Multidrug Resistance Gene (P-Glycoprotein) Expression in the Human Fetus

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P-glycoprotein, a transmembrane protein associated with multidrug resistance in cancer cells, is also expressed in normal tissues. To get more insight into the physiologic role of mdr1/P-glycoprotein, we investigated its expression in human fetal tissues after 7 to 38 weeks of gestation by an immunohistochemical technique, using three different monoclonal antibodies, and by a sensitive RNAse protection assay. Expression of mdr1-mRNA could already be demonstrated in the embryonal phase of human development, after 7 weeks of gestation. Comparing the adult with the fetal tissue distribution, differences were found in specific organs, such as adrenal, intestine, respiratory epithelium, and brain capillaries. In the fetal zone cells of the fetal adrenal cortex no staining was observed by immunohistochemistry, whereas the definitive zone showed increasing expression throughout gestation. Prenatal intestine did not show staining of the epithelium, although definite mdr1-mRNA expression was observed in late specimens. Interestingly, respiratory epithelium of main bronchi and pharynx, not expressing P-gp in adults, did stain positive. Expression of P-gp in brain capillaries was not observed before the third trimester of pregnancy, whereas in kidney and liver, mdr1-mRNA expression and staining for P-glycoprotein were detected in early fetal life (11 to 14 weeks). These findings suggest a pivotal role of P-glycoprotein in physiology of various organs already in early phases of human development and may help to identify its physiologic substrates. (Am J Pathol 1992, 141:1063–1072)

Human P-glycoprotein (P-gp) is a 170-kd integral plasma membrane protein, encoded by the *mdr1*-gene, which functions as an energy-dependent pump for antineoplastic agents in multidrug resistant (MDR) cancer cells *in vitro*.[1,2] The anticancer drugs pumped by P-gp are all derived from natural occurring toxins. Two structurally similar, but functionally different P-gp isoforms (*mdr1* and *mdr3*) have been identified in humans, but only *mdr1* confers the multidrug resistance phenotype.[3] Human P-glycoprotein shows a remarkable homology to a superfamily of transport proteins encountered from bacteria to humans.[4] Bacterial transport proteins, the chloroquine-resistance protein in plasmodium falciparum (malaria), the STE6 gene responsible for the secretion of a-factor mating pheromone in yeast, and the anion transporting glycoprotein deficient in human cystic fibrosis, all belong to this superfamily.[4,5] The conservation of similar molecules across phylogenetically distant species suggests a fundamental role of P-gp as a transport protein. Interestingly, with RNA *in situ* hybridization and immunohistochemistry techniques, P-gp was shown to be expressed in several normal human tissues[6–9]: organs with an excretory function (colon, kidney, liver),[7] the adrenal gland,[10] the placental trophoblast,[11] and the vessels at blood–tissue barrier sites.[9,11] The peculiar distribution of P-gp in normal tissues suggests an excretory, protective or hormone handling role.[9] The physiologic substrates of P-gp are still largely unknown, however, and they might well vary from organ to organ in which P-gp is expressed.

The study of the expression pattern of P-gp in early fetal material and its modification during development might shed light on its physiologic role in the body. We therefore investigated P-gp expression in human tissues at different developmental stages of fetal life using immunohistochemistry with a panel of three different monoclonal antibodies (MAbs) against P-gp, and a sensitive RNAse protection assay.

Materials and Methods

Tissue Handling

Embryonal and fetal material, from 7 to 20 weeks' gestation, was collected after elective uterine dilatation and evacuation. Tissues obtained from newborns (25 weeks of gestation to 4 months postpartum) that were either stillborn or died after birth also were studied. The procurement of human material was approved by the institutional ethical committee. A specimen of each tissue was snap frozen and stored in liquid nitrogen until use. The length of gestation was calculated as number of weeks of amenorrhea minus 2 weeks. Immunohistochemistry for P-gp expression was performed on all organ specimens. RNAse protection assay was used to confirm the presence or absence of mdr1-mRNA expression in selected cases. Because of the small amount of tissue available, RNAse protection only was used in a whole embryo after 7 weeks of gestation.

Antibodies

A panel of three anti-P-gp MAbs (C-219, MRK-16 and JSB-1), which recognize different epitopes of the P-gp molecule, was used. C-219,[12] an IgG2b, was provided by Centocor (Tongeren, Belgium) and used at a working dilution of 1:100, resulting in a final concentration of 10 μg/ml. MRK-16,[13] provided by Dr. T. Tsuruo, Tokyo, Japan, is an IgG2a antibody that recognizes an epitope on the extracellular side of P-gp; MRK-16 was used at a dilution of 1:400, giving a final concentration of 4 μg/ml. JSB-1, an IgG1 antibody, raised in our laboratory,[14] was used as ascites at a 1:100 dilution; JSB-1 is believed to recognize a conformational epitope of the P-gp molecule (V. Ling, personal communication), most probably at the inner side of the plasma membrane, because some permeabilization is required for the reaction with JSB-1 to occur. JSB-1 and MRK-16 have been shown to be mdr1-specific, whereas C-219 cross-reacts with mdr3/P-gp.[15] As a control antibody, a mouse myeloma immunoglobulin (IgG1) was used in a dilution of 1:100, giving a final concentration of 10 μg/ml.

Immunohistochemistry

For immunohisto/cytochemistry, cryostat sections (5 μ) and cytospin preparations of tumor cell lines were air dried and fixed in cold acetone (10 minutes, 0°C) before staining. Tissue sections or cytospin preparations were incubated with the MAbs for 1 hour at room temperature. The immunohistochemical method was an avidine-biotine complex (ABC) immunoperoxidase method (Histostain-SP kit, Zymed Laboratories Inc., San Francisco, California), as described in detail earlier.[9] The slides were developed with amino-ethyl carbazole (AEC), rinsed with deionized water, counterstained with hematoxylin, and mounted with aquamount. Phosphate-buffered saline (pH 7.4) was used for antibody dilution and for all earlier wash steps. Cytospin preparations of SW-1573, a chemosensitive non–small-cell lung carcinoma cell line, originally isolated by Dr. A. Leibovitz (Scott and White Clinic, Temple, TX) and the human epidermoid carcinoma cell line KB-3-1[16] were used as negative controls. The MDR sublines SW-1573/2R160[17] and KB-ChR-8-5,[16,18] derived from the parental cell lines by in vitro exposure to doxorubicin and colchicine, respectively, were used as positive controls for mdr1/P-gp expression. Staining results were judged and scored independently by three of us (PvdV, CJLMM and CvK). Cases in which agreement was not obtained after first evaluation were rediscussed and concordance achieved. Cytospins or tissue sections were scored for each MAb based on intensity of staining (+, weak; + +, definite; + + +, strong) of each cell type. A description of type of staining (membranous or intracytoplasmatic staining) and preferential localization of the positive cells in the tissue specimens was given whenever possible. A specimen was considered P-gp positive only if staining was observed with *all three* anti-P-gp MAbs.

RNAse Protection Assay

Frozen fetal specimens were pulverized in a microdismembrator[19] and subsequently total cellular RNA was extracted by homogenization in guanidinium isothiocyanate followed by centrifugation in cesium chloride gradient.[20] An NP40 procedure was used to isolate cytoplasmic RNA from control cell lines.[20] RNAse protection was performed as described earlier.[21,22] Briefly, RNA samples (10 μg) were hybridized with a [32P]CTP-labeled antisense RNA probe, specific for mdr1-mRNA, which was obtained by transcription of a 301 nucleotide cDNA fragment (positions 3500 through 3801) with SP6 RNA polymerase.[23] A probe for γ-actin was included as an internal control for determination of RNA loading, which was performed by densitometric scanning. Separation of RNA samples was performed on a polyacrylamide/urea gel (19:1), followed by autoradiography (exposure to Kodak XS film overnight at −70°C). The parental cell line KB-3-1 was used as negative control, and the MDR sublines KBChR-8 and KBChR-8-5 with a 10-fold and 30-fold increase in mdr1-mRNA expression relative to KB-3-1,[18] respectively, were used as positive controls. Compared with the parental KB-3-1 cells,

KBChᴿ-8 cells are less than twofold, and KBChᴿ-8-5 cells are threefold to sixfold resistant to colchicine, doxorubicin, and vinblastine.[18]

Results

Sensitivity and Accuracy of the Methods

By immunohistochemistry, clear expression of P-gp was observed in KB-Chᴿ-8-5 cells (Figure 1), but not in KBChᴿ-8 cells, whereas expression of *mdr1*-mRNA could just be detected in KBChᴿ-8 cells (10 µg) with RNAse protection assay (Figure 2). With this technique, a clear *mdr1*-mRNA expression was observed in KBChᴿ-8-5 cells (Figure 2A, B, C). Therefore, individual cells with less P-gp expression than KBChᴿ-8-5 cells (Figure 1) may be missed by immunohistochemistry in tissue specimens. The RNAse protection assay, however, does not allow study of the heterogeneity of expression, or to identify small subpopulations of positive cells. In most cases, with the immunohistochemistry technique employed, concordant staining with all the three antibodies was observed, although some specific discrepancies were seen. In general, C-219 produced clearer staining, and less background in tissue sections than the other two MAbs.

