HIGH-RESOLUTION AUTORADIOGRAPHY OF DRUGS IN FIXED TISSUES

Kenneth R. Smith, Jr.*
with the technical assistance of Kenneth Arnold†

Washington University School of Medicine, St. Louis, Missouri

Many investigations have explored the disposition of radioactive drugs in body tissues. In most studies drugs were localized by measuring radioactivity in the tissues or by autoradiography of slices of frozen tissues. Few drugs have been traced to their cellular location, and only a few papers on electron-microscopic autoradiography of drugs (*1–6*) have been published. Since most autoradiographic work has been on frozen sections or frozen-dried material, little is yet known about the fate of drugs during fixation of the tissue. Inasmuch as chlormerodrin has been shown to remain in tissues during fixation (*7*), it has been used in the present work as well as atropine, diphenylhydantoin and various barbiturates about which less is known. It is the purpose of this study to determine the fate and cellular localization of these drugs during preparation of tissues for electron microscopy using routine fixatives and procedures.

MATERIAL AND METHODS

To make estimations of the loss or preservation of drugs during preparation of tissues, it was necessary to determine weights of the various organs before and after fixation and dehydration. Brain, kidney, liver, salivary gland and muscle were removed from three mice immediately after decapitation, cut into 1–2-mm cubes, weighed and fixed in 1% Dalton's chrome-osmium solution (*8*), in White's osmium solution (*9*) or in 6% glutaraldehyde in 0.1 M phosphate buffer containing 10% sucrose and 5 mg% $CaCl_2$. The tissues were fixed for 1 hr in OsO_4, then dehydrated through a graded series of ethanol solutions. Glutaraldehyde fixation took place for 2 hr at 4°C, after which the blocks were washed in the same buffer solution without glutaraldehyde overnight at 4°C and then dehydrated in ethanol. After they were removed from absolute ethanol, the blocks were placed in toluene for 1–2 hr. The toluene was then decanted and the tissues left at room temperature for 2 hr before they were weighed again. By comparing percentages of the loss of weight during fixation and dehydration versus simple desiccation, the weight of fresh tissue represented by a given weight of fixed and dehydrated tissue was estimated and the percentage of drug lost during processing of the tissues was determined (Tables 1 and 2).

Chlormerodrin‡ containing ^{197}Hg or ^{203}Hg with specific activities of 200–2,000 μc/mg was given to 21 mice (normal Swiss albino ranging in age from a few hours to several months; or C3H mice). One of the C3H mice had a spontaneous sarcoma and four had tumors in the brain secondary to implanted methylcholanthrene pellets. The doses of drug varied from 50 to 800 μc and from 0.05 to 2.5 mg. The elapsed times from injection to decapitation were 15 min to 7 hr. Fixatives used included 5–10% formalin, 10% formolsaline, 10% neutral buffered formol, 3% and 6% glutaraldehyde, 0.1 M phosphate buffer containing 10% sucrose and 5 mg% $CaCl_2$, 1% OsO_4 in Dalton's solution or in White's saline and 2.5% $KMnO_4$ in phosphate buffer. Postfixation in osmium tetroxide occasionally was carried out after glutaraldehyde fixation. After fixation, tissues were dehydrated in graded ethanol solutions, placed in toluene for 1 hr and then embedded in paraffin, methacrylate or an epoxy resin (Epon 812 or DOW) (*10*).

Radioactivity of weighed specimens in the fresh state and after fixation and dehydration was measured in a well scintillation counter (Picker Spectro-

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TABLE 1. EFFECT OF FIXATION AND DEHYDRATION ON WEIGHT OF TISSUE (% FRESH WEIGHT)

	Desiccated	Glutaraldehyde fixed and dehydrated	OsO_4 fixed and dehydrated
Brain	25%	22%	40%
Kidney	27%	30%	40%
Liver	35%	40%	50%
Salivary gland	32%	35%	65%
Muscle	30%	27%	33%

Scaler III). Sections were cut on microtomes and floated onto glass slides coated with gelatin in chrome-alum solution (*11*). Paraffin sections were 5–10-micron thick and epoxy sections were 0.8–1.5-micron thick. For autoradiography the mounted sections were dipped into Ilford G5 emulsion diluted 1:1 with water. Slides were stored at room temperature in light-tight boxes containing silica gel, developed after suitable intervals in Kodak D72 developer, fixed and washed. They were examined by phase microscopy and by routine microscopy after staining with Giemsa or azure blue. Electron-microscopic autoradiography observations were made on several kidneys. Thin sections of epoxy-embedded tissue were cut on a Porter-Blum microtome and floated onto copper grids. The grids were attached to lucite disks with cellophane tape and spun on the center post of a centrifuge while four drops of Ilford L4 emulsion diluted 1:2 with water were allowed to fall on them (*12*). Grids were placed in a dark box for several weeks and examined after developing in Microdol-X and staining with uranyl acetate in 50% ethanol.

