this fraction by affinity chromatography, using a Sepharose column containing covalently bound human H-FABP. The protein contents of the affinity purified antibodies were 1.6, 1.2 and 0.1 mg/ml for the anti-bovine H-FABP, anti-bovine MDGI and anti-human H-FABP respectively.

Western blot analysis

Mouse tissue extracts were obtained by pulverisation of frozen tissue pieces under liquid nitrogen in a mortar, suspended in 5% SDS and solubilized for 10 min at 100° C. The extracts were cleared by centrifugation at 14000 rpm for 15 min and the protein concentration estimated with bicinchoninic acid using the bicinchoninic acid (BCA) kit (Pierce, Rockford, Ill., USA). Aliquots of each tissue extract containing 60 µg of protein or 200 ng of each the purified proteins were applied to a 15% polyacrylamide gel and analysed as described (Binas et al. 1992). Immunochemical detection of the separated proteins on nitrocellulose was performed as described earlier (Binas et al. 1992). In brief, the nitrocellulose blot was first incubated in 1% bovine serum albumin (BSA; w/v) in 50 m*M* TRIS-buffered 150 m*M* NaCl, pH 7.4, containing 0.1% Tween 20 (TBS/Tween) for 20 min, and afterwards at 4° C overnight with the corresponding primary antibody at a concentration of 3.0 µg protein/ml in TBS/Tween. After washing in TBS/Tween twice for 15 min, the blot was incubated for 3 h with anti-rabbit IgG conjugated to alkaline phosphatase (Dakopatts, Copenhagen, Denmark) at a dilution of 1:500 in TBS/Tween, then washed again with TBS/Tween three times for 10 min, and finally stained with nitroblue tetrazolium/1-bromo-2-chloroindolyl phosphate as substrate (Binas et al. 1992).

Immunohistochemistry

Paraffin sections of 4 µm in thickness were mounted on poly-L-lysine coated glass slides and processed routinely. After incubation for 15 min in hydrogen peroxide (1.0%, w/v), followed by washing with PBS, the sections were incubated for 20 min in PBS sup-

Table 1 Results [a] of Western blot analysis of various fatty acid-binding proteins (FABPs) and mouse tissue extracts using four different antisera (*MDGI* Mammary derived growth inhibitor, *H-FABP* heart-type FABP, *L-FABP* liver-type FABP, *I-FABP* intestinal-type FABP)

Proteins	Antibodies			
	Anti-bovine MDGI	Anti-bovine H-FABP	Anti-human H-FABP	Anti-human rec. H-FABP [b]
FABPs:				
Bovine MDGI	++	++	++	(+)
Bovine H-FABP	++	++	++	(+)
Rat L-FABP	–	–	–	–
Bovine I-FABP	–	–	–	(+)
Human H-FABP	++	++	++	++
Tissue extracts (mouse):				
Lactating mammary gland	++	++	++	+
Skin	–	–	–	–
Liver	–	–	–	–
Skeletal muscle	++	++	++	+
Heart	++	++	++	++
Brain	(+)	(+)	(+)	–
Adipose tissue	++	++	+	++

[a] –, Negative; (+), faint reaction; +, weak raction; ++, strong reaction
[b] Recombinant H-FABP

plemented with BSA (1.0%, w/v). After further washing with PBS, the sections were incubated for 2 h in the primary antisera. Anti-bovine MDGI, anti-human H-FABP and anti-human H-FABP from skeletal muscle were diluted 1:100, and anti-bovine H-FABP was diluted 1:50 in 1% BSA (w/v) in PBS. After the excess of primary antibodies was removed by washing with PBS, the sections were incubated for 45 min with biotinylated sheep anti-rabbit IgG (Amersham, Oxford, UK), followed by washing with PBS. The sections were next treated with streptavidin-conjugated horseradish peroxidase for 15 min. Finally, the sections were extensively washed with PBS, treated with diaminobenzidine (DAB) for 5 min, followed by rinsing in distilled water. The sections were counterstained with haematoxylin, dehydrated and mounted routinely. Sections incubated with unspecific primary antibody or with antiserum that was pre-absorbed with the respective antigen served as negative controls.