Embryo

RNAse protection assay showed definite *mdr1*-mRNA expression in a whole embryo at the end of the embryonal period (7 weeks of gestation) (Figure 2A).

Adrenal

Fetal adrenals after 11 to 38 weeks of gestation were examined. No expression of P-gp was found in the fetal zone of the adrenal cortex (Figure 3A), whereas clear P-gp expression was detected in cells of the definitive zone of the cortex (Figure 3B, C). This observation was evident in the third trimester of pregnancy (Figure 3B, C), although scattered P-gp expressing cells were detectable in the adrenal of a fetus of 14 weeks of gestation. The number of P-gp–expressing cells increased with the length of gestation, and clear staining was observed after 25 to 28 weeks. At full-term pregnancy (38 weeks), strong expression in the cells of the definitive zone was observed (Figure 3C), even though differentiation of the adrenal cortex into the three zones (glomerulosa, fasciculata, reticularis) had not occurred by that stage. In the adrenal of a 4-month-old baby in which the three zones of the adrenal cortex could be distinguished, clear expression of P-gp was found, predominantly in the developing zona reticularis and fasciculata (Figure 3D). No expression was detected in the medulla of fetal, child (Figure 3D), and adult adrenal.

Kidney

Positive staining for P-gp was observed at the apical side of tubules and in the Bowman's capsule as early as after 11 weeks of gestation (Table 1, Figure 3E, F). No preferential staining in either proximal or distal tubules could be distinguished, although the number of P-gp–expressing tubules at this stage of kidney formation was small. Ex-
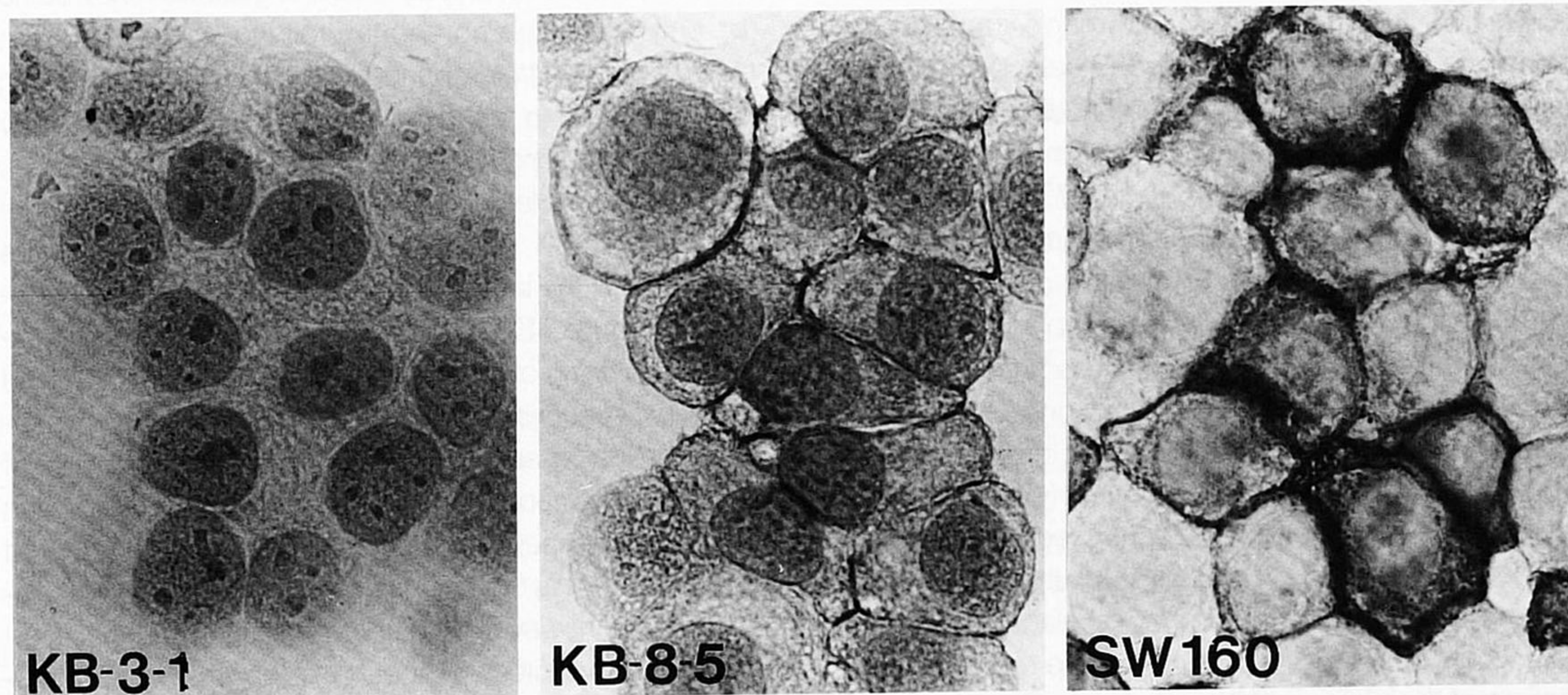

