Pharmacokinetics of Trichloroethanol and Metabolites and Interconversions among Variously Referenced Pharmacokinetic Parameters

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Abstract □ The pharmacokinetics of intravenously administered trichloroethanol, chloral hydrate, sodium trichloroacetate, and trichloroethanol glucuronide were studied in the dog with developed GC analyses of blood, urine, and feces, and analyzed with aid of analog and digital computation. Trichloroethanol pharmacokinetics were evaluated at multiple-dosage levels and showed distribution from a central compartment assignable to extracellular body water into readily available and less available tissues assignable to total body water and lipoidal and other tissues. The major route of removal of trichloroethanol in the dog was by conjugation to the glucuronide, and the first-order rate constants of this metabolic route were dose dependent but did not appear to be a consequence of enzymic saturation or change in apparent volumes of distribution with dose. A possible explanation is that the hepatic lipid–water ratio may vary with the dose of trichloroethanol. The formed trichloroethanol glucuronide, after release from the liver, was rapidly distributed in the extracellular body water of the dog and rapidly eliminated in the urine by glomerular filtration and tubular secretion. Biliary elimination of the glucuronide accounted for about 5% of an intravenous dose of trichloroethanol. Saturation of the bile secretory mechanism was demonstrated at higher trichloroethanol doses, *e.g.*, 100 mg./kg., but the absence of trichloroethanol and its glucuronide in the feces may be explained by hydrolysis of the biliary glucuronide in the GI tract with subsequent reabsorption of the formed trichloroethanol, *i.e.*, an enterohepatic shunt. No assayable trichloroacetic acid was observed in the blood or urine of dogs dosed with trichloroethanol. A long apparent disposition half-life of 75 hr. was observed after intravenous administration of sodium trichloroacetate; this can be explained by the extensive tissue binding of trichloroacetate which gave high apparent volumes of distribution. Intravenously administered chloral hydrate was rapidly and quantitatively converted to trichloroethanol in the dog, with an apparent half-life of 3 min. Protein binding of trichloroethanol and its glucuronide (about 35%) was determined. Although the glucuronide did not partition into the red blood cells, the trichloroethanol did so instantaneously, with a partition coefficient of 2.1 in favor of the red blood cells. Since apparent volumes of distribution and microscopic pharmacokinetic rate constants have quantitative values that vary widely, depending on whether they are referenced to total blood, total plasma, or unbound drug in plasma concentrations, a detailed analysis of the conversions among these variously referenced constants was made and applied to the data of these studies. Estimates of the microscopic rate constants and apparent volumes of distribution referenced to unbound drug in plasma have the most valid physiological significances. These are complex functions of the hematocrit, the degree of protein binding, the true volume of plasma or blood in the animal, and the red blood cell/plasma partition coefficient when the drug is assayed per milliliter of whole blood or per milliliter of plasma. The awareness of these facts will necessitate extensive recalculation of many pharmacokinetic constants now given in the literature.

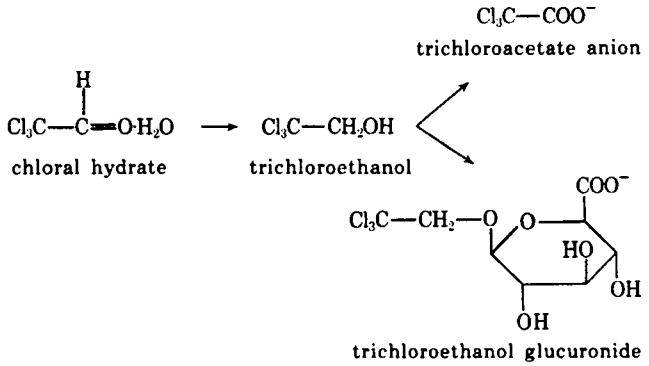
Keyphrases □ Trichloroethanol—pharmacokinetics and metabolites after intravenous administration, dogs □ Chloral hydrate—pharmacokinetics and metabolites after intravenous administration, dogs □ Pharmacokinetics—trichloroethanol, chloral hydrate, and metabolites after intravenous administration, dogs □ GLC—analysis, trichloroethanol and metabolites

The hypnotic effect of 2,2,2-trichloroethanol was first demonstrated in 1882 (1). It was previously reported that the product isolated from the urine of patients dosed with chloral hydrate was a conjugation product of trichloroethanol and glucuronic acid (2). The conjugated glucuronic acid was measured in rabbit urine, and it was concluded that chloral hydrate and trichloroethanol are both converted to the conjugated product in the same proportions (3).