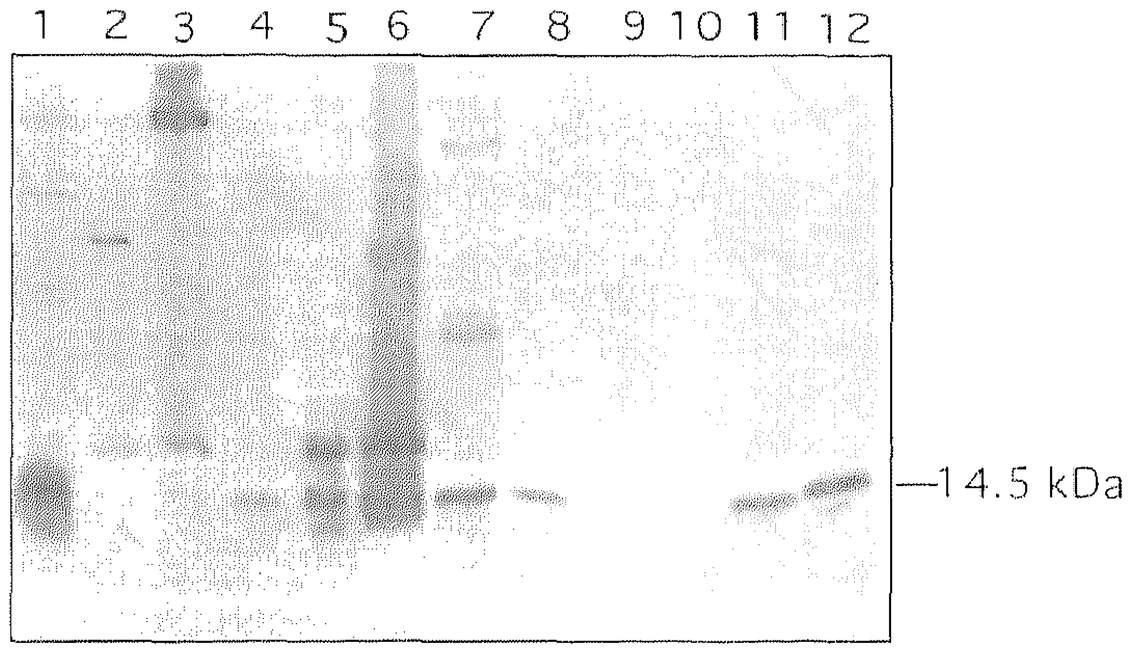


Fig. 1 Western blot of various fatty-acid binding proteins (FABPs) and mouse tissue extracts analysed with anti-bovine heart-type (H)-FABP antiserum. Tissue extracts (*lanes 1–7*) and purified or recombinant proteins (*lanes 8–12*) were obtained as described in the "Materials and methods". *Lane 1*, Mammary adipose tissue; *lane 2*, skin; *lane 3*, liver; *lane 4*, brain; *lane 5*, skeletal muscle; *lane 6*, heart; *lane 7*, lactating mammary gland; *lane 8*, bovine mammary derived growth inhibitor (MDGI); *lane 9*, rat liver-type (L)-FABP; *lane 10*, bovine intestinal-type (I)-FABP; *lane 11*, bovine H-FABP; *lane 12*, human H-FABP. Strong reactivity was obtained with extracts of skeletal muscle, heart, and lactating mammary gland (*lanes 5–7*) and with bovine MDGI, bovine H-FABP and human H-FABP. The staining of mouse mammary adipose tissue and possibly of brain extracts has to be considered as cross-reactive