KB-3-1 **KB-8-5** **SW160**

Figure 1. *Immunocytochemical staining for P-gp with Mab JSB-1 in control cell lines (cytospin preparations, acetone fixation, magnification ×1000). Left: Absence of staining in mdr1-mRNA negative KB-3-1 carcinoma cells. Middle: Definite (+ +) membranous staining in 3–6-fold multidrug resistant KB-Chᴿ-8-5 (KB-8-5) carcinoma cells. Right: Strong (+ + +) membranous staining in highly (40–500-fold) multidrug resistant SW-1573/2R160 human squamous cell lung carcinoma cells. Similar staining patterns were observed with C-219 and MRK-16 in these cell lines.*
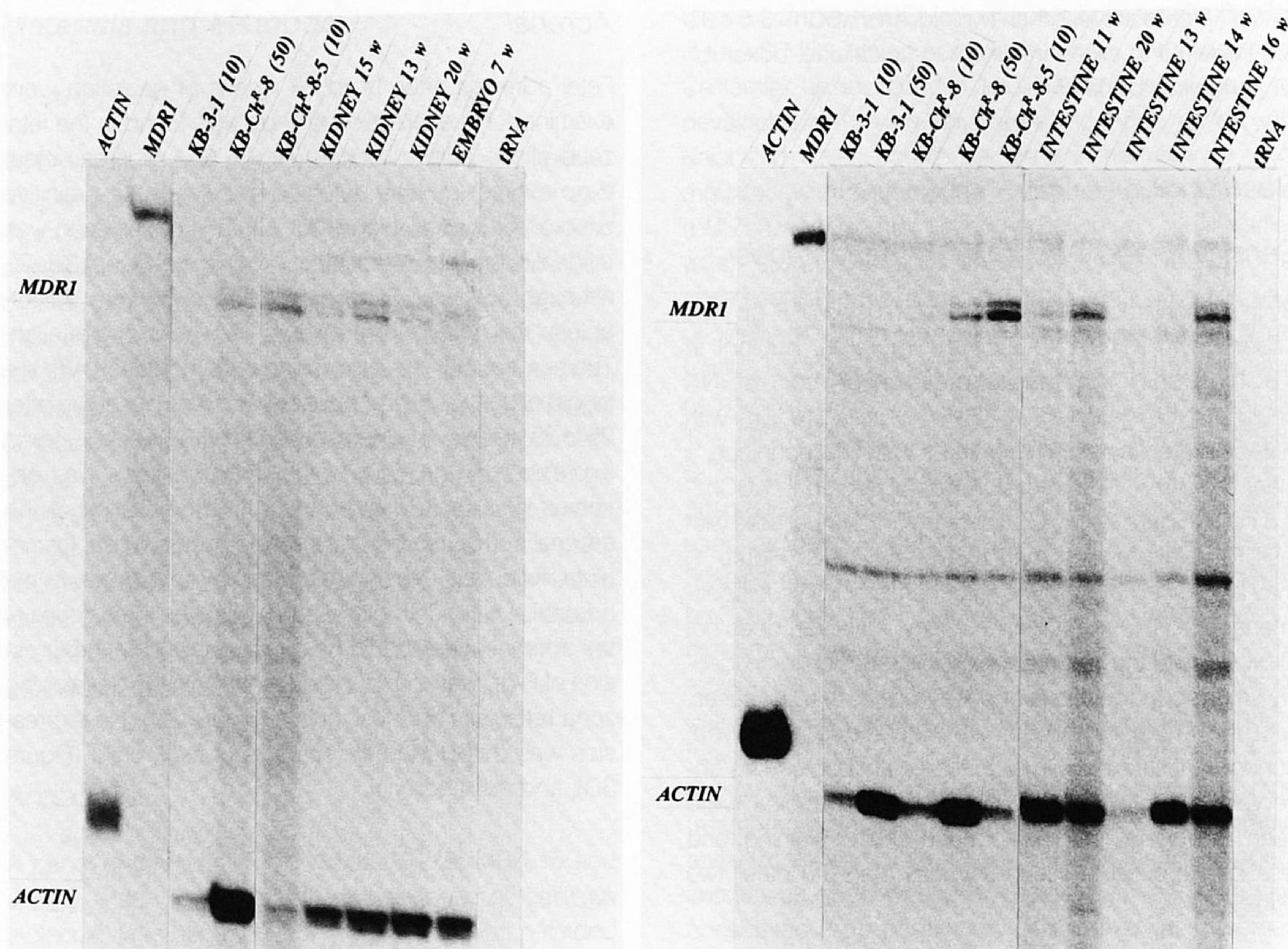
Figure 2. mdr1-*mRNA expression in fetal human tissues. RNAse protection assay was performed as described in Materials and Methods on 10 μg of total cytoplasmic RNA from each tissue specimen. The positions of protected fragments for* mdr1 *and* γ-*actin are indicated. The amount of RNA of control cell lines (μg) is indicated between brackets. A: Control cell lines, kidney specimens (13, 15, 20 weeks), embryo (7 weeks). B: Control cell lines. Intestinal specimens (11, 13, 14, 16, 20 weeks). Lanes with probes and t-RNA are added as internal controls.*

pression appeared to be related to maturity of nephrons (Figure 3F); in fact, in the two earliest specimens (11 and 13 weeks), clear staining of Bowman's capsule was found (Fig. 3E), which disappeared in later stages of development (Table 1). Glomeruli and metanephric blastema were negative (Figure 3E, F). *Mdr1*-mRNA expression levels of 4 kidney specimens (13, 15, 17, 20 weeks) were lower than those found in KB-Ch^R-8-5 cells (Figure 2A), as shown by densitometric scanning.

Digestive System

Squamous epithelium of the tongue (14 weeks) (not shown) and the esophagus (28, 38 weeks) (Figure 4A) did not express P-gp, whereas weak but distinct positive staining was observed in esophageal glands (38 weeks) (results not shown). Clear staining of the respiratory epithelium of the pharynx (14 weeks) and of a pharyngeal recess (Figure 4B) was observed. Striated muscles in this area (tongue, pharyngeal/esophageal area) showed strong staining with MAb C-219, but not with the other two

MAbs. Epithelial cells of five fetal intestinal specimens after 11, 13, 14, 16, and 20 weeks of gestation showed no or only weak membranous staining (Figure 4C, E), whereas strong heterogeneous staining of the epithelium was observed in the stomach and the colon of a 7-day old premature born neonates (28 weeks) (Figure 4D). Clear intracytoplasmic staining of the paranuclear Golgi area with JSB-1 but not with the other two MAbs was observed in the intestinal specimen of 14 weeks of gestation (Figure 4E). In general, however, strong background staining of goblet cells in the epithelium of all fetal intestinal specimens hampered judgment of these specimens. Weak reactivity of MRK-16 and JSB-1 but not of C-219 with smooth muscles in the wall of the stomach and the intestine was observed (Table 1), as reported previously.[9] This did not interfere with evaluation of staining results, however. RNAse protection assay was performed on five intestinal specimens on which also immunohistochemistry was done. No or very weak expression of *mdr1*-mRNA (< KB-Ch^R-8) was seen in intestinal specimens after 11, 13, and 14 weeks, whereas a clear *mdr1*-mRNA expression (< KB-Ch^R-8-5) was observed

in fetal intestinal specimens at a later stage of fetal development (16 and 20 weeks) (Figure 2B).

Fetal liver specimens (14 to 28 weeks) showed staining for P-gp in bile canaliculi already after 14 weeks of gestation. No apparent difference in staining intensity was observed between the specimens of different fetal stages (Figure 4F, G; Table 1), although the patterns of staining of bile canaliculi followed the architectural changes occurring during fetal liver development (Figure 4F, G). The characteristic platelike arrangement of hepatocytes was only observed in the latest fetal specimens (25, 28 weeks) (Figure 4G). RNAse protection performed in one specimen (16 weeks) confirmed the presence of *mdr1*-mRNA (not shown). No staining of the liver parenchyme and the larger bile ducts was detected (Figure 4F,G), whereas staining of bile ductules was observed in only one specimen (25 weeks).

No expression of P-gp was seen in two fetal pancreas specimens (18, 28 weeks) (Table 1).

Lung

In fetal lung tissue (14 to 20 weeks), neither developing pneumocytes nor bronchioli showed staining (Table 1). Interestingly, definite apical membrane staining of respiratory epithelium of main bronchi was detected in two specimens (17, 20 weeks) with the three MAbs. No expression of *mdr1*-mRNA was observed, however, in the 20-weeks specimen (not shown).

Heart

Very strong staining of the fetal heart specimens (9, 14, 20, 28 weeks) was observed with MAb C-219, but not with the other two MAbs (Fig 3Ga and b). No expression of *mdr1*-mRNA was seen with RNAse protection in one specimen (14 weeks) (not shown).

Brain

No staining of embryonal and fetal brain cells was observed (7, 14, 28 weeks). Staining of small meningeal vessels (Figure 3H) and of capillaries in the brain was only observed in the specimen after 28 weeks, but not in earlier specimens.

Discussion

Human P-glycoprotein was discovered in the attempt to identify mechanisms of multidrug resistance to antineo-

plastic agents in cancer cell lines. It was shown to act as an efflux pump for antineoplastic agents derived from natural toxins in MDR cells. On the basis of its expression pattern in adult human tissues, *mdr1*/P-gp has been suggested to play a role also in transport of (xenobiotic) toxins and endogenous substances. No physiologic substrate of P-gp has so far been definitely identified, however. Here we demonstrate that P-gp is expressed in early stages of human development and that the expression correlates with specific functional changes occurring in developing fetal organs. These findings support a hypothetical role of this protein in important physiologic process taking place in early developmental stages.

