LOCALIZATION OF DRUGS IN TISSUES WITH RESPECT TO THEIR PHYSIOLOGICAL ACTIONS

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The title of this review may imply that only drugs whose localizations and mechanisms of action are both known will be considered. There are few drugs, however, that can qualify for this honor and enjoy such extensive study or importance. Therefore, it might be useful to categorize the patterns of distribution of drugs, anticipating that from this order there may emerge later an association of this distribution with the mechanism of action.

The localization of drugs in tissues may be studied by a variety of techniques. The older, and unfortunately more common, technique is removal of an organ or tissue from an animal that has received a drug and analysis of the amount of drug in the sample. The organ or tissue is treated as a homogeneous system and the amount of drug found is converted to concentration simply by dividing by the weight of the tissue. Occasionally, concessions are made, in an attempt to obtain more useful information (or to substantiate the investigator's thesis), and the concentration is expressed as amount per gram of total tissue water, extracellular water, intracellular water, total protein, RNA, DNA, fat, glycogen, or other stock. Little is gained by these maneuvers if the actual cellular and subcellular distribution of the drug in the organ at the time of removal is not known. It is not sufficient evidence that the drug is confined to a specific compartment or associated with a specific macromolecule simply because its concentration in the organ agrees with some reference value for being confined to a specific compartment or associated with a specific molecule. Many deceptive incidents of apparent agreement are to be found in the literature.

Another, newer technique which is being used in some, but all too few, laboratories is autoradiography. For drugs, which are usually small, soluble molecules, careful attention must be taken to avoid translocation or removal of the drug from the site at which it was present at the time of sacrifice of the animal. The only successful technique that is currently available at the organ and tissue level was developed by Ullberg (1–3). The most successful technique at the cellular and subcellular level was developed by Stumpf (4–6). Although attempts have been made at the electron microscope level of resolution for autoradiography of soluble substances, there is no truly successful method currently available (7).

Autoradiography of drugs in whole animals, organs, or cells, when care is taken to prevent translocation or removal of the drug or its metabolites, offers by far the best information on the localization of drugs in biological material. The

structural organization of living systems certainly does not stop at the boundary between organs and it most likely continues within cells, subcellular organelles, and even macromolecules. Inhomogeneity of drug distribution is frequently seen within organs by autoradiography. The increased uptake of compounds in the adrenal cortex (8–10) or medulla (11) but not the other might have been missed by an analysis only of the whole adrenal. Pancreatic islets (12, 13), bronchi (14), corpora lutea (9, 10, 15, 16), proximal kidney tubules (17), and retina (18–20) are other examples of inhomogeneities within organs where increased drug uptake has been found by autoradiography and most likely would have been missed by whole tissue analysis. Sites such as the middle ear (20), spinal ganglia (21), and rodent yolk sac epithelium (22, 23) which are rarely sampled in whole tissue techniques have been found to concentrate certain drugs by whole-body autoradiography.

It may very well be that an increased uptake of drugs is occurring in subcellular organelles or in macromolecules of certain cells and we are totally unaware because of inadequate methods. It would not be surprising if most of the localizations related to the mechanism of action of a drug are at a molecular level and not detectable by most currently available techniques. The physiologically important interaction of a drug with a specific cellular molecule might account for only a small fraction of the total amount of the drug present in the cell. Interactions detected by electron spin resonance (ESR) (24) or nuclear magnetic resonance (NMR) (25) may be particularly valuable in the ultimate elucidation of the mechanism of action of drugs.

Hypothetical Relationship between Localization of a Drug and its Physiological Action

Many factors may account for an increased concentration of a drug at a particular site in living biological systems. It is important to consider the causes of an increased concentration and whether or not there might be a relationship between these and the physiological action of that drug.

What are the ways in which the presence of a molecule exerts an effect on other molecules in that system?

Dilution or decrease in concentration of essential substances.—It is possible that a drug can act by diluting other substances below the concentration necessary for continuing normal function. Obviously, abnormalities of water metabolism can fall in this category.

Osmotically active substances may be used as diuretics, cathartics, or plasma expanders if placed in the water compartment to effect the proper osmotic gradient. In this situation, a decreasing concentration may correlate with decreasing activity.

There has been some speculation that replacement of certain ions with others may account for their action by simply reducing the concentration of the ion normally present. This was suggested as the mechanism of action of the bromide ion since the concentration of the chloride ion fell with the administration of

bromides. However, the replacement of chloride with nitrate did not produce the effect and that mechanism was therefore considered unlikely (26). Replacement is, a priori, with a molecule that differs and therefore cannot have the same interactions with all the compounds that the replaced molecule did.

