0022-1554/90/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 38, No. 12, pp. 1805–1814, 1990
Printed in U.S.A.

Original Article

Light Microscopic Autoradiography for Study of Early Changes in the Distribution of Water-soluble Materials[1]

TADAFUMI KAWAMOTO

Department of Biochemistry, School of Dental Medicine, Tsurumi University, Tsurumi, Yokohama, Japan.

Received for publication February 27, 1990 and in revised form July 11, 1990; accepted July 20, 1990 (0A1918).

An approach using autoradiography for the study of early changes in the distribution of water-soluble materials and the chemography involved was investigated. Radioactive calcium chloride (^{45}Ca) was injected into the iliac vein of a rat. Ten seconds after the injection the rat was frozen in hexane (−90°C). The frozen rat was embedded in 5% sodium carboxymethyl cellulose and blocked in the coolant. A sheet of plastic tape coated with a synthetic rubber glue was fastened to the trimmed block surface, and whole-body sections 2–10 μm thick were cut with a disposable microtome knife. Selected sections were freeze-dried and then covered with a dried autoradiographic emulsion film about 1 μm thick. The autoradiograph clearly showed the distribution of radioactive calcium in the calcification zone of long bones. The samples chosen to assess chemographic artifacts showed positive and negative chemographies on most of the tissues when these were kept at 23°C, and although both chemographic effects were significantly reduced when the samples were kept at −20°C, cells in several tissues still exhibited positive and negative chemographies. The technique can be used for the study of any animal whose size is suitable for whole-body freeze-sectioning. (*J Histochem Cytochem 38:1805–1814, 1990*)

KEY WORDS: Autoradiography; Soluble material; Chemography.

Introduction

Autoradiography has been widely utilized to study the distribution of drugs and metabolites in the body. The procedure can be used to examine the distribution of both insoluble materials and soluble materials. The conventional histological procedure, consisting of fixation, graded solvent dehydration, and embedding, can be employed for the former purpose but is not suitable for the latter because the soluble tracer is lost by diffusion from its original site. In consequence, a number of techniques have been evolved to avoid the diffusion of soluble tracer during the preparation of specimens (5,6,9,11,12,22,24,27). In most cases, after the administration of a tracer, tissues were dissected from the animal and freeze-sectioned, and the sections were used for the light microscopic autoradiography. However, because the distribution pattern of tracers may change during dissection of the tissue, the only way to avoid the problem is to use a whole-body sectioning technique accompanied by direct dry mounting of a photographic emulsion film. However, the autoradiograph prepared by this procedure often yields false results caused by a chemical reaction between the specimen and the photographic emulsion (a process known as chemography).

In the present study, an attempt was made to prevent, as far as possible, the migration of diffusible radioactive substances and to identify chemographic changes occurring in autoradiographs of freeze-dried sections prepared by this method.

[1] Supported in part by Research Grant No. 62771509 from the Ministry of Education of Japan.

Materials and Methods

Preparation of Frozen Sections. In this study, ^{45}Ca was selected as a typical water-soluble tracer. A 9-day-old rat of the Wistar strain (weighing about 20 g) was anesthetized with ether, and 5 μCi of $^{45}CaCl_2$/g body weight in a volume of 0.1 ml of saline solution was injected into the iliac vein. After exposure for 10 sec, the animal was rapidly frozen by immersion in hexane at −90°C cooled with a cooling apparatus (Neocool Bath, Model BD-51; Yamato Scientific Co; Tokyo, Japan). The frozen animal was wiped with gauze and immersed in a brass container (3 × 4 × 12 cm) filled with 5% sodium carboxymethyl cellulose solution (CMC). As soon as possible, the container was put into the coolant and the CMC was completely frozen. The frozen block was then taken out of the container and attached to a sample holder with CMC. The holder was fastened to the microtome stage with screws and left in the cryostat (−20°C) (LKB 2258 PMV; Rockville, MD) to acclimatize for more than 3 hr before sectioning. The block was trimmed with a disposable microtome blade (8 cm in length; Type CK35; Feather Co, Gifuken, Japan) attached to the blade holder (Figure 1). The exposed tissue surface was covered with a specially prepared adhesive plastic tape and placed in close contact with the block surface by rubbing the tape with a cotton wad. The tape was prepared by placing a sheet of polyvinylidene chloride film (Saran Wrap, 5 μm thick) on an acrylic board wetted with 100% ethanol, and then coated with a synthetic rubber glue (Nenchakunori; Azia Gensi Co., Gifuken, Japan) which was diluted by adding two or three parts of normal hexane to one part of the glue. After drying,

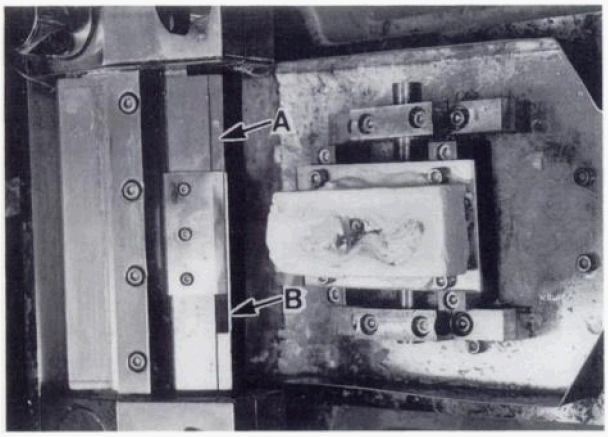

Figure 1. Microtome with a carboxymethyl cellulose block and a disposable microtome blade installed. Arrow A indicates a blade holder and arrow B indicates a disposable microtome blade.

the prepared film was cut with a rotary cutter (Type S; Olfa Co, Osaka, Japan) or surgical knife to an appropriate size. The frozen sections (2–10 μm thick) supported by the adhesive tape were cut with a cutting angle of 30° and a cutting speed of less than 0.5 m/min. The cut sections were placed on a metal plate cooled in the cryostat, and the section surface was covered with a sheet of glassine paper (Hakuai Co; Tokyo, Japan) and pressed with a pre-cooled rubber roller to ensure a close contact between the section and the tape. The paper was then removed from the section.