Although the adrenal cortex is the tissue with the highest expression of P-gp in the adult,[6-10] the cells of the fetal zone of the fetal adrenal cortex did not express P-gp, as already described.[10,24] We observed expression of P-gp in cells of the definitive zone or neocortex of the fetal adrenal cortex, however. The development of the fetal adrenal cortex starts in the 4th through 5th week of gestation from mesothelial cells medial to the urogenital ridge, giving rise first to the fetal zone, which comprises 80% of the gland during fetal life, and later on, in the 6th to 8th week, to the definitive cortex or neocortex, the precursor of the adult adrenal cortex.[25] The fetal zone undergoes rapid involution within the first few weeks of postnatal life. Fetal zone cells play an important role in maintaining pregnancy, by secreting a precursor of placental estrogens (dehydroepiandosterone sulphate),[26] whereas, because of a deficiency of the enzyme 3 β-hydroxysteroid dehydrogenase (3 β-HSD), they cannot secrete detectable amounts of mineralocorticoid and glucocorticoids.[26,27] In the neocortex or definitive zone, the activity of 3 β-HSD is markedly higher and associated with the capacity to synthesize cortisol[26,28]; thereby the activity of this enzyme, relatively low during midgestation (threefold compared with fetal zone cells),[28-30] increases throughout the third trimester.[28,30] The expression pattern of P-gp in definitive zone cells of the fetal adrenal throughout gestation correlates with the capacity of the fetal adrenal to secrete specific steroid hormones, as seen in our study. We have recently demonstrated that cortisol is pumped actively by MDR cells (manuscript in preparation), and this finding adds further support to the ability of P-gp to secrete specific steroid hormones in the adrenals, in correlation to the stages of fetal adrenal development.