Results

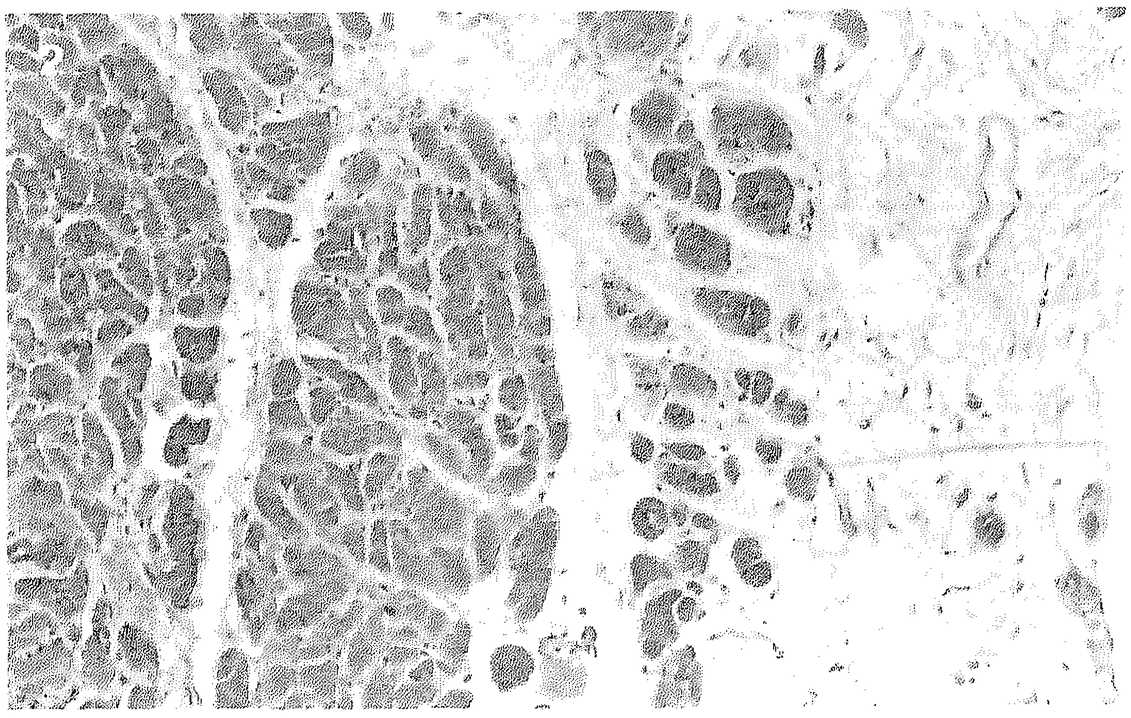
Western blot analysis

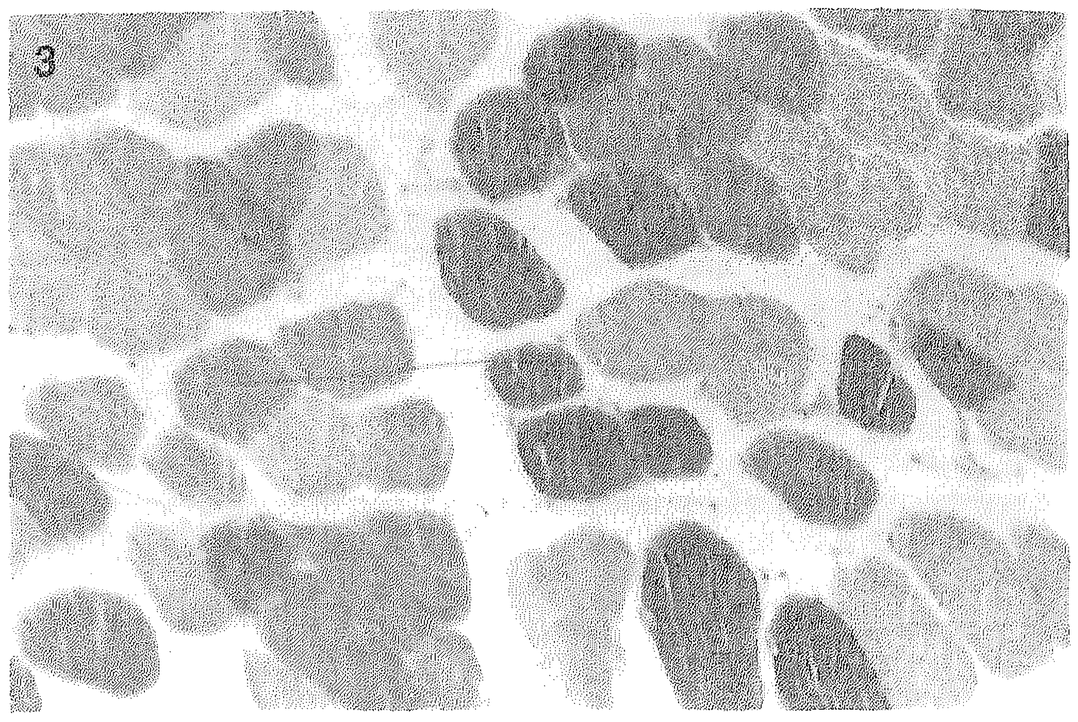
For investigating the specificity of the various antibodies, purified FABPs and aqueous extracts of mouse tissues were employed. The spectrum of the antigens and the results are listed in Table 1. A Western blot demonstrating the reactivity of anti-bovine H-FABP antiserum with various recombinant FABPs and mouse tissue extracts is given as example (Fig. 1). The bovine and human FABPs from mammary gland, heart and skeletal muscle gave positive reactions with all the heterologous and homologous antibodies. Likewise, the murine tissue extracts of lactating mammary gland, skeletal muscle and heart showed positive reactivity. Weak immunostaining was noted using brain extracts. FABPs and tissue