There are other mechanisms for reducing the concentration of substances that are essential for the physiological action. For example, EDTA, penicillamine, and BAL can reduce the concentration of certain ions or molecules by combining with them and enhancing their renal elimination. In this instance, the drug (EDTA, etc.) is interacting with a cellular constituent and the example falls into the categories discussed in the next section.

Perhaps the most subtle and interesting mechanism for its effect on the concentration of water is that proposed for clathrate crystal formation by the volatile general anesthetics (27). The hydrophobic anesthetics reduce the activity of solvent water to an extent that stabilizes some of the water in a highly structured or crystal form. In this instance, the solvent water concentration is reduced; the effect on the concentration of anesthetic at this site is difficult to predict.

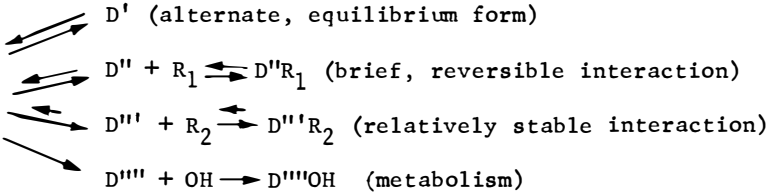
Other ways to reduce the concentration of normal cellular constituents, without a direct interaction with another molecule, are either to increase the amount of the solvent (water) or to change the properties of that solvent (structure, ionic strength, pH, or temperature). If the action of the drug is to increase the amount of water or change its properties, then there must be an interaction with cellular substances that maintain water homeostasis. Such is, then, an interaction with other cellular substituents and not simple, unreactive dilution.

Interaction with cellular constituents.—If the drug interacts with any cellular constituent, then as a result the drug is changed in some manner. The question is the degree of change in the drug as a result of the interaction.

Any interaction that is insufficient in duration, magnitude, or numbers of drug molecules will have a correspondingly small effect on the total concentration of the drug in that phase. The magnitude of the change is important only in that another form or species of the drug be produced which therefore increases the total concentration of the drug. Of course, multiple forms of the drug can be produced; each of these increases the total concentration of the drug correspondingly. The extent of the change in the form of the drug can be as weak as a dipole-induced dipole bond, or can be a complete disintegration of the molecule.

Obviously, the longer the duration of this change in the form of the drug (from milliseconds to permanent change), the greater is the initial reduction in the concentration of the original form of the drug. Then, redistribution of the original form of the drug returns it to the same chemical potential in all compartments and consequently the total drug concentration increases in the environment of the interaction.

The situation is illustrated in Figure 1. The drug, as administered, is represented by D and four altered forms or species are represented by D′, D″, D‴, and D⁗. Different degrees of change in the drug are illustrated. The least permanent form, D′, might be, for example, the ionized or un-ionized form of a drug that is a weak



Total drug concentration = D or any combination including

$$D+D'+D''+D''R_1+D'''+D'''R_2+D''''+D''''OH$$

FIG. 1. Schematic representation of a drug molecule (D) and four of its species which interact with biological substituents.

acid or base. The most permanent form, D''''OH, might be the hydroxylated, inactive metabolite. Although D′ and D'''' might be the forms of the drug that produce the physiological response, the interaction with the biological molecule to produce the response is illustrated for D″ and D‴ with receptors R_1 and R_2.

The purpose of this discussion is to lead up to the fact that the total concentration of drug at any site depends on both the extent of interaction with R_1, R_2 or OH *and* the method used for determining the concentration of drug. It is the nonspecific nature of analytical methods and the reversibility of most of the interactions (e.g., with R_1 and R_2) that in large part accounts for concentration gradients in biological tissues. The chemical potential of each of the forms (D, D′, D″, D‴, D'''') is the same throughout the entire living system.

Other explanations for concentration gradients are that a change in the solvent's properties or a phase change requires a change in concentration in order to maintain the same chemical potential in the new solvent or phase. Examples are the increased concentration of lipid soluble molecules such as DDT (8), Δ^9-THC (10), and thiopental (29, 30) in fat. Another example might be the increased concentration of the ionized form of a weak acid in a water compartment with a higher pH value. These are examples of increased concentration that involve interactions with solvent molecules but not receptor molecules.