The P-gp staining observed in mature tubules of the fetal kidney already at 11 weeks of gestation suggests that P-gp may play an important role in the excretory function of this organ. In addition, expression observed by RNAse protection assay provides further evidence that the staining represents the *mdr1*/P-gp isoform. In fact, the definitive fetal kidney or metanephros, which
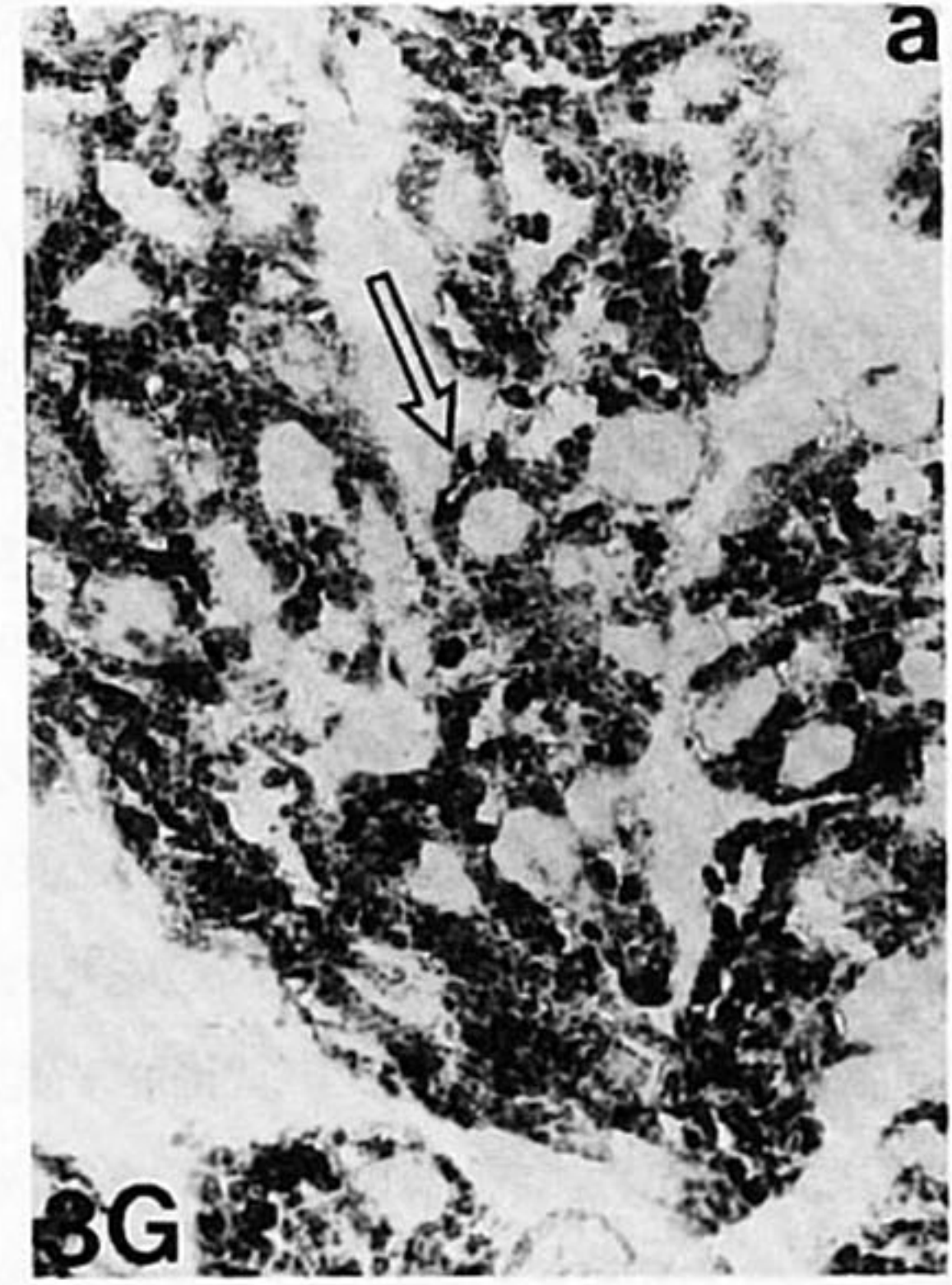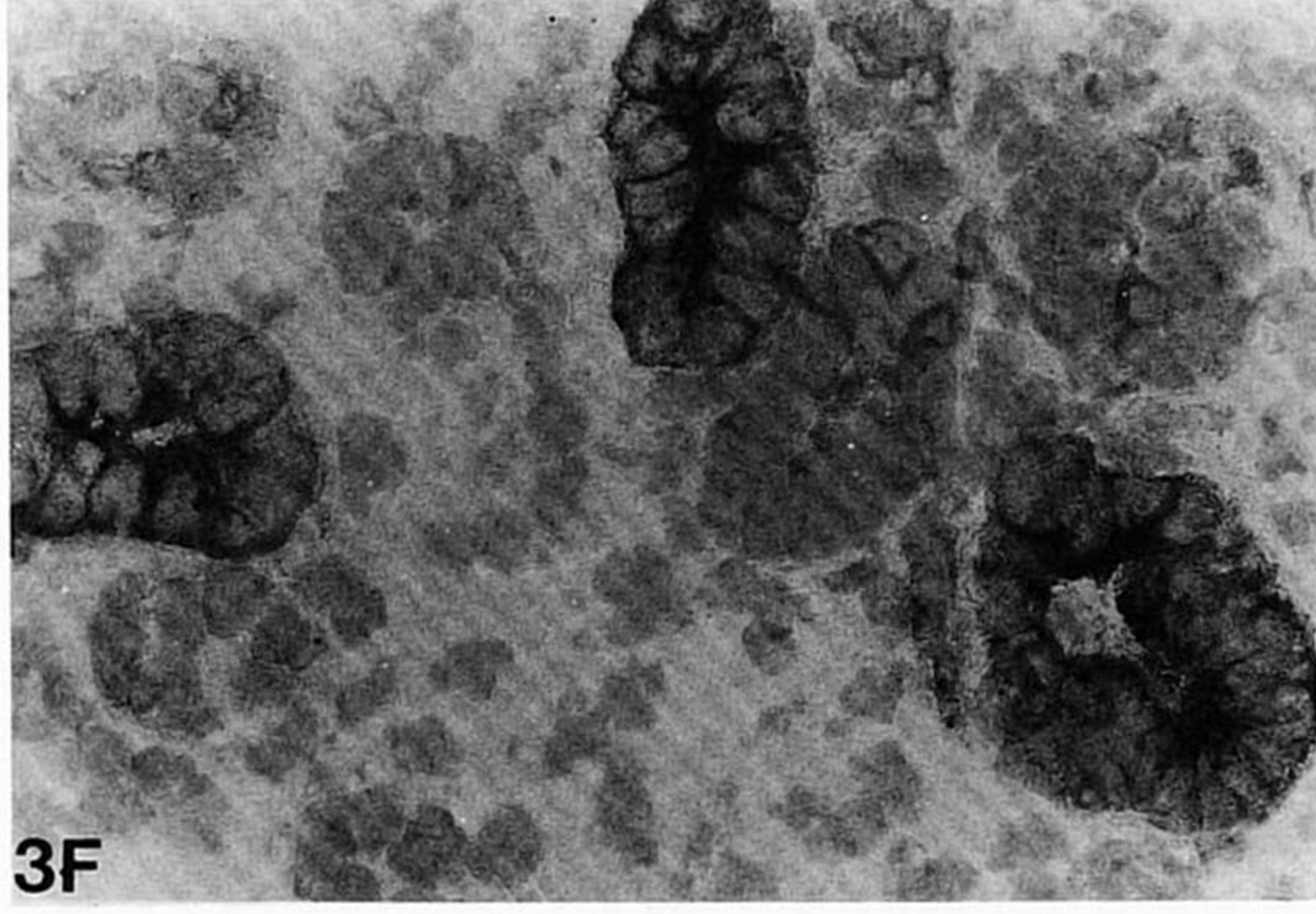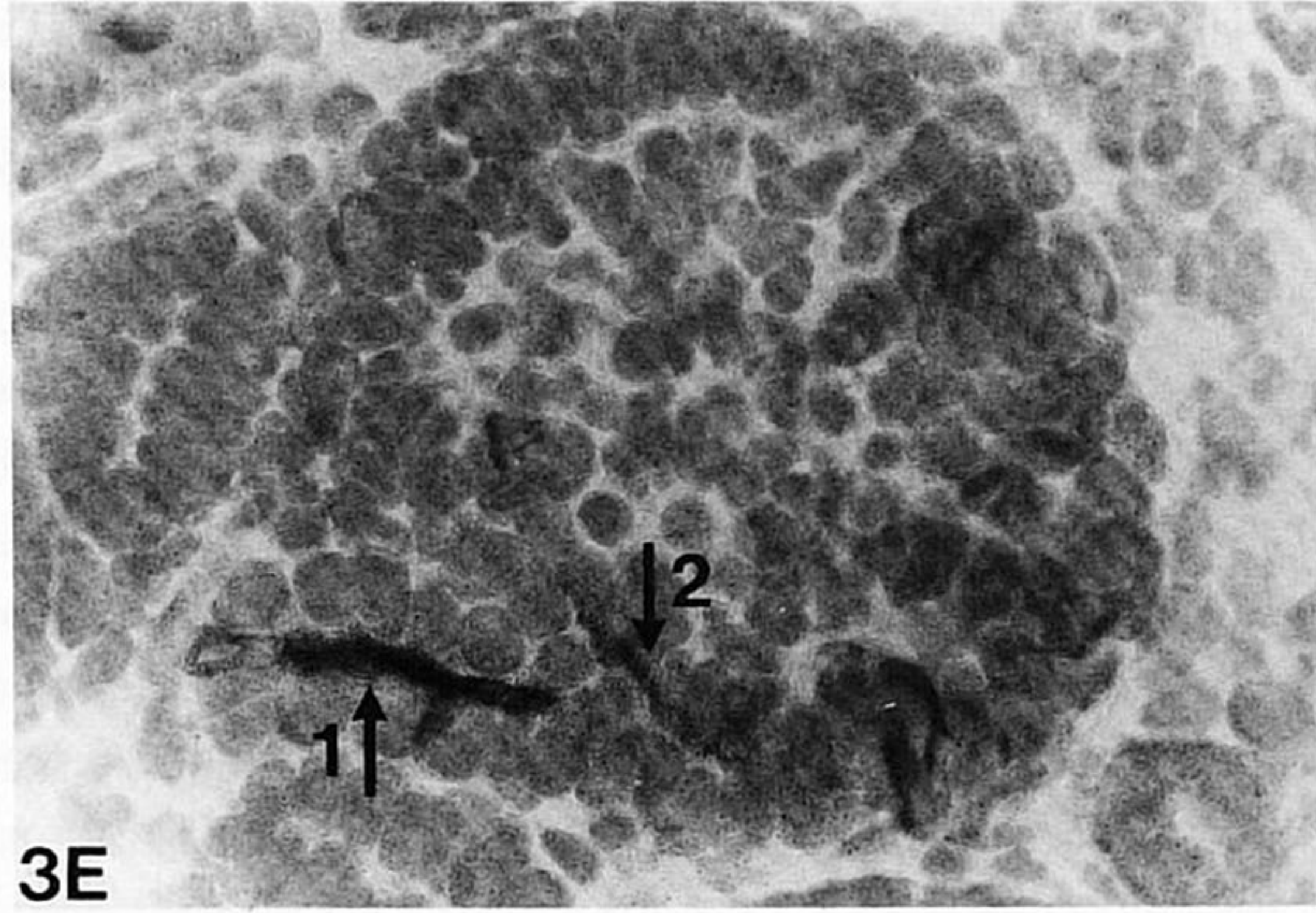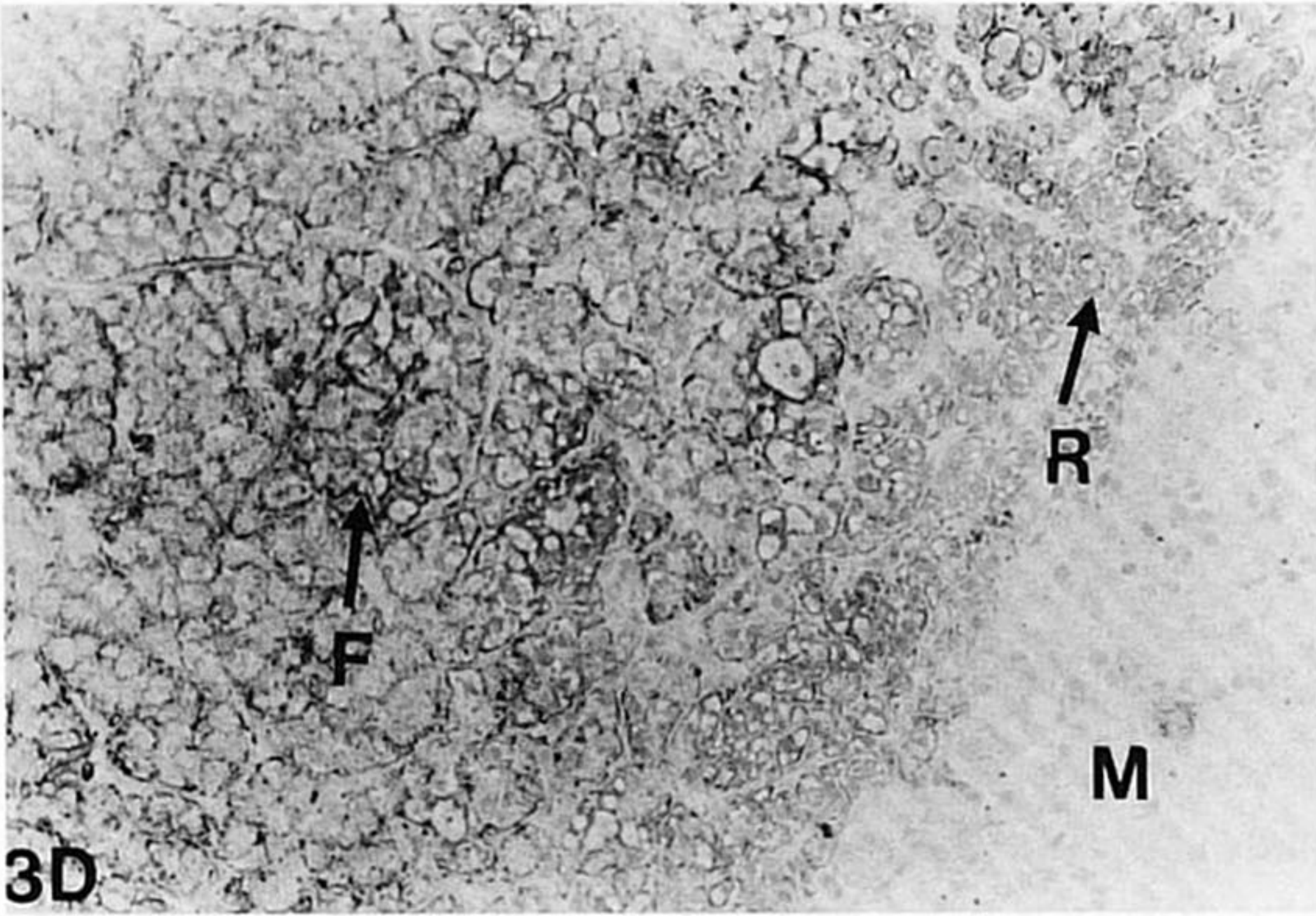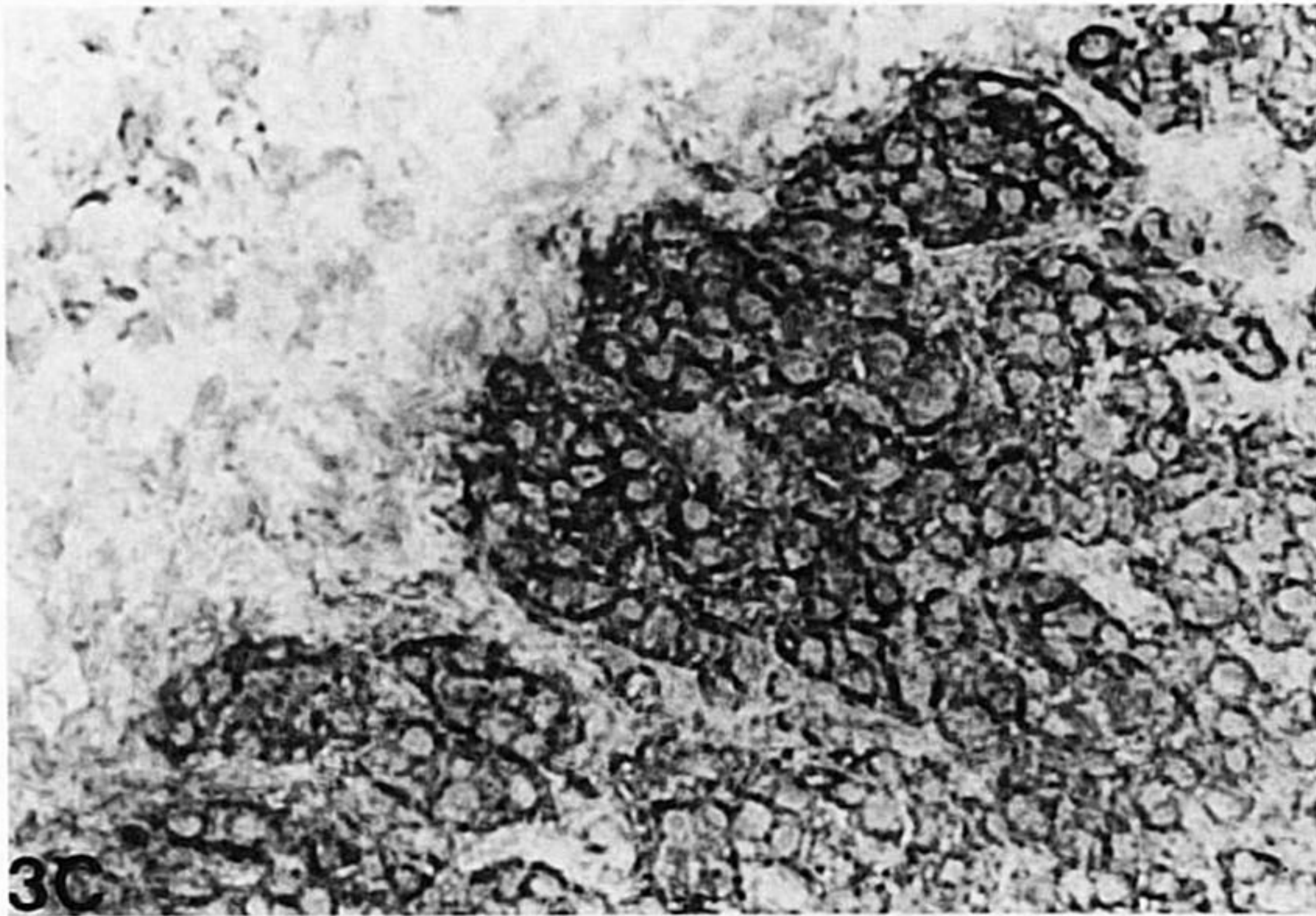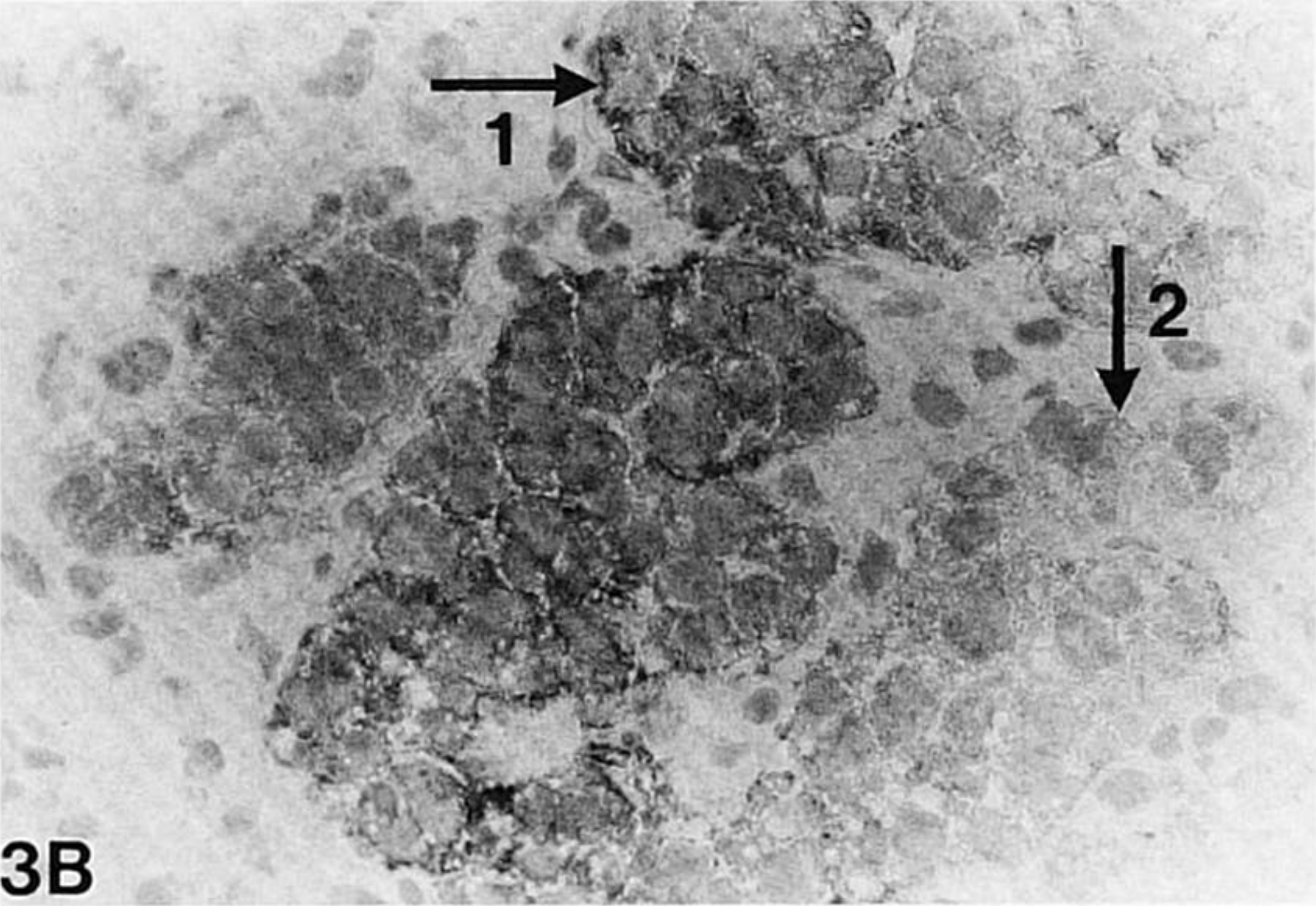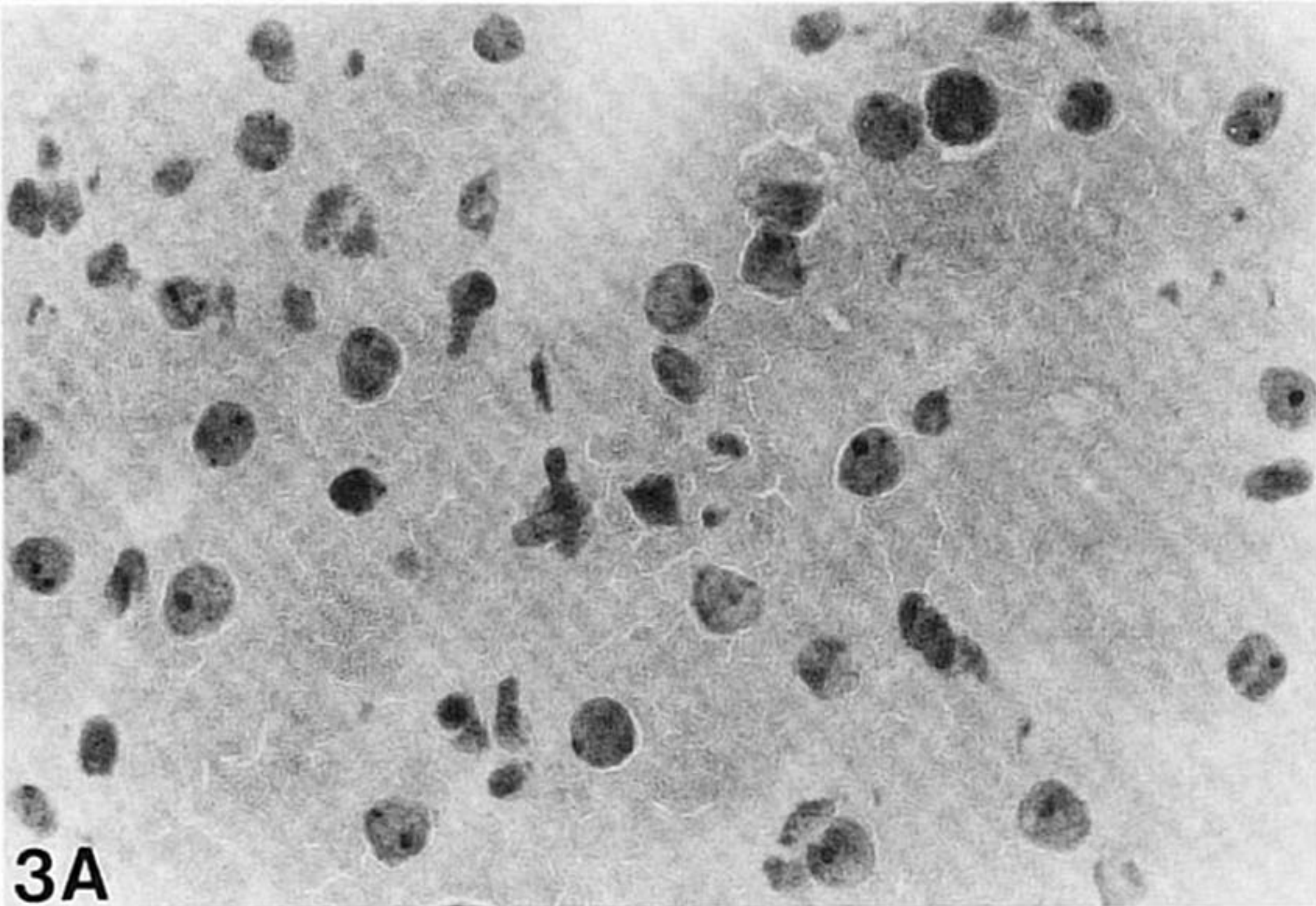
Figure 3. *Immunohistochemical localizations of P-glycoprotein in frozen sections of fetal human tissues. Three different Mabs were used to detect P-gp in fetal tissues as described in Materials and Methods. A: Absence of reactivity with C-219 in the fetal zone of the adrenal (14 weeks). Note the bulky polygonal cells with abundant cytoplasm of these steroidogenic cells. B: Clear membrane accentuated staining (arrow 1) with C-219 of definitive zone cells of the fetal adrenal (28 weeks), enveloping the fetal zone (arrow 2). Definitive zone cells show thickening and evidence of steroidogenesis from the 25th week of gestation. C: Strong staining with C-219 of the definitive zone after full term pregnancy (38 weeks). D: Neonatal adrenal (4 months) with C-219, showing staining in the zona fasciculata (F) and in the developing zona reticularis (R) but not in the adrenal medulla (M). E: Fetal kidney (11 weeks), showing clear immunoreactivity of C-219 with the apical surface of proximal tubular epithelium (arrow 1) and weaker but distinct reactivity with Bowman's capsule epithelium (arrow 2). Note the lack of reactivity with the glomerulus. F: Fetal kidney (13 weeks) with C-219, clearly showing apical staining of mature tubules and weaker staining in less differentiated tubules. Note the absence of staining in metanephric blastema. G: Fetal heart (C-219), with very strong granular intracytoplasmic staining of cardiac muscle fibers (arrow) after 9 weeks (a), and clear intracytoplasmic z-band pattern (arrow) after full term pregnancy (b),. H: Meningeal vessels of the fetal brain (28 weeks) with JSB-1, clearly showing positivity of endothelial cells. Magnifications: A,B,E,F,Ga,H ×630; C,Gb ×400; D ×200.*

