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Tissue- and Cell Type-Specific Expression of Cytochrome P450 1A1 and Cytochrome P450 1A2 mRNA in the Mouse Localized *in situ* Hybridization

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ABSTRACT. We used *in situ* hybridization to examine organ- and cell type-specific constitutive and 3-methylcholanthrene (3MC)-inducible cytochrome P450 (CYP)1A1 and CYP1A2 mRNA expression in various tissues of the C57BL/6N mouse. *In situ* hybridization was carried out 10 hr after the mice had received intraperitoneal 3MC, or vehicle alone. We detected levels of 3MC-induced CYP1A1 mRNA in: liver (centrilobular, more so than periportal, regions); lung (Clara Type II cells much more than Type I epithelial cells); brain, especially endothelial cells lining the vascular surface of the choroid plexus; the digestive tract (duodenum > jejunum > ileum > colon > esophagus > stomach—in particular, the villous epithelium, plus cells surrounding glands in the lamina propria); renal corpuscles of the kidney; the ovary (medulla more so than cortex); and the endothelial cells of blood vessels throughout the animal. Constitutive CYP1A1 mRNA was not detectable by *in situ* hybridization in any of these tissues. In contrast, constitutive CYP1A2 mRNA was measurable in liver, and 3MC-inducible CYP1A2 mRNA was observed only in liver, lung, and duodenum (having cell-type locations similar to those of CYP1A1); the other above-mentioned tissues were negative for CYP1A2 mRNA. These data demonstrate the striking differences in tissue- and cell type-specific expression between the two members of the mouse *Cypla* subfamily. Because of the ubiquitous nature of 3MC-inducible CYP1A1 throughout the animal rather than just "portals of entry," these results support our hypothesis that CYP1A1, induced by particular endogenous signals in various tissues and cell types, might participate in one or more critical life processes—in addition to its well-established role of metabolism of polycyclic hydrocarbons, certain drugs, and other environmental pollutants. BIOCHEM PHARMACOL **58**;3:525–537, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. [*Ah*] gene battery; Ah receptor; CYP1A1 mRNA; CYP1A2 mRNA; tissue-specific P450 expression; cell type-specific P450 expression; liver; lung; brain; choroid plexus; duodenum; jejunum; ileum; colon; esophagus; stomach; kidney; ovary; endothelial cell CYP1A1; *in situ* hybridization

CYP1A1§ and CYP1A2 are two of the four dozen to six dozen individual members of the *CYP* gene superfamily in mammals [1, 2]. The CYP1A1 and CYP1A2 enzymes have been demonstrated to play a key role in expression of all genes in the [*Ah*] battery [3; reviewed in Refs. 4, 5]. The mouse [*Ah*] gene battery consists of at least six dioxin-inducible genes—two Phase I cytochrome P450 genes, *Cyp1a1* and *Cyp1a2*, and four Phase II genes: *Nqo1* [NAD(P)H:quinone oxidoreductase]; *Aldh3a1* (cytosolic aldehyde dehydrogenase, ALDH3c; *Ahd4*); *Ugt1a6* (UDP glucuronosyltransferase 1A6); and *Gsta1* (glutathione transferase Ya).‖ Although all six genes are up-regulated by ligands such as dioxin that bind to the Ah receptor (AHR) and all four [*Ah*] Phase II genes are induced by oxidative stress, the criterion for "membership" in the mouse [*Ah*] battery is their CYP1A1/1A2 metabolism-dependent down-regulation of transcription of each of these six genes [reviewed in Refs. 4, 5]. The endogenous ligand for the AHR is not yet known, but it has been suggested [6] and recently supported with experimental data [7] that the endogenous AHR ligand might be a CYP1A1 substrate.

We believe that the reason for the evolution of this gene

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§ *Abbreviations:* CYP, cytochrome P450; AHR, Ah receptor; 3MC, 3-methylcholanthrene; RT-PCR, reverse transcription-polymerase chain reaction; 1X SSC, 0.15 M NaCl, 0.015 M sodium citrate; and SP6 and T7, viral promoter DNA motifs and corresponding polymerases.

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‖ By convention, mouse genes include (italicized) the first letter capitalized and all other letters lowercase (e.g. *Cyp1a1*, *Gsta1*), and genes of other mammals include all capital letters italicized, whereas the mRNA, cDNA, or enzyme (gene product) of all mammals including mouse are all capital letters and not in italics (e.g. CYP1A1, GSTA1).

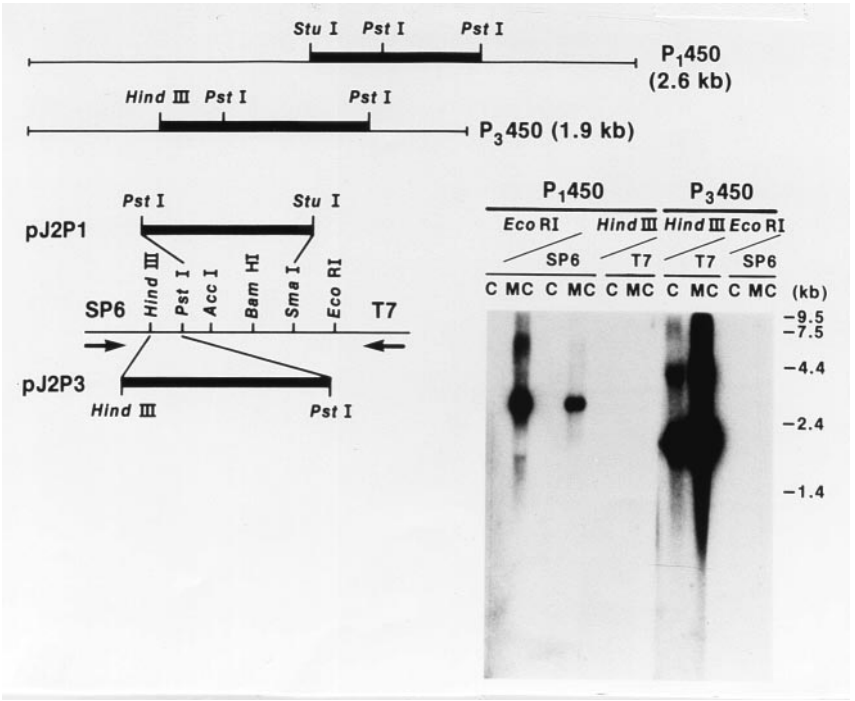

FIG. 1. Specificity of the CYP1A1 (P_1-450) and CYP1A2 (P_3-450) cDNA probes used in this study. Including poly(A^+) tails, CYP1A1 and CYP1A2 mRNAs (top) are about 2.9 and 2.2 kb, respectively. Polylinker vectors containing the SP6-driven and T7-driven CYP1A1 probe (pJ2P1) and CYP1A2 probe (pJ2P3) are shown at lower left. In the Northern blot using C57BL/6N mouse liver at lower right, 3MC-inducible (MC) but not constitutive (C) CYP1A1 mRNA (2.9 kb) is illustrated in lanes 2 and 4. Constitutive and very high levels of 3MC-inducible CYP1A2 mRNA (2.2 kb) are shown in lanes 7 and 8, respectively. The other spurious bands are commonly seen with these strong SP6 and T7 promoters. Each lane contained total RNA (10 μg for lanes 1, 2, 5, 6, 7, 8, 9, and 10, and 2 μg for lanes 3 and 4). Note that this photograph represents a composite of images from separate hybridizations.

battery during the past 400 million years or longer is most likely the co-participation of these genes in important cellular processes—rather than simply the metabolic detoxification of foreign chemicals. If the CYP1A1 or CYP1A2, or both enzymes, were present in numerous mammalian tissues, especially tissues and cell types that are not "portals of entry" for noxious foreign chemicals, such data would be consistent with our hypothesis that the [*Ah*] gene battery might exist in order to perform one or more important endogenous functions. In the present *in situ* hybridization study, we show that polycyclic hydrocarbon-inducible CYP1A1 mRNA is ubiquitous in mouse tissues, whereas localization of CYP1A2 mRNA is limited to the liver and digestive tract. We believe that these results reinforce our hypothesis. CYP1A2 appears to exist at portals of entry primarily for the metabolism of foreign chemicals (plant metabolites, drugs, arylamines). On the other hand, CYP1A1 exists in many tissues and cell types in addition to portals of entry; if the AHR is activated by its endogenous ligand, the resultant induction of *Cyp1a1* gene expression might lead to one or more critical life responses (e.g. cell division or migration, apoptosis, mitosis).