Therefore, interactions of a drug or any of its species with a molecule to produce a physiologic action will increase the concentration of the interacting species at that site; after re-equilibrium of the other species of the drug, there will also be an increase in the apparent total concentration of drug if the analytical method does not discriminate between the various species of the drug. It is equally important to be aware that nonphysiologic-action concentrations can be produced by the same mechanism with nonphysiologic-action molecules or by solvent effects (e.g., pH gradients, fat, etc.).

The problem, then, in its simplest terms, is just twofold. The first is to distinguish increased concentrations due to nonphysiologic-action mechanisms from those

due to physiologic-action mechanisms. The second problem is to distinguish which of the species of the drug is in fact increased in concentration at that site. In many ways, solution of the second problem may solve or help solve the first problem.

Sites of Increased Concentration of Drugs seen by Autoradiography

Sites of increased concentration which most likely are not due to their primary physiologic action.—There are several sites of increased drug uptake which are seen so frequently by such obvious tissues that certain generalizations may be made. Several recent reviews of studies of the autoradiographic distribution of drugs have appeared from which patterns of distribution are seen (19–21, 31, 32). The liver, contents of the gall bladder, and contents of the upper intestine will quite obviously show increased concentration of drug if there is metabolism of the drug in the liver and secretion of the drug into bile. Secretion of unchanged drug by the liver into bile may also account for this increase. In this and other secretory mechanisms, the cells or membranes lining the route of secretion must be functionally impermeable to the secreted species of the drug.

The kidney, ureter, urinary bladder, salivary glands, salivary ducts, pancreas, etc. also commonly have increased concentrations for this same reason.

One must be careful, however, not to assume that all increased drug concentrations in these tissues are due to simple metabolism and/or secretion. Some drugs localize in these tissues due to, or presumably due to, their physiological action. Examples will be given below.

Bone, cartilage, and aorta accumulate a variety of drugs and ions including urea, inulin (20), cations, and anions (32, 33). Although many of these may be direct binding interactions, the localization of some substances in these tissues may be only apparent. The apparent localizations are due to the slow rate of equilibration of drugs in the water of these tissues with the water in other compartments such as plasma. Elimination of the drug from plasma (e.g., by renal secretion, etc.) at a faster rate than its diffusion from bone or aortic wall creates a concentration gradient that is interpreted in an autoradiogram as increased uptake in bone or aorta. The same phenomenon explains apparent uptake in the brain and fetus for some compounds (33).

Specific affinities of calcified or calcifiable tissues for certain drugs and ions may be due to the nonspecific binding by collagen or hydroxyapatite. The importance of the affinity of these two common biological materials for drugs has been widely overlooked in drug disposition studies. Whole-body autoradiography has demonstrated impressively the wide variety of substances that accumulate in calcifiable tissue and emphasized the advantages of this method over tissue removal methods for drug distribution studies.

Most of these affinities for calcifiable tissues are probably not due to their physiological action; they are probably more analogous to binding of drugs to albumin. However, it should be obvious that some of these substances may be affecting the rate or extent of calcification of these tissues.

Sites of increased concentration that are due, or probably due, to their primary physiologic action.—Most of the recent reviews cited above have emphasized the association between localization and probable mechanism of action. These may be summarized into drugs involved in the metabolism or action of: (*a*) steroid hormones, (*b*) vitamins, (*c*) adrenergic substances, (*d*) other hormones, and (*e*) a few miscellaneous drugs.

Probably the most impressive correlation between localization and physiological action of drugs is to be found with the steroid hormones. Appelgren (14) studied the sites of steroid hormone metabolism by administering labeled precursors and determining their distributions in mice by autoradiography. The adrenal cortex had an intense and persistent localization of radioactivity after administration of ^{14}C-4-cholesterol and the corpora lutea had the remarkable accumulation after ^{14}C-4-pregnenolone. Obviously, the conversion of these precursors to other compounds in part, at least, accounts for the radioactivity in these sites. There are a surprisingly large number of other substances that accumulate in sites of steroid hormone metabolism. A list of compounds compiled from reports of autoradiographic studies is given in Table 1; the list was assembled more or less at random and no attempt was made to be comprehensive.

TABLE 1. Compounds that Localize in Sites of Steroid Hormone Metabolism in the Mouse.

