Anesthesiology
V 37, No 3, Sept 1972

Studies with Halothane:

I. The Distribution and Excretion of Halothane Metabolites in Animals

John C. Topham, Ph.D., and Susan Longshaw

The distribution and excretion of nonvolatile radioactivity in mice after administration of a single dose of ^{14}C-halothane by inhalation (10 minutes of anesthesia) and iv are compared. The ratio of the concentration of nonvolatile radioactivity in liver to that in muscle progressively decreased in a period of four days. The liver/muscle ratio initially was higher after inhalation than after iv administration, but it declined rapidly to a ratio lower than that observed after iv dosing. After inhalation, high levels of nonvolatile radioactivity were present in the nasal mucosa, lung, and thyroid.

After a single five-hour period of anesthesia with halothane, all the tissues, but not bile and urine, contained similar concentrations of nonvolatile metabolites. Following numerous five-hour periods of anesthesia, nonvolatile metabolites did not accumulate. In mice, more halothane was converted to nonvolatile metabolites during the 23 hours after recovery than during a one-hour period of anesthesia. The rate of metabolism of halothane to nonvolatile metabolites was highest during anesthesia. Unchanged halothane was found in the bile of the rat and dog, and nonvolatile metabolites of halothane were excreted in the bile of mice and rats.

After iv administration of ^{14}C-trifluoroacetic acid to mice, the distribution of radioactivity was similar to the distribution of nonvolatile radioactivity after iv administration of halothane. (Key words: Halothane; Metabolism; Distribution; Excretion.)

IN DISTRIBUTION and excretion, nonvolatile metabolites of anesthetics may differ from the parent compounds because of differences in the physical properties of the molecules. The metabolic fate of halothane and the distribution and excretion of the resultant metabolites have been the subjects of debate for several years. It was originally thought that halothane was not metabolized.[1] Only minimal cleavage of the carbon–fluorine bonds in halothane occurs *in vivo*[2] and, although several minor metabolites have been observed,[3, 4] salts of trifluoroacetic acid constitute the major metabolite of halothane (80+ per cent of radioactivity in urine of several species) and remain the only nonvolatile metabolites that have been identified[5-7] unequivocally, apart from chloride,[8] bromide,[9] and trifluoroacetyl ethanolamide.[4, 7] Unidentified nonvolatile metabolites have been demonstrated in mouse liver following administration of halothane[7] iv[3, 4] or by inhalation. Other workers have suggested that more metabolism of halothane may occur after recovery than during anesthesia with halothane.[10] Many of the data[2, 3, 5, 8] on the metabolism, distribution, and excretion of halothane were derived from studies with subanesthetic concentrations administered by a variety of routes which may have little relevance to the behavior of anesthetic concentrations of halothane administered by inhalation.

The present study was designed: 1) to compare the distribution and excretion of nonvolatile metabolites after iv and inhalation administration of halothane and iv administration of trifluoroacetic acid; 2) to determine the rates of formation of nonvolatile metabolites during and after anesthesia with halothane; 3) to study the biliary excretion of unchanged halothane during anesthesia.

Material and Methods

Male mice (15–25 g body weight, specific pathogen-free Swiss-derived Alderley Park strain), male rats (200–250 g body weight, specific pathogen-free Wistar-derived Alderley Park strain), and male beagle dogs (10 kg body weight) were used.

Received from the Research Department, I.C.I. (Pharmaceuticals Division), P. O. Box 25, Alderley Park, Macclesfield, Cheshire, England. Accepted for publication February 9, 1972.

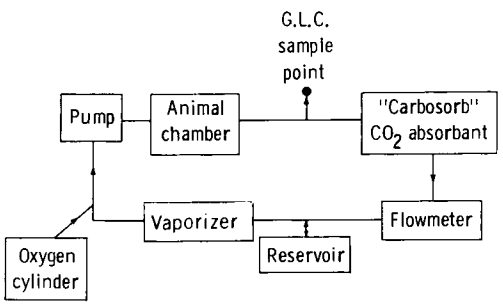

FIG. 1. Closed-circuit anesthetic apparatus. The vaporizer was a Goldman (British Oxygen Co., Ltd., London) charged with 1.0 ml ^{14}C-halothane. The gas-flow rate was 800–900 ml/min.

^{14}C-1-halothane * and ^{14}C-1-trifluoroacetic acid † were obtained from commercial sources. Radio–gas chromatography and thin-layer chromatography/autoradiography established the high purity (>99.9 per cent) of the materials. For iv administration ^{14}C-halothane was dissolved in saline solution: each animal received 2.5 mg halothane (specific activity 8.12 μCi per mg)/kg body weight. For inhalation adminstration to individual animals the ^{14}C-halothane was diluted with two volumes of unlabelled halothane; approximately 20 μCi (equivalent to about 2 per cent halothane v/v oxygen) were used to anesthetize each mouse, as described by Cohen and Hood.[3, 11] For simultaneous anesthesia of a group of mice with ^{14}C-halothane, a closed-circuit apparatus (fig. 1) was constructed. The halothane concentration was maintained between 1 and 2.5 per cent throughout anesthesia by monitoring the circulating gas and estimating halothane concentrations with a gas chromatograph ‡ fitted with an automatic sampling device and a thermistor katharometer detector. Halothane (Fluothane §) was administered by a Mk2 Fluotec to the animals with cannulated bile ducts. ^{14}C-trifluoroacetic acid was administered intravenously in saline solution as the sodium salt, each animal receiving 8.5 mg trifluoroacetic acid (specific activity 17.55 μCi/mg)/kg body weight. Whole-body autoradiography was carried out as described by Ullberg.[12] The tissue sections were routinely exposed to film for 10 weeks, except where otherwise indicated. Routine handling of the 40-μ tissue sections was found to result in complete loss of volatile radioactivity. This was established by comparison of autoradiograms of nonvolatile radioactivity obtained from sections prepared routinely with those obtained from similar (and the same) sections heated to 85 C under a vacuum for five days before exposure to film. The autoradiogram of volatile radioactivity was prepared as described by Cohen and Hood,[3] by exposure of the tissue section to film for a week. This autoradiogram is derived from both volatile and nonvolatile radioactivity, but because of the large concentration difference (a tenfold increase in exposure time is necessary for satisfactory visualization of nonvolatile metabolites; see fig. 3) the contribution of nonvolatile radioactivity to the autoradiogram (fig. 2) is negligible.