MATERIALS AND METHODS

Treatment of Animals and Collection of Samples

Six female C57BL/6N mice (aged 4–5 weeks) were purchased from the NIH Animal Facilities and acclimated to our mouse colony for 1 week. Three mice were then treated intraperitoneally with 3MC (200 mg/kg body weight) for 10 hr; three controls received the vehicle alone (corn oil, 25 mL/kg). The groups of three mice were killed by CO_2 asphyxiation, following which the liver, lung, esophagus, stomach, duodenum, jejunum, ileum, colon, brain, kidney, and ovary were separately collected. Induced hepatic, pulmonary, and renal CYP1A1 and hepatic and pulmonary CYP1A2 mRNA are known to be maximal in C57BL/6N mice 10 hr following 3MC treatment [8].

Generation of CYP1A1- and CYP1A2-specific RNA Probes

Improper design of the cDNA clones could lead to cross-hybridization of CYP1A2 mRNA with the CYP1A1 probe, and cross-hybridization of CYP1A1 mRNA with the CYP1A2 probe [8, 9]. Cross-hybridization was shown not to exist (Fig. 1). We subcloned 3′-specific fragments of mouse CYP1A1 and CYP1A2 cDNAs into the pGEM-3 vector [10]; these subclones represent a 733-bp *Stu* I-*Pst* I fragment (pJ2P1) and a 900-bp *Hind* III-*Pst* I fragment (pJ2P3), respectively. These vectors were linearized with *Eco* RI (SP6-directed pJ2P1) or *Hind* III (T7-directed pJ2P3) prior to generation of the specific antisense probe. Linearization and use of the opposite promoters produced the sense probe (Fig. 1). We prepared the ^{35}S-labeled single-stranded RNA probes (2×10^9 cpm/μg), using uridine 5′-(α[^{35}S]thio)

triphosphate (800 Ci/mmol) and SP6 and T7 polymerases, as described [10–12]. The radiolabeled RNA probes were purified from free label by ethanol precipitation with 10 μg of yeast tRNA (GIBCO BRL) as carrier. Figure 1 shows that SP6-driven pJ2P1 detects 3MC-inducible but no constitutive CYP1A1 mRNA (lanes 1–4), whereas T7-driven pJ2P1 detects absolutely no CYP1A2 mRNA (lanes 5 and 6); in contrast, T7-driven pJ2P3 detects significant amounts of basal CYP1A2 mRNA and large amounts of 3MC-inducible CYP1A2 mRNA (lanes 7 and 8), whilst SP6-driven pJ2P3 detects absolutely no CYP1A1 mRNA (last two lanes). The amount of 3MC-induced CYP1A2 mRNA is 3- to 5-fold greater than the maximal amount of 3MC-induced CYP1A1 mRNA, consistent with many earlier studies in C57BL/6 mouse liver [reviewed in Ref. 13].

In Situ *Hybridization*

All the above-mentioned tissues were handled in the same manner. The tissues were fixed in 4% (w/v) paraformaldehyde in isotonic PBS for 2 hr, followed by washing out the formaldehyde in PBS. Further processing included dehydration, followed by embedding in paraffin. For dehydrating, each sample was soaked in 30%, then 50%, then 70%, then 95%, and finally 100% ethanol. The samples were next treated with ethanol:xylene (1:1), then xylene:xylene (1:1), and finally xylene:paraffin (1:1) at 58°. After three exchanges in paraffin, each tissue was embedded in fresh paraffin at 58°. The tissue blocks were then stored at 4° until preparation of the slides. The tissues were sectioned (3–4 μm) and collected on polylysine-coated slides. The samples on the slides were first treated twice in xylene to remove the paraffin and then hydrated (soaking in 100%, then 95%, then 70%, and then 50% ethanol). Each slide was next treated with 2X SSC for 30 min at 68°, then dipped in water, digested with proteinase K (2 μg/mL in 50 mM Tris–HCl, pH 7.5, plus 5 mM ethylenediaminetetraacetic acid) for 15 min, rinsed in glycine (2 mg/mL PBS) to stop the proteinase action, washed in PBS, and post-fixed in 4% paraformaldehyde for 15 min. After washing, each slide was acetylated (0.1 M triethanolamine, pH 8.0, in 0.75 mL acetic anhydride) for 10 min, rinsed in PBS, dehydrated again in ethanol as described above, and air-dried. Hybridization was performed as described [10]. Probe size was decreased to about 100 bp by alkaline hydrolysis [14]. After overnight hybridization at 50°, the slides were initially washed for 2–3 hr in 50% formamide/5X SSC, followed by a stringent wash for 1 hr in 0.1X SSC at 60°. The slides were then treated with RNase A (1 μg/mL) in 2X SSC for 10 min at room temperature. Dithiothreitol (10 mM) was included in all of the wash solutions. After RNase treatment, the slides were rinsed with 2X SSC, dehydrated, and air-dried. The slides were dipped in emulsion (Kodak NTB2) diluted 1:2 in distilled water, and then exposed at 4° for 5 days and developed with Kodak D-19. After photography in dark-field illumination, the same sections were stained with Giemsa solution and photographed in bright-field illumination [10]. Ten slides of each of the organs for each of the six mice were examined; more than 300 photographs were taken. Among all the tissues examined, *in situ* hybridization data were qualitatively similar in each of the three 3MC-treated mice, and *in situ* hybridization data were similar in each of the three control mice.

RESULTS AND DISCUSSION

The liver, lung, esophagus, stomach, duodenum, jejunum, ileum, colon, brain, kidney, and ovary were examined. We carried out *in situ* hybridization with ^{35}S-labeled CYP1A1- and CYP1A2-specific probes in order to determine organ- and cell type-specific expression of constitutive and 3MC-inducible CYP1A1 and CYP1A2 mRNA.