	Sites of Localization				
		Ovary			
Compound	Adrenal Cortex	Corpora Lutea	Follicles	Testes	References
Phenobarbital-^{14}C	+	+			(20)
Thiopental-^{35}S	+	+			(29)
Meprobamate-^{3}H and ^{14}C	+	+		−	(34, 35)
Carisoprodol-^{14}C		+			(35)
Tybamate-^{14}C	+	+		−	(35)
Diphenylhydantoin-^{14}C	+	+	−		(9)
Chlorpromazine-^{35}S	+	−	+	+	(36–38)
Imipramine-^{14}C	+				(39)
Amitriptyline-^{14}C	+			+	(40)
Oxypertine-^{3}H	−			+	(41)
Diazepam-^{14}C	+	+	−		(42)
Chlordiazepoxide-^{14}C	+	+	−		(42, 43)
Chlorcyclizine-^{14}C	+	+			(31)
Secergan-^{35}S	−			+	(44)
LSD-^{14}C	+			+	(45)

TABLE 1. (Continued)

Compound	Sites of Localization				References
		Ovary			
	Adrenal Cortex	Corpora Lutea	Follicles	Testes	
Δ^9-THC-^{14}C	+	+			(10)
Propranolol-^{14}C			+		(46)
Tabun-^{32}P		+			(47)
Dimethyl mercury-^{203}Hg	+				(48)
p-Aminosalicylic Acid-^{14}C	+				(49)
DDT-^{14}C		+p			(28)
Dieldrin-^{14}C		+p			(28)
Cholesterol-^{14}C	+p	+		+p	(14)
Pregnenolone-^{14}C	+	+		+	(14)
Progesterone-^{14}C	+	+		+	(14, 31)
Corticosterone-^{14}C	+				(23)
Hydrocortisone-^{14}C	+				(23)
Cortisone-^{14}C	+				(22)
Testosterone-^{14}C	+p	+		+	(50)
Estradiol-^{3}H and ^{14}C	+p		+	+	(51, Waddell, unpublished)
Estrone-^{14}C	+p		+	+	(51)
Diethylstilbestrol-^{14}C	+	+	+	+	(52)
Polydiethylstilbestrol-^{14}C	+p	+	+	+p	(53)
F6060-^{14}C	+	+p		+	(15, 16)
F6066-^{14}C	+	+p		+	(15, 16)
Thiamine-^{35}S	+	+p		+p	(54)
Nicotinamide-^{14}C	+	+	+		(Waddell, unpublished)
Ascorbic Acid-^{14}C	+	+p		+p	(12)
Dehydroascorbic Acid-^{14}C		+p		+p	(12)
α-Tocopherol-^{14}C		+	+		(19)
Cyanocobalamin-^{58}Co, ^{57}Co, ^{60}Co	+p	+	+	+p	(55, 56)
Selenomethionine-^{75}Se		+	+		(57)

+ > blood; − < blood; +p > blood and persists > 24 hrs; blank = either not studied or not reported

TABLE 2. Ions that Localize in Sites of Steroid Hormone Metabolism in the Mouse

		Sites of Localization				
			Ovary			
Ion	Conjugate Ion	Adrenal Cortex	Corpora Lutea	Follicles	Testes	Reference
$^{65}Zn^{+2}$	Cl^-				+	(58)
$^{75}SeO_3{}^{-2}$	Na^+	+	−	−	−	(59)
$^{91}Y^{+3}$	Cl^-		−	+	−	(60)
$^{109}Cd^{+2}$	Cl^-	+p			+p	(61)
$^{131}I^-$, $^{125}I^-$	Na^+		−	+		(62, 63)
$^{137}Cs^+$	Cl^-		+	−	+p	(64)
$^{144}Ce^{+3}$	Cl^-	+	+	−		(65)
$^{147}Pm^{+3}$	Cl^-	+	−	+		(65)
$^{160}Tb^{+3}$	Cl^-	+	+	−		(66)
$^{166}Ho^{+3}$	Cl^-	+	+	−		(66)
$^{169}Yb^{+3}$	Cl^-		+	−		(66)
$^{203}Hg^{+2}$	Cl^-	+p			+p	(67)
$^{239}Pu^{+6}$	Citrate	+	+p	+p	+p	(68)
$^{241}Am^{+3}$	NO_3^-	+p	same	+	+	(69)

+ > blood; − < blood; same = to blood; +p > blood and persists > 24 hrs.

A similar list for ions is shown in Table 2. Clearly, most of these substances cannot be converted to steroid hormones. But the possibility exists that they could be interacting with the enzymes for steroid metabolism and either inhibiting or enhancing their activity. Their physiological action, in part at least, could be mediated indirectly through an alteration of the concentration of endogenous steroids. The intriguing dissimilarities between some of the sites such as corpora lutea and follicles suggests interaction with different enzymes in some instances. Further clarification of the explanation of the distribution of these many substances in sites of steroid hormone metabolism awaits experiments at the biochemical and molecular level.