Butler (4) and Marshall and Owens (5) noted that the CNS depression which follows the ingestion of chloral hydrate is due principally, if not entirely, to the trichloroethanol formed by reduction *in vivo*. The same investigators (4–6) demonstrated that the principal metabolic products of trichloroethanol are trichloroacetic acid and trichloroethanol glucuronide (Scheme I). Those metabolites did not appear to have hypnotic activity.

The major route of trichloroethanol metabolism in dogs was glucuronidation, and more than 60% of the dose was found in the urine as the glucuronide (5). This was more than twice that found in the urine of human subjects (5). Some of the glucuronide formed from an intravenous dose of trichloroethanol was secreted and concentrated in the bile of dogs (6). A small fraction of the dose was excreted unconjugated. Comparison of the species difference in trichloroacetate anion formation was stated to be difficult due to its probable metabolic destruction by the dog (5).

This paper presents the results of studies on the pharmacokinetics of trichloroethanol and its metabolites in the dog at various doses administered intravenously. These pharmacokinetic studies were based on analyses expressed in terms of total concentrations of drugs or metabolites in blood that give apparent volumes of distribution and microscopic rate constants referenced to such concentrations. These are different values than would be obtained if the analyses were expressed in terms of total concentrations or unbound concentrations in plasma. The latter values would be most consistent with the physiological referents and the physicochemical realities of drug disposition in the



Scheme I

body. The literature is replete with the presentation of such parameters variously referenced. Since the calculation of one set of reference parameters from another is necessary to compare those of the same and different drugs within and among species, rigorous expressions of such mathematical conversions are developed in a special section and applied to the pharmacokinetic data of this paper. The protein binding and red blood cell partitions necessary for these applications are determined.

The complete model for the pharmacokinetics of trichloroethanol and its metabolites that will be outlined here did not spring into being intuitively as a full-fledged entity. It had to be developed by sequential processes of systems analyses that included graphical analyses, analog simulation, and digital computation in accordance with a consistent model. A section of this paper delineates the systematic technological patterns and sequences of such logical analyses used in model construction and quantification.

The pharmacokinetic studies of trichloroethanol at various intravenously administered dose levels clearly show that the drug distributes by first-order processes into both a shallow and a deep compartment and that the only significant route of removal of trichloroethanol in the dog is by conjugation to the glucuronide. The apparent first-order rate constants of trichloroethanol are dose dependent. This does not appear to be due to changes in the apparent volumes of distribution of the major compartments, nor is it a consequence of enzyme saturation. The latter is denied by the facts that first-order processes persist at all dose levels and that simultaneous challenging *in vivo* with the potent glucuronidation inhibitor sulfobromophthalein indicated a large capacity for glucuronidation. However, an excessively large dose administered did decrease the rate of glucuronidation.

It is shown that the rate of appearance of trichloroethanol glucuronide in the body did not reflect the rate of loss of trichloroethanol. The glucuronide's appearance was delayed, and the maximum blood level was lower than would be predicted from the instantaneous loss of the alcohol. This implies an intermediate storage depot which can be assigned to the liver. The results of balance studies across the liver of a surgically modified dog are consistent with this hypothesis. It is shown that the phenomenon of trichloroethanol dose-dependent pharmacokinetics can be rationalized by postulating that the hepatic lipid–water ratio varies with the dose of trichloroethanol and that this effect on microscopic redistribution of trichloroethanol in the liver from an aqueous metabolic biophase modifies the metabolism rate.