3MC-inducible CYP1A1 mRNA and Both Constitutive and Inducible CYP1A2 mRNA in Liver

Figure 2 shows the hybridization pattern in the liver of 3MC-treated mice. We observed a lower, but significant, expression of constitutive CYP1A2 mRNA in control mice (data not illustrated). The abundance of the hybridization signal indicates that it is the hepatocytes (rather than Kupfer cells or mesenchymal cells) that predominantly express the 3MC-induced CYP1A1 mRNA and both the basal and 3MC-inducible CYP1A2 transcripts. We found that the pattern of hybridization distribution of all three of these mouse liver transcripts was very similar. Accumulation of CYP1A1/1A2 mRNA was fairly uniform throughout the liver, with somewhat higher expression in centrilobular regions (around the central vein) than in periportal regions or around bile ducts. The relative grain accumulation was higher for CYP1A2 mRNA (Fig. 2c) than for CYP1A1 (Fig. 2b)—which is consistent with the relative intensities of the bands in Fig. 1, as well as several semi-quantitative studies on transcription rates, mRNA levels, and protein immunoprecipitation concentrations of these two genes [8, reviewed in Ref. 13]. These data confirm numerous previous studies on polycyclic hydrocarbon-inducible CYP1A1 and both constitutive and inducible CYP1A2 localization and distribution within mammalian liver [13, 15–19, reviewed in Refs. 20–24].

3MC-inducible CYP1A1 and CYP1A2 mRNA in Mouse Lung

In the lung we detected considerably higher levels of 3MC-inducible CYP1A1 mRNA (Fig. 3b) than 3MC-inducible CYP1A2 mRNA (Fig. 3d), and basal mRNA levels were not detected for either CYP1A1 or CYP1A2 (data not illustrated). Upon higher magnification (Fig. 3c), we found that 3MC-inducible CYP1A1 (as well as CYP1A2, not shown) was located primarily in Clara Type II cells and endothelial cells around blood vessels, compared with much less in the squamous alveolar epithelial

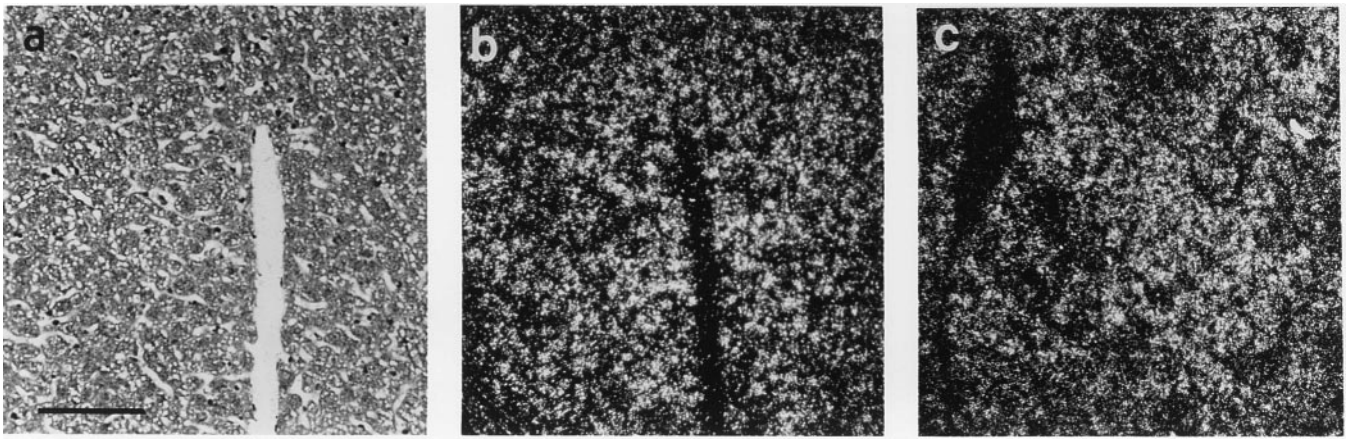

FIG. 2. Localization of inducible CYP1A1/1A2 mRNA in the liver of a 3MC-treated mouse [magnification 250X]. (a) Giemsa-stained bright-field photomicrograph of a representative liver section. The rod-like structure right of center is a longitudinal section of a central vein. Bar at bottom left = 100 μm. (b) Dark-field picture of the same field as that in a, hybridized with the CYP1A1-specific probe. (c) Dark-field photomicrograph of a field similar to that in a, hybridized with the CYP1A2-specific probe. A central vein can be seen at upper left.

(Type I) cells in the respiratory bronchioles. These data are consistent with dozens of studies of polycyclic hydrocarbon- and dioxin-inducible CYP1A1 and CYP1A2 mRNA expression in the lung of laboratory animals, much of which is relevant to cigarette smoke-induced lung cancer [reviewed in Refs. 13, 20–24]. It is noteworthy that, whereas rodent lung exhibits polycyclic hydrocarbon-inducible CYP1A2, no evidence for CYP1A2 mRNA or protein has been detected in human lung [21–25].

3MC-inducible CYP1A1, but not CYP1A2, mRNA Detected in Mouse Brain

We found that 3MC-inducible CYP1A1 mRNA levels were very high in the highly vascular choroid plexus (Fig. 4, c and d) of the brain, distributed in the lateral, third, and fourth ventricles. Basal CYP1A1 mRNA was not detected anywhere in the brain (Fig. 4b), nor was 3MC-inducible or constitutive CYP1A2 mRNA (data not illustrated). At higher magnification (Fig. 4, e and f), it can be seen that the silver grains are located only in specific cells of the choroid plexus; due to their location and number, these cells are very likely to be the endothelial cells lining the vascular surface of the choroid plexus, known to serve as part of the blood–brain barrier, located physically in the area separating the lumen of the ventricle from the lumen of the blood capillaries. No 3MC-induced CYP1A1 transcripts associated with the choroid epithelial cells were observed; it is possible that the closed penetration of the endothelial cells and the continuous basement membrane might prevent significant amounts of the inducer 3MC from reaching these epithelial cells. Our results extend what is already known from several studies of polycyclic hydrocarbon- and dioxin-inducible CYP1A1 enzyme activity [26] as well as mRNA and protein [27], but not CYP1A2 expression, in the brain of rodents [reviewed in Refs. 20–24]. It is worth noting that, whereas rodent brain exhibits no basal or polycyclic hydrocarbon-inducible CYP1A2, there is evidence for CYP1A2 mRNA and protein detected in human brain [23, 28, 29].

3MC-inducible CYP1A1/1A2 mRNA in the Digestive Tract

Wattenberg's study of the distribution of polycyclic hydrocarbon-inducible benzo[*a*]pyrene 3-hydroxylase activity (now known largely as CYP1A1 expression) throughout the rat digestive tract [reviewed in Ref. 30] was among the first lines of evidence to demonstrate extrahepatic CYP1A1 activity. Our *in situ* hybridization analysis in the mouse (Fig. 5) revealed the highest 3MC-inducible CYP1A1 mRNA in duodenum, less in the jejunum and ileum, even less in the colon and esophagus, and lowest amounts in the stomach—very consistent with the distribution of enzyme activity reported by Wattenberg's laboratory long ago [30].

ESOPHAGUS AND STOMACH. Figure 5, a and b shows that stratified squamous epithelial cells of the esophagus, more so than the gastric mucosa, exhibited measurable 3MC-inducible CYP1A1 mRNA levels. At higher magnification (Fig. 6a), the grains can be seen localized to these epithelial cells, whereas the lamina propria and layers of muscle cells show no evidence of CYP1A1 transcripts. We detected no constitutive CYP1A1 or CYP1A2 mRNA, nor was 3MC-inducible CYP1A2 mRNA detectable in the esophagus or stomach.