A steroidal isoxazole (17β-hydroxy-4,4,17α-trimethyl-androst-5-en-[2,3d]-isoxazole) competitively inhibits 3β-hydroxysteroid dehydrogenase and $\Delta^{5\text{-}4}$,3-ketosteroid isomerase. The inhibition is potent, persistent, and selective (70). The distribution of this ^{14}C-labeled isoxazole was studied in mice by whole-body autoradiography (Waddell, unpublished). The intense and selective accumulation in sites where these enzymes are present is shown in Figure 2 and is remarkably similar to the distribution of many of the substances in Tables 1 and 2.

Appelgren (14) noted an unusual localization of ^{14}C-progesterone and ^{14}C-pregnenolone in bronchi of mice which correlated with the histochemical

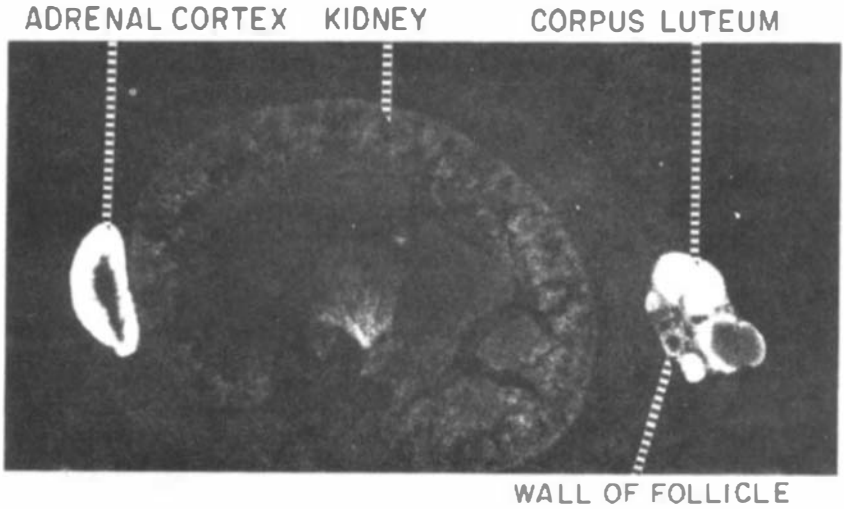


FIG. 2. Print of an autoradiogram of a 20 μ section from an A/JAX mouse that received the ^{14}C-isoxazole subcutaneously on day 15 of gestation and was frozen 24 hours later. White areas correspond to radioactivity.

distribution of "secondary alcohol dehydrogenase". Figure 3 is a print of auto radiograms of the distribution of ^{14}C-progesterone and of the ^{14}C-isoxazole in the thoracic region of mice. Both of the compounds localize in the walls of the bronchi. These observations suggest that some steroid metabolism occurs in the bronchi, at least in this species.

There are also several reports on the localization of hormonally active steroids in target tissues. The natural estrogens, estradiol and estrone, as well as the synthetic estrogen diethylstilbestrol, accumulate in vaginal mucosa and the uterine wall (52). The endometrial cells had the highest concentration within the uterine wall; the nuclei had a higher activity than the cytoplasm (51). Later studies reported that nuclei of both myometrial and endometrial cells had high amounts of radioactivity (71, 72).

Although biochemical methods have identified some of the characteristics of the macromolecules that bind estrogens, androgens, progesterone, glucocorticoids, and aldosterone (see 73 for recent reviews), the histochemical localization of the cells containing these binding molecules has been made for only a few substances. Aldosterone was localized by autoradiography in the nuclei of mucosal epithelial cells of the toad bladder (74).

The most extensive studies at the cellular and subcellular level have been those of Stumpf and associates (see 75 for review). Estradiol was found in nuclei of oviduct, uterus, vagina, epithelium of mammary tumors, pituitary, and brain; however, not all cells were labeled. Unlabeled cells were found adjacent to heavily labeled cells. Radioactivity from ^{3}H-testosterone was found in the nuclei of epithelial cells of the prostate, seminal vesicles, epididymus, and coagulation