Autoradiography. The corners of the tape which mounted the section were fixed on a pre-cooled glass slide with double-sided adhesive tape in the cryostat. Sections were then freeze-dried for more than 12 hr, and then placed in a desiccator containing silica gel within the cryostat to prevent the occurrence of condensation on the section when they were removed from the cryostat. After this the desiccator was removed from the cryostat and allowed to reach room temperature. For examination of the distribution of tracer in the whole body, sections 5–10 μm thick were selected and the section surface was then covered with a plastic film (Diafoil, 4 μm thick; Diafoil Co, Tokyo, Japan) to prevent chemography. A piece of X-ray film (double-coated film, Fuji Type 100; Fuji Photo Co, Tokyo, Japan) was placed in contact with the Diafoil covering the section. The specimens were then stored in a light-tight box containing silica gel and placed in a refrigerator at 4°C. For subsequent development the film was wetted with water, and then carefully floated on the developer (Rendol; Fuji Photo Co) for 5 min at 20°C (9). After development, the film was submerged in the photographic fixative (Fuji Fix, Fuji Photo Co.) for 10 min. A serial section adjacent to that used for autoradiography was stained with hematoxylin and eosin for combined examination of the histology and the distribution of tracer, and mounted with glycerin.

For light microscopic autoradiography, sections 2–5 μm thick were used, and dried thin films of autoradiographic emulsion used in light microscopic autoradiography were prepared according to the previously reported method (9). In brief, 0.4 ml of 2% aqueous solution of sodium dioctylsulfosuccinate was added to 5 g of autoradiographic emulsion (Sakura NR-M2; Konica Co, Japan) melted at 40°C, and the solution was mixed by stirring. After being left for about 1 hr, a small amount of the emulsion was collected by dipping the tip of a glass tube (1.3 cm in diameter) into the emulsion and a bubble was carefully blown to a diameter of approximately 6 cm. The bubble was allowed to air-dry until it began to wrinkle, and two glass slides with a section mounted on each were placed so that each section side faced the bubble. The glass slides were then moved together until the bubble burst. This resulted in an emulsion film on each section, which was made to adhere closely to the section by breathing moist air on it. For exposure, the emulsion covered samples were stored at −20°C in a light-tight box containing silica gel. After exposure the emulsion on the section was developed for 5 min at 20°C and fixed. After rinsing with water, the section was stained with hematoxylin and eosin. Then the section mounted on the plastic tape was trimmed with a surgical knife and separated from the glass slide. It was then re-mounted between a glass slide and a coverslip with glycerin, or dehydrated with graded ethanol and mounted with entellan.

To examine the thickness of the emulsion film thus produced, after drying the film was embedded in Epok 812 and placed in a 60°C oven to polymerize. Ultra-thin sections of the film were cut with a diamond knife using an Ultrotome (LKB; Ultrotome V 2088) and then coated with a carbon film by evaporation. The distribution of silver halide grains in the film and the thickness of the film were examined by means of a transmission electron microscope (2000EX; JEOL). The relationship between the radioactivity and the autoradiographic optical density was examined with standard samples containing various amounts of ^{45}Ca. The samples were prepared by the following procedures. Six 5% gelatin solutions containing different amounts of radioactive calcium were prepared. Each solution was put into small cavities (a diameter of 4 mm) made in a frozen carboxymethyl cellulose block (CMC) and frozen in hexane (−90°C). The CMC block was mounted to the sample holder of the cryotome. Eighteen serial sections were cut and each section was fixed on the glass slides with double-sided adhesive tape and freeze-dried. The sections were subdivided into three groups of six sections and were placed in contact with glass slides covered with one to three layers of emulsion. Two sections from each group were then exposed for 2, 3, or 4 days. After exposure the film and the section were separated, and the film was developed for 5 min at 20°C and fixed. The radioactivity of each sample trimmed from respective areas of the section was measured with a liquid scintillation counter. The autoradiographic optical density of the film was measured with a microdensitometer (Joyce Loebl 3CS; Gateshead, UK).

Chemographic artifacts occurring in the autoradiograph were assessed with the sections from an animal untreated with radioactive tracer. The sections were prepared by the same procedures and divided into two groups. One group was covered with unexposed emulsion film to test positive chemography, and the other was covered with emulsion film fogged by a 10-sec standard exposure to light to test negative chemography. Both groups were further subdivided into three groups and stored separately in light-tight boxes. One of these groups was exposed for 5 weeks at room temperature (about 23°C); the others were exposed for 5 and 10 weeks at 4°C and for 10 weeks at −20°C. After exposure, the films were developed for 5 min at 20°C, fixed, and mounted under a coverslip with glycerin, and were examined by light microscopy.

Results

Quality of Emulsion Film

The quality of the prepared emulsion film used in the light microscopic autoradiography is shown in transmission electron micrographs (Figure 2). The photographs show that the thickness of single layer was about 1 μm (Figure 2A). Figure 2B shows two thicknesses of the film, and two layers of the film were brought into full contact. The silver halide grains were distributed uniformly in the film (Figure 2C). Figure 3 shows the results of standard samples exposed for 3 days. The intensity of the autoradiographic optical density and the radioactivity showed an approximately proportional relationship when the proper exposure period was selected (Figure 3).

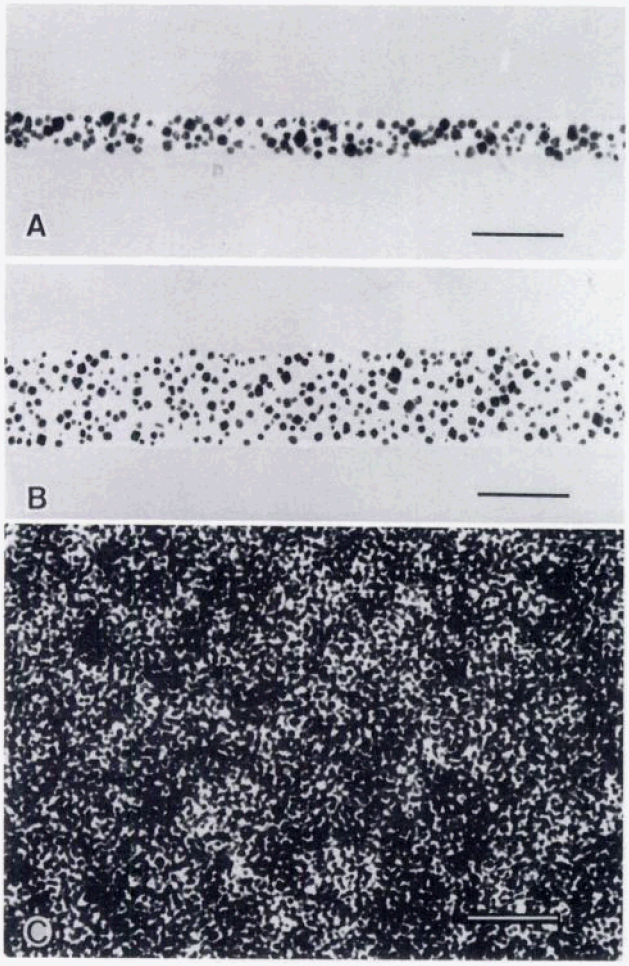

Figure 2. Electron micrographs of an emulsion film. **A** and **B** show cross-sections of a single and double layer of emulsion film prepared from one and two bubbles, respectively, and **C** shows the distribution of silver halide grains in the emulsion film prepared from one bubble. Bars = 2 μm.