DUODENUM. The level of 3MC-induced CYP1A1 mRNA in the duodenum (Fig. 5, c and d) was comparable to that seen in the liver (Fig. 2b). As was observed in esophagus, at higher magnification (Fig. 6b) the grains are localized to the villous epithelial cells of the duodenum and much less in cells of the underlying lamina propria. Duodenal glands located at the base of the villi, surrounded by

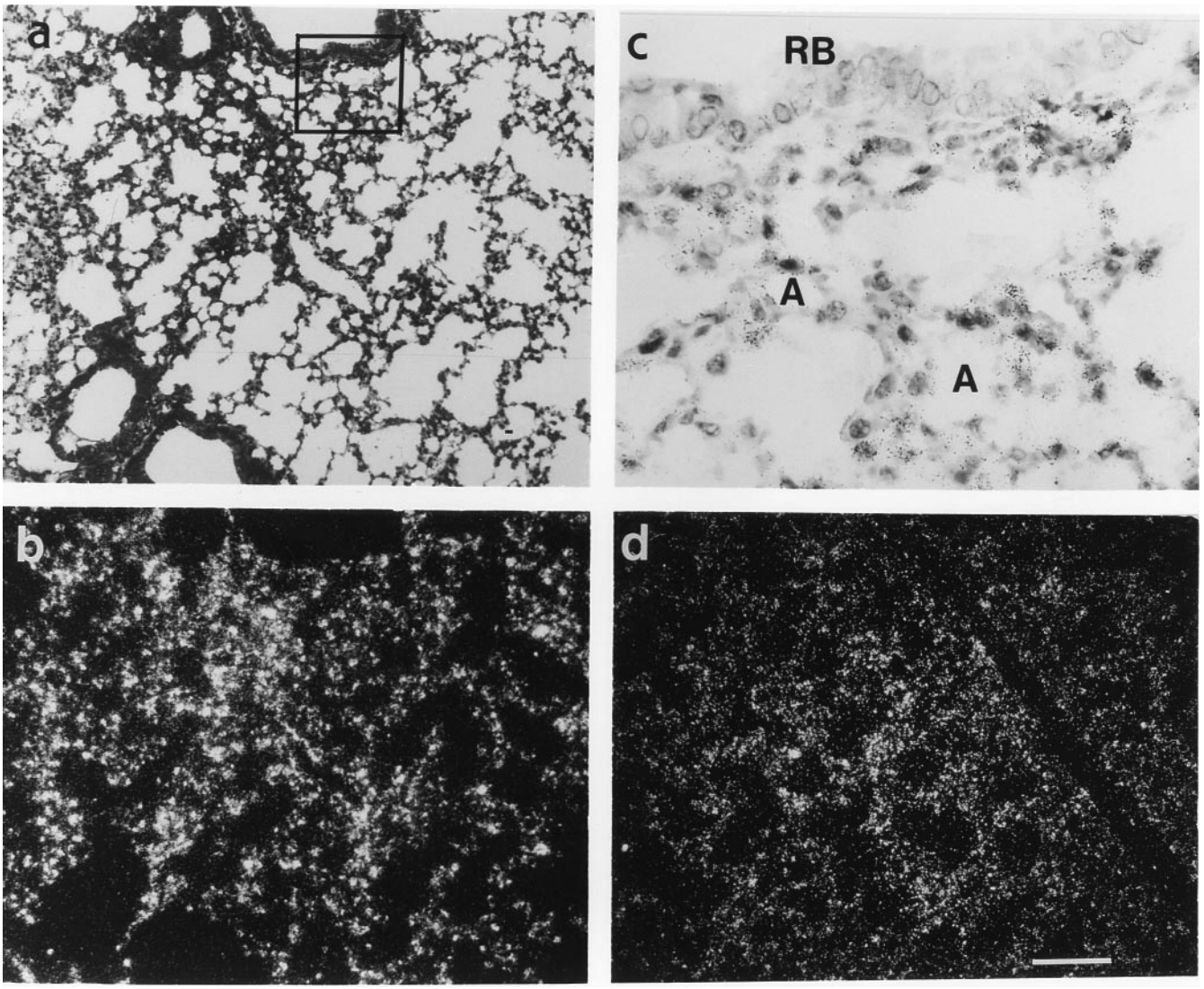

FIG. 3. Distribution of inducible CYP1A1/1A2 mRNA in the lung of a 3MC-treated mouse. (a) Bright-field Giemsa-stained photomicrograph [magnification 100X] of lung. (b) Dark-field picture of the same field as that in a, hybridized with the CYP1A1-specific probe. (c) Bright-field higher power magnification [630X] of boxed area shown in a, hybridized with the CYP1A1-specific probe (visible as silver grains). RB, respiratory bronchiole. A, alveolar sac. (d) Dark-field photomicrograph [magnification 100X] of a field similar to that in a, hybridized with the CYP1A2-specific probe. Bar at lower right = 200 μm.

cells from the lamina propria, are moderately labeled (Fig. 5b). Interestingly, we also found 3MC-inducible CYP1A2 mRNA in the duodenum, distributed similarly to that of 3MC-inducible CYP1A1 mRNA, but at levels considerably lower than that of CYP1A1 mRNA (data not illustrated). Constitutive CYP1A1 or CYP1A2 transcripts were not detected in the duodenum. The route of administration of benzo[*a*]pyrene (oral vs intraperitoneal) was recently shown to have strikingly different effects on the pattern of inducible CYP1A1 in the mouse small intestine [31]. Under tissue culture conditions, bilirubin has been suggested [32, 33] to be the endogenous ligand, or one of the endogenous ligands, of the AHR, which is involved in CYP1A1 and CYP1A2 basal and inducible expression. The fact that we found no duodenal CYP1A1 or CYP1A2 mRNA in untreated mice at the ampulla of Vater (junction of the bile duct and pancreatic duct just proximal to the site of their entrance into the duodenum), where bilirubin and bile acids enter the gastrointestinal tract, might suggest that bilirubin in the intact animal is not an important physiological ligand of the AHR. It should also be noted, however, that the major portion of bilirubin reaching the duodenum is normally conjugated with glucuronide and therefore would be incapable of acting as an AHR ligand.

JEJUNUM AND ILEUM. The pattern of distribution of 3MC-induced CYP1A1 mRNA in the jejunum (not shown) and ileum (Fig. 5, e and f) was very similar to that seen in the duodenum, but at considerably less intensity. At higher magnification, the grains were localized to the villous epithelial cells rather than the lamina propria of the jejunum and ileum (not illustrated). We found considerable