It is explained that the renal clearance of glucuronide that decreases with the higher glucuronide concentration in blood, which results from higher doses of trichloroethanol, can be rationalized by postulating the saturation of the tubular secretory pathway for glucuronide.

The pharmacokinetic study on intravenously administered trichloroethanol glucuronide clearly shows that it is primarily eliminated by filtration and tubular secretion. Although about 5% of the dose is biliary excreted, the lack of significant glucuronide in the feces implicates extensive GI solvolysis and reabsorption of the resultant trichloroethanol. It is demonstrated that saturation of the bile secretory mechanism for glucuronide occurs at higher trichloroethanol doses as well as increased bile flow rates.

The pharmacokinetic studies on intravenously administered trichloroacetic acid demonstrate long dose-independent apparent half-lives of elimination, which can be rationalized by the high apparent volumes of distribution assignable to extensive tissue binding.

The pharmacokinetic studies of intravenously administered chloral hydrate demonstrate that it is rapidly and quantitatively converted to trichloroethanol in the dog, with an apparent half-life of 3 min.

EXPERIMENTAL

Materials—The following were used: glacial acetic acid[1], chloral hydrate USP[1], 5% trichloroacetic acid solution[1], chlorobutanol USP[1], anhydrous ether[2], anhydrous sodium acetate (analytical reagent)[3], mepesulfate (sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside)[4], and sulfosalicylic acid[5]. The β-glucuronidase was bovine liver B grade and had 50,000 units/vial or 360 Fishman units/mg.[6]. Sulfobromophthalein injection was 50 mg./ml.[7]. The heparin solution had 10,000 USP units/ml.[8]. The sodium pentobarbital[9] was in a sterile solution of 50 mg./ml. The trichloroethanol[6,10] was redistilled at 153° before use. The trichloroethanol glucuronide was the sodium salt of 95% purity on assay (7) and was isolated from the urine of dogs given repeated parenteral doses of trichloroethanol. The isolation procedure was described by Seto and Schultze (8). The acidified glucuronide melts at 142° and its triacetyl methyl ester melts at 158° (8).

Analytical Methods—Sensitive and specific GC assays for trichloroethanol, its chlorohydrate precursor, and its metabolites, trichloroethanol glucuronide and trichloroacetic acid, were described previously (7). These methods were modified as described below.

A gas chromatograph[11] equipped with an electron-capture detector and a recorder[12] was used. A pulse interval of 15 μsec. gave linear calibration curves for all physiologically encountered concentrations of trichloroacetic acid, trichloroethanol, and its glucuronide.

The columns used for all studies were either 1.23- or 2.46-m. (4- or 8-ft.) stainless steel tubes, 0.63-cm. (0.25-in.) diameter, containing 20% Carbowax 20M on 60–80-mesh Chromosorb W.

Conditions—The temperature conditions used for assay of trichloroethanol and trichloroacetic acid were: injection port, 160°; column, 135°; and electron-capture detector, 190°. The carrier gas (helium) flow rate was 60 ml./min. (at 50 psig.), while the purge gas (90% argon–10% methane) was fixed at 140 ml./min. (at 30 psig.).

Blood and Urine Assays—Trichloroethanol, chloral hydrate, and trichloroacetic acid were extracted from 1.00 ml. of whole blood or urine admixed with 1.00 ml. of distilled water and 1.00 ml. of a 10% solution of sulfosalicylic acid containing 0.72 mg. of chlorobutanol/100 ml. The extraction was effected with 2.0 ml. ether after protein precipitation with the sulfosalicylic acid. Chlorobutanol was used as an internal standard. The two-phase mixture, in an injection vial of 6- or 10-ml. capacity sealed with a rubber stopper and an aluminum crimped cap, was agitated for 1 min. on a mixer[13] and centrifuged[14]

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[1] Fisher Scientific Co., Fair Lawn, N. J.
[2] J. T. Baker Chemical Co., Phillipsburg, N. J.
[3] Mallinckrodt Chemical Works, New York, N. Y.
[4] Hoffmann-La Roche Inc., Nutley, N. J.
[5] Merck & Co., Rahway, N. J.
[6] Calbiochem, Los Angeles, Calif.
[7] Hynson, Westcott and Dunning, Baltimore, Md.
[8] Organon Inc., West Orange, N. J.
[9] Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.
[10] Aldrich Chemical Co., Inc., Milwaukee, Wis.
[11] F & M model 700, Hewlett-Packard Co., Waltham, Mass.
[12] Minneapolis-Honeywell Apparatus Controls Division, Minneapolis, Minn.
[13] Vortex Jr. Mixer, Scientific Industries, Inc., Queens Village, N. Y.
[14] 1/2 HP International centrifuge (model SBR), International Equipment Co., Boston, Mass.