Tritiated atropine alkaloid,* generally labeled, with specific activity of 500 μc/mg was injected intraperitoneally into three albino mice in doses of 500–1,000 μc (1–2 mg) and tissues were removed 10–150 min later using pentobarbital anesthesia for two and decapitation without anesthesia for the third. Two cats were anesthetized with pentobarbital and then given, respectively, 500 and 7,000 μc (1 mg and 14 mg) atropine intravenously. Tissues were removed 20–30 min later. They were fixed in 1% OsO_4 or in 6% glutaraldehyde and dehydrated and embedded in epoxy resin. Aliquots of tissue were removed before and after fixation and dehydration and were dissolved in hydroxide of Hyamine® (p-(diisobutyl-cresoxyethoxyethyl) dimethylbenzyl-ammonium hydroxide) or hydrolyzed in 0.1 N HCl at 90°C for 3 hr (*13,14*). Portions of these solutions were suspended in scintillation-counting mixtures and counted in a Tricarb Liquid Scintillation counter. Autoradiography was done as for chlormerodrin.

5,5 Diphenylhydantoin-4-^{14}C† with specific activity of 25 μc/mg was injected intraperitoneally into two albino mice in doses of 50 and 100 μc (1 and 4 mg). The mice were decapitated 2 and 3 hr later and tissues removed, fixed in OsO_4 or glutaraldehyde and embedded in epoxy resin as above. Portions of tissues were emulsified in concentrated hydrochloric acid for 24–72 hr before and after fixation and dehydration. Aliquots were placed on planchets, dried quickly at 60°C and counted in a gas-flow counter. Other portions of the tissues were dissolved in hydroxide of Hyamine® or hydrolyzed in 0.1 N HCl and counted in a liquid scintillation counter as described above. Autoradiograms were prepared as above.

TABLE 2. ^{203}Hg- AND ^{197}Hg-CHLORMERODRIN

	Fresh tissue (cpm/mg)	% drug retained in fixed tissue	
		GI*	OsO_4†
Brain (6 mice)	20–210	100	50
Tumor (2 mice)	20–1,600	90	60
Kidney (7 mice)	1,100–18,000	80	35
Liver (4 mice)	470–11,000	70	20
Salivary gland (4 mice)	700–1,800	85	40
Muscle (4 mice)	225–890	40	10

* GI = 6% glutaraldehyde fixation with dehydration through ethanol and toluene.
† OsO_4 = 1% OsO_4 fixation with dehydration.

Barbituric-2-^{14}C acid† with specific activity of 20 μc/mg was given to two mice in doses of 100 and 200 μc (5 and 10 mg) intraperitoneally. They were decapitated 30 min and 4½ hr later. Tissues were removed, fixed in OsO_4 and glutaraldehyde and embedded in epoxy resin. Portions were removed, emulsified in concentrated hydrochloric acid and counted on planchets as described above. Autoradiograms were made.

5-Ethyl-5-phenylbarbituric-2-^{14}C acid† (phenobarbital) with specific activity of 7 μc/mg was given intraperitoneally to one mouse in a dose of 100 μc (15 mg). Tissues were taken 18 min later, fixed in OsO_4 or glutaraldehyde and embedded as above.

* Obtained from Nuclear Chicago Co., Chicago, Ill.

† Obtained from New England Nuclear Corp., Boston, Mass.

Portions were removed, emulsified in concentrated hydrochloric acid and counted on planchets.

5-Ethyl-5 (1 methylbutyl) barbituric-2-^{14}C acid (pentobarbitol)* with specific activity of 24 μc/mg was given to one mouse in a dose of 100 μc intraperitoneally. Tissues were removed 14 min later and treated as for barbituric acid and phenobarbital.

RESULTS

Table 1 shows the effect of fixation and dehydration on the weight of different tissues of three adult albino mice. The percentages do not indicate how much tissue remains after fixation, but rather the weight of tissue plus fixative. This percentage indicates what each milligram of fixed and dehydrated tissue represents compared to fresh tissue, (wt. tissue after processing/wt. fresh tissue) × 100%, and so can be used to determine how much radioactive drug is lost between the fresh state and the time when it is embedded.