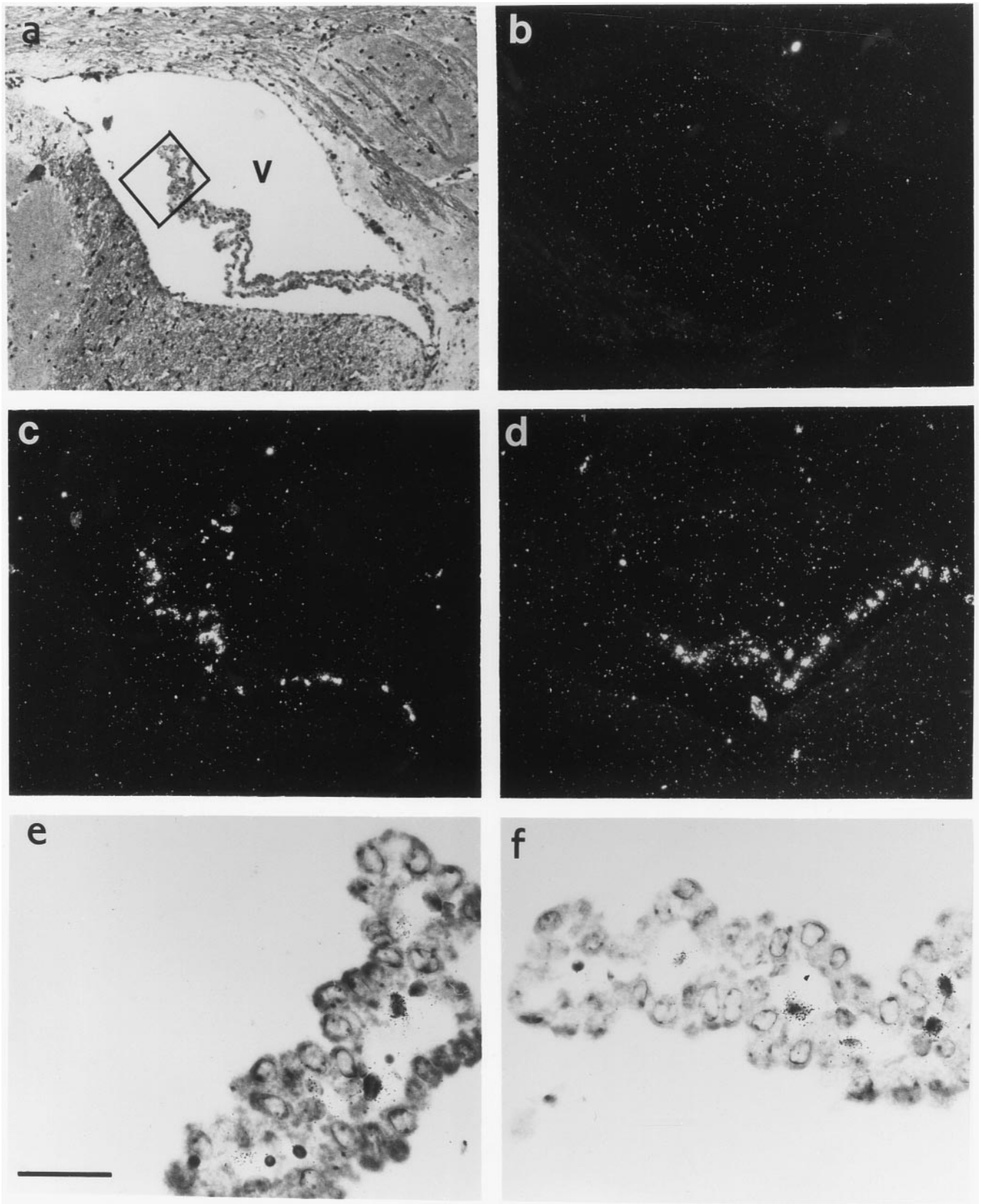

FIG. 4. Localization of inducible CYP1A1 but not CYP1A2 mRNA in the brain of a 3MC-treated mouse. (a) Giemsa-stained bright-field [magnification 100X] of the choroid plexus within the lateral ventricle (V). (b) Dark-field picture of the same area as in a, hybridized with the CYP1A2-specific probe [magnification 100X]. (c) Dark-field photomicrograph [magnification 100X] of the same area as in a, hybridized with the CYP1A1-specific probe. (d) Dark-field picture [magnification 100X] of an area of the distal portion of the choroid plexus similar to that in a, again hybridized with the CYP1A1-specific probe. (e) and (f) Bright-field higher magnifications [630X] of two areas in the distal portion of the choroid plexus [from *box* seen in a]. The bar at lower left in e = 25 μm.

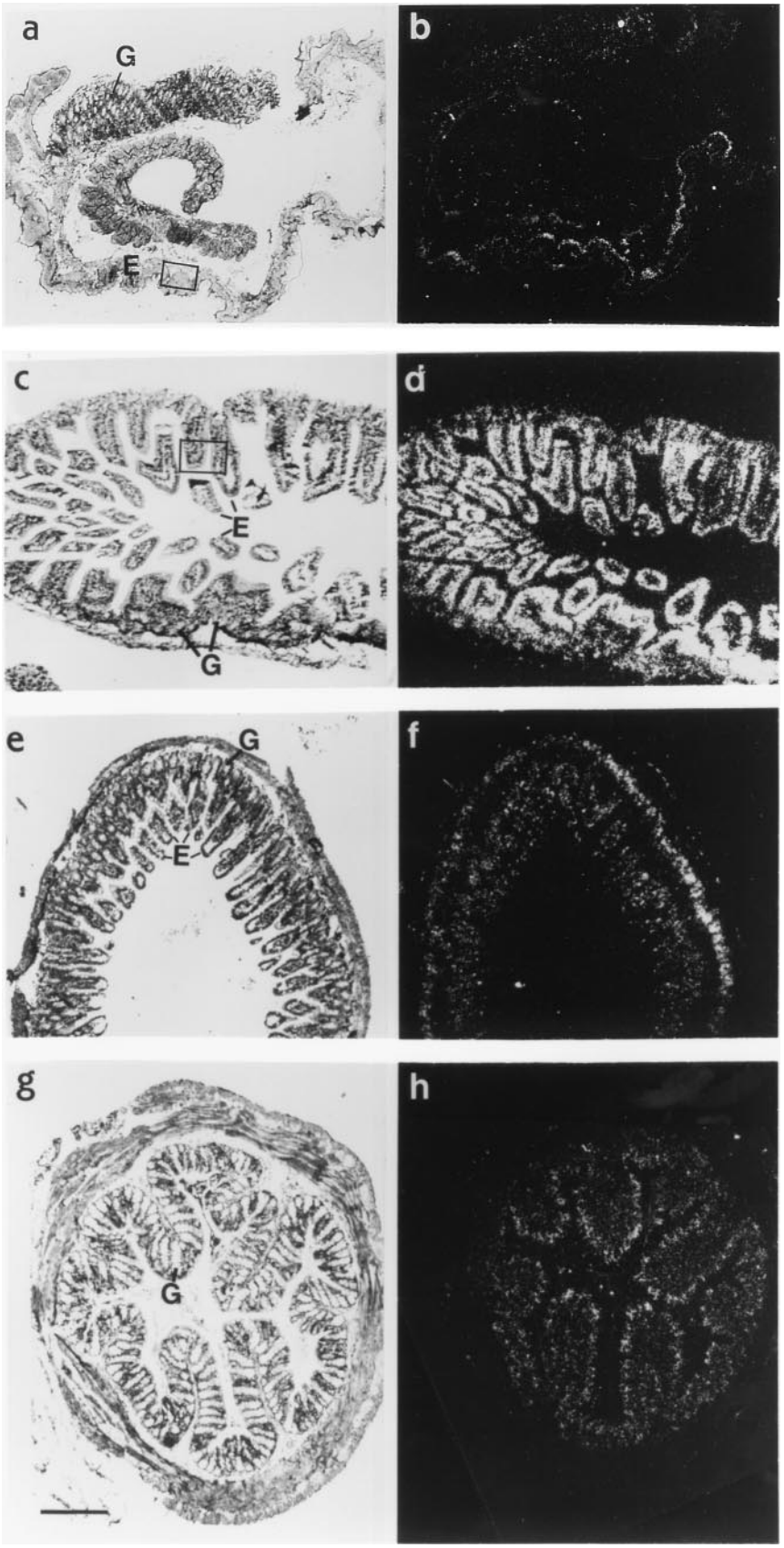

FIG. 5. Localization of inducible CYP1A1 mRNA in the digestive tract of a 3MC-treated mouse. (a) Giemsa-stained bright-field photomicrograph [magnification 50X] of the junction of the esophagus and stomach. E, epithelial cells. G, gastric glands. (b) Dark-field picture of the same as that shown in a, hybridized with the CYP1A1-specific probe. (c) Bright-field picture [magnification 50X] of the duodenum. E, epithelium. G, duodenal basal glands. (d) Dark-field picture of the same as that shown in c, hybridized with the CYP1A1-specific probe. (e) Bright-field photomicrograph [magnification 50X] of the ileum. E, epithelial cells. G, ileal glands. (f) Dark-field picture of the same as that shown in e, hybridized with the CYP1A1-specific probe. (g) Bright-field picture [magnification 25X] of the colon. G, large tubular glands of colon. Bar at *lower left* = 250 μm. (h) Dark-field photomicrograph of the same as that shown in g, hybridized with the CYP1A1-specific probe.