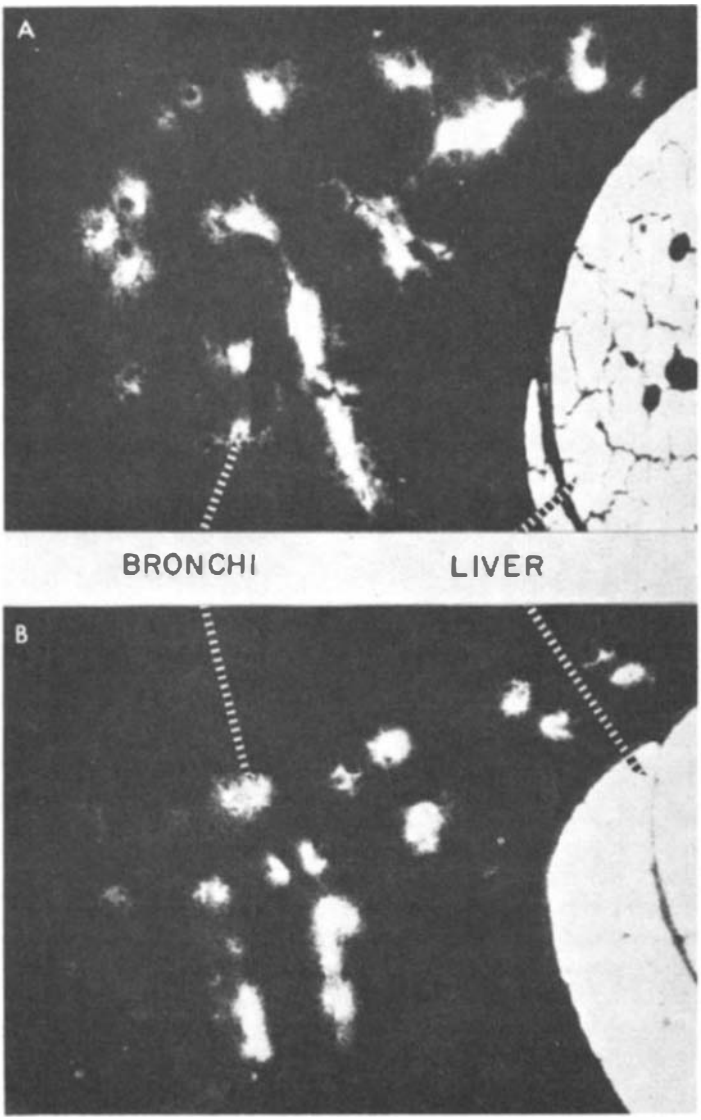


FIG. 3. Prints of autoradiograms of 20 μ sections from pregnant A/JAX mice frozen 3 hours after subcutaneous injection. A. The mouse received ^{14}C-progesterone on day 12.5 of gestation. B. The mouse received the ^{14}C-isoxazole on day 15 of gestation.

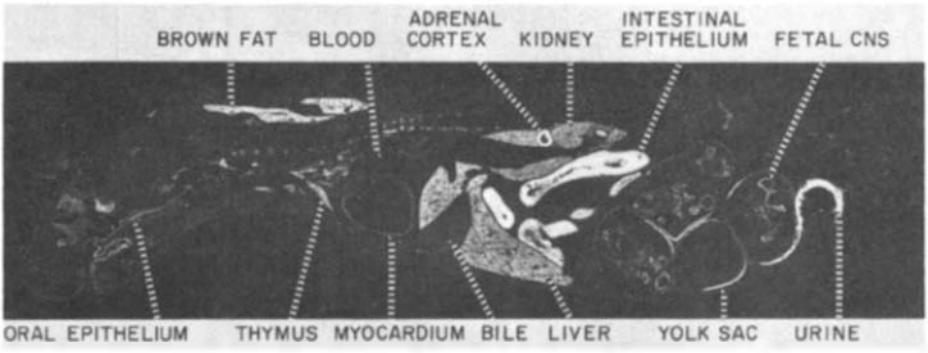


FIG. 4. Print of a whole-body autoradiogram of a 20 μ section from an A/JAX mouse injected intravenously with ^{14}C-nicotinamide on day 12.5 of gestation and frozen 3 hours later.

gland, and in neurons of the brain. Estradiol concentrated in identical sites in the brain of both male and female rats and these sites were widespread. Therefore, instead of the concept of "sex centers" in the brain, estrogen-neuron systems are proposed, which regulate gonadotropin synthesis and secretion as well as sex behavior. Since androgens did not localize in the pituitary, it was proposed that androgens, in contrast to estrogens, do not exert a feedback effect on the pituitary. Very little information is available on the autoradiographic distribution of ^{3}H-progesterone at the cellular and subcellular level although ^{14}C-fluocinolone acetonide has been found to accumulate in the pituitary (76).