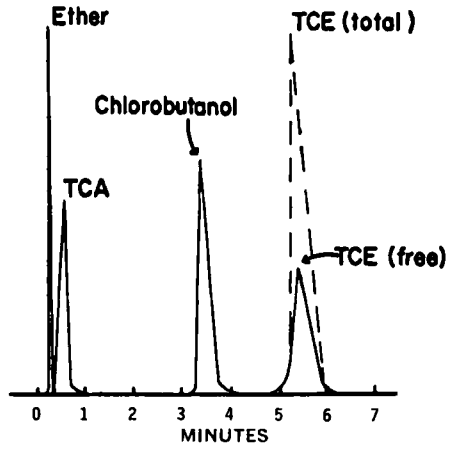


Figure 1—*Typical chromatogram obtained after injection of a 5-μl. aliquot of a 2-ml. ether extract of an acidified 1-ml. blood sample which contained trichloroethanol (TCE), trichloroacetic acid (TCA), and trichloroethanol glucuronide (TCE-G). The solid curve, TCE (free), is a measure of the free trichloroethanol present in the sample, while the broken curve, TCE (total), is a measure of the total trichloroethanol obtained after glucuronidase hydrolysis of the trichloroethanol glucuronide present in the sample. The chlorobutanol was added to the blood sample as an internal standard and permitted calculation of peak height ratios.*

for 2–5 min. until the ether–water emulsion separated and two distinct phases reappeared. The peak height ratios of the peak assigned to the substrate to the peak assigned to the chlorobutanol were used as assay parameters after the injection of a 5-μl. aliquot of the ether solution (Fig. 1).

Trichloroethanol glucuronide in 1.0 ml. of blood or of appropriately diluted urine (1:10, 1:100, or 1:1000) was hydrolyzed with 360 Fishman units (1 mg.) of β-glucuronidase (1.0 ml. of 0.1 *M* pH 4.5 acetate buffer with 1.0 mg. of β-glucuronidase) by incubating at 37°, pH 4.5, for 48 hr., and the trichloroethanol formed was assayed as already described. The difference in the trichloroethanol–chlorobutanol peak height ratios obtained before and after enzymatic hydrolysis was the trichloroethanol glucuronide–chlorobutanol peak height ratio.

The optimum time for complete hydrolysis of trichloroethanol glucuronide by β-glucuronidase was determined by adding a small amount of the purified sodium salt of the glucuronide to urine. One-milliliter aliquots of this urine were treated and incubated as already described for 30, 60, 94, 125, and 180 min. and for 5.0, 8.7, 19.8, 24.5, 27, 31, 50, and 147 hr. Each sample removed at the specified time was immediately refrigerated. The refrigerated samples were chromatographically analyzed for trichloroethanol on the day following the taking of the last sample.

Calibration curves for the substrate were established by adding appropriately graded amounts to the pertinent biological fluid and assaying by the described methods. These methods differed from those previously described (7) in that chlorobutanol was included in the aqueous solution of sulfosalicylic acid added rather than in the ether used for extraction. Also, in the current assay, trichloroacetic acid was directly extracted from acidified blood or urine and subsequently gas chromatographically analyzed in the ether solvent. The previous method cited (7) used an alkaline decarboxylation which yielded assayable chloroform.

The reliability of the assays for trichloroethanol was determined by conducting these analyses on blood (from four different dogs) to which 2.94 mcg./ml. of trichloroethanol had been added. Six samples of similarly spiked, freshly drawn heparinized blood were placed at 5° and analyzed at daily intervals against calibration curves prepared daily.