starts to develop in the 5th week of gestation, is able to produce urine already at the end of the embryonal period (8 to 9 weeks).[31] In this respect, the observed expression of P-gp in Bowman's capsule in early kidney specimens (11,13 weeks) may point at an active secretory role of these cells in the primitive kidney, a function that may disappear after flattening of the capsule cells during further nephrogenesis. Interestingly, the staining pattern of P-gp in fetal kidney resembles that of childhood nephroblastoma,[32] where expression could be detected in areas of tubular differentiation, whereas blastemal cells, from which this malignancy originates,[33] were negative.[32] This tumor is highly sensitive to natural chemotherapeutic agents.[34]

Weak or no staining for P-gp was observed in fetal intestinal epithelium, pancreatic ducts, and bile ductules of the liver. This clearly differs from the adult situation, in which expression of P-gp can readily be demonstrated in

Table 1. *Reactivity of Fetal Tissues with Anti–P-gp Antibodies*

	P-glycoprotein expression										
Gestation time (weeks)	7	11	13	16	18	20	25	28	38	4m	Adult*
Kidney											
Glomeruli	−		−	−	−	−	−	−	−		−
Bowman's capsule	+ +	+ +	−	−	−	−	−	−	−	−	−
Tubules	+ +	+ +	+ +	+ +	+	+ +	+	+ +	+ +	+ +	+ +
Digestive tract											
Epithelium											
Oesophagus								−	−		+
Stomach								+			+
Intestine	−	−	−			−		+ +			+ +
Muscles											
Striated		+ + +†					+ + +†				+ + +†
Smooth		+‡		+‡				+‡			+‡
Liver											
Bile canaliculi		+ +	+ +	+			+ +	+ +			+ +
Bile ductules								+ +			+ +
Parenchyme		−	−	−		−	−				−
Pancreas											
Acini				−			−				+
Ducts			−				−				+
Islets			−				−				−
Adrenal											
Adrenal cortex		13/14									
Definitive zone		+/+		+		+ +	+ +	+ +	+ + +	+ + +	+ + +
Fetal zone	−	−/−		−		−	−	−	−		−
Adrenal medulla		−/−									
Lung			14	15	17						
Pneumocytes			−	−	−	−					−
Bronchioli			−	−	−	−					−
Main bronchi					+ +	+ +					
Brain											
Parenchyma	−	−						−			−
Vessels	−	−						+			+
Heart	9‖										
Muscle	+ + +†	+ + +†				+ + +†		+ + +†			+ + +†