Nonvolatile radioactivity in dried (24 hours at 80 C under a vacuum) powders of whole-animal homogenates and tissue samples was determined in duplicate by total combustion of the samples in a Packard Sample Oxidizer and absorption of $^{14}CO_2$ in scintillator cocktail I ¶ prior to liquid scintillation counting. If the samples had not been dried prior to analysis, the measured radioactivity would have included volatile and nonvolatile radioactivity. Nonvolatile radioactivity in urine and feces was determined by counting 1 ml of an aqueous slurry in 10 ml of scintillator cocktail II ** in duplicate. Halothane in bile samples was collected under acid-washed heptane directly from the cannula, extracted into the organic layer, and estimated by gas chromatog-

* New England Nuclear Corp., Boston, Mass. (C^*F_3 CHClBr). This ^{14}C-halothane was labelled on the same carbon atom as that obtained from the same source by other workers (personal communications from E. N. Cohen). Due to differences in nomenclature, this ^{14}C-halothane has been described as halothane-2^{14}C, as well as ^{14}C-1-halothane.

† Duphar Medical, Basingstoke, England.

‡ Hook and Tucker, London, England.

§ Fluothane is a trade mark, property of I.C.I. Ltd.

¶ Scintillator cocktail I contains 4 ml ethanolamine + 9 ml methanol + 6 ml toluene containing 2 per cent butyl P.B.D. [2-(4-t-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxiazole] (Ciba (A.R.L.), Duxford, Cambridge, England).

** Scintillator cocktail II cointains 0.6 per cent butyl P.B.D. in a mixture of 1 part Triton ×100 and 2 parts toluene.

TABLE 1. Distributions of Nonvolatile Radioactivity in Mice after an iv Dose of ^{14}C-halothane: Concentrations*

Survival Time	Nonvolatile Radioactivity as μg Halothane/g Wet Tissue			
	Muscle	Liver	Lung	Brain
10 minutes	0.09	0.30	0.11	0.15
2 hours	0.03	0.08	0.06	0.03
26 hours	0.03	0.07	0.05	0.03
4 days	Trace	Trace	Trace	Trace

* Tissue samples were taken from animals used in the whole-body autoradiographic study. Nonvolatile was radioactivity determined in duplicate samples, as described in Materials and Methods.

TABLE 2. Distributions of Nonvolatile Radioactivity in Mice after an iv Dose of ^{14}C-halothane: Tissue/Muscle Ratios

Survival Time	Ratio of Concentration of Nonvolatile Radioactivity in Tissue to That in Muscle		
	Liver/Muscle	Lung/Muscle	Brain/Muscle
10 minutes	3.3	1.2	1.7
2 hours	2.7	2.0	1.0
26 hours	2.3	1.7	1.0

TABLE 3. Distributions of Nonvolatile Radioactivity in Mice after 10 Minutes of Anesthesia with ^{14}C-halothane: Concentrations*

Survival Time	Nonvolatile Radioactivity as μg Halothane/g Wet Tissue		
	Muscle	Liver	Brain
10 minutes	0.53	10.7	1.36
2 hours	18.4	78.8	17.4
26 hours	29.8	71.2	33.5
4 days	5.53	16.0	4.80

*Tissue samples were taken from animals used in the whole-body autoradiographic study. Nonvolatile radioactivity was determined in duplicate samples, as described in Materials and Methods.

TABLE 4. Distributions of Nonvolatile Radioactivity in Mice after 10 Minutes of Anesthesia with ^{14}C-halothane: Tissue/Muscle Ratios

Survival Time	Ratio of Concentration of Nonvolatile Radioactivity in Tissue to That in Muscle	
	Liver/Muscle	Brain/Muscle
10 minutes	20.2	2.6
2 hours	4.3	0.9
26 hours	2.4	1.1
4 days	2.9	0.9

raphy (F & M instrument fitted with a 6-foot × 3/10-inch column packed with Porapak 80-100, injection temperature 175 C, column temperature 150 C; carrier gas argon: 90 per cent argon purge + 10 per cent methane gas; flow rate 60 ml/min; detector, electron-capture 150-μCi tritium foil, detector temperature 200 C; sample size: 1 μl in acid-washed heptane).

The quantitative data in tables 1–4 support the semiquantitative whole-body autoradiographic data. Fuller data on tissue distribution and biliary excretion of nonvolatile metabolites of halothane will be presented in the second paper in this series,[7] together with results of metabolic studies in animals and man. The data in tables 1–4 are expressed both as absolute values and as tissue/muscle ratios to demonstrate the large differences between the distribution and excretion of nonvolatile metabolites after iv and inhalation administration of halothane. The tissue/muscle ratio was chosen as a convenient index because muscle tissue does not appear to have any specific or specialized function in the metabolism, storage, or elimination of halothane or its nonvolatile metabolites.

Because it is not possible to include all the tissues in an animal in a single 40-μ section, some of the data discussed are not directly illustrated in the figures (*e.g.* although radioactivity was present in the eye of the mouse two hours after iv administration of trifluoroacetic acid, this organ is not shown in fig. 8, but the eye is clearly shown in fig. 9, which refers to the distribution of radioactivity 26 hours after administration). Because of the limitations of photographic reproduction of whole-body autoradiograms, some differences between concentrations of radioactivity readily observable on the original autoradiograms are not as evident in the figures (*e.g.*, the very high concentration of nonvolatile radioactivity in bile in fig. 8 and the low concentration of nonvolatile radioactivity illustrated in fig. 10).