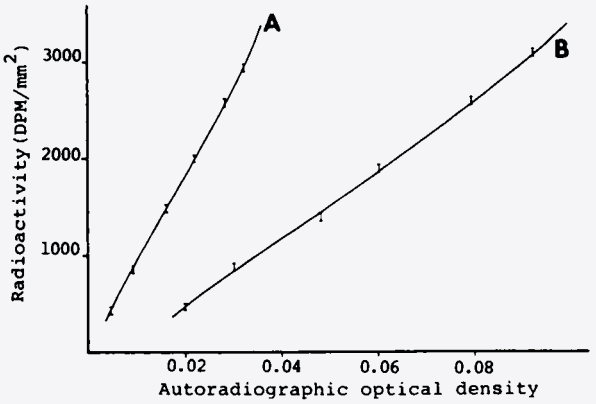

Figure 3. Relationship between radioactivity and autoradiographic optical density of emulsion film developed. A single layer (**A**) (about 1 μm thick) or (**B**) double layers (about 2 μm thick) of emulsion film were exposed on samples containing various amounts of ^{45}Ca.

Autoradiography

The following results were accomplished in serial whole-body sections of 6 μm thickness. Figure 4A shows an HE-stained serial section adjacent to the section in Figure 4B. Figure 4B shows the whole-body autoradiograph obtained from a section of a 9-day-old rat sacrificed 10 sec after an IV injection of $^{45}CaCl_2$, and Figure 4C shows an autoradiograph which was exposed for a shorter period than Figure 4B, because the radioactivity in the organs within the abdominal cavity is very intense. Tissues and organs were readily distinguished and well preserved. The tracer was also well localized. The highest radioactivity was found in the heart and blood vessels, and the radioactive calcium had already circulated around the body. The calcium was incorporated into the hard tissues such as bone, dentin, and enamel. High radioactivity was revealed in the lung, parenchyma of the liver, and in submandibular and labial glands. In tooth germ, the activity in the stellate reticulum was much higher than that in the dental papilla. The activity in brain and fat tissue was very low. No autoradiographically detectable radioactivity was found in the urine or in the contents of the small intestine and stomach. Detailed localization of the radioactivity was examined in whole-body sections 3 μm thick, and the distribution in the long bones and the cortex of kidney are shown as a demonstration in Figures 5 and 6. Both autoradiographs showed that the tracer was well localized in the tissues. In the bone, very high radioactivity was observed on the surface of trabeculae and extracellular spaces, whereas the radioactivity in osteoblasts adjacent to the trabeculae and other cells was very low (Figures 5A and 5B), and the radioactivity in the cortex of the kidney was observed in the glomerulus and renal tubules (Figures 6A and 6B).

Chemography

Figure 7 shows the positive chemography caused at 23°C or −20°C. The chemography appeared in most of the tissues in the sections kept for 5 weeks at about 23°C. Intense chemography was particularly observed in the retina, bone, cementum, dentin, and enamel of the forming side of the lower and upper incisor at low magnification (Figures 7A and 7B). The chemography was significantly decreased when the sample was kept for 5 weeks at 4°C, and was not recognized in these tissues. However, the chemography was observed at high magnification when the sample was kept for 10 weeks. Intensive chemography was seen over the cells of several tissues such as bone marrow, spleen, lung, liver, muscle, and tooth germs. Although the chemography caused by most of these tissues was not apparent when the sample was kept for 10 weeks at −20°C, the cells of lung (Figure 7C), bone marrow (Figure 7D), the papillary layer of tooth germs (Figure 7E), and muscle (Figure 7F) still caused chemography.

Figure 8 shows the negative chemography caused at 4°C and −20°C. When the samples were kept for 5 weeks at 23°C, most tissues showed negative chemography. Although the chemography was significantly decreased when the sample was kept at 4°C or −20°C, it was recognized in some areas. The most intensive negative chemography was observed in the blood, the feces in the large