extracts from liver, intestine, and skin were negative. The immunostaining of adipose tissue extracts may be due to cross-reactivity, since the homology between H-FABP and adipose tissue (A)-FABP extends to more than 60% (Veerkamp et al. 1991). Except for the anti-human recombinant H-FABP which was less reactive, all the antisera reacted with equal intensity.

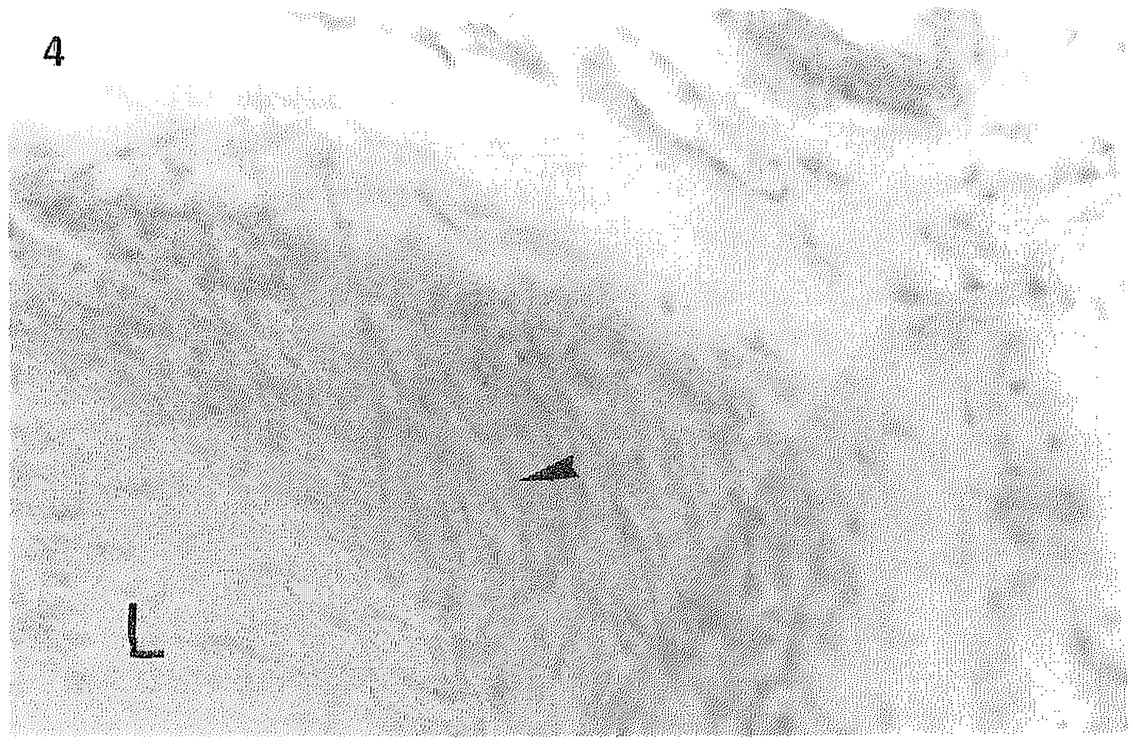
Immunohistochemistry

Myocardium, skeletal and smooth muscles

In accordance with the results of the immunoblotting, all antisera showed strong species-non-specific reactivity with myocardium (Fig. 2) of both ventricles and atria and with skeletal muscles. Red striated muscle was more intensely stained than white striated muscle. Fibres within an individual muscle showed quite heterogeneous im-