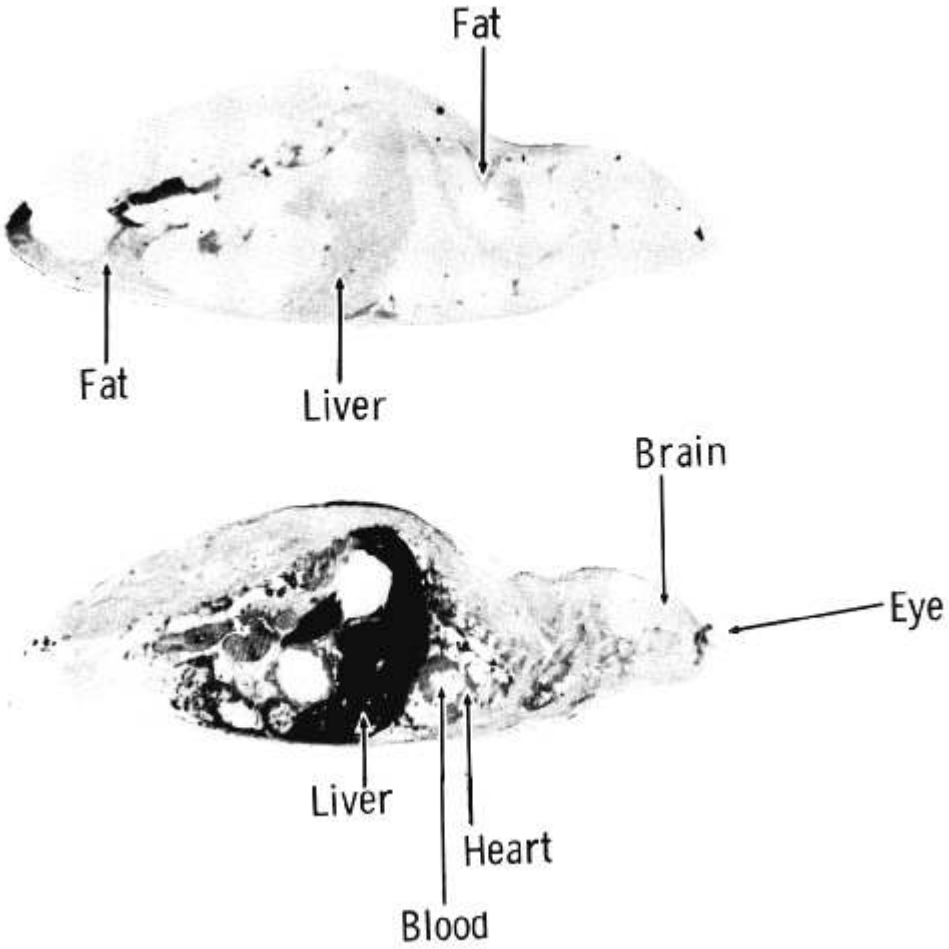

FIG. 2 (*above*). Distribution of volatile radioactivity in a mouse 10 minutes after an iv dose of ^{14}C-halothane (exposure time 1 week).
FIG. 3. Distribution of nonvolatile radioactivity in the same mouse 10 minutes after an iv dose of ^{14}C-halothane.

Results

HALOTHANE IV STUDY

Mice were killed 10 minutes, 1 hour, 2 hours, 1 day, and 4 days after iv administration of ^{14}C-halothane. Rats were killed 1 hour and 1 day after iv administration of ^{14}C-halothane High levels of volatile radioactivity were present in fat and liver 10 minutes after administration of halothane (fig. 2). Nonvolatile radioactivity in the same animal (fig. 3) was widely distributed throughout all the tissues, including brain, but a relatively high level was present in the liver. One and two hours after administration of halothane, the distributions of nonvolatile radioactivity in the mouse were similar to that observed earlier, except that the levels had declined in all tissues and the ratio of the concentration of nonvolatile radioactivity in the liver to that in muscle (the liver/muscle ratio of nonvolatile radioactivity) was reduced. After a day, all the tissue levels in the mouse had declined further, and only the bladder contained a high level of nonvolatile radioactivity. Four days after halothane administration, barely detectable levels were present in the mouse.

The distribution of nonvolatile radioactivity

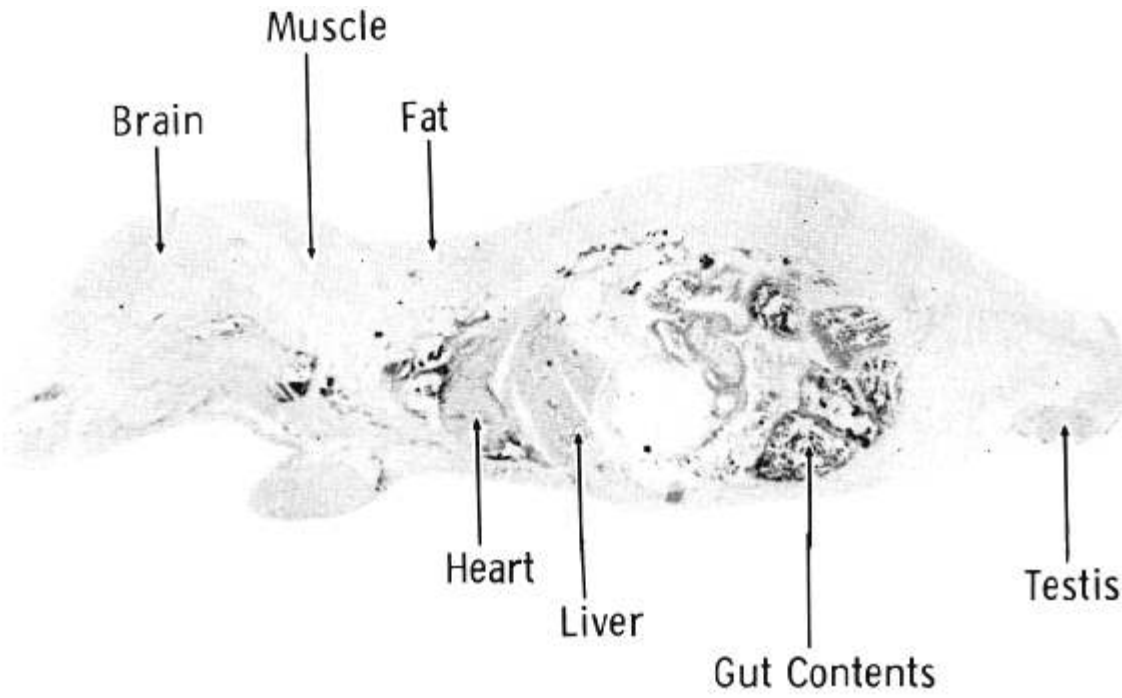

FIG. 4. Distribution of nonvolatile radioactivity in a rat one day after an iv dose of ^{14}C-halothane.

in the rat (fig. 4) was identical to that in the mouse, except that the levels of nonvolatile radioactivity were about twice those in the mouse at the corresponding times. The semiquantitative observations in mice were confirmed by quantitative determination of the concentrations of nonvolatile radioactivity in tissue samples from these mice (table 1).†† The levels of nonvolatile radioactivity in muscle, liver, lung, and brain were highest 10 minutes after administration, and declined to barely detectable levels after four days. The liver/muscle ratio of nonvolatile radioactivity declined for as long as a day after iv administration (table 2).

HALOTHANE INHALATION STUDY (10-MINUTE ANESTHESIA)

Mice were killed immediately after 10 minutes of anesthesia, and 2 hours, 5 hours, 1 day, 4 days, and 14 days later. Immediately after anesthesia the highest levels of nonvolatile radioactivity were present in the nasal mucosa and lung, but relatively high levels were also present in the liver and the intestine wall and contents (fig. 5). Very little nonvolatile radioactivity was present elsewhere in the animal. Two hours after administration of halothane (fig. 6), nonvolatile radioactivity was present in all tissues, with high levels in the liver, bile, intestinal contents, and urine. Although not shown in figure 6, high levels of nonvolatile radioactivity were also present in the thyroid and the lens and retina of the eye. The higher level of nonvolatile radioactivity present in the animal killed 2 hours after administration was expected because of metabolism of halothane retained in the animal after anesthesia has ended. Halothane, of course, is not shown on these autoradiograms of nonvolatile radioactivity. Similar patterns of distribution were observed five hours and one day after anesthesia. After four days (fig. 7), considerable excretion of nonvolatile radioactivity had occurred. The level in the liver remained quite high, although

†† Tissues for quantitative analysis were not available from all animals because after an adequate number of sections had been obtained for autoradiography the remaining tissue was sometimes insufficient for satisfactory quantitative analysis.