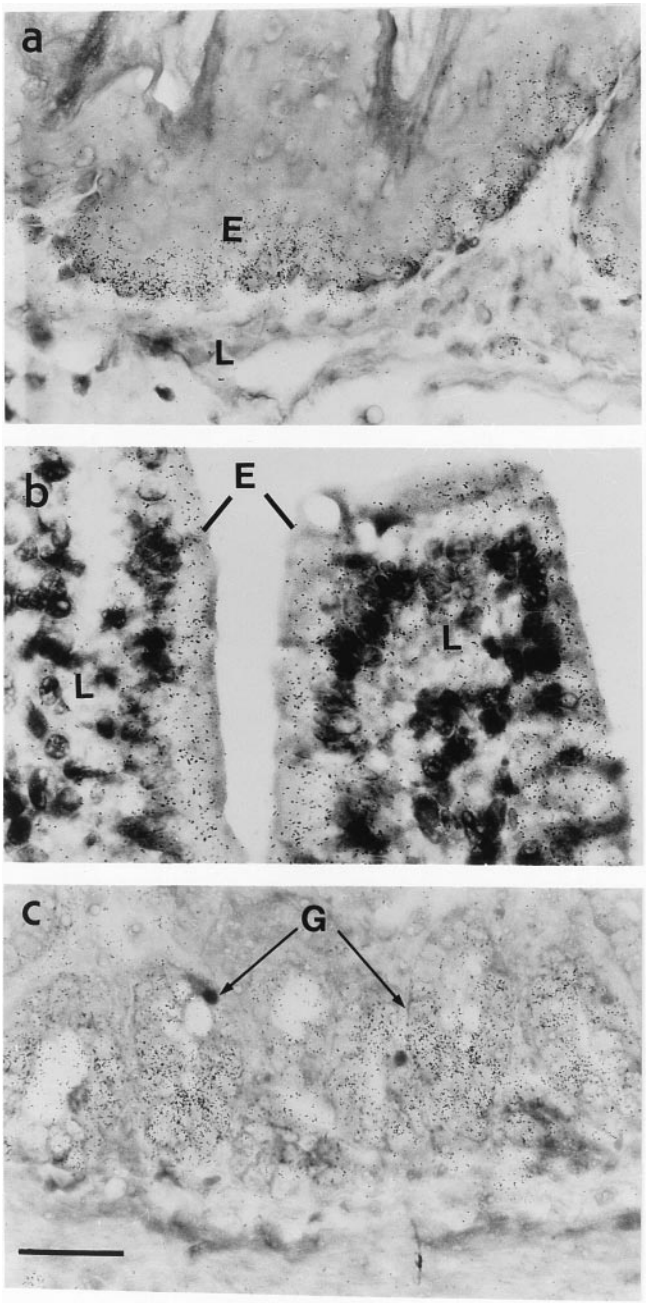

FIG. 6. Further localization of 3MC-inducible CYP1A1 mRNA in the digestive tract. (a) Bright-field photomicrograph [magnification 630X] of the portion of esophagus located in the box shown in Fig. 5a. E, stratified squamous epithelium. L, lamina propria. (b) Bright-field picture [magnification 630X] of the portion of duodenum located in the *box* shown in Fig. 5c. E, duodenal epithelium. L, lamina propria. (c) Bright-field photomicrograph [magnification 630X] of the portion of duodenal basal glands [(G) shown in Fig. 4c] located at the base of the villus. G, glandular cells at the base of the duodenal villus. Bar at *lower left* = 25 μm.

labeling of endothelial cells surrounding the glands at the base of villi of the ileum (Fig. 6c) and jejunum (not shown). We were unable to detect 3MC-inducible CYP1A2 mRNA or constitutive CYP1A1 or CYP1A2 mRNA in the jejunum or ileum.

COLON. 3MC-inducible CYP1A1 mRNA levels were found in the colon (Fig. 5, g and h) with a pattern of distribution similar to that of esophagus, stomach, duodenum, jejunum, and ileum, i.e. expression primarily in the mucosa and endothelial cells surrounding the underlying glands, but not in the lamina propria or underlying muscle layers. Again, no 3MC-inducible CYP1A2 mRNA nor constitutive CYP1A1 or CYP1A2 transcripts were detected in the colon. These data are in agreement with other reports of polycyclic hydrocarbon-inducible CYP1A1 enzyme activity [30], as well as mRNA or protein, throughout the digestive tract of laboratory animals—with expression highest in the duodenal mucosa—and of polycyclic hydrocarbon-inducible CYP1A2 expression at least in the duodenum [34–38; reviewed in Refs. 20–24]. In the lower digestive tract it is well known that, over our lifetime, hundreds of thousands of mucosal cells are generated from stem cells surrounding the underlying glands and migrate toward the lumen over a 2- to 3-day period before undergoing apoptosis and sloughing into the intestinal lumen. Intestinal malignancies arise largely from the stem cells. Interestingly, the villous cells much more so than the stem cells in the mouse [Figs. 5 and 6] appear to exhibit the highest levels of 3MC-inducible CYP1A1 mRNA in the lower digestive tract. Given the time that it takes for a stem cell to migrate to the tip of a villus, and the 10-hr treatment with 3MC in the present experiment, the CYP1A1 mRNA induction that we observed must have occurred in large part directly in the villous cells rather than in the precursor stem cells. As stated above, orally administered 3MC instead of intraperitoneally administered 3MC, and the choice of different time points, might have given a considerably different pattern of CYP1A1 mRNA induction in these epithelial cells of the gastrointestinal tract [31].

3MC-inducible CYP1A1 mRNA in Mouse Kidney

The expression pattern of 3MC-inducible CYP1A1 transcripts in the kidney (Fig. 7a) showed a very low expression overall, with high expression areas distributed quite evenly throughout the cortex. Upon higher magnification (Fig. 7b), the renal corpuscle, including the vascular endothelium, was found to be the site of highest CYP1A1 expression. Renal corpuscles consist of anastomosing afferent and efferent blood capillaries and other supporting cells and are the site for filtering the plasma. Water and ions are able to pass through these corpuscles, whereas large particles are retained in this filtering device and removed by appropriate means. It is likely that CYP1A1 expression is high in the renal corpuscle because of its direct contact with 3MC being passed from the blood into the urine. However, the filtration process in the kidney is passive, and the tubular filtrate is essentially an ultrafiltrate of plasma; hence, it is not clear why the cells of the corpuscle appear to have much more 3MC-inducible CYP1A1 mRNA than the epithelial cells of the proximal tubule, which would be