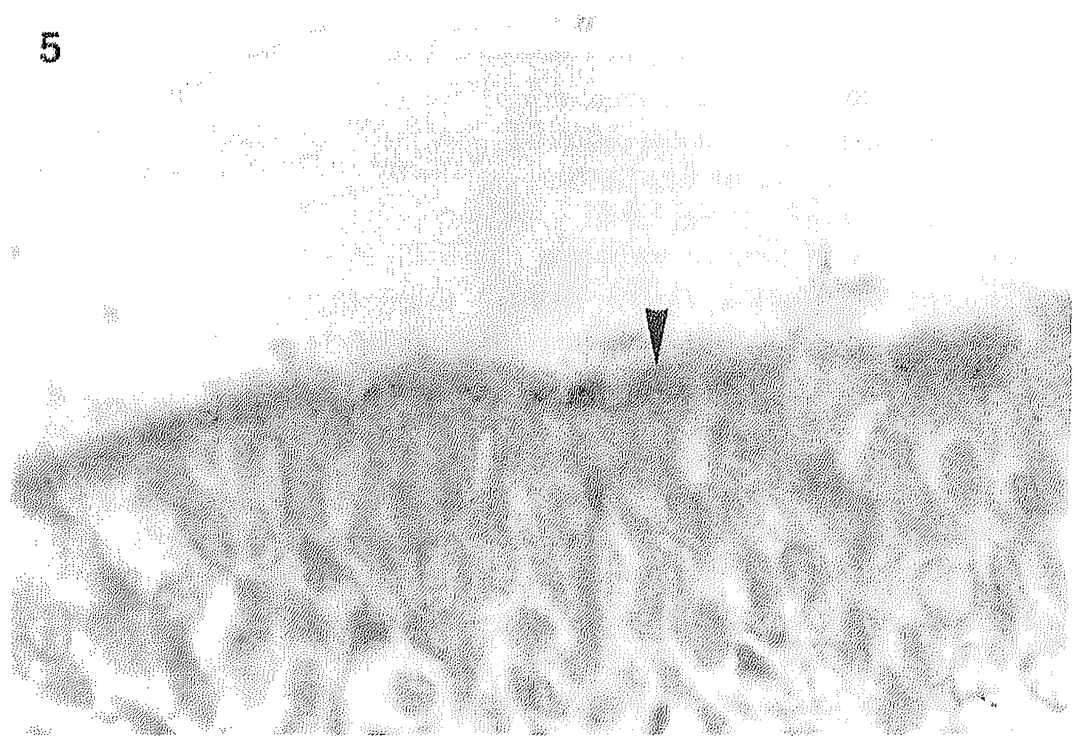


Fig. 2 Myocardium of the human left ventricle, strongly reactive with anti-human H-FABP antiserum. Note immunonegativity of the subendocardial connective tissue and the smaller blood vessels. ×110

Fig. 3 Human striated muscle of the M. dorsalis longus, demonstrating different reactivity of white and red muscle fibres with anti-human H-FABP antiserum. ×110

Fig. 4 Large artery of the human perigenital fat tissue showing faint H-FABP expression (*arrowhead*) with anti-human H-FABP antiserum (*L* Lumen). ×220

Fig. 5 Epithelial layer of a large bronchus of the human lung. Distinct immunoreactivity of the apical cell compartments with anti-human H-FABP antiserum (*arrowhead*). ×550

munolabelling (Fig. 3). In contrast, the smooth muscle system as checked in intestinal wall, trachea and uterus gave only faint reactions. Smooth muscle of the aorta and walls of the larger arteries were also weakly positive (Fig. 4).

Gastrointestinal tract

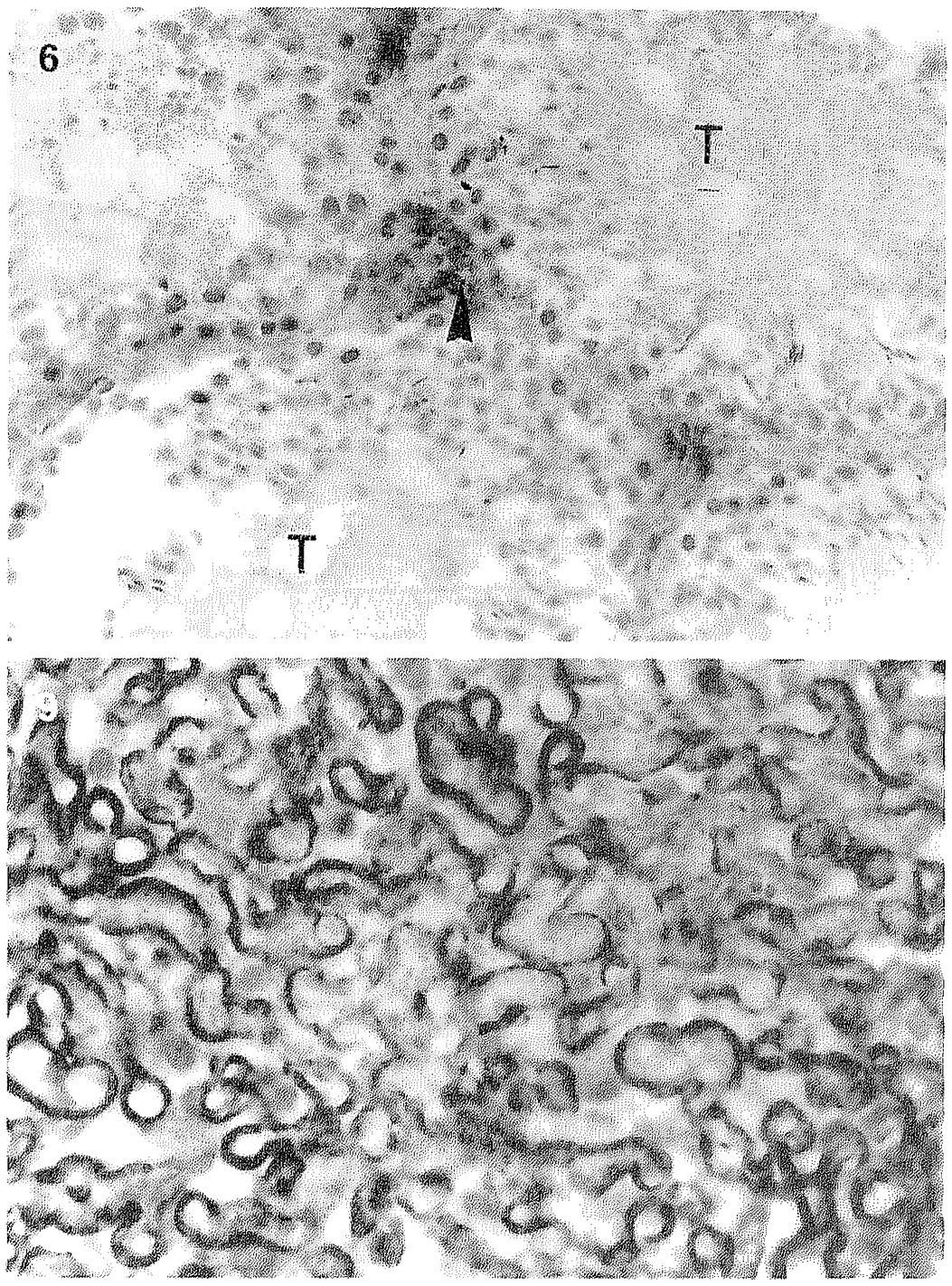
In human and murine stomach only the parietal cells demonstrated distinct immunostaining. In addition, the squamous epithelium of the murine pre-stomach was strongly reactive. The epithelium of the intestinal tract showed clear immunoreactivity for MDGI/H-FABP with a generally decreasing gradient from duodenum to rectum, sparing Brunner's glands, Lieberkühn's crypts and the mucous glands of colon and rectum. Expression of MDGI/H-FABP increased from the base to the tips of the intestinal villi, but was irregularly expressed by the cells on the villous surface, while explicitly excluding the goblet cells. The serous parts of all the human and murine salivary glands showed moderate expression in the epithelial cells lining the inter- and intralobular ducts, whereas the mucinous parts were thoroughly negative. Likewise, human and murine liver, including intra- and extrahepatic bile ducts, as well as excretory pancreas showed no reactivity.