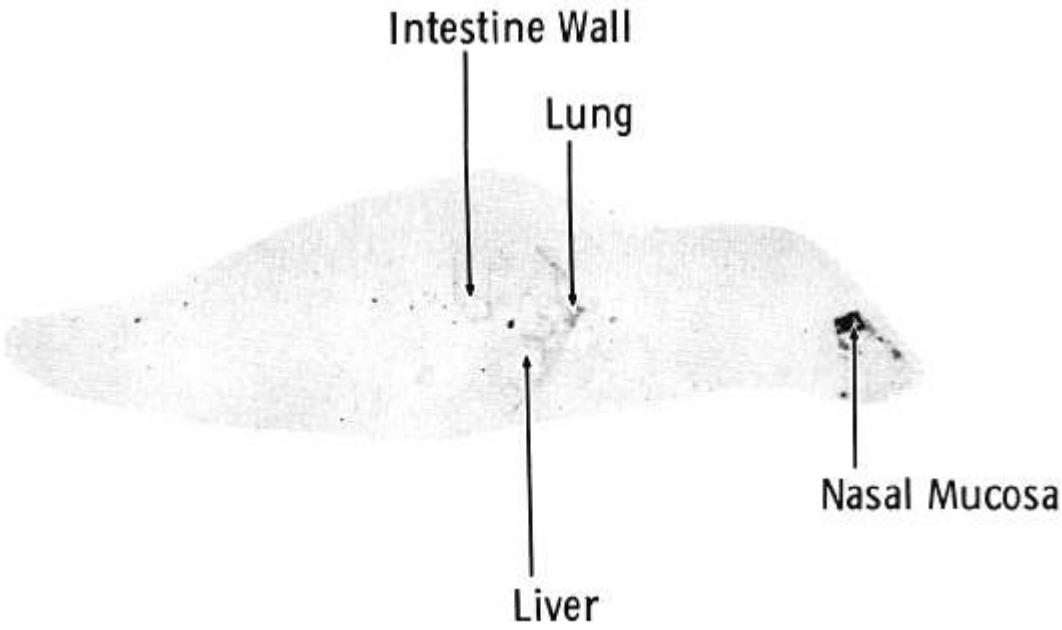

FIG. 5. Distribution of nonvolatile radioactivity in a mouse immediately after 10 minutes of anesthesia with ^{14}C-halothane.

the highest level was present in the thyroid. Only by increasing the sensitivity, by doubling the period of exposure of the sections to the film, could traces of nonvolatile radioactivity associated with the thyroid, liver, and kidney be detected 14 days after anesthesia. Only low levels of nonvolatile radioactivity were present in fecal pellets at any time.

These semiquantitative studies were confirmed by quantitative analysis of the nonvolatile radioactivity in tissue samples from these mice (table 3). The levels in muscle, liver, and brain were highest 2 hours and 1 day after anesthesia. The liver/muscle ratio declined rapidly between 10 minutes and two hours after anesthesia (table 4). After two hours, the liver/muscle ratio declined more slowly after anesthesia than after iv administration of halothane.

Trifluoroacetic-acid Study

Mice were killed 2 hours, 1 day, 4 days, and 11 days after iv administration of the sodium salt of ^{14}C-trifluoroacetic acid. Two hours after administration (fig. 8), radioactivity was present in all tissues, with slightly higher levels in blood, bile, intestine contents, and eye than in other tissues. A day after administration (fig. 9), extensive excretion had occurred, but the residual radioactivity was distributed throughout all the tissues. The labelling of the intestinal mucosa had become more marked, and slightly higher levels were maintained in the eye, bile, and urine. Four days after administration, the distribution of activity was similar to that observed after 1 day. Only by increasing the sensitivity, by doubling the period of exposure of the section to the film, could traces of radioactivity be detected, in the urine only, 11 days after administration of ^{14}C-trifluoroacetic acid.

Distribution of Nonvolatile Radioactivity in Mice after Numerous Periods of Anesthesia with ^{14}C-halothane

^{14}C-halothane was diluted with Fluothane to a specific activity of 2.07 μCi/g and used to anesthetize a group of mice for five-hour periods in a closed system (fig. 1). The concentration of ^{14}C-halothane in the chamber was maintained between 2.5 and 1.0 per cent v/v during anesthesia. The animals were anesthetized on days 1, 2, 3, 4, 7, 8, 9, 10, 15, 16, 17, 18, 21, 22, 24, and 25. Individual animals

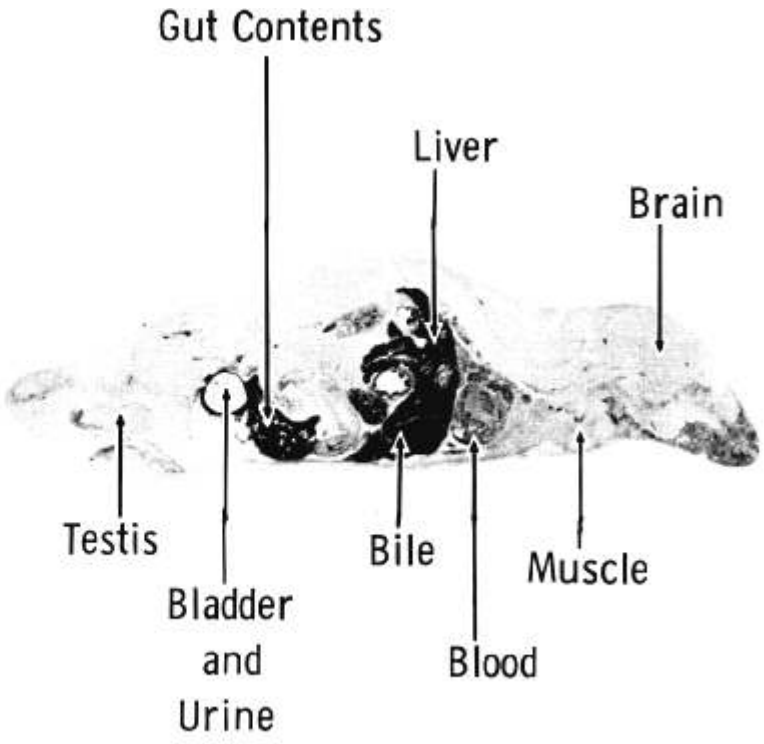

FIG. 6. Distribution of nonvolatile radioactivity in a mouse two hours after 10 minutes of anesthesia with ^{14}C-halothane.