Tissues in the first column were weighed, heated at 105°C for 4 hr and then weighed again. Tissues in the second column were fixed in 6% glutaraldehyde for 2 hr, washed in buffer for 12 hr, then dehydrated through ethanol and toluene and allowed to dry at room temperature. Tissues in the third column were fixed in 1% OsO_4 for 1 hr, then dehydrated through ethanol and toluene and allowed to dry at room temperature. All percentages were consistent within ±7% except for liver fixed in glutaraldehyde. Here the length of dehydration was inversely proportional to the final weight of tissue since lipid and glycogen apparently were not well enough stabilized by the fixative to withstand dehydration. This experiment indicates that when brain is fixed in glutaraldehyde and dehydrated, each milligram of tissue represents 5 mg of fresh tissue, but when fixed in OsO_4 each milligram represents 2.5 mg. Other tissues have roughly the same pattern but lose less weight so that each milligram of salivary gland fixed in glutaraldehyde represents 3 mg of fresh tissue, and each milligram of OsO_4-fixed salivary gland represents 1.5 mg of fresh tissue.

Table 2 shows the amounts of ^{203}Hg- or ^{197}Hg-chlormerodrin in tissues of mice which were given 100–800 μc (0.5–1.0 mg) chlormerodrin i.p. 14–45 min before decapitation. The percentages are calculated

$$\frac{\text{cpm/mg fixed tissue}}{\text{cpm/mg fresh tissue}} \times \frac{\text{wt. fixed tissue}}{\text{wt. fresh tissue}} \times 100\%.$$

* Obtained from New England Nuclear Corp., Boston, Mass.

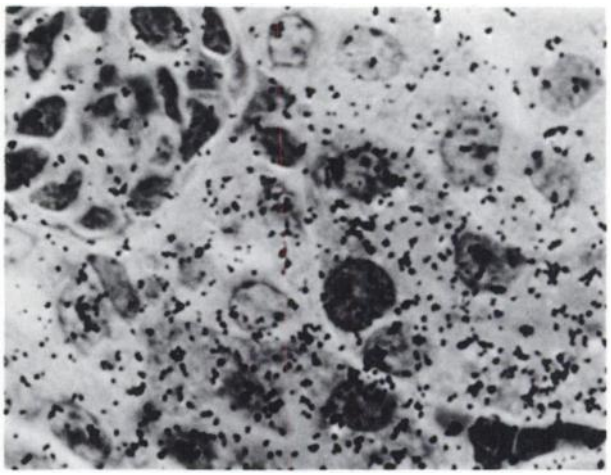

FIG. 1. Kidney of 3-day-old mouse that received 170 μc (1.0 mg) ^{197}Hg-chlormerodrin 2 hr before decapitation. Some label is present in all portions of nephron, but concentration is greater in convoluted tubules C than in distal tubules D or glomerulus G. Glutaraldehyde fixation × 2,400.

By using the percentages in Table 1, the amount of radioactivity retained in fixed and dehydrated tissue can be compared with the amount of radioactvity in fresh tissue. The reciprocals of the percentages in Table 1 represent the amount of radioactivity expected in fixed and dehydrated tissues if no radioactivity were lost during processing. The actual percentages are in Table 2, and a comparison of these indicates that kidney fixed in glutaraldehyde retained 80% of the labeled chlormerodrin but after

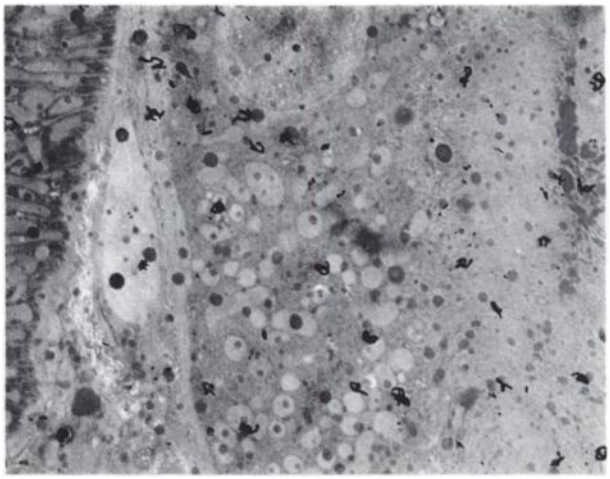

FIG. 2. Electron micrograph is of kidney of adult mouse that received 100 μc (0.05 mg) ^{203}Hg-chlormerodrin 30 min before death. Silver grains are scattered equally over all portions of proximal convoluted tubular cells. Other micrographs have label over nuclei as well as cytoplasm. Glutaraldehyde fixation × 18,000.