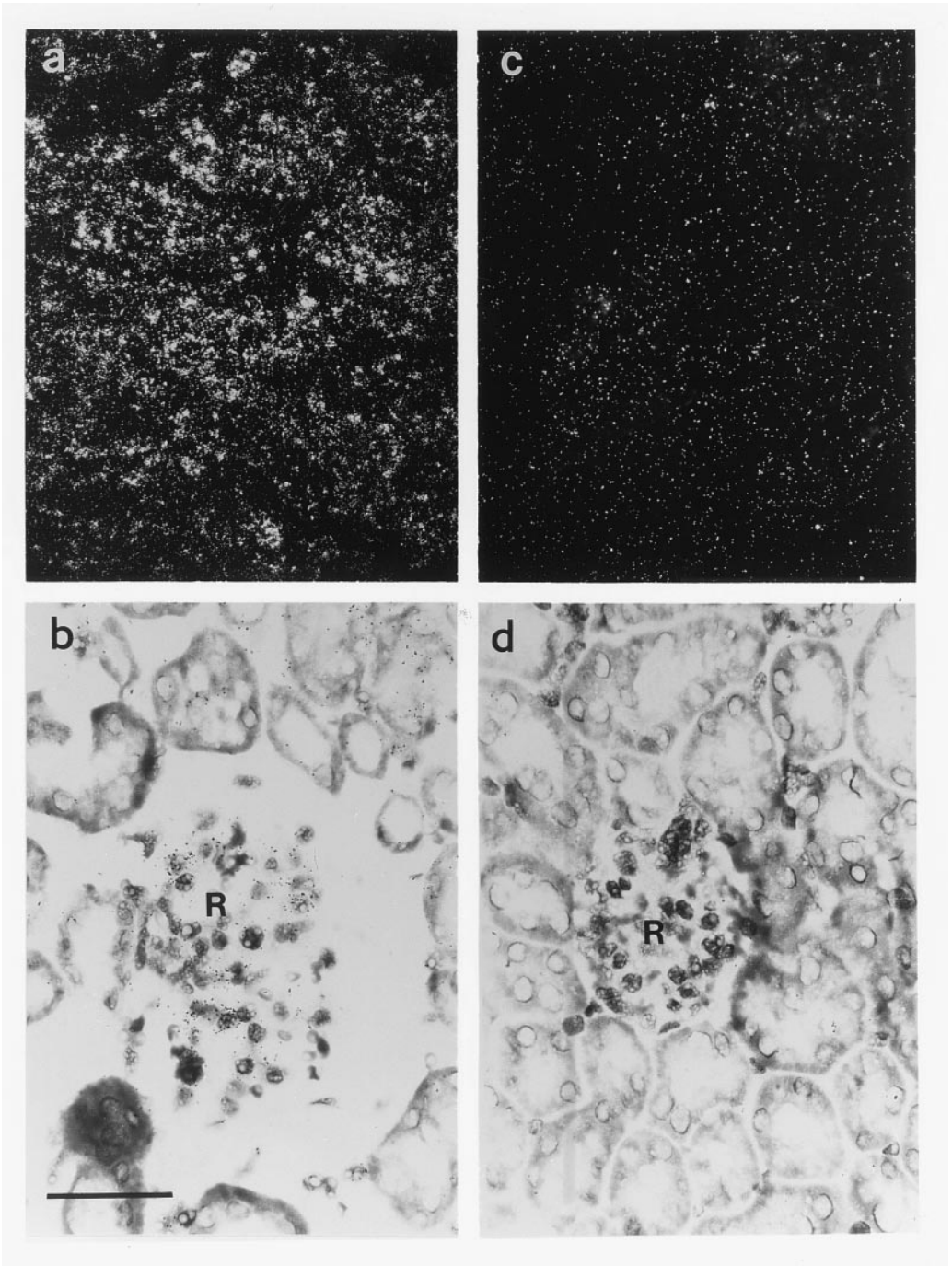

FIG. 7. Localization of CYP1A1 mRNA in the kidney of a control and a 3MC-treated mouse. (a) Dark-field photomicrograph [magnification 50X] of the renal cortex from a 3MC-treated mouse, hybridized with the CYP1A1-specific probe. (b) Bright-field picture [magnification 1000X] of one high-CYP1A1-expressing area from a. R, renal corpuscle. Bar at lower left = 25 μm. (c) Dark-field picture [magnification 50X] of the renal cortex from a control mouse, hybridized with the CYP1A1-specific probe. (d) Higher magnification [630X] of another renal corpuscle similar to that illustrated in b, showing in a control mouse no detectable constitutive CYP1A1 mRNA.

exposed to even higher concentrations of inducer due to the active concentration of the filtrate as it passes along the length of the lumen of the tubule. 3MC-inducible CYP1A1 mRNA was also detectable in the urinary epithelium of the renal calyx, ureter, and bladder (not illustrated). Constitutive CYP1A1 mRNA was not detectable in the kidney (Fig. 7, c and d). No basal or 3MC-inducible CYP1A2 was found in the kidney. These results are consistent with other studies showing expression of polycyclic hydrocarbon- or dioxin-inducible CYP1A1, but not basal CYP1A1 or basal or inducible CYP1A2, in the kidney [reviewed in Refs. 20–24].

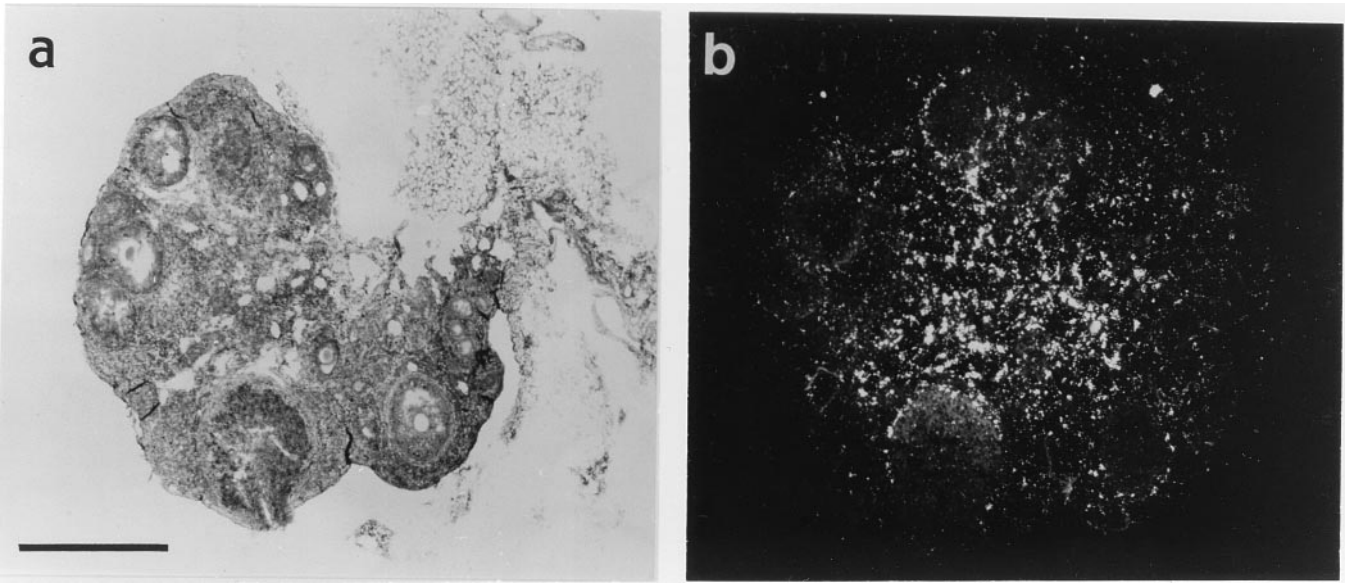

FIG. 8. Localization of 3MC-inducible CYP1A1 mRNA in the ovary. (a) Bright-field photomicrograph [magnification 50X] of the entire ovary from a 3MC-treated mouse. Bar at lower left = 500 μm. (b) Dark-field picture [magnification 50X] of the same section as that shown in a, hybridized with the CYP1A1-specific probe.