Excretory system

In both human and murine kidney MDGI/H-FABP appeared in the distal tubules and, though less pronounced, in the collecting tubules. The glomeruli were completely immunonegative. The urothel of renal pelvis and urinary bladder reacted only weakly with the antisera.

Respiratory system

In the respiratory tract of humans the cylindrical epithelial layer of the trachea and the larger bronchi demonstrated strong immunoreactivity, which was confined to



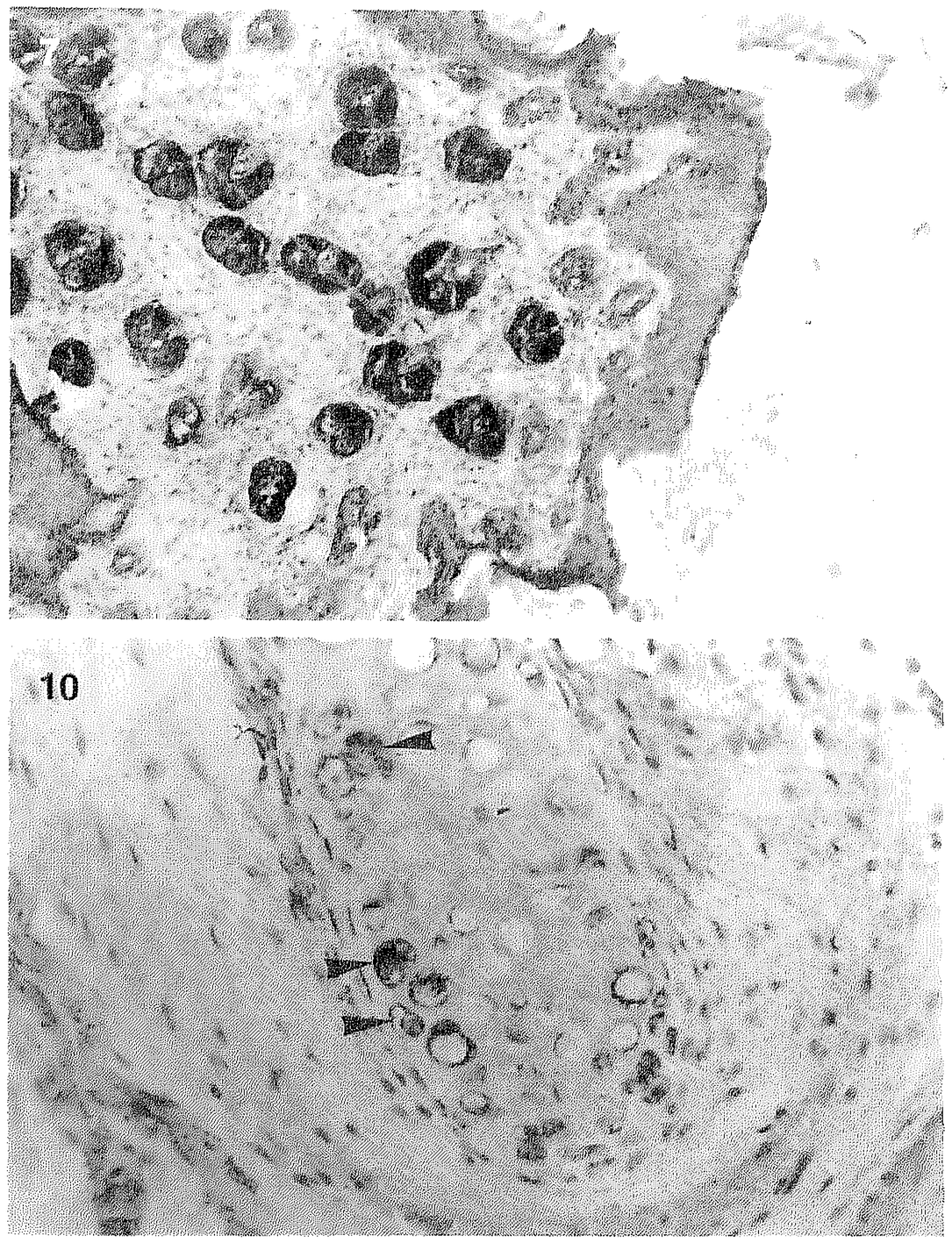


Fig. 6 Mouse testes with distinctly immunolabelled interstitial Leydig cells (*arrowhead*). Reactivity with anti-bovine MDGI antiserum (*T* Seminiferous tubules). ×500

Fig. 7 Mouse ear with strongly immunostained sebaceous glands after treatment with anti-bovine MDGI antiserum. ×60

Fig. 9 Syncytiotrophoblastic meshwork of mouse placenta, strongly expressing H-FABP as shown by immunostaining with anti-bovine MDGI antiserum. ×240

Fig. 10 Laryngeal cartilage of the mouse, demonstrating chondroblasts distinctly immunolabelled with anti-human H-FABP antiserum against skeletal muscle (*arrowhead*). ×600

the luminal surface of the cells (Fig. 5). The squamous epithelium of the larynx was negative. In mice, tracheal and bronchial epithelium was only discontinuously immunolabelled, but reacted at the level of the alveoli. Mesothelial cells of the pleura failed to react in both species investigated.

Endocrine system

Among the endocrine organs, both human and murine adrenals exhibited moderate expression of H-FABP mainly in the zona reticularis and quantitatively declining towards the cortex. Moreover, the corpora lutea of the murine ovaries were regularly stained. The testicular Leydig cells of both species exhibited distinct MDGI/H-FABP expression (Fig. 6). The islets of Langerhans of the pancreas and the cells of the adenohypophysis of mouse and humans were completely immunonegative.

Skin and mammary gland

Keratinocytes and cells of Langerhans of the epidermis showed no immunostaining either in humans or in mice. The sebaceous glands which surround the hair sheaths were, however, strongly immunolabelled with all four antiser employed (Fig. 7). The mammary gland of adult human females failed to express MDGI/H-FABP. In female mice the MDGI/H-FABP content of the mammary gland was strongly related to the gestational status; whereas the epithelium of the mammary gland was immunonegative in virgin and adult non-pregnant mice, the immunoreactivity increased during pregnancy in parallel to the formation of alveolar lipid droplets (Fig. 8A), resulting in maximum staining during the lactation period. Although MDGI/H-FABP was predominantly expressed in the lipid droplet-containing cells lining the alveoli, the epithelial layer of ductules and of small and large ducts occasionally also showed minor immunoreactivity. The antigen was intracellularly concentrated around the lipid droplets. Furthermore, the membranes of the milk fat globules within the lumina were also positively immunolabelled (Fig. 8B).