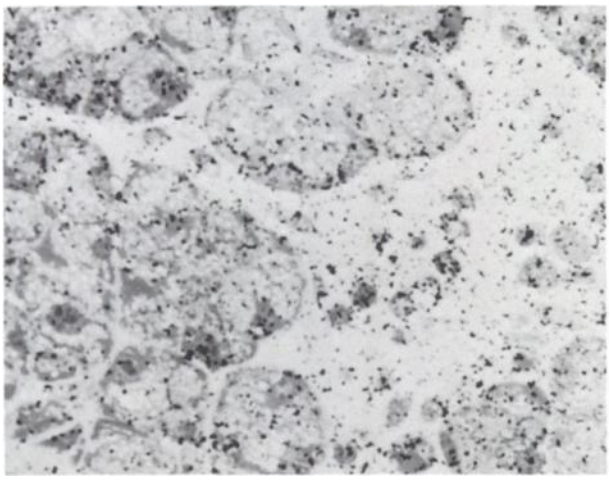

FIG. 3. Submaxillary salivary gland of mouse that received 800 μc (0.35 mg) ^{203}Hg-chlormerodrin 30 min before death. Connective tissue contains slightly more label than does parenchyma. Some connective tissue cells concentrate chlormerodrin. In parenchymal cells, secretory granules are labeled considerably less than remainder of cytoplasm and nuclei. Only black granules are developed silver grains; lighter granules are emulsion artefacts. Glutaraldehyde fixation × 800.

OsO_4-fixation the kidney retained only 35%. Brain retained 100% when fixed in glutaraldehyde and 80% after fixation in OsO_4, etc. However, autoradiograms of OsO_4-fixed tissues contained less label than would be indicated by these percentages, and it is believed that the tissues lost even more chlormerodrin while being sectioned, placed on slides and coated with emulsion. In addition, localization of the drug was poor compared to the discretely labeled areas seen in glutaraldehyde-fixed tissue, indicating that OsO_4 loosened the tissue binding of chlormerodrin. Therefore OsO_4 fixation was considered unsuitable for this drug, and all observations were made on glutaraldehyde-fixed tissue without addition of OsO_4.

Autoradiograms of kidney indicated that connective tissue, blood vessels, glomeruli, loops of Henle and collecting ducts contained little label compared to convoluted tubules (Fig. 1), as has been shown by Taugner *et al* (*15*), Miller *et al* (*16*) and Wedeen and Goldstein (*7*). In newborn animals this same differential concentration of drug was present but to a lesser degree than in adults. Electron-microscopic autoradiography of kidney showed similar amounts of label over all parts of tubular cells—nucleus, brush border, base of cell, etc.—without preferential concentration over any organelle (Fig. 2). Autoradiograms of salivary gland had more label over stroma than parenchyma with concentration over blood vessels and some fibroblasts or macrophages (Fig. 3). In parenchymal cells the secretory products had almost no label whereas the remainder of cytoplasm and the nuclei contained a considerable amount of chlormerodrin. In the liver, label was heavy and diffuse with no preferential concentration. Skeletal muscle had more label over fibers than connective tissue. The label was more concentrated over blood vessels than elsewhere. Some muscle fibers contained considerably more label than others (Fig. 4). Longitudinal and transverse sections showed the label to be distributed diffusely over the fibers except for increased concentration at the immediate vicinity of blood vessels.

In normal brain chlormerodrin was predominantly within blood vessels unless large doses were given. The label actually present in brain was concentrated near blood vessels with no localization over specific cells or neuropil (Fig. 5). Distribution was the same in newborn and 3-day-old mice as in adults (concentration of radioactivity in fresh brain of the newborn was about the same as in adult brain). The brains of two animals with methylcholanthrene-induced brain tumors were studied. One brain resembled the normal in being sparsely labeled, but the other had a marked increase in label diffusely scattered over cells and neuropil throughout the hemisphere bearing the tumor.