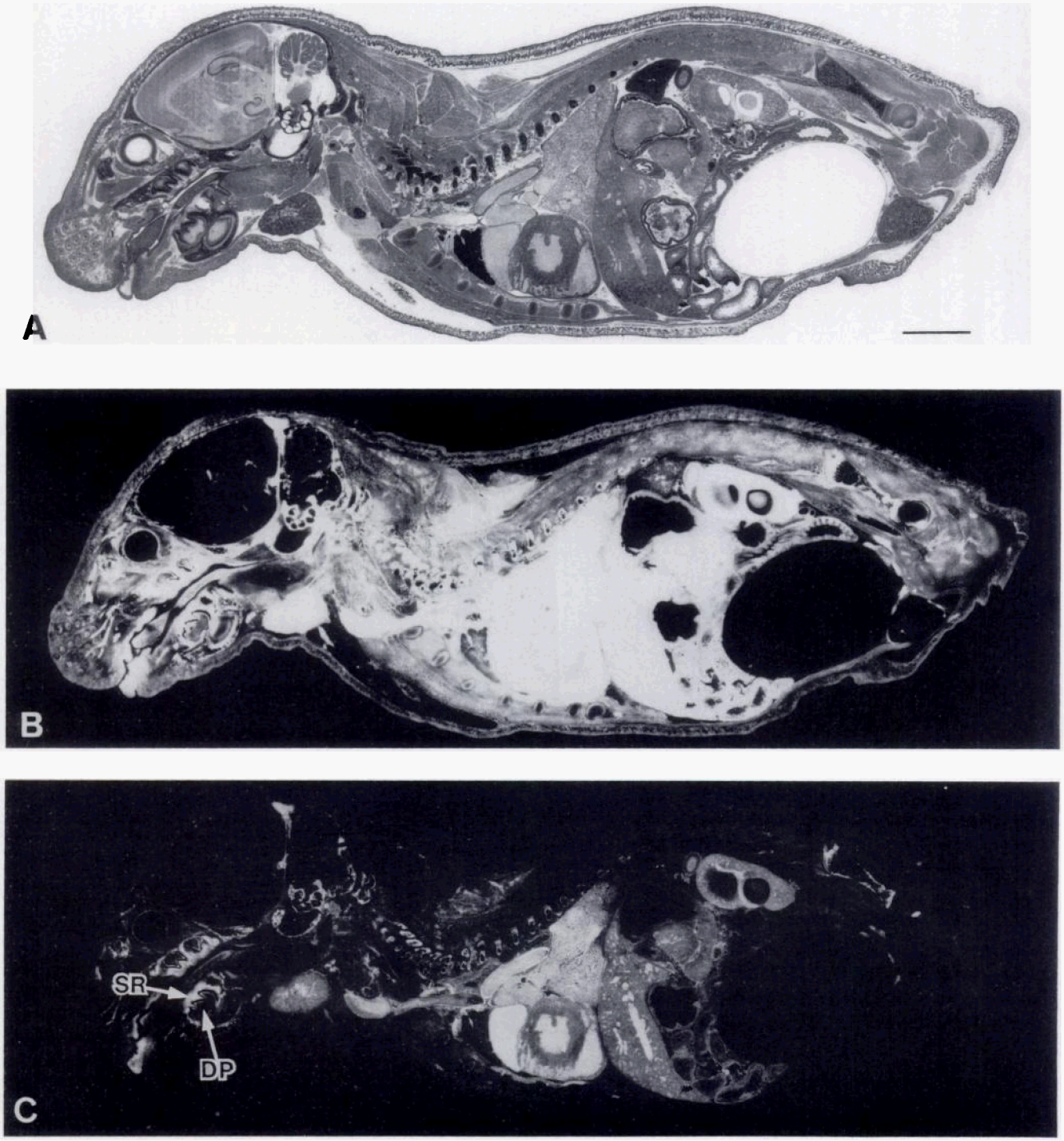

Figure 4. Serial whole-body sections of a 9-day-old rat sacrificed 10 sec after an IV injection of $^{45}CaCl_2$. (**A**) Section stained with hematoxylin and eosin. (**B, C**). Whole-body autoradiograph of the same section; **C** was exposed for a shorter period than **B**. White areas in the autoradiograph correspond to high radioactivity. SR, stellate reticulum; DP, dental papilla. Bar = 5 mm.

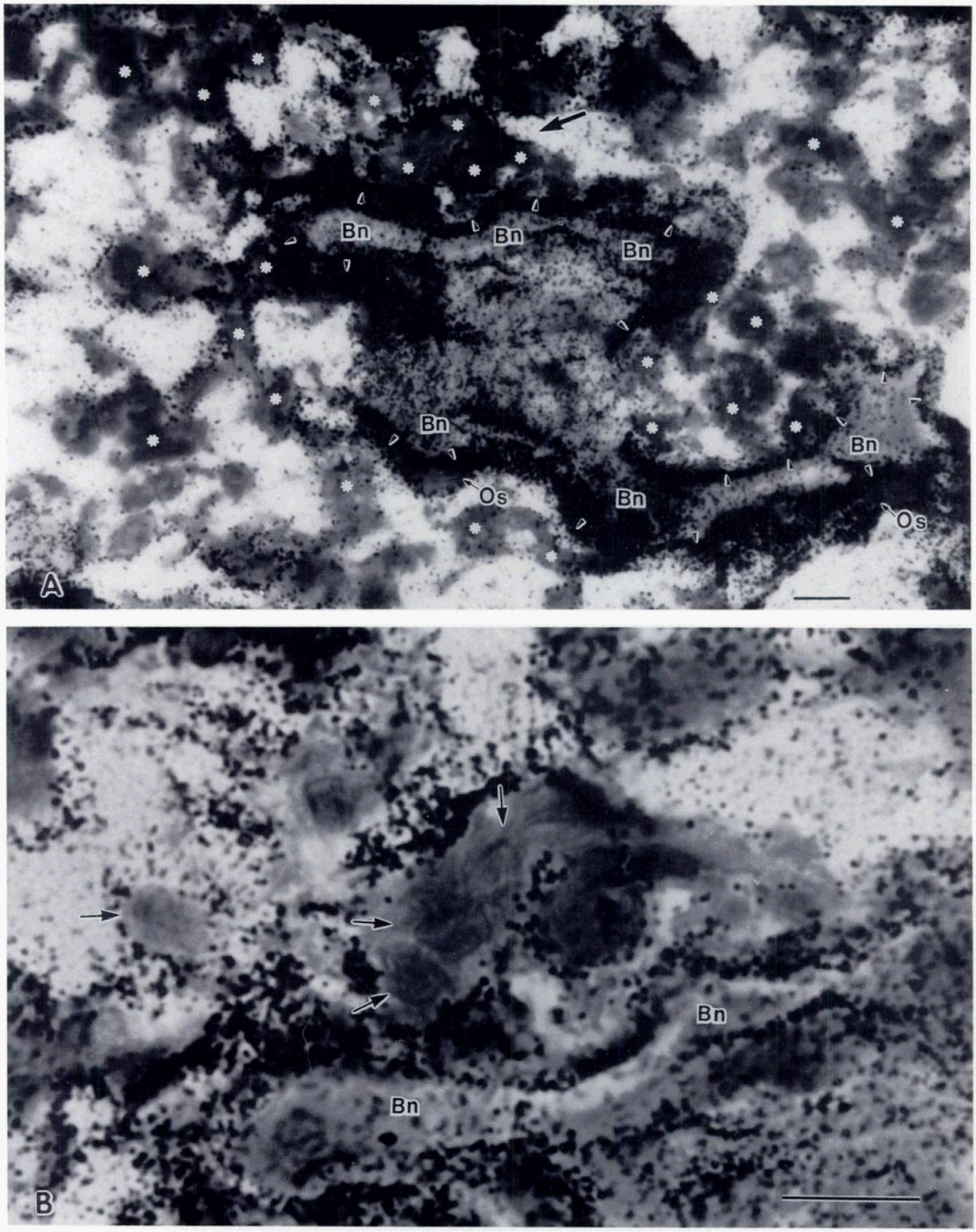

Figure 5. Light microscopic autoradiographs of long bone of the same animal used for the whole-body autoradiography. (**A**) Trabeculae in calcification zone; (**B**) the portion indicated with an arrow in **A**. Arrowheads in **A** indicate the surface of bone, and arrows in **B** indicate the cells. Bn, bone; Os, osteoblast; *, cells. Bars = 10 μm.