3MC-inducible CYP1A1 mRNA in Mouse Ovary

Expression of 3MC-inducible CYP1A1 mRNA was detectable throughout the ovary (Fig. 8), but especially the inner portion (medulla), which consists of large blood and lymphatic vessels, nerves, and smooth muscle and interstitial cells. Polycyclic hydrocarbon-induced ovarian toxicity (death of oocytes) has been demonstrated to be correlated with AHR-mediated CYP1A1 activity [39], suggesting the likelihood that these authors would have been successful in detecting 3MC-inducible CYP1A1 mRNA in that study. Inducible CYP1A1 mRNA and protein have been reported in rat ovary [40]. Moreover, using the highly sensitive RT-PCR technique, we have recently reported detectable levels of CYP1A1 transcript in the mature oocyte of the mouse [41].

We did not examine mouse testis. Inducible CYP1A1 activity or protein has been demonstrated in rat [42–44] and mouse [43] testis. Furthermore, benzo[*a*]pyrene has been shown to have a strong effect on fertility in male as well as female mice [45], suggesting the presence of polycyclic hydrocarbon-inducible CYP1A1 that is capable of generating toxic reactive intermediates from the substrate benzo[*a*]pyrene.

3MC-inducible CYP1A1 mRNA Detected in Endothelial Cells

We found 3MC-inducible CYP1A1 mRNA not only in endothelial cells of the choroid plexus (Fig. 4, d and e) but also in endothelial cells of blood vessels throughout all tissues examined, including the cerebrum and cerebellum (data not shown). Polycyclic hydrocarbon-inducible CYP1A1 has been detected in fish heart endothelium [46], rat aorta [47], cultured human umbilical vein endothelial cells [48], and blood vessel endothelial cells of β-naphthoflavone-treated rat and mice [49]. Given the possible role of CYP1A1 metabolism in the arachidonic acid cascade [5, 50, 51], it is likely that CYP1A1—induced via the AHR that has been activated by either its endogenous ligand or an exogenous ligand—in vascular endothelial cells might participate in cardiovascular diseases. For example, there is an increased risk of coronary artery disease in rats [52] and human populations [53] exposed to dioxin.

CYP1B1 mRNA: No Cross-hybridization with These CYP1A1- or CYP1A2-specific Probes

After this study had been concluded, CYP1B1 was discovered [1, 2] and found to be inducible by polycyclic hydrocarbons and to exhibit substrate specificity similar to that of CYP1A1 [54, 55]. Like CYP1A1, CYP1B1 is ubiquitously expressed, but shows tissue specificity differing from that of CYP1A1 [54, 56]. Using the CYP1A1- and CYP1A2-specific probes developed for the present study, and the hybridization conditions used, we were unable to detect any cross-hybridization with CYP1B1 transcripts.

In Situ *Hybridization* versus *RT-PCR*

The detection level of transcripts of the *in situ* hybridization assay used in the current study has been claimed to be as low as approximately 50 molecules of mRNA per cell [11, 12]. It should be emphasized that it is possible, indeed even likely, that mRNA concentrations below this still might exist (and carry out one or more critical life functions [5]) in some of these tissues that we have recorded as "undetectable." In other words, *in situ* hybridization appears able to detect 3MC-inducible CYP1A1 mRNA, which repre-

sents the result of enhancer activity. On the other hand, RT-PCR is more sensitive than *in situ* hybridization and can detect activity of the proximal promoter when enhancer activity is absent. For example, with RT-PCR we were able to detect constitutive CYP1A1 mRNA in the liver and mature oocyte of the untreated mouse [41]. Also, it should be emphasized that measurement of transcript levels does not necessarily guarantee a direct correlation with the level of functional enzyme in each of these cell types, although some of the studies cited herein did include immunohistochemical evidence of CYP1A1 and/or CYP1A2 protein, in addition to or instead of detection of mRNA levels. Finally, the level of inducer in contact with a particular tissue or cell type is very important; 3MC as an oral dose or intratracheal dose would obviously be more likely to induce higher levels of CYP1A1 mRNA in the gastrointestinal tract and lung, respectively, than 3MC as an intraperitoneal dose.

In conclusion, *in situ* hybridization with ^{35}S-labeled CYP1A1- and CYP1A2-specific probes was carried out in order to determine cell type-specific expression of constitutive and 3MC-inducible CYP1A1 and CYP1A2 mRNA. Whereas we found no constitutive CYP1A1 mRNA in any tissue, we did find detectable levels of 3MC-inducible CYP1A1 mRNA in liver, lung, brain, esophagus, stomach, duodenum, jejunum, ileum, colon, kidney, ovary, and in the endothelial cells of blood vessels of all tissues examined. In contrast, 3MC-inducible CYP1A2 mRNA was observed only in the liver, lung and duodenum; none was found in any of the other above-mentioned tissues, and constitutive CYP1A2 mRNA was detected only in liver—none was detectable in lung or duodenum.

There appear to be interesting distinct human–mouse differences in the tissue specificity of CYP1A2 expression. We found 3MC-inducible CYP1A2 mRNA in mouse lung, whereas CYP1A2 has not been detected in human lung [21–23]. One might argue that 3MC treatment (200 mg/kg body weight) of rodents is an excessive dose compared with environmental exposures to humans; however, no detectable CYP1A2 has been found in explants or primary cultures or established cell lines, derived from normal human lung or lung tumors, following treatment of these cultures with high doses of CYP1A inducers. CYP1A2 has been detected in human brain [28] and colon [38], whereas we did not detect CYP1A2 mRNA in these tissues of the mouse in the present study; however, these species dissimilarities could reflect the difference in sensitivity between RT-PCR [28, 38] and *in situ* hybridization—or, in the case of the gastrointestinal tract, the use of intraperitoneal instead of oral administration of 3MC.

We did not examine the skin or eye in the present report. It is well known, however, that polycyclic hydrocarbon-inducible CYP1A1—but not CYP1A2—exists in mammalian skin [21–23]. β-Naphthoflavone-inducible CYP1A1 and CYP1A2 mRNA [57] and protein [58] have been demonstrated in the mouse eye.

The results of the present study corroborate dozens of previous studies from numerous laboratories, many of which have been cited herein. These data demonstrate the dramatic differences in tissue- and cell type-specific expression between the two members of the mouse *Cyp1a* gene subfamily. Furthermore, because of the ubiquitous nature of 3MC-inducible CYP1A1 but not CYP1A2 expression throughout the animal, these data support our hypothesis that CYP1A1 might play one or more important roles in critical life processes, in addition to its well-established role of metabolism of polycyclic hydrocarbons, certain drugs, and other environmental pollutants. In response to important intracellular signals (e.g. during differentiation, inflammation), increased amounts of an endogenous ligand might lead to the AHR-mediated induction of genes that are necessary for the appropriate response (e.g. cell division, apoptosis, migration). The data of this present report are also consistent with the suggestion that, whereas the *Cyp1a2(-/-)* knockout mouse has been shown to be viable and fertile with normal longevity [59], a conventional *Cyp1a1(-/-)* knockout mouse line might be either generally in poor health or actually lethal during early embryogenesis.

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