Of the four neoplasms examined, two had a diffuse distribution of chlormerodrin (one spontaneous sarcoma and one methylcholanthrene induced small cell tumor in the brain). The other two tumors were

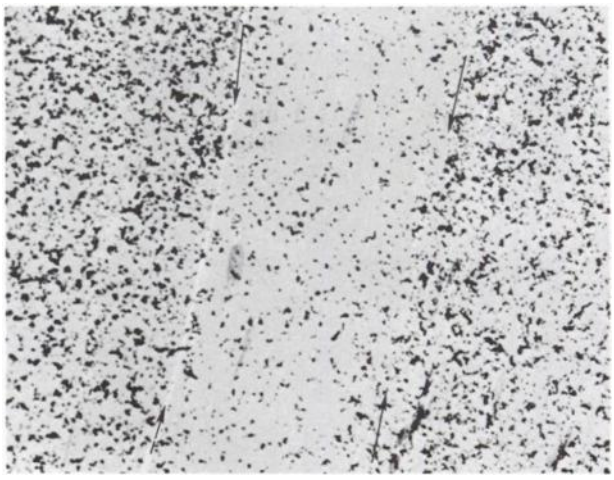

FIG. 4. Skeletal muscle from same mouse as in Fig. 3. Portions of three muscle fibers fill this micrograph (margins of fibers are indicated by arrows). There is less label over center fiber than over two adjacent fibers. Transverse sections also demonstrate variation from fiber to fiber. Glutaraldehyde fixation × 900.

TABLE 3. ^{3}H-ATROPINE

	Fresh tissue (cpm/mg)	% drug retained in fixed tissue	
		Gl*	OsO_4†
Cerebrum, Hypothalamus, Medulla	110–360	2	30
Kidney	900–24,000	5	25
Liver	600–23,000	30	30
Salivary gland	1,000–7,200	2	8
Muscle	550	1	2

* Gl = 6% glutaraldehyde fixation with dehydration through ethanol and toluene.
† OsO_4 = 1% OsO_4 fixation with dehydration.

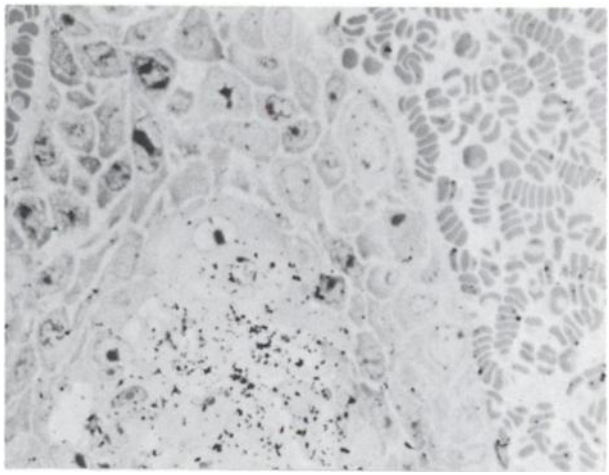

FIG. 6. This is malignant undifferentiated neoplasm induced by implanting methylcholanthrene pellet intracranially in C3H mouse. Mouse was given 700 μc (0.33 mg) ^{203}Hg-chlormerodrin 14 min before death. Drug is almost entirely confined to certain groups of pale cells (which may be degenerating). Well-preserved, actively dividing cells contain little label. Blood vessels within tumor contain less drug than do pale, poorly demarcated neoplastic cells. Glutaraldehyde fixation × 1,200.

methylcholanthrene-induced mixed gliomas with label over certain cells but not others (Fig. 6).

Table 3 shows the amount of ^{3}H-atropine retained in tissues from two cats and one mouse. One cat received 500 μc (1 mg) atropine ^{3}H i.v. 20 min before tissues were removed, one cat received 7,000 μc (14 mg) atropine i.v. 30 min before tissues were removed and the mouse received 1,000 μc (2 mg) atropine i.p. 10 min before tissues were removed. With osmium fixation the tissues retained up to 15 times as much drug as with glutaraldehyde fixation although the maximum retained was 30%.

Autoradiography of liver, kidney, salivary gland, superior cervical ganglion, brain and adrenal showed no preferential localization within particular cells except that neurons of the cervical ganglion of the mouse had more label over them than did nerves and satellite cells (Fig. 7). Label was scattered diffusely over all parts of the ganglion cells. All ganglion cells appeared to be labeled equally, unlike the observations of Appelgren, Hansson and Schmiterlöw (*17*) with ^{14}C-nicotine in cervical ganglia of cats. They found that some neurons contain more drug than others.