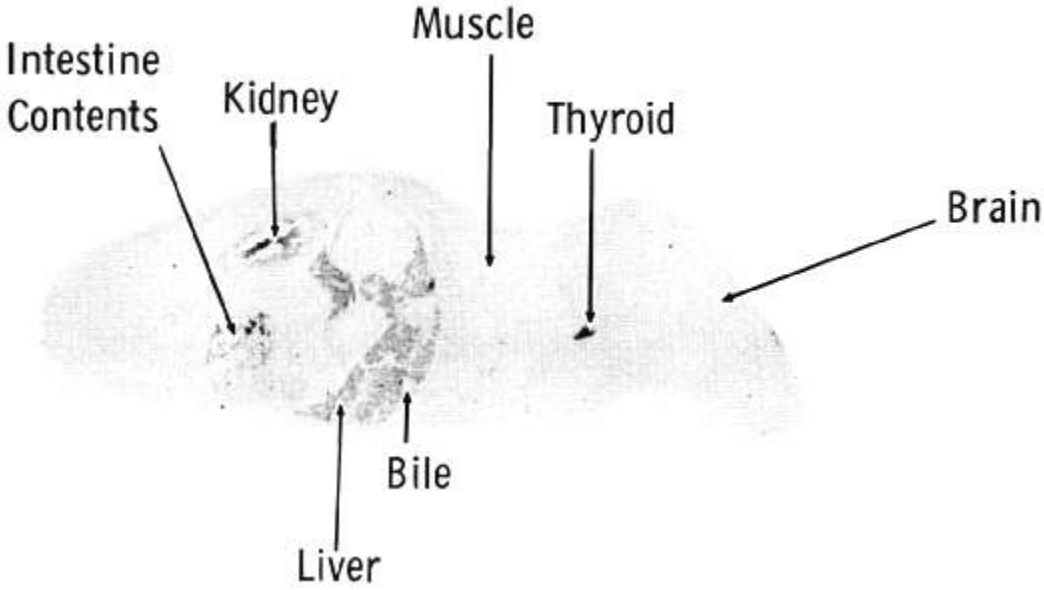

FIG. 7. Distribution of nonvolatile radioactivity in a mouse four days after 10 minutes of anesthesia with ^{14}C-halothane.

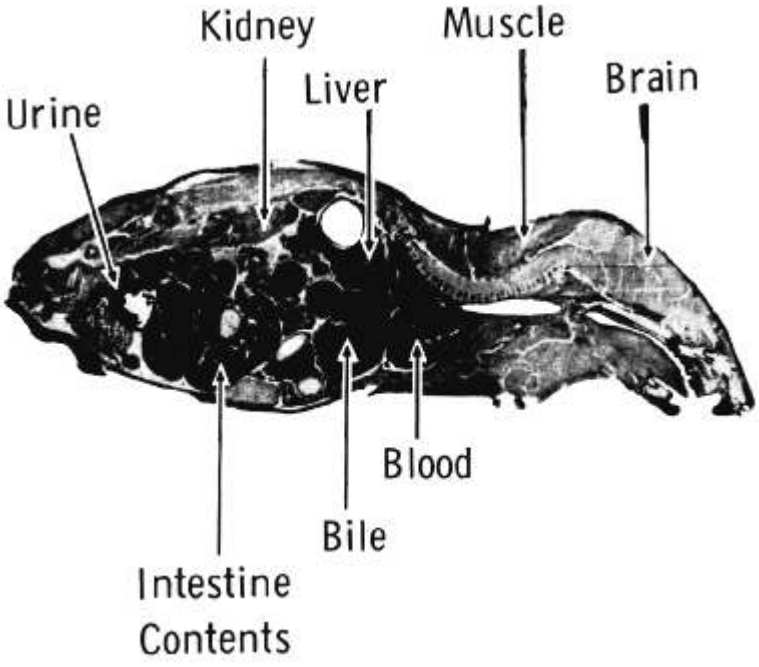

FIG. 8. Distribution of radioactivity in a mouse two hours after an iv dose of ^{14}C-trifluoroacetic acid.

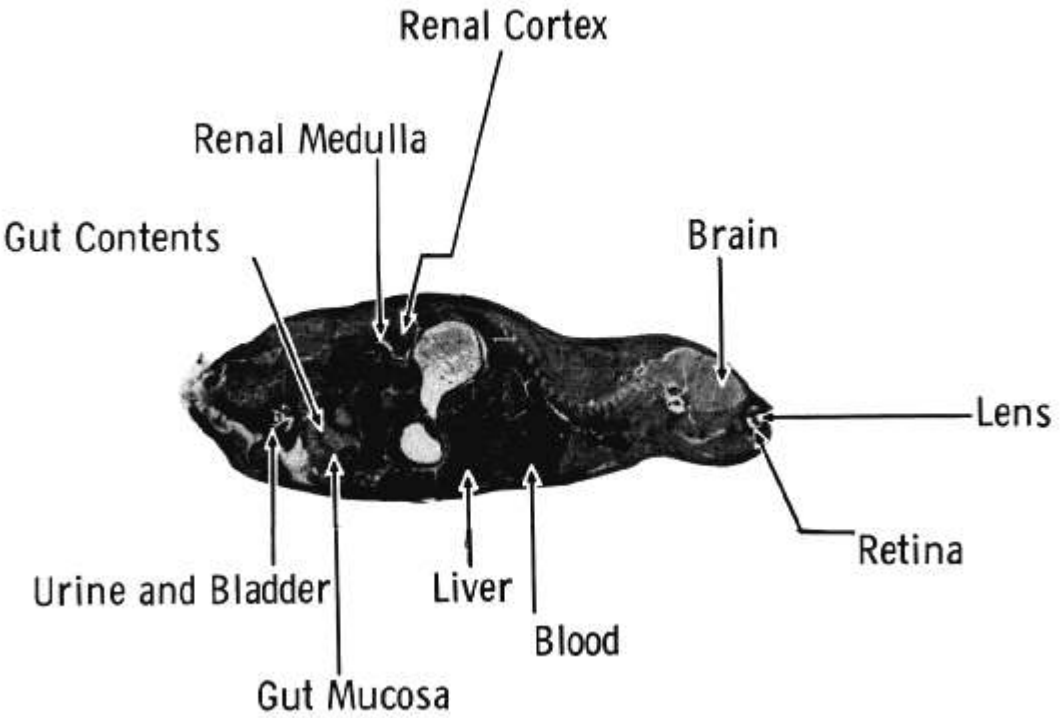

FIG. 9. Distribution of radioactivity in a mouse 26 hours after an iv dose of ^{14}C-trifluoroacetic acid.