* Staining results in adult human tissues reported earlier.[7,9,11]
† Strong staining with C-219, but not with JSB-1 and MRK-16.
‡ Weak staining with JSB-1 and clear staining with MRK-16 but absence of staining with C-219.
‖ Numbers in the table indicate intermediate gestation times.
− = negative; + = weak positivity or positivity in a minority of cells (see text), + + = definite positivity, + + + = strong positivity.
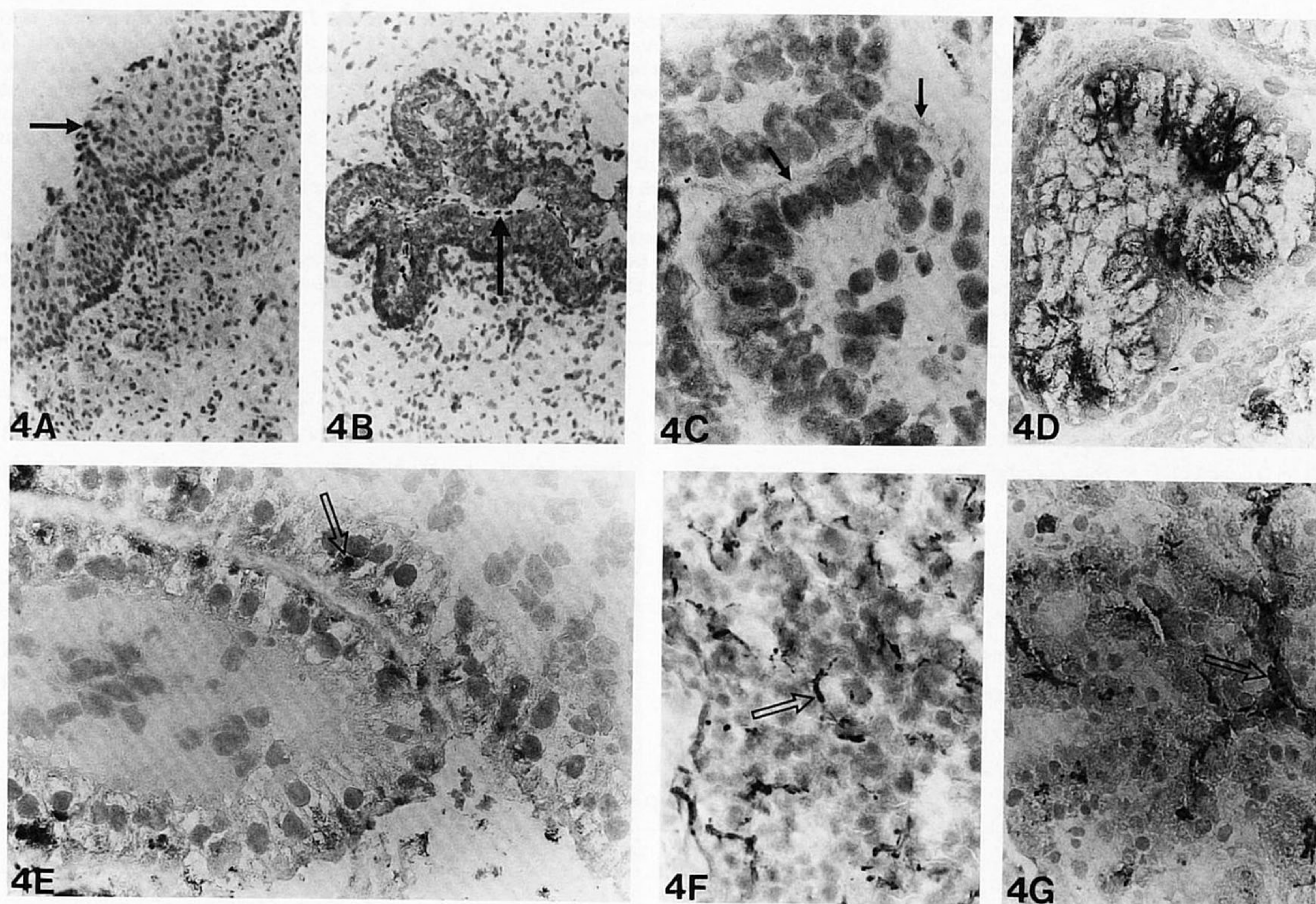
Figure 4. Immunohistochemistochemical staining for P-gp in frozen tissue sections of fetal human tissues of the gastrointestinal tract. A: Fetal esophagus with C-219 (28 weeks), showing absence of staining in the squamous epithelial lining (arrow). B: Respiratory epithelium of a pharyngeal fold (14 weeks) with C-219, showing clear staining of the luminal side of the plasma membrane (arrow). C: Intestine (11 weeks) with C-219, not showing any staining with the panel of antibodies (arrows). D: Stomach (28 weeks) with MRK-16 of a 7-day-old premature born neonate, showing heterogeneous staining of the gastric epithelium. E: Intestine (14 weeks), showing clear intracytoplasmic reactivity of the Golgi area with JSB-1 (open arrow). This staining was not found with C-219 and MRK-16. No apical membrane staining, readily detectable with this technique in normal adult intestinal epithelium, was observed at this stage. F,G: Liver (14 and 28 weeks) with C-219, showing staining patterns of bile canaliculi (open arrows), reflecting the different stages of development of the fetal liver. Note the lack of a clear difference in staining intensity between the two specimens and the absence of staining in the liver parenchyme. Magnifications A ×200; B,F,G ×400; C,D,E ×630.

these organs.[6-9] This observation might reflect the different prenatal function of the digestive tract, where absorption of toxic substances from the amniotic fluid, rather than digestion of food and excretion of toxins, takes place. Interestingly, later fetal intestinal samples showed increasing expression of mdr1-mRNA. Early expression of P-gp in bile canaliculi of fetal liver but not in bile ductules would support the hypothesis of a role for P-gp in bile excretion, a process already operative at the 12th week of gestation. Because many normal metabolic functions of the liver are performed by the placenta, however, the production of bile constituents and their excretion by the fetal liver is very low[35]; in addition, transport of cholic acids was recently shown not to be mediated by P-gp.[36] Therefore, the physiologic relevance of P-gp in the liver remains speculative.

Expression of P-gp is barely or not detectable in both pneumocytes and the epithelium of the bronchial tree in the adult.[9,11] Before birth, however, the lungs are filled with liquid from the lungs (surfactant) and tracheal glands as well as with amniotic fluid, which contains fetal excre-

tion products. Therefore, the expression of a P-gp in respiratory epithelium of fetal bronchi and the pharyngeal area might play a role in preventing toxins to reenter the fetus and to protect the developing lungs. The absence of a detectable message of mdr1-mRNA is probably due to the inability of RNAse protection assay to detect the very small subpopulation of positive bronchial cells in the fetal lung specimen. The dilution effect characteristic of bulky techniques, like the RNAse protection assay, has already been described in tumor material.[37]

The interpretation of the strong staining with MAb C-219 but not with other anti–P-gp MAbs observed in human[9,38] and in chinese hamster[39] cardiac, skeletal, and upper esophageal muscles, in the absence of mdr1-mRNA expression, remains a matter of debate. The cardiovascular system is the first functional embryonal system (3 weeks of gestation) to develop. In the fetal heart, very strong staining of cardiac muscle fibers was observed already after 9 weeks of gestation exclusively with C-219, indicating recognition of an important functional antigen by C-219. Although the finding of a 200-kd band

with C-219 immunoblotting and specificity of staining for type I fibers in striated muscles[9,38] strongly suggests cross-reaction with a myosin isoform, it was reported that known myosin isoforms did not show the amino acid sequence recognized by C-219.[39] Because C-219 also recognizes *mdr3*/P-gp,[15] the possibility of *mdr3* expression in striated and cardiac muscle cannot be ruled out. However, *mdr1* expression in the heart is definitely unlikely, in view of the lack of staining with *mdr1* specific MAbs JSB-1 and MRK-16,[15] and the absence of *mdr1*-mRNA expression by RNAse protection assay.

Capillary endothelium in several adult nonexcretory tissues like the testis and the central nervous system were reported to express P-gp.[9,11] The demonstration of clear staining in capillaries of the fetal brain in the third trimester of gestation but not in earlier specimens supports the idea that P-gp is an operative part of this highly specialized capillary system, and that increasing P-gp expression along with maturation of this system may play a role in the protection of the fetal brain from damage by drugs or toxic compounds in late gestation.

In conclusion, the expression of P-gp in various fetal tissues at an early stage of development, and its distribution pattern further support a role as an efflux pump for toxic substances or steroid hormones. A correlation was found between the expression of P-gp and the developing fetal function of several organs, like the adrenal, the lung, and the intestine. These results may help in discovering the physiologic substrates of P-gp in different organs.

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