The highly selective accumulation of vitamin A in the retina of the eye is a dramatic example of localization with respect to function (19); it presumably indicates an active involvement of vitamin A in the metabolism of rhodopsin. The accumulation of other vitamins, thiamine-^{35}S (54), O-butyrylthiamine-^{35}S (77), ascorbic acid-^{14}C (12), α-tocopherol-^{14}C (19), cyanocobalamin-^{57,58,60}Co (19, 55, 56) and nicotinamide-^{14}C (Waddell, unpublished), has been seen in tissues with active metabolism involving that vitamin. Liver, kidney, myocardium, Harder's gland, intestine, brain, adrenal, brown fat, and fetal yolk sac epithelium are typical tissues with such metabolic rates. An example may be seen in Figure 4 which is a print of an autoradiogram showing the distribution of nicotinamide-^{14}C in epithelial tissues of both mother and fetus, brown fat, and central nervous system. Figure 5 is a detail from a whole-body autoradiogram showing the accumulation of radioactivity from nicotinamide-^{14}C in maternal ovary and fetal brown fat and retina. The active metabolism of this vitamin in these tissues undoubtedly accounts for this localization.

Labeled dihydroxyphenylalanine (dopa) has been studied extensively by autoradiography (11). This compound is converted to catechol amines and the distribution of radioactivity in adrenal medulla, sympathetic ganglia, and spinal ganglia is obviously due to the metabolism of the compound to adrenergic amines.

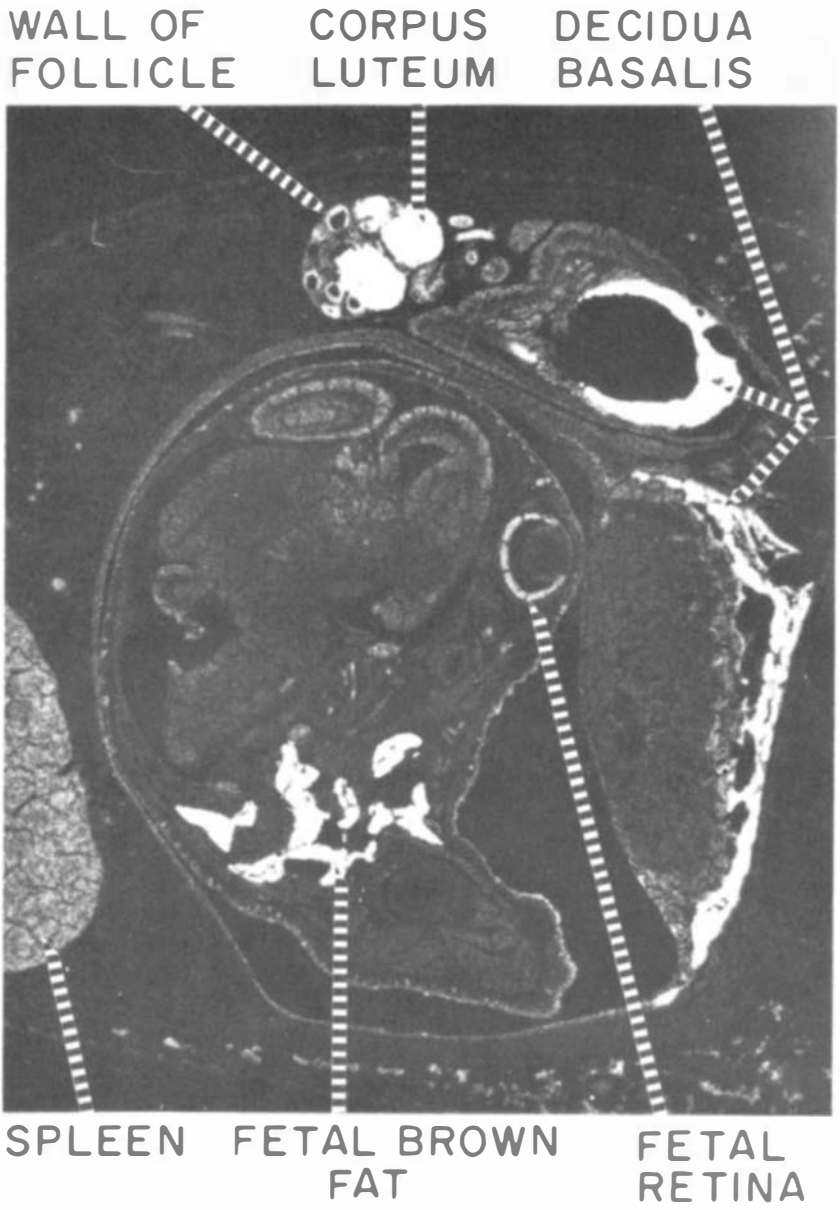


FIG. 5. Detail of an autoradiogram of a 20 μ section from an A/JAX mouse injected intravenously with ^{14}C-nicotinamide on day 17.5 of gestation and frozen 1 hour later.