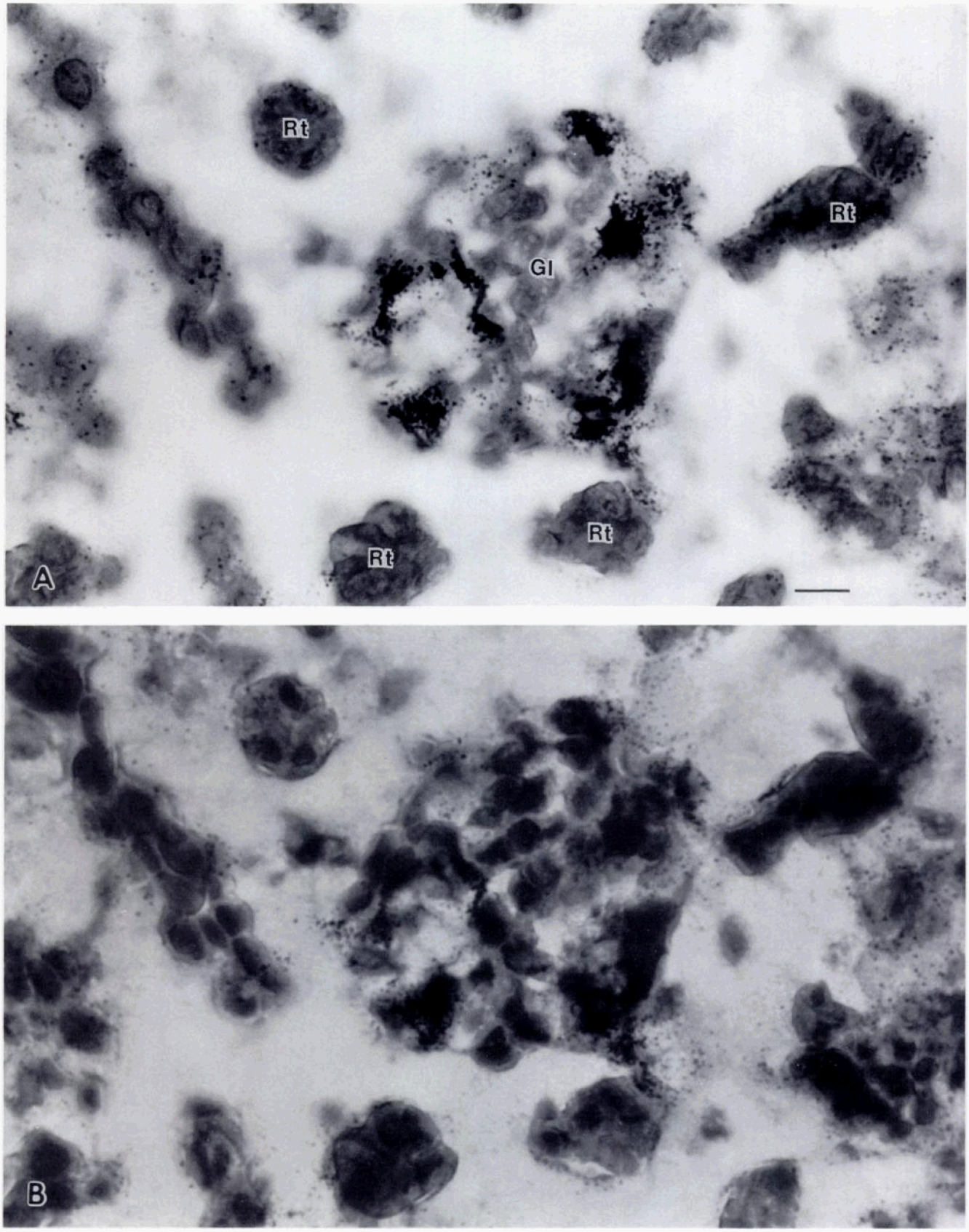

Figure 6. Light microscopic autoradiographs of the kidney of the same animal used for the whole-body autoradiography. (**A, B**) The glomerulus and proximal renal tubules of kidney. **A** is focused on the silver grains and **B** is focused on the tissue. Rt, renal tubule; Gl, glomerulus. Bar = 10 μm.