Protein Binding—Equilibrium dialysis experiments were run with trichloroethanol and its glucuronide to determine protein binding to dog plasma. Two-milliliter samples of dog plasma were separated from whole dog blood by centrifugation and placed inside sealed dialysis sacs[15]. Dialyses were run at 5° for 48 hr. against 10.0-ml. solutions (5, 50, and 500 mcg. trichloroethanol/ml.) in pH 7.4 phosphate buffer. A control dialysis was run at each trichloroethanol concentration with a sac containing only 2.0 ml. of buffer. Aliquots were removed from the solutions outside and inside the dialysis sacs, appropriate dilutions were made, samples were assayed, and the fractional binding was calculated.

Trichloroethanol glucuronide binding to plasma protein was estimated by dialysis of pooled blood plasma containing the glucuronide against pH 7.4 buffer.

Determination of Red Blood Cell/Plasma Distribution of Drug—Since trichloroethanol has high solubility in nonaqueous solvents (9), it was possible that a distribution between red blood cells and plasma would occur when the drug was introduced into a volume of whole blood. Aliquots (0.1 ml.) of trichloroethanol solutions were mixed with 10.0 ml. of heparinized dog blood to give final concentrations of 0.294 and 2.94 mcg./ml., and 1.0 ml. of each blood sample was removed for assay. The remaining 9.0-ml. portions were centrifuged until complete separation of red blood cells occurred and a clear, colorless plasma layer remained. A 1.0-ml. plasma sample was then removed from each tube and assayed. The hematocrit was determined with a hematocrit tube.

Three further determinations were carried out on pooled blood samples obtained from the pharmacokinetic experiments on two other dogs which had been administered 50 mg./kg. of trichloroethanol intravenously. One dog had received 50 mg./kg. of sulfobromophthalein in combination.

Similar studies on red blood cell/plasma distribution were conducted with trichloroethanol glucuronide.

Pharmacokinetics of Trichloroethanol—Female mongrel dogs, 10–20 kg., were fasted for 24 hr. with water *ad libitum*. At 8:30 a.m. on Day 0 (the day of the experiment), the animal was weighed and anesthetized with an intravenous or intramuscular injection of pentobarbital, 30 mg./kg.

One of three pairs of veins was used for cannulation and blood sampling: the external jugular veins of the neck, the cephalic veins of the forelegs, or the lateral saphenous veins of the hind legs. The cannula was frequently flushed with physiological saline solution but was filled with whole blood before 5-ml. samples were taken. These samples were put in 10.0-ml. injectable vials containing 0.1 ml. of 1% mepesulfate solution to prevent blood clot formation.

At least 1 hr. after the pentobarbital was administered, control urine and blood samples were taken. When the animals showed signs of movement and diminished depth of anesthesia, a volume of 5.0% trichloroethanol solution equivalent to the desired dose was injected within 40 sec. The cannula was flushed with 10–20 ml. of physiological saline within an additional 20 sec.

A typical sampling schedule after the time of the saline flush of the trichloroethanol dose was: 2, 4, 6, 8, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min. and then at 30-min. intervals to 720 min. Further samples were taken during the 24–120-hr. interval in several experiments.

Urine samples were collected at least hourly *via* an indwelling urethral catheter in 100-ml. graduated cylinders for 12 hr. on Day 0. When possible, feces were collected after 12 hr. Total urine and feces were collected at t = 24 hr. and at 24-hr. intervals thereafter until no further traces of trichloroethanol or its metabolites were found upon assay.

A minimal period of 14 days was allowed between any two trichloroethanol pharmacokinetic experiments on the same animal. Samples were analyzed for concentrations of trichloroacetic acid, trichloroethanol, and its glucuronide.

Each of four female dogs, A, B, C, and D, received various doses of trichloroethanol ranging from 10 to 150 mg./kg. All studies were grouped within 4 months to avoid possible age, weight, or seasonal effects on the animals.