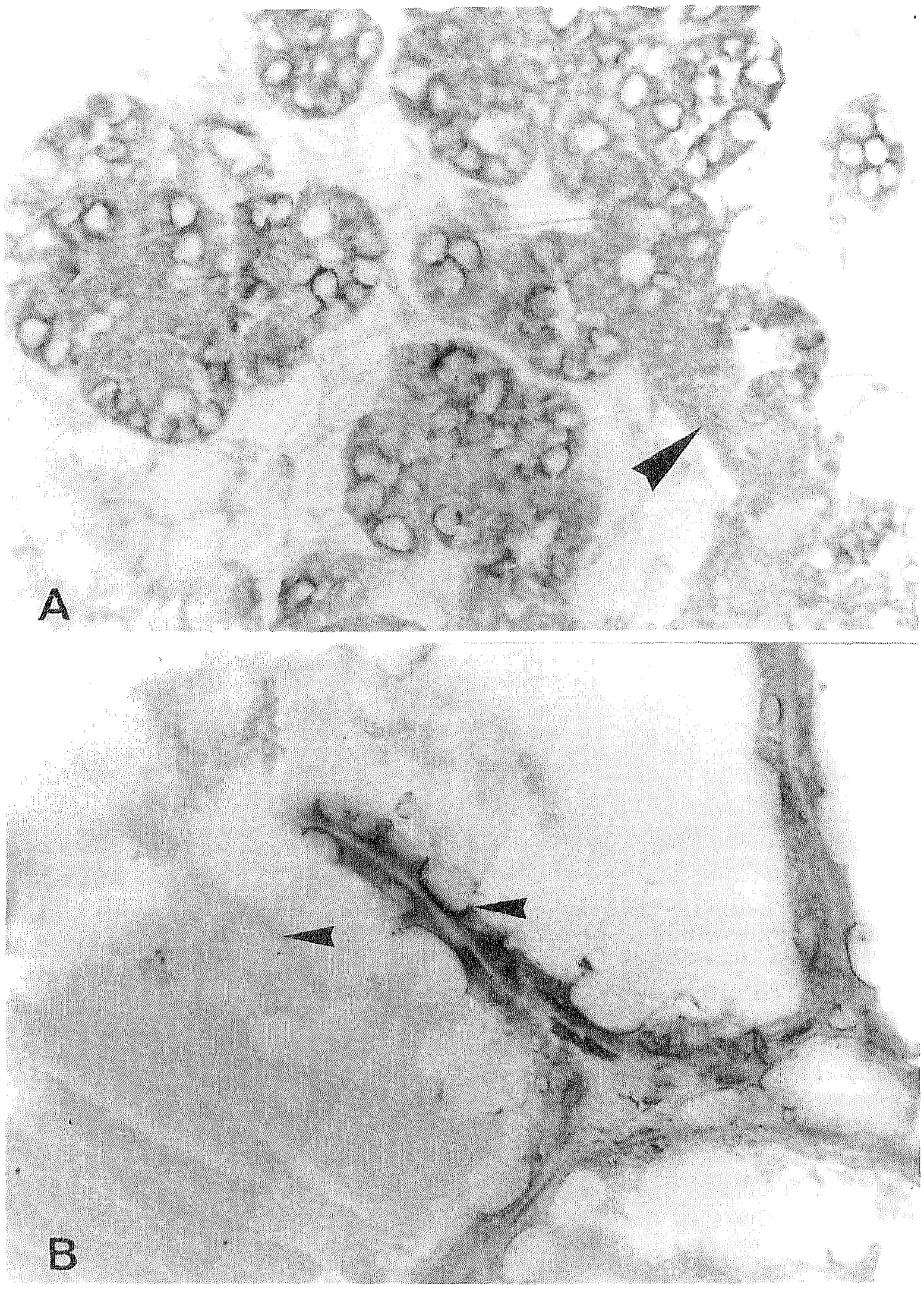


Fig. 8A, B MDGI/H-FABP expression in mouse mammary gland as shown with anti-bovine MDGI antiserum. **A** Mammary gland of a late-pregnant mouse (day 17 of gestation). Reactivity is concentrated around the intracellular lipid droplets of the alveolar epithelial cell layer whereas the ductuli (*arrowhead*) fail to express H-FABP. **B** Mammary gland of a lactating mouse. Distinct reactivity of the alveolar epithelial cells and the intra- and extracellular milk fat droplet membranes (*arrowheads*). **A** ×500; **B** ×750

Reproductive organs

The endometrium in human as well as murine females showed faint to moderate immunostaining. Granulosa cells and oocytes in the ovaries did not react with the antisera. In contrast the Fallopian tube showed strong but discontinuous immunoreactivity of the epithelial layer in both species. In males of both species the cells involved in spermatogenesis failed to express MDGI/H-FABP. However, the epithelial cells lining the seminiferous duct and seminal vesicles showed a faint immunoreaction. The epithelium of the human prostate showed no positive immunostaining. MDGI/H-FABP was detected in the full-term human and murine placenta, mainly expressed in syncytiotrophoblastic and decidual cells (Fig. 9).

Miscellaneous

The human and murine haemopoietic and lymphopoietic systems completely failed to show positive immunostaining with the antisera employed. Osteoblasts and osteoclasts were also not immunolabelled. Distinct cytoplasmic immunoreactivity was, however, discerned in certain chondroblasts in the laryngeal and tracheal cartilage (Fig. 10). The cells of the white and, even more

strongly, the brown fat tissue showed variable submembranous immunostaining. The immunoreactivity of the endothelial cells in all human and murine organs studied could not be detected with certainty. Control incubations performed with unspecific antibody or antisera pre-absorbed with the respective antigen all showed negative results.

In order to recognise a possible influence of the paraffin embedding procedure on H-FABP immunoreactivity, sections of native and paraffin-embedded material of several mouse organs were compared. The results showed no spatial differences in immunoreactivity between native and processed tissue. Furthermore, a post-mortem autolysis period up to 12 h failed to influence the localisation and intensity of immunoreactivity as revealed in control experiments with mouse tissue, thus excluding the possibility of autolysis effects on the immunohistochemical results. In general, all the organs and tissues from mouse and humans gave a quantitatively similar staining reaction with the anti-bovine MDGI and anti-human H-FABP antisera, but less intensively with the anti-bovine H-FABP antiserum.

Discussion

Among the members of the FABP family, H-FABP is most widely distributed within the organism. Whereas expression of liver (L-FABP), adipose tissue (A-FABP), intestinal (I-FABP), ileal (Il-FABP) and epidermal (E-FABP)-type FABPS is mainly confined to their respective organs, H-FABP is found in a variety of organs and tissues. Previous immunochemical studies using rat tissue extracts demonstrated the appearance of H-FABP in heart and skeletal muscle, lung, placenta, testes, ovaries, kidneys and stomach (Bass and Manning 1986; Kanda et al. 1989; Paulussen et al. 1989). Recent immunohistochemical data indicated, moreover, the expression of H-FABP in human and murine mammary gland (Binas et al. 1992; Watanabe et al. 1991, 1993) and in human adrenals, stomach, kidney, heart muscle, striated muscle, placenta, testes, ovaries and salivary glands (Kanda et al. 1989; Watanabe et al. 1991, 1993).

The above results were confirmed by the immunohistochemical study described in this paper. In addition, we detectd H-FABP in intestinal epithelium, male and female genital tract, urothel and skin appendages. As far as comparable data on mouse, rat and human tissues are available, there are no fundamental species-specific differences in the cytolocalisation of H-FABP. However, in contrast to Watanabe et al. (1993), we failed to demonstrate immunolabelling of thyroid epithelia and found strong immunoreactivity in the oviduct of mice and humans; these differencs remain to be explained. In our study, endothelial cells failed to demonstrate reliable immunostaining in all organs with the antisera employed in contrast to previous immunohistochemical data (Fournier and Rahim 1985; Paulussen et al. 1990; Robers et al. 1993). Recent electron microscopic studies indicated the absence of H-FABP in endothelial cells of the myocardium, but its presence in capillary endothelial cells of pregnant cows using our anti-bovine MDGI antiserum (Breter and Erdmann 1994; Van Nieuwenhoven et al. 1975), further studies are required on the distribution of H-FABP/MDGI in endothelium.