Table 4 contains the data for a mouse injected i.p. with 100 μc (4.3 mg) ^{14}C-dilantin 2 hr before decapitation. More drug was retained with OsO_4 fixation than with glutaraldehye except in the kidney. Autoradiography of tissues from two mice showed diffuse scattering of label over liver, kidney and muscle. Connective tissue and ducts of salivary gland showed more label than parenchymal cells. In parenchymal cells there was less label over the secretory products than over other parts of the secretory cells. In cerebellum and medulla, label was distrib-

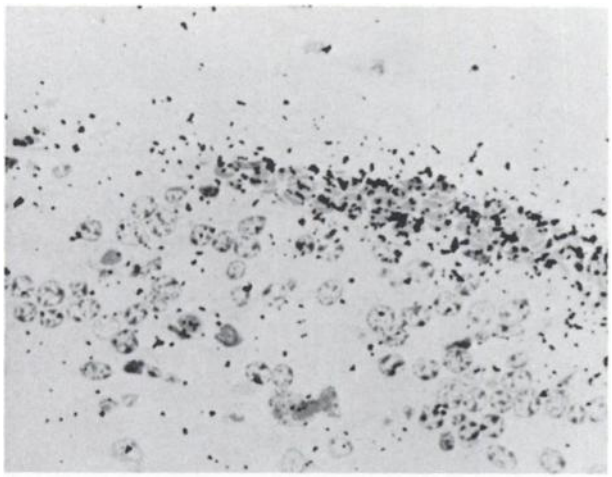

FIG. 5. Cerebellar cortex from mouse of Figs. 3 and 4. Molecular layer is at top, granular layer at bottom. Blood vessel is coursing between layers and contains much more label than does surrounding brain. Label outside blood vessels is diffusely distributed. Same pattern of distribution is seen in all regions of central nervous system which have been examined. Glutaraldehyde fixation × 1,200.

TABLE 4. ^{14}C-DILANTIN

	Fresh (cpm/mg)	% drug retained in fixed tissue	
		Gl	OsO_4
Cerebrum	1,500	20	50
Kidney	2,000	30	30
Liver	2,600	30	45
Salivary gland	1,600	45	55
Muscle	840	35	80

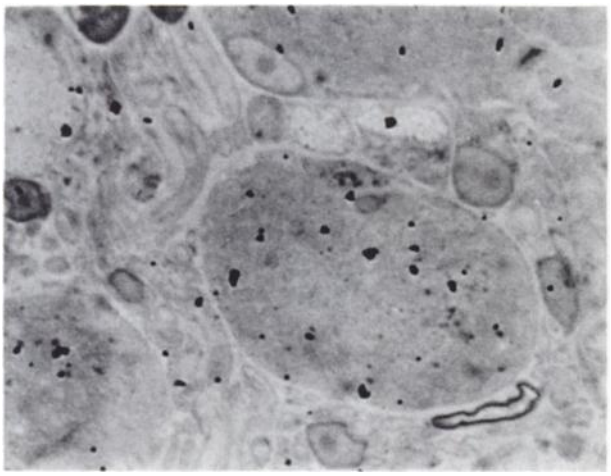

FIG. 7. Superior cervical ganglion of mouse that received 500 μc (1.0 mg) ^{3}H-atropine 1½ hr before removal of ganglion. Neurons contain more atropine than do satellite cells or nerves. Label is diffuse within neurons, and most neurons of the ganglion are equally labeled. OsO_4 fixation × 2,400.

uted diffusely over white and gray matter with no preferential concentration. Label was distributed evenly over neurons, glial cells and neuropil. In the cerebrum there also seemed to be equal labeling of white and gray matter, but in the gray matter the label was more concentrated over neuropil than the perikarya of neurons. Glial cells had approximately the same concentration of label as did surrounding tissue (Fig. 8).

The results of two experiments with mice injected with ^{14}C-barbituric acid are seen in Table 5. One mouse received 150 μc (7.5 mg) ^{14}C-barbital 30 min before decapitation. A second mouse received 100 μc (5.2 mg) 4 hr before decapitation but had excreted almost all the drug with 30 cpm/mg in the kidney and 0 cpm/mg in the brain. Neither mouse was anesthetized by this dose, and the drug was excreted rapidly in the urine. In the second mouse autoradiography showed a heavy diffuse label over all parts of the kidney and a small amount of label in the cerebral neuropil, especially over and around

TABLE 5. ^{14}C-BARBITURIC ACID

	Fresh (cpm/mg)	% drug retained in fixed tissue	
		GI	OsO_4
Brain	56	65	20
Kidney	3,500	45	35
Liver	730	—	21

TABLE 6. ^{14}C-PHENOBARBITAL

	Fresh (cpm/mg)	% drug retained in fixed tissue	
		GI	OsO_4
Brain	140	1	1
Kidney	130	10	2

blood vessels. No preferential concentration was present over specific cells surrounding vessels (Fig. 9).