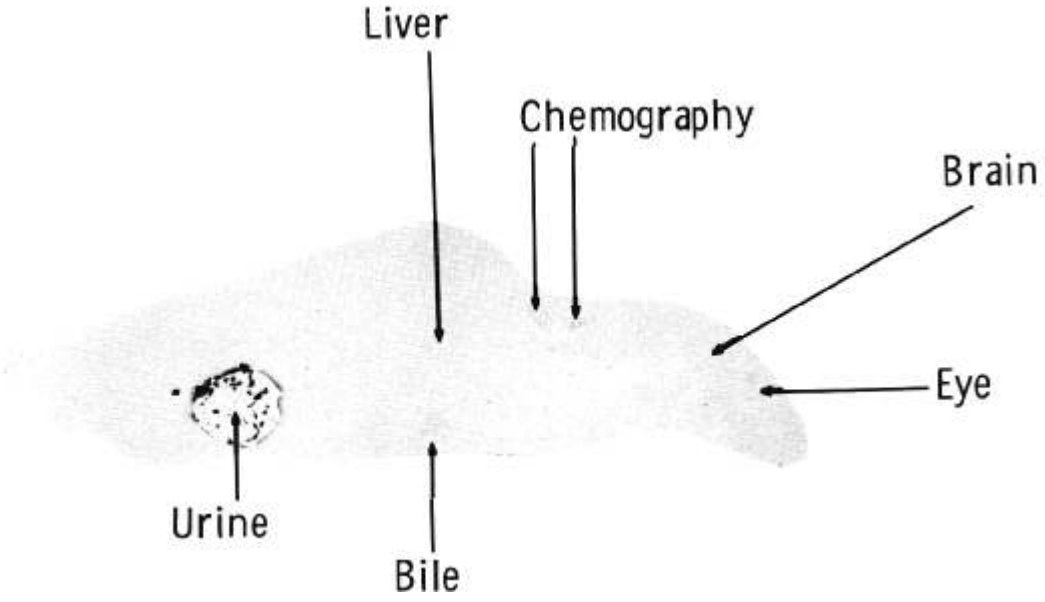

FIG. 10. Distribution of nonvolatile radioactivity in a mouse killed immediately after the tenth five-hour period of anesthesia with ^{14}C-halothane (12 months of exposure of section to film).

were killed for whole-body autoradiography immediately after anesthesia on days 1 (one period of anesthesia), 9 (seven periods of anesthesia), 16 (ten periods of anesthesia), and 25 (15 periods of anesthesia). Additional animals were also killed for whole-body autoradiography an hour after recovery from anesthesia on day 1 and day 9. Because of the numbers of animals used, statistical analysis of these results was not appropriate; however, the data clearly support the conclusions and the interpretation of the results presented.

Because of the low specific activity of the halothane used, it was necessary to expose the sections to film for 12 months to obtain satisfactory autoradiograms. Because of the possibility of chemographic artifacts, sections of untreated animals were also exposed to film for 12 months. No chemography that could be confused with the blackening caused by radioactivity was observed.

No differences between distributions of nonvolatile radioactivity in animals killed immediately after anesthesia and those killed an hour later were found. The quantities and distributions of nonvolatile radioactivity were the same in all mice examined, whether they had been subjected to 1, 7, 10, or 15 periods of anesthesia.

Nonvolatile radioactivity was present in all tissues, including brain, but the highest concentrations were present in the bile and urine (in the bladder and kidney). The levels in the blood, liver, eye, and lung were only slightly higher than those in muscle (fig. 10).

RATE OF HALOTHANE METABOLISM IN MICE

Thirteen male mice were anesthetized with ^{14}C-halothane (specific activity 6.85 μCi/g) (fig. 1) and killed in groups of three immediately after anesthesia (*i.e.*, before recovery), and 1, 3, and 23 hours after the end of anesthesia (four were killed after 23 hours). After anesthesia all animals were housed individually and any urine and feces collected. The total amount of halothane metabolized was assessed by analysis of nonvolatile metabolites in total-body homogenates plus excreta (table 5).

If the contribution of metabolism at the shorter survival times is allowed for, the decrease in the rate of conversion of halothane to nonvolatile metabolites following anesthesia becomes more obvious (table 6).

TABLE 5. Conversion of Halothane (1–2 Per Cent in the Circulating Gas) to Nonvolatile Metabolites by Mice during an Hour of Anesthesia and in the Following 23 Hours

Survival Time	μg Nonvolatile Metabolites* Formed/g Body Weight Mean (Range)	μg Nonvolatile Metabolites* Formed/g Body Weight /Hour Mean (Range)
1 hour (period of anesthesia)	49 (37–56)	49 (37–56)
2 hours (period of anesthesia + 1 hour)	86 (72–102)	43 (36–51)
4 hours (period of anesthesia + 3 hours)	140 (132–163)	36 (33–41)
24 hours (period of anesthesia + 23 hours)	318 (268–366)	13 (11–15)

* Expressed as μg halothane metabolized.

TABLE 6. Rate of Conversion of Halothane to Nonvolatile Metabolites during and after a One-hour Period of Anesthesia

	μg Nonvolatile Metabolites* Formed/g Body Weight /Hour (Mean)	μg Nonvolatile Metabolites* Formed/g Body Weight (Mean)
First hour (during anesthesia)	49	49
Second hour (after recovery)	37	37
Third and fourth hours (after recovery) (mean)	30	60
Fifth–24th hours (after recovery) (mean)	9	180

* Expressed as μg halothane metabolized.

EXCRETION OF UNCHANGED HALOTHANE INTO DOG AND RAT BILE

Serial bile samples were collected via a Portex tubing bile-duct cannula from three male rats anesthetized with halothane, 1.0 per cent. Unchanged halothane was progressively excreted into the bile (table 7). Similar serial bile samples were collected via a glass bile-duct cannula from two male dogs anesthetized with halothane, 0.5 per cent. Unchanged halothane was again progressively excreted into the bile (table 8).

These data suggest that the amount of unchanged halothane excreted in the bile of rats and dogs increases during anesthesia. In both species, individual variations in the rates of halothane excretion in bile appeared to be partly owing to individual differences in the rates of bile secretion. Excretion of unchanged halothane in bile was, of course, additional to the excretion of any metabolites of halothane in bile.