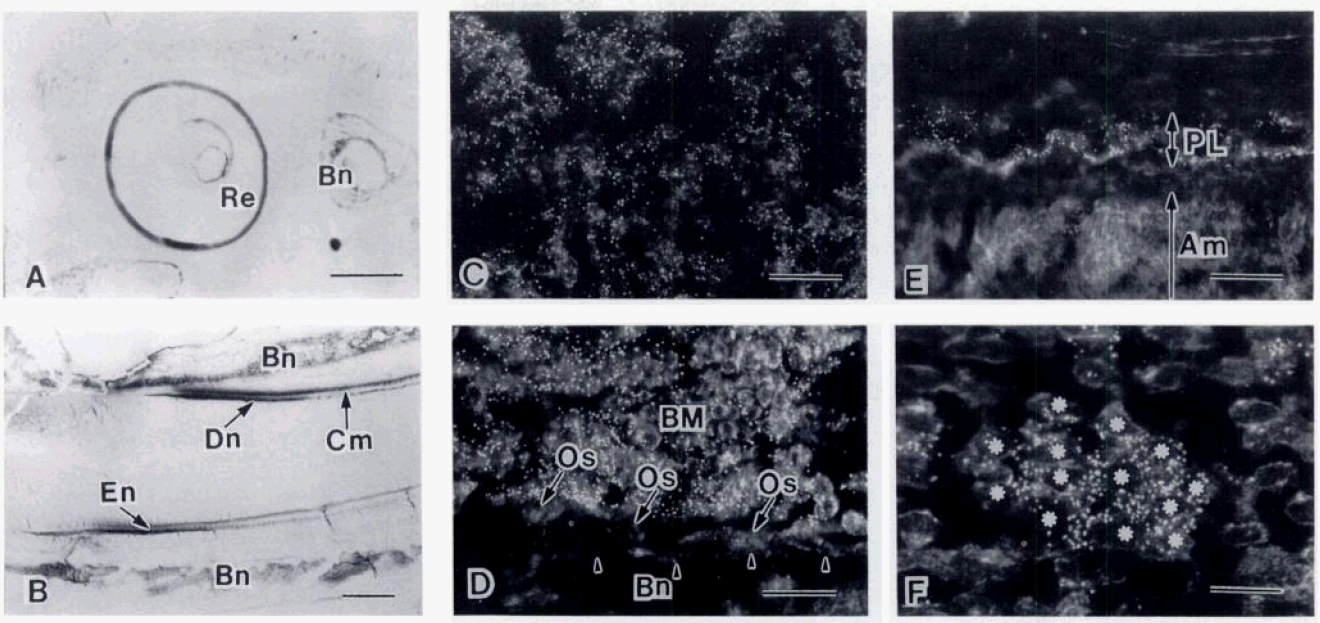

Figure 7. Positive chemography. **(A, B)** Samples were kept for 5 weeks at about 23° C; **(C, D, E, F)** samples were kept for 10 weeks at −20°C. **(A)** Eyeball; **(B)** proximal side of lower incisor; **(C)** lung; **(D)** bone marrow; **(E)** papillary layer in the zone of pre-secretion and initial secretion of ameloblasts in a lower incisor; **(F)** cross-section of tendon muscle. **A** and **B** are transmission and **C, D, E,** and **F** are darkfield illuminations. Arrowheads in **D** indicate the surface of the bone. Re, retina; Bn, bone; BM, bone marrow; En, enamel; Dn, dentine; Cm, cementum; Am, ameloblastic layer; PL, papillary layer; Os, osteoblast; *, muscle fiber. Bars; **A** = 1 mm; **B** = 0.2 mm; **C–E** = 33 μm.

intestine, and the goblet cells of the intestine (Figures 8A and 8B). In addition, intense chemography was detected over the enamel in the maturation phase and appeared to relate to the cyclical activity of the ameloblastic layer of this phase (Figure 8A). In long bone, relatively intense chemography was observed in the calcification zone of bone. Chemography was also observed in cells of the bone marrow (Figure 8C), liver (Figure 8D), proximal tubules of kidney (Figure 8E), and spleen (Figure 8F).

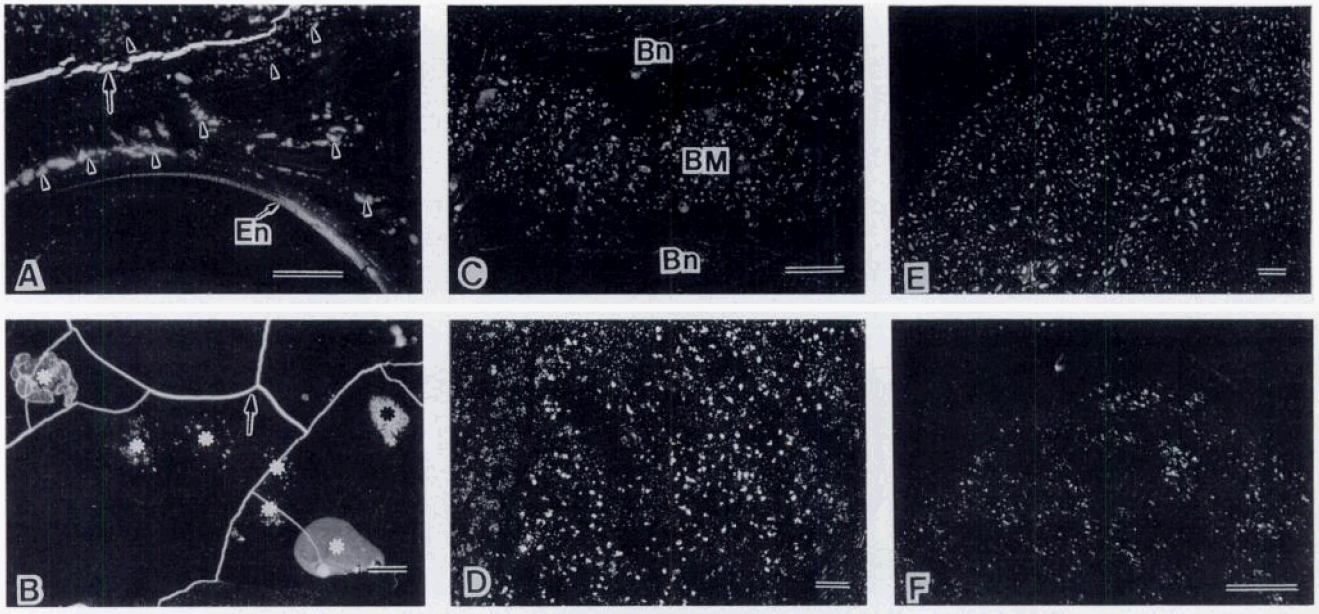

Figure 8. Negative chemography caused on fogged emulsion film. **A, B** Samples were kept for 10 weeks at 4°C; **(C–F)** samples were kept for 10 weeks at −20°C. White areas correspond to intensive negative chemography. **(A)** Upper incisor; **(B)** abdominal cavity; **(C)** bone marrow; **(D)** liver; **(E)** cortex of kidney; **(F)** spleen. Arrowheads in **A** indicate the blood vessel, and arrows in **A** and **B** indicate cracks in the emulsion film. *, small and large intestine; BM, bone marrow; Bn, bone. Bars: **A,B,D,F** = 0.5 mm; **C,E** = 100 μm.

Discussion

When the distribution of drugs or metabolites in the body is studied by autoradiography, it is often necessary to detect not only the distribution of water-soluble tracer but also the early changes in their distribution after administration of the tracer. With respect to the whole body level, the tape sectioning technique initiated by Ullberg (23) in 1954 has been widely used as the standard technique (12,25). For light microscopic autoradiography, various attempts have been reported by many researchers (1,6,7,11,17,20, 21,24,26,27).

Kawamoto and Shimizu (9) described procedures for the preparation of frozen sections mounted on a glass slide and for the application of a dried thin film of autoradiographic emulsion on the section. Their method is available to examine early changes in the distribution of tracer, but it is not adequate to demonstrate the precise location of tracer at high magnifications because the sections routinely prepared by the method were too thick (about 10 μm) for detailed observation (10). To make thin, well-preserved whole-body sections, it was necessary to select a sharp knife and section supporting material. In the present work, a disposable microtome blade of the Feather type was used, and the blade holder for the cryomicrotome (LKB; 2258 PMV) was specially designed and made of stainless steel. The blade can be attached to the holder and changed to a new blade without any alteration in the sectioning conditions. By use of this type of blade, whole-body sections of 2 μm thickness can be cut, although the quality of the section is influenced by the shape and size of the CMC block. The width