Tables 6 and 7 show the retention of ^{14}C-phenobarbital and ^{14}C-pentobarbital in tissues of mice. In Table 6 one mouse received 100 μc (15 mg) ^{14}C-phenobarbital i.p. 20 min before decapitation. In Table 7 one mouse received 100 μc (3.8 mg) ^{14}C-pentobarbital i.p. 15 min before decapitation. No label was present over the tissues of either of these mice.

DISCUSSION

This investigation has demonstrated that high-resolution autoradiography of some drugs in tissues prepared for electron microscopy is practicable. For the more energetic beta particles of ^{203}Hg and ^{14}C, as well as for tritium, resolution obtained was on the order of several microns in light-microscopic preparations. With this resolution drugs can be localized to certain parts of cells as well as to individual cells and groups of cells.

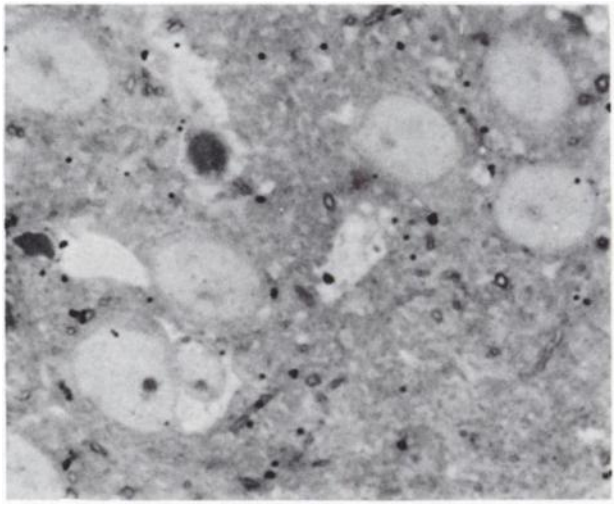

FIG. 8. Cerebral cortex of mouse that received 100 μc (4.3 mg) ^{14}C-dilantin 2 hr before death. Neuropil seems to contain more label than do perikarya of neurons in cerebrum of animal. OsO_4 fixation × 2,900.

TABLE 7. ^{14}C-PENTOBARBITAL

	Fresh (cpm/mg)	% drug retained in fixed tissue	
		GI	OsO_4
Brain	800	0.2	2
Kidney	1,200	2	3
Liver	1,400	0.5	2

Certain features of this method should be kept in mind to prevent erroneous conclusions. The first is that only drugs which are bound to tissues and which survive fixation, dehydration, embedding and sectioning can be seen in the autoradiograms. Whereas almost 100% of the chlormerodrin is retained in some tissues, for other drugs the visible silver grains are produced by only 1–50% of the drug present in the tissue at the time the animals were killed.

Certain drugs are loosened from tissue more by one fixative than by another. Osmium tetroxide was found to cause tissues to lose most of their chlormerodrin before embedding as well as to lead to a diffuse distribution of the remaining drug. In contrast dilantin and atropine were retained better by osmium fixation than by glutaraldehyde. Fischman and Gershon (*18*) have made a detailed study of the preservation and loss of ^{14}C-serotonin and ^{45}Ca from intestine and skeletal muscle using various formalin, osmium tetroxide and alcoholic fixatives as well as oxalate for precipitation of calcium. They found 3–60% of serotonin and 15–90% of calcium retained, depending upon conditions of fixation. Tubbs, Crevasse and Wheat (*5*) observed that 8–22% of tritiated digoxin was retained in myocardium during processing, whereas Smith and Fozzard (*3,4*) obtained a retention of 70% of this drug with acetone dehydration. Wedeen and Goldstein (*7*) found 90% chlormerodrin still in kidney after formalin fixation and paraffin embedding. For most drugs, however, the amount lost during processing still is unknown.