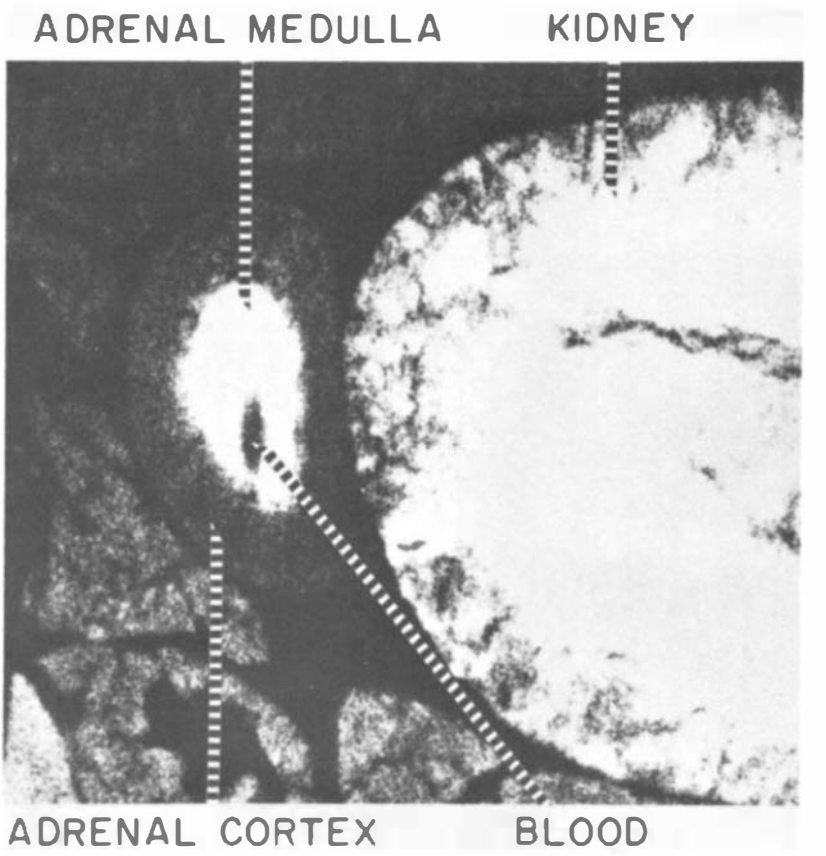


FIG. 6. Detail of an autoradiogram of a 20 μ section from an A/JAX mouse injected intravenously with ^{14}C-mescaline on day 12.5 of gestation and frozen 20 minutes later.

The distribution of the labeled hallucinogenic agent, mescaline-^{14}C, was studied by autoradiography in mice (Waddell & Marlowe, unpublished). Figure 6 shows the selective accumulation of radioactivity from this compound in the adrenal medulla; it suggests that the similar chemical structures of mescaline and endogenous adrenergic amines may allow the hallucinogen to interfere with the metabolism of endogenous amines.

The almost specific localization of thiouracil-^{14}C and thiourea-^{14}C in the thyroid (21) and alloxan-2-^{14}C in islets in the pancreas (13) are examples of agents that interfere with normal hormonal function and are found at their site of action.

Several depolarizing and curarizing drugs were studied in mice by autoradiography by Waser (78). There was a high, and in some cases, specific accumulation of radioactivity at the motor endplates in skeletal muscle. The autoradiograms

record a beautiful correlation between site of action and localization. A similar functional distribution is to be seen in the accumulation of ^{3}H-atropine in the ciliary body and iris of the eye (79).

Other compounds that localize in the eye with respect to their physiological, or at least toxic, action are chlorpromazine-^{35}S and chloroquine-^{14}C in the pigment layer (80) and dimethylsulphoxide-^{14}C (DMSO-^{14}C) in the lens (21). These may be related to their light sensitivity and retinal and lens toxicities.

Dimethylchlortetracycline (DMCTC) localizes in bone and teeth (81); if it is given before the administration of ^{45}Ca, the uptake of ^{45}Ca by bone and teeth is suppressed. This suggests that the defect in calcification seen when tetracyclines are given to pregnant women is due to localization of the antibiotic at these sites which interferes with the normal mechanism for calcification.

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