In central and peripheral nervous system at least three FABPs have been immunochemically demonstrated: MDGI, a closely related protein (Schoentgen et al. 1989), and a further FABP tentatively designated X-FABP (Kurtz et al. 1994) and brain lipid-binding protein, respectively (Feng et al. 1994). Because our antisera showed weak immunostaining with mouse brain extracts and, with regard to the sequence homologies of the cerebral FABPs, could be expected to react with either of them, the investigation of nervous tissue was omitted in this study.

The results showed that the antisera employed were not cross reactive with L-FABP in immunoblots and tissue sections. Likewise, cross reactivity with I-FABP, L-FABP and Il-FABP in the intestine could be excluded for they show a different localisation, exhibiting continuous and increasing expression from the base to the top of the intestinal villi (Iseki and Kondo 1990; Shields et al. 1986; Sweetser et al. 1988). Non cross-reactivity of the antisera employed with L-FABP was also shown by immunostaining of kidney tissue. L-FABP is located in the proximal tubuli, while H-FABP is located in the distal tubuli of the kidney (Maatman et al. 1991). Positive immunostaining of the white and brown fat tissue, on the other hand, must be attributed to cross reactivity, since the homology between A-FABP and H-FABP is about 60% (Veerkamp et al. 1991). The antisera also reacted with murine fat tissue extracts in the immunoblot.

With regard to Western blot analysis and immunohistochemistry, the results with the polyclonal antisera against bovine and human FABPs show that (i) H-FABPs of bovine, human and murine tissue share a high degree of identity of immunological reactivity and, thus, may contain highly conserved epitopes; (ii) MDGI and H-FABP are quite similar, if not identical proteins with respect to immunological reactivity. According to the lipid-binding properties of this family of proteins, the involvement of FABPs in lipid metabolism has been generally inferred. However, as already may be deduced from its spatial distribution, H-FABP has to be regarded as a multifunctional protein (Spener and Börchers 1992). A number of functions have been proposed (Glatz et al. 1993; Glatz and Van der Vusse 1990; Kaikaus et al. 1990; Veerkamp et al. 1991, 1992; Yang et al. 1994): (1) providing a reserve pool for cytoplasmic fatty acids; (2) promoting cellular uptake of fatty acids; (3) modulating enzyme activities involved in fatty acid metabolism; (4) enabling intracellular, targeted trafficking of fatty acids; (5) serving as an intermediate in intracellular signalling pathways; and (6) inhibiting cell growth and promoting cell differentiation. The physiological significance of these proposed functions in vivo has still to be substantiated.

With regard to the biochemical data referred to above, MDGI/H-FABP is localised in three types of cytological structures:

1. Cell systems with a high rate of β-oxidation. This applies to muscle fibres of the myocardium and the red striated muscles and to a lesser degree also to white striated and smooth muscles. Previously, in rat heart and skeletal muscles a relation has been reported between the muscle H-FABP content and the palmitate oxidation capacity (Veerkamp and Van Moerkerk 1993) or the 3-hydroxyacyl CoA dehydrogenase activity (Garnier et al. 1993). On the other hand, in quantitative studies on the physiological impact on H-FABP in these organs, a close correlation between β-oxidation and H-FABP content was lacking (Veerkamp and Van Moerkerk 1993).
2. Cells known to have intensive fat metabolism, especially a high rate of steroid synthesis. This applies to cells of the endocrine system (adrenal cortex, corpora lutea, Leydig cells), the sebaceous glands, placenta and epithelia of the mammary gland of pregnant and lactating mice.
3. Cells, for the most part of entodermic origin, with resorptive (enterocytes) or reabsorptive functions (distal renal tubuli, urothelium, ducts of salivary glands, epithelium of both male and female genital tracts). In contrast, excretory cell systems (salivary glands, pancreas, gastrointestinal tract, proximal renal tubuli) are clearly negative, except for the merocrine (mammary gland) or holocrine epithelia (sebaceous glands).

From a cell kinetic point of view it becomes evident that all cells expressing H-FABP are highly differentiated. They comprise (i) terminally differentiated, irreversibly postmitotic cells lacking the potency for further division (e.g. muscle cells), and (ii) differentiated daughter cells from unequally dividing stem cells of ectodermal and entodermal epithelia (e.g. enterocytes). This has been demonstrated by electron microscopy in the bovine mammary gland where MDGI/H-FABP is heterogenously expressed in alveolar cells, apparently sparing the stem cells (Erdmann and Breter 1993). Comparison with autoradiographic data from the mouse (Schultze 1968) emphasizes that the cells expressing H-FABP frequently are mitotically inactive and have a generation time of more than 150 h (e.g. epithelia of the pre-stomach, mammary gland alveoli and uterus mucosa). Considered altogether, these data strongly support existing evidence that H-FABP is associated with the regulation of proliferation and differentiation of certain cell systems. Involvement in these processes is experimentally based on findings concerning up-regulation of the cellular H-FABP content by differentiation-promoting hormonal stimuli, e.g. by prolactin (Binas et al. 1992) and antiprogestins (Li et al. 1994), or down-regulation by mitotic agents such as epidermal growth factor and transforming growth factor-α (Spitzer et al. 1994) as shown in mouse mammary gland explant cultures. Most recent experiments have directly confirmed the growth inhibitory and differentiation promoting properties of H-type FABPs when added to primary mammary epithelial cell and organ cultures of mouse mammary gland (Yang et al. 1994) or primary murine cerebral cell cultures (Feng et al. 1994). Future studies have to elucidate whether H-FABP, in this context, may act as a signalling intermediate, as implied by data suggesting the insulin-dependent phosphorylation of H-FABP (Nielsen and Spener 1993).

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