Pharmacokinetics of Trichloroethanol Glucuronide—Two experiments were performed with intravenous administration of a solution of sodium trichloroethanol glucuronide (100 and 450 mg.) in an anesthetized dog. Blood and urine samples were taken at the intervals previously described for experiments with trichloroethanol and assayed for both trichloroethanol and its glucuronide.

Pharmacokinetics of Trichloroacetic Acid—Twenty milliliters of a 5% trichloroacetic acid solution (1.0 g.) was brought to pH 7.0 with sodium hydroxide and intravenously administered to an anesthetized dog. Blood samples were removed at regular intervals for the first 12 hr. of the experiment, at t = 24 hr., and at 24-hr. intervals thereafter until t = 480 hr.; these samples were assayed for tri-

[15] 0.94-cm. (0.37-in.) flat width, Visking Co., Chicago, Ill.

chloroacetic acid. A second experiment was performed with a 100-mg. dose of trichloroacetic acid.

Pharmacokinetics of Chloral Hydrate—A dose of 500 mg. chloral hydrate (dissolved in physiological saline) was administered through an indwelling jugular vein catheter to a lightly anesthetized dog. Blood samples were taken at intervals and urine samples were taken hourly for a total of 2 hr. and assayed for trichloroethanol, its glucuronide, and for chloral hydrate and trichloroacetic acid.

Studies on Biliary Excretion of Trichloroethanol and Its Glucuronide—Two dogs were anesthetized and laparotomized, and the common bile duct was exposed and cannulated. The renal circulation was not disturbed. The incision was closed with wound clips and covered with gauze pads wet with warm physiological saline solution. Trichloroethanol was injected 1 hr. after surgery was completed.

To obtain maximal data from these experiments, two doses of trichloroethanol were administered in each experiment. In the first experiment, 150 mg. of trichloroethanol (11.6 mg./kg.) was given at $t = 0$ and 600 mg. (46 mg./kg.) at $t = 245$ min. In the second experiment, 500 mg. of trichloroethanol was given at $t = 0$ and 1000 mg. at $t = 370$ min.

Blood samples were collected from the jugular vein and urine samples were collected from a urethral cannula. Bile was collected hourly and assayed for trichloroethanol and its glucuronide in the same manner as urine.

At the end of each experiment, the animal was sacrificed. Gallbladder bile was removed for assay from the second animal but not from the first.

Possible *In Vivo* Inhibition of Trichloroethanol Glucuronidation by Sulfobromophthalein—Dog E, a 10-kg. female, was anesthetized and 600 mg. of trichloroethanol was administered intravenously at $t = 0$. At $t = 250$ min., a second dose of 600 mg. trichloroethanol was given along with 100 mg. of sulfobromophthalein (10 mg./kg.). In addition, a constant intravenous drip of approximately 0.6 mg. sulfobromophthalein/min. was also maintained until $t = 490$ min. The dose of sulfobromophthalein used was twice that recommended (10) for studies on hepatic function.

In a second experiment with the same dog, 600 mg. of trichloroethanol was given at $t = 0$ and samples were taken for 300 min. At $t = 320$ min., a second 600-mg. dose of trichloroethanol was given, which was followed immediately by a dose of 500 mg. sulfobromophthalein. In addition, a constant intravenous infusion of 3 mg. sulfobromophthalein/min. was maintained until $t = 500$ min.

In both experiments, blood and urine samples were taken at regular intervals and assayed for trichloroethanol and its glucuronide.

Hepatic Storage of Trichloroethanol and/or Its Glucuronide—A major problem in the *in vivo* evaluation of hepatic storage of a drug and/or its metabolites by sampling blood flow across the liver is due to the dilution of hepatic venous return by vena cava blood from the lower extremities and kidneys. Whereas blood entering the liver may be easily sampled by catheterization of the hepatic portal vein, hepatic output occurs *via* very short hepatic veins, which immediately merge with the inferior vena cava at the level of the diaphragm in the dog. The vena caval dilution must be circumvented to sample hepatic output *via* the hepatic veins. One approach that was tried was to shunt the vena caval flow into the hepatic portal vein by surgery so that the total blood flow from the lower trunk entered the liver through the hepatic portal vein and all blood exited the liver as hepatic venous output. This surgical approach was attempted on two dogs; one died during surgery and the other expired shortly thereafter.