Discussion

The whole-body autoradiographic studies with halothane and trifluoroacetic acid showed that in mice: 1) The distribution of nonvolatile metabolites was dissimilar to that of halothane, confirming the observations of Cohen and Hood.[3] 2) At similar times after a single administration, the liver/muscle ratios of nonvolatile metabolites of halothane were higher in six mice that received halothane by inhalation during a 10-minute period of anesthesia than in five mice that received halothane iv. This difference may be the result of different distributions of the larger amounts of metabolites formed after the much higher dose (×100 approx.) of halothane administered by inhalation, because the proportions of various metabolites formed after halothane administration depend on the size of the dose.[7] 3) After a single administration of halothane (iv or 10 minutes of anesthesia), the liver/muscle ratio of nonvolatile radioactivity decreased for periods lasting as long as four days. The very high liver/muscle ratio of nonvolatile radioactivity observed immediately after 10 minutes of anesthesia may be the result of incomplete equilibration of metabolites formed in the liver with other tissues. 4) The initial very rapid decline of the liver/muscle ratio of nonvolatile radioactivity after a single 10-minute period of anesthesia was reduced to a rate somewhat slower than that observed after iv administration of halothane, presumably because of efficient metabolism of halothane that was slowly released from storage depots. Because of the larger dose administered by inhalation, much greater amounts of halothane would be present in storage depots than after iv administration. 5) The similarity of the levels of nonvolatile radioactivity in tissues two

TABLE 7. Excretion of Halothane in Rat Bile during Anesthesia

Sample Period	μg Halothane Excreted*			μg Halothane Excreted/Hour*		
	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3
0–0.5 hours	6.9	1.2	2.0	13.8	2.4	4.0
0.5–1.0 hours	10.4	4.9	3.6	20.8	9.8	7.2
1.0–1.5 hours	14.4	8.1	4.6	28.8	16.2	9.2
1.5–2.0 hours	11.1	8.9	7.8	22.2	17.8	15.6
2.0–3.0 hours	33.8	12.2	18.0	33.8	12.2	18.0
3.0–4.0 hours	28.3	37.2		28.3	37.2	

* Individual variation in the rates of halothane excretion appeared to be partly owing to differences in the rates of bile secretion.

TABLE 8. Excretion of Halothane in Dog Bile during Anesthesia

Sample Period	μg Halothane Excreted*		μg Halothane Excreted/ Hour*		Total μg Halothane Excreted*	
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
0–1.0 hours	0	3.0	0	3.0	0	3.0
1.0–1.5 hours	0	5.7	0	11.4	0	8.7
1.5–2.5 hours	1.2	16.0	1.2	16.0	1.2	24.7
2.5–4.0 hours	16	60.8	10.7	40.6	17.2	85.5
4.0–4.5 hours	12.5	50.4	25.0	100.8	29.7	135.9

* Individual variation in the rates of halothane excretion appeared to be partly owing to differences in the rates of bile secretion.

hours and one day after a single halothane administration (iv or 10 minutes of anesthesia, tables 1 and 3) may be owing to a balance between the elimination of nonvolatile metabolites and their efficient formation from residual halothane slowly released from storage depots. 6) Immediately after a 10-minute period of inhalation of halothane, high levels of nonvolatile radioactivity were present in the nasal mucosa and lung. 7) Nonvolatile radioactivity was retained in the thyroid after 10 minutes of inhalation but not after iv administration or prolonged inhalation of halothane or iv administration of trifluoroacetic acid (figs. 4, 7, 9, and 10). This finding may result from effects similar to those mentioned in 2, above. 8) The distribution of radioactivity following administration of trifluoroacetic acid was qualitatively similar to the distribution of nonvolatile radioactivity after iv administration of halothane, except that relatively high levels of nonvolatile radioactivity were not observed in the liver. (Trifluoroacetic acid would be present as salts at physiologic *p*H and, therefore, would not be volatile under our experimental conditions.) 9) Excretion of nonvolatile radioactivity occurred in urine and bile, but since little was present in the fecal pellets, enterohepatic recirculation probably occurred. This conclusion is supported by other metabolic and excretion data.[7] Enterohepatic recirculation may be largely responsible for the long half-life (50 hours approx. in several species) of nonvolatile radioactivity observed after halothane or trifluoroacetic acid administration [7, 13, 14] and for the accumulation of nonvolatile radioactivity during repeated administration of halothane.[15] 10) After a single five-hour period of anesthesia with halothane, the distributions of nonvolatile metabolites of halothane were remarkably uniform in all animals. After numerous five-hour periods there was no change in distribution, indicating that under these conditions no detectable accumulation of nonvolatile metabolites of halothane occurred in mice. The reasons for these results may be similar to those mentioned in 2, 5, and 9 above. Cohen [15] demonstrated progressive increases in the concentrations of nonvolatile metabolites in the livers of mice after as many as five weekly injections of halothane; these mice were killed seven days after each injection; this procedure and the different amounts of halothane administered iv may be reasons for the differences between Cohen's results [15] and ours.

We cannot suggest explanations for every observation that we have reported, but have limited our discussion to those interpretations that seem most reasonable and to those results that appear to be of the greatest interest.

Our results are broadly similar to those reported by Cohen and Hood.[3] However, they did not observe the initial high levels of nonvolatile radioactivity in the nasal mucosa and lung, or (probably because their observation period of two hours was too short) the retention of nonvolatile radioactivity in the thyroid or enterohepatic recirculation after anesthesia. Cohen and Hood [3] expressed their data on concentrations of nonvolatile radioactivity (metabolites) in tissues as percentage of total radioactivity (halothane plus metabolites) in the tissue. As halothane is eliminated (unchanged in exhaled air)[1] much more rapidly than the nonvolatile metabolites are excreted in urine and feces, inevitably the percentage of total

radioactivity present as nonvolatile radioactivity in tissues increases progressively after anesthesia. Cohen and Hood [3] also demonstrated that the levels of nonvolatile metabolites in all tissues increased in the postanesthetic period. We have confirmed this observation and demonstrated that the differences between the levels of nonvolatile radioactivity in tissues are progressively reduced for as long as four days after halothane administration, and that selective retention in the liver does not occur, although after inhalation of halothane the absolute levels of nonvolatile radioactivity in the liver remain higher than those in other tissues (table 3), and the liver/muscle ratio (table 4) shows little change between one and four days.

The different distributions of nonvolatile radioactivity observed in the present study after iv and inhalation administration of halothane illustrate the hazards of interpreting data based on iv administration of a compound that should always be given by another route clinically. Apart from effects directly related to the routes of administration, these differences could be owing to variations in the sensitivity of the assay methods, resulting from the different radiochemical doses (and specific activities) used, or to the large variation in chemical doses employed.