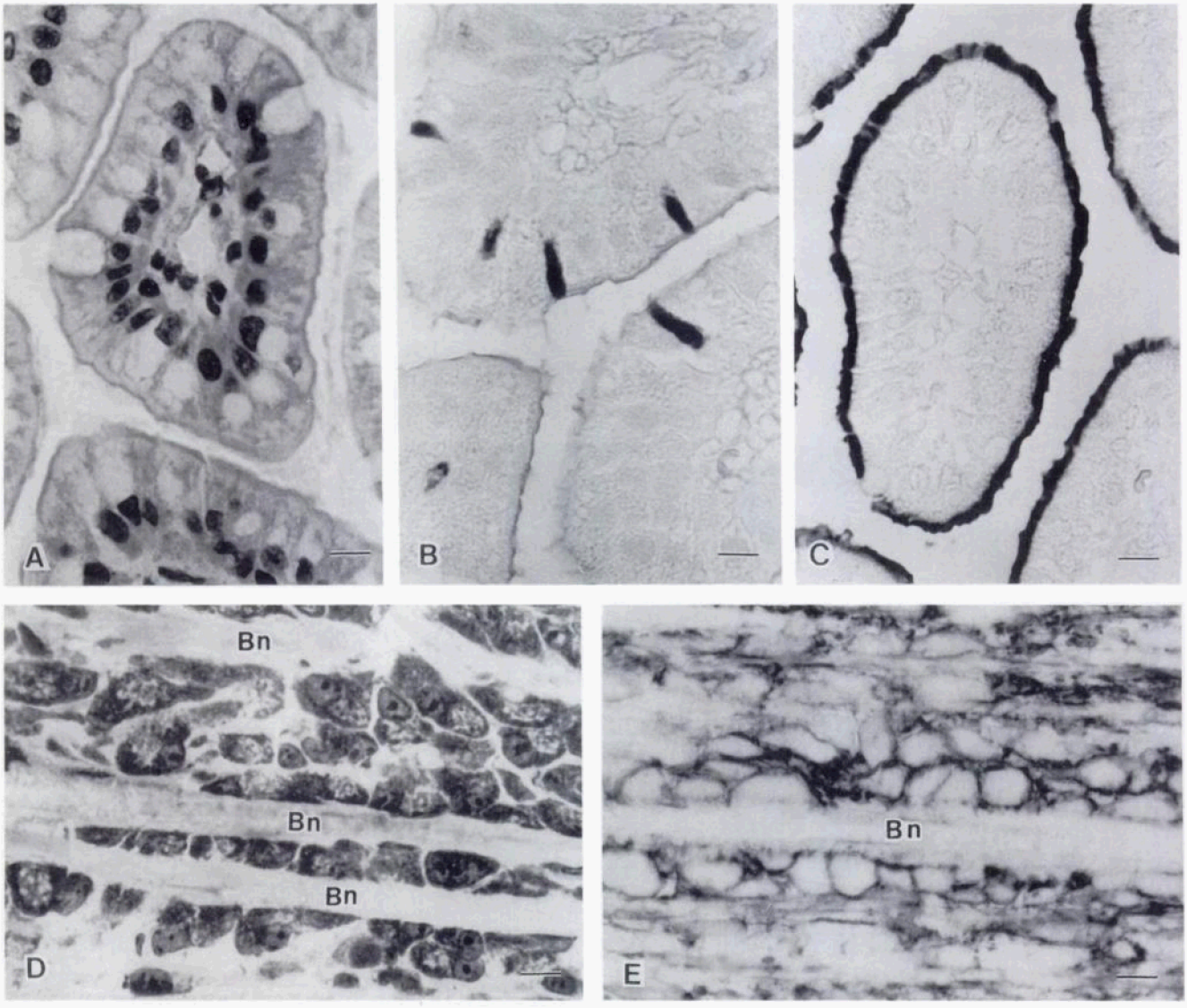

Figure 9. (A–C) Small intestine; (D,E) calcification zone of long bone. (A) HE staining; (B) Alcian blue staining at pH 2.5; (C,E) alkaline phosphatase activity; (D) toluidine blue staining. Osteoblasts are located on the bone surface in D and E. Bn, bone. Bars = 10 μm.

of the block facing the knife edge can significantly affect the quality, whereas the cutting distance has a lesser influence on the quality of the section. The best sections can be cut from a narrow block, and the quality of the section is much better than that cut with a conventional microtome knife. The plastic block usually used to prevent the separation between section and tape at the knife edge during cutting is not advisable, because the pressure involved affects the thickness of the resulting section.

As a supporting material, a film that is thin, flexible, and transparent, with high adhesive power, is the most desirable for this purpose. Five kinds of adhesive tape, such as Scotch 810 (4), adhesive cellophane tape, and cellophane, polyethylene and polyvinylidene chloride film (8) coated with synthetic adhesive glue, were tested. Among them, commercially available Scotch 810 and cellophane tape were not suitable because their adhesivity was not great enough to preserve the detailed structure of the section, and cellophane film could not be used because the film was influenced by the solvents during the staining process. Although both polyethylene and polyvinylidene plastic films fit the purpose, polyvinylidene chloride film is much better than polyethylene film because the latter is easily stretched.

The combination of a disposable microtome blade and Saran Wrap adhesive tape (polyvinylidene chloride film) permits frozen sections 2–20 μm thick to be cut, and more than 95% of the whole-body serial sections of 5 μm thickness from a block size of 4 × 12 cm were satisfactorily preserved. The sections can be used in almost all histological and histochemical studies because the adhesive tape supporting the section is not affected by the staining processes. Histological demonstration is shown in Figures 3A and 9A–9D, and the appearance of tissue is superior in comparison with those previously recorded by the author (9). In addition, the distribution of mucopolysaccharide demonstrated by the method of Mowry (14) and of alkaline phosphatase activity demonstrated by the method of Burstone and Keyes (2) is shown in Figures 9B, 9C, and 9E. That the enzymatic activity is localized on the cell membranes of the osteoblasts is clearly shown. When the technique is applied to make whole-body sections of, for example, an adult mouse, one may encounter difficulty in cutting the fully calcified tissues, such as enamel and dentin. In this case, it is recommended that such hard tissues be ground away from the cutting surface of the CMC block using a dental burr. A whole-body section of an adult mouse is shown in Figure 10. The organs and tissues are well preserved. Although ice crystal artifacts can be minimized by accelerating the rate of freezing, this artifact never can be avoided in such large samples as young rats or adult mice. In fact, the histology, especially in the middle part of the section, revealed damage to the detailed cell structure, but the morphology was preserved well enough for cell identification.