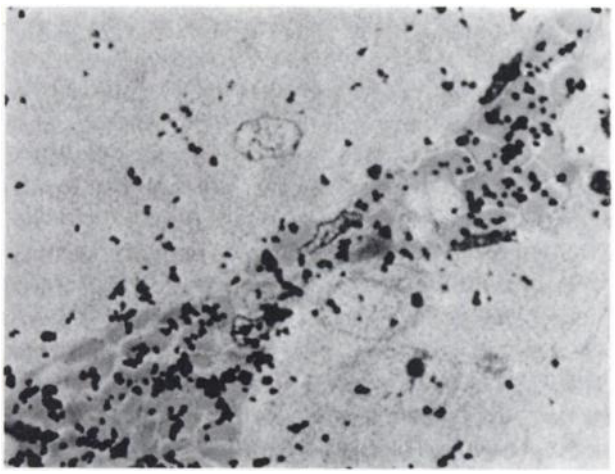

FIG. 9. Cerebral cortex of mouse that received 150 μc (7.5 mg) ^{14}C-barbituric acid 30 min before death. Drug is concentrated within and immediately surrounding blood vessels. Glutaraldehyde fixation × 2,500.

A second factor is that labeled drugs may be displaced during processing, and thus the autoradiogram may not represent a true picture of the position of the drug in life. This possibility must be considered when most of drug is lost during processing and the resultant autoradiogram is diffusely labeled. Mitchell, King, Marrian and Chipperfield (*19*) found that fixation of tissues containing tritiated TRA119 (a highly tritiated drug used for parenteral radiation therapy of malignant neoplasms) in Susa solution resulted in diffusion of this drug and that they had to use frozen sections to obtain accurate localization in autoradiograms. When little drug is lost during fixation and dehydration and when label is found over certain parts of cells or over one group of cells and not over an adjacent group, it is less likely that a significant shifting of drug has occurred. To confirm findings in fixed tissues, studies must be done on frozen sections without use of solvents or by microchemical analysis at the cellular level.

Still a third feature of this type of study is that specific activities of most labeled drugs are so low that physiological doses do not contain enough radioactivity to give good autoradiograms—especially for electron microscopic work. Therefore to obtain a significant label over thin sections of kidney, a toxic dose of chlormerodrin was administered and the tubular cells were flooded with more drug than ever would be used for therapy or for scintillation scanning. This problem cannot be resolved until drugs of higher specific activity are prepared.

A fourth point to be remembered is that concentration of a drug in a certain part of an autoradiogram does not mean that this necessarily represents the site of physiological action of the drug.

In spite of these limitations, informative studies have been published concerning the location of drugs in fixed tissues, and new information has been presented in the present work. By means of electron-microscopic autoradiography, Kayes, Maunsbach and Ullberg (*1*) observed that iodide is concentrated 25-fold in colloid in comparison to the follicular cells of the thyroid 1 hr after carrier-free ^{125}I-ionic was given intravenously to rats, extending the observations of Leblond and Gross (*20*).

Wolfe, Potter, Richardson and Axelrod (*2*) demonstrated that tritiated noradrenaline was concentrated over the granules of sympathetic nerve endings in the pineal body of rats 2 hr after intravenous infusion. Chlormerodrin and mersalyl tagged with ^{203}Hg have been seen to be concentrated within proximal tubules of the kidneys of rats, mice and dogs 15 min to 2 hr after injection (*7,15,16*). The site of localization of chlormerodrin within the tubule varied in different species. Tritiated cholesterol was found to have a characteristic rate of diffusion through the layers of the aortic wall in rats and rabbits (*21*). Digoxin was demonstrated to have a fairly diffuse distribution within myocardium of frogs and dogs, but A bands of myofibrils contained a higher concentration of the drug than did any other component of the myocardium (*3,5*). Other drugs have been localized at the cellular level by rapid freezing techniques, but the results will not be reviewed here since this paper is concerned with drugs in fixed tissues.

The localization of chlormerodrin within specific renal tubular cells may be of value in understanding the action of mercurial diuretics. The fact that some tumors have uptake of chlormerodrin within all cells and that other tumors have only a few cells which concentrate this drug helps to explain why some tumors are readily located by scintillation scanning while others are not. The concentration of dilantin in cerebral neuropil in contrast to its diffuse distribution within cerebellum is of interest considering its anticonvulsive efficacy.

The observation of barbituric acid essentially confined to the blood vessels in the brain of one mouse which showed no physiological effect of the drug may explain this lack of sedative action, although the absence of pentobarbital and phenobarbital within fixed tissues of anesthetized mice indicates that these drugs do not have to be firmly bound to tissues to have a profound physiological action. Further studies of anesthetized and unanesthetized animals need to be done.

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