These problems are particularly pertinent to the metabolism of volatile anesthetics, because many published data relate to studies with subanesthetic doses (with widely differing specific activities) administered by a variety of routes.[2, 3, 5, 8, 11, 15] Quantitative aspects of work on the metabolism, distribution, and excretion after ip or iv or oral dosing have little relevance to the behavior of effective doses of volatile anesthetics administered by inhalation.

Sawyer *et al.*[10] have suggested that high concentrations of halothane may inhibit its own metabolism. Our data obtained with mice clearly showed that, although quantitatively more metabolism of halothane occurred after anesthesia, the rate of metabolism was highest during anesthesia and declined progressively during the 23 hours after recovery. Substantial amounts of nonvolatile metabolites were formed during the fifth to twenty-third hours following anesthesia, presumably by efficient metabolism of low concentrations of halothane that are slowly released from storage depots.

Examples of the excretion of volatile compounds unchanged directly into bile are known [16, 17]; however, the direct excretion of halothane into bile has not been studied. We have demonstrated that significant quantities of halothane are excreted unchanged into the bile of the rat and dog, and that the rate of biliary excretion of halothane increases during inhalation of a constant concentration of halothane for several hours. Hepatic extraction of anesthetics has been equated with their metabolism by Sawyer *et al.*[10] and Halsey *et al.*[18] in their studies with miniature swine. The excretion of unchanged halothane in bile of the rat and dog indicates that hepatic extraction in these species results from both metabolism of halothane and biliary excretion of unchanged halothane. Similar estimations have not been made for miniature swine, but such data are needed before hepatic extraction is equated with metabolism of halothane in this species.

The authors thank Dr. D. M. Foulkes and Dr. M. J. Winrow for valuable discussions, and Dr. J. S. L. Fowler and Mr. R. D. Broad for the surgical preparation of the animals with bile-duct cannulae.

References

1. Duncan WAM, Raventos J: The pharmacokinetics of halothane (Fluothane) anaesthesia. Br J Anaesth 31:302–315, 1959
2. Van Dyke RA, Chenoweth MB, Larsen ER: Synthesis and metabolism of halothane-1-^{14}C. Nature 204:471–472, 1964
3. Cohen EN, Hood N: Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse: III. Halothane. ANESTHESIOLOGY 31:553–559, 1969
4. Cohen EN: Metabolism of the volatile anesthetics. ANESTHESIOLOGY 35:193–202, 1971
5. Stier A: Trifluoroacetic acid as a metabolite of halothane. Biochem Pharmacol 13:1544, 1964
6. Stier A, Gerth I: Zum Stoffwechel von Halothane. Arch Exp Pathol Pharmakol 250:284, 1965
7. Winrow MJ, Hedgecox E, Foulkes DM: Studies with halothane. II (in preparation)
8. Van Dyke RA, Chenoweth MB, Van Poznak A: Conversion *in vivo* of several anesthetics to $^{14}CO_2$ and chloride. Biochem Pharmacol 13: 1237–1247, 1964
9. Stier A, Alter H, Hessler O et al: Urinary

excretion of bromide in halothane anesthesia. Anesth Analg (Cleve) 43:723–728, 1964
10. Sawyer DC, Eger EI II, Bahlman SH, et al: Concentration dependence of hepatic halothane metabolism. ANESTHESIOLOGY 34:230–235, 1971
11. Cohen EN, Hood N: Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. I. Chloroform. ANESTHESIOLOGY 30:306–314, 1969
12. Ullberg S: Studies on the distribution and fate of S^{35} labelled benzylpenicillin in the body. Acta Radiol suppl 118:1–110, 1954
13. Winrow MJ, Strunin L, Simpson R: Studies with halothane: III (in preparation)
14. Cascorbi HF, Blake DA, Helrich M: Differences in the biotransformation of halothane in man. ANESTHESIOLOGY 32:119–123, 1970
15. Cohen EN: Metabolism of halothane-2-^{14}C in the mouse. ANESTHESIOLOGY 31:560–565, 1969
16. Fowler JSL: Carbon tetrachloride in sheep and in *Fasciola Hepatica*. *Br J Pharmacol* 39: 599–607, 1970
17. Langley PF: Whole-body Autoradiography of Water-Soluble Compounds Using the Sawbench Technique. Symposium on Autoradiography, Nottingham, 1971
18. Halsey MJ, Sawyer DC, Eger EI, II, et al: Hepatic metabolism of halothane, methoxyflurane, cyclopropane, Ethrane and forane in miniature swine. ANESTHESIOLOGY 35: 43–47, 1971

Drugs and Their Actions

LEVODOPA AND HEMODYNAMIC FUNCTION The effects of levodopa, dopamine, and epinephrine on myocardial contractility, blood pressure, and heart rate were evaluated in man during acute (two weeks) and chronic (three months) treatment of Parkinson's disease. During the first two weeks, levodopa in doses of 1 to 1.5 g exerted a positive inotropic effect, maximal at 30–60 minutes and significant for 90 minutes after 1 g and 120 minutes after 1.5 g. Blood pressure was reduced minimally and heart rate was unaffected. Propranolol, 10 mg, prevented the inotropic effect. The effect also disappeared after three months. The authors conclude that levodopa exerts its positive inotropic effect via beta-adrenergic receptors; the tolerance developed after three months is not attributable to impaired responsiveness of the heart, since dopamine and epinephrine exert similar effects during both acute and chronic administration. (*Whitsett, T. L., and Goldberg, L. I.: Effects of Levodopa on Systolic Preejection Period, Blood Pressure, and Heart Rate during Acute and Chronic Treatment of Parkinson's Disease, Circulation 45: 97–196, 1972.*)

RITODRINE AND UTERINE MOTILITY Unwanted uterine activity is encountered in premature labor, in selected instances of labor associated with fetal distress, and in uterine hypertonus resulting from injudicious use of oxytocic preparations. Appropriate pharmacologic treatment of premature labor might reduce neonatal mortality and the incidence of neurologic abnormality. The uterine and cardiovascular responses to ritodrine hydrochloride were studied in 16 patients immediately post partum and in 17 patients during term labor. Data obtained from electronic monitoring of maternal and fetal responses to ritodrine were compared with those of the control series. Except for its effect on maternal heart rate (tachycardia), ritodrine is an effective inhibitor of uterine motility with minimal cardiovascular activity. It appears promising for the treatment of unwanted uterine activity. (*Barden, T. P.: Effect of Ritodrine on Human Uterine Motility and Cardiovascular Responses in Term Labor and the Early Postpartum State, Am. J. Obstet. Gynecol. 112: 645–652, 1972.*)