For light microscopic autoradiography, soluble tracers can diffuse during the autoradiographic procedure, such as during mounting of the emulsion and exposure. Various emulsion films (1,3,9,13,16,22) and methods (1,5,9,11,13,22,26) for mounting the film have been reported, and stripping film and the photographic emulsion coated on a glass slide have been most widely used. However, as the results of various trials, the film formed by a bubbling technique was most suitable for covering a large area and for enabling a close contact between the sections without serious diffusion of water-soluble tracer (9). In this study, sections of 3 μm thickness were prepared and emulsion films of 1 μm thickness were mounted on the section. The light microscopic autoradiograph thus prepared demonstrated that ^{45}Ca was well localized in the tissues (Figures 5 and 6). It can be readily observed in the autoradiograph that the radioactivity in the osteoblasts and cells in the space between the trabeculae was very low, although in the extracellular spaces and the surface of the trabeculae it was very high.

It has been known that latent images can be created in autoradiography by direct chemical action of the specimen on the emulsion (15,18). These artifacts can be recognized by examining control specimens identical to the experimental ones but non-radioactive, exposed to emulsion in the same way. Roger and John (19) have reported that the most intensive negative chemography occurs over the salivary gland and pancreas. In the present study, positive and

Figure 10. Whole-body section of 12-week old mouse stained with HE. Enamel and dentine was ground off with a dental burr. Bar = 5 mm.

negative chemographies at different temperatures were examined using fresh whole-body sections. When the samples were exposed at room temperature, many tissues caused severe positive and/or negative chemography. Although these chemographies were significantly reduced when the sample was exposed at low temperature (4°C or −20°C), the chemographies were still detectable over the cells of several tissues; relatively intense positive chemography was found in the bone marrow, lung, and muscle (Figures 7C–7F), and negative chemography was also recognized in blood, spleen, bone marrow, proximal tubule of kidney, and feces of the large intestine (Figure 8). In my experience therefore, when the distribution of tracer in the tissues showing intense chemography is to be studied, I recommend exposure for a period of less than 5 weeks at below −20°C.

Acknowledgments

I wish to express my gratitude to Professor M. Shimizu for frequent stimulating and helpful discussions. I am also indebted to Professor R. W. Fearnfead for his valuable comments and advice.

Literature Cited

1. Appleton TC: Autoradiography of soluble labelled compounds. J R Microsc Soc 83:277, 1964
2. Burstone MG, Keyes PH: Study on calcification. 1. The effect on inhibition of enzyme activity on developing bone and dentine. Am J Pathol 13:1229, 1957
3. Caro LG: Electron microscopic radioautography of thin sections: the Golgi zone as a site of protein concentration in pancreatic acinar cells. J Biophys Biochem Cytol 10:37, 1961
4. Farebrother DA, Wood NC: Permanent preparations of stained whole-body sections using 'Trycolac'. J Microsc 97:373, 1973
5. Grant LD, Stumpf WE: Combined autoradiography and formaldehyde-induced fluorescence methods for localization of radioactively labeled substances in relation to monoamine neurons. J Histochem Cytochem 29:75, 1981
6. Hammarstrum L, Applegren LE, Ullberg S: Improved method for light microscopy autoradiography with isotopes in water-soluble form. Exp Cell Res 37:608, 1965
7. Ison EJ, Sheridan PJ: Autoradiography of diffusible substances—a practical approach. Am J Med Technol 47:38, 1981
8. Kawamoto T, Shimizu M: Changes in the mode of calcium and phosphate transport during rat incisal enamel formation. Calcif Tissue Int 46:406, 1990
9. Kawamoto T, Shimizu M: A method for preparing whole-body sections suitable for autoradiographic, histological and histochemical studies. Stain Technol 61:169, 1986
10. Kawamoto T, Shimizu M: Distribution of calcium and phosphate in cells of the enamel organ in the rat lower incisor. Adv Dent Res 1:236, 1974
11. Kinter WB, Leape LL, Cohen JJ: Autoradiographic study of diodrast-I^{131} transport in Necturus kidney. Am J Physiol 199:931, 1960
12. Larsson B, Ullberg S: Whole-body autoradiography. J Histochem Cytochem 29:216, 1981
13. Miller OL, Stone GE, Precott DM: Autoradiography of soluble material. J Cell Biol 23:654, 1964
14. Mowry RW: Special value of prestaining polyanions with basic dyes of contrasting color (Alcian blue) before staining with aldehyde fuchsin with particular reference to pancreatic islet B cells and the diagnosis of nesidoblastosis. J Histochem Cytochem 23:322, 1975
15. Mulvaney BD: Chemography of lysosome-like structures in olfactory epithelium. J Cell Biol 51:568, 1971
16. Nagata T, Nawa T: A modification of dry-mounting technique for radioautography of water-soluble compounds. Histochemie 7:370, 1966
17. Pilgrim CH, Wagner HJ: Improving the resolution of the 2-deoxy-D-glucose method. J Histochem Cytochem 29:190, 1981
18. Roger AW: Techniques of autoradiography. Elsevier/North-Holland, 1979, 95, 116
19. Roger AW, John PN: Latent image stability in autoradiographs of diffusible substances. In Roth LJ, Stumpf WE, eds. The autoradiography of diffusible substances. New York, Academic Press, 1969, 51
20. Roth LJ, Diab IM, Watanabe M, Dinerstein RJ: A correlative radioautographic, fluorescent, and histochemical technique for cytopharmacology. Mol Pharmacol 10:986, 1974
21. Stumpf WE, Roth LJ: High resolution autoradiography with dry mounted, freeze-dried frozen sections: comparative study of six methods using two diffusible compounds, ^{3}H-estradiol and ^{3}H-mesobilirubinogen. J Histochem Cytochem 14:274, 1966
22. Stumpf WE, Roth LJ: Vacuum freeze-drying of frozen sections for dry-mounting, high resolution autoradiography. Stain Technol 39:219, 1964
23. Ullberg S: Studies on distribution and fate of S^{35}-labelled benzylpenicillin in body. Acta Radiol (suppl) 118:1, 1954
24. Ullberg S, Applelgren LE: Experience in locating drugs at different levels of resolution. In Roth LJ, Stumpf WE, eds. The autoradiography of diffusible substances. New York, Academic Press, 1969, 279
25. Ullberg S, Larsson B: Whole body autoradiography. Methods Enzymol 77:10, 1981
26. Wedeen RP: Autoradiography of freeze-dried sections: studies of concentration transport in the kidney. Prog Nucl Med 2:147, 1972
27. Wilsk KR, Ross R: Autoradiographic localization of lipid- and water-soluble compounds: a new approach. J Histochem Cytochem 13:38, 1965