An alternative solution to the problem was provided by the work of Harris and Riegelman (11). A collateral circulation between a slowly occluded inferior vena cava and the azygous vein was promoted. Slow occlusion was achieved by insertion of a small segment of Tygon tubing in the vena cava 2 weeks prior to the hepatic storage or clearance experiments. An irritation set up by the tubing caused a slow clot and fibrin formation to occlude the vessel. The slow occlusion allowed establishment of the aforementioned collateral circulation. Rapid occlusion of vena caval flow causes death by cardiac arrest. Insertion of the Tygon tubing was performed with sterile surgical technique. The X-rays were taken after cannulation of the inferior vena cava *via* the lateral saphenous vein of the hind leg and clearly showed the occlusion. The radiopaque medium was a 10% solution of meglumine diatrizoate[16] rapidly injected simultaneously with X-irradiation.

One week after X-ray, the animal was anesthetized with pentobarbital and laparotomized and the hepatic portal vein was cannulated *via* a splenic vein. The remaining splenic veins were tied off as was the hepatic artery. Omission of the latter step allows a 25% error to occur due to dilution of hepatic venous flow with hepatic arterial flow. The hepatic venous return was sampled with a cannula inserted in the external jugular vein through the right atrium and into the inferior vena cava (which now only contains hepatic venous blood). After injection of a 50-mg./kg. dose of trichloroethanol *via* the hepatic portal vein, blood samples were simultaneously removed from both cannulas at regular intervals and assayed for trichloroethanol and its glucuronide.

CALCULATIONS, ANALYSIS, AND CURVE FITTING

Calculation of Red Blood Cell/Plasma Distribution Coefficient—The derivation of an expression for the calculation of a red blood cell/plasma distribution coefficient is as follows. The amount of drug, A, in whole blood, A_B, is:

$$A_B = A_P{}^u + A_P{}^b + A_{RBC} \quad \text{(Eq. 1)}$$

where $A_P{}^u$ and $A_P{}^b$ are the amounts of drug in plasma, unbound and bound to plasma proteins, respectively. The amount of drug in red blood cells is represented by A_{RBC}.

When the amount of drug in whole blood is converted to concentration, Eq. 2 becomes:

$$A_B/V_{B_{true}} = A_P{}^u/V_{B_{true}} + A_P{}^b/V_{B_{true}} + A_{RBC}/V_{B_{true}} \quad \text{(Eq. 2)}$$

where $V_{B_{true}}$ is the true volume of whole blood in the animal body.

When $V_{P_{true}}$ and V_{RBC} are the respective true volumes of plasma and red blood cells in the animal body:

$$\frac{A_B}{V_{B_{true}}} = \frac{A_P{}^u/V_{P_{true}}}{V_{B_{true}}/V_{P_{true}}} + \frac{A_P{}^b/V_{P_{true}}}{V_{B_{true}}/V_{P_{true}}} + \frac{A_{RBC}/V_{RBC}}{V_{B_{true}}/V_{RBC}} \quad \text{(Eq. 3)}$$

or:

$$[A_B] = \frac{[A_P{}^u]}{V_{B_{true}}/V_{P_{true}}} + \frac{[A_P{}^b]}{V_{B_{true}}/V_{P_{true}}} + \frac{[A_{RBC}]}{V_{B_{true}}/V_{RBC}} \quad \text{(Eq. 4)}$$

Since the hematocrit (H) may be defined as:

$$H = V_{RBC}/V_{B_{true}} \quad \text{(Eq. 5)}$$

therefore:

$$1 - H = V_{P_{true}}/V_{B_{true}} \quad \text{(Eq. 6)}$$

Substitution of Eqs. 5 and 6 into Eq. 4 yields:

$$[A_B] = [A_P{}^u](1 - H) + [A_P{}^b](1 - H) + [A_{RBC}]H \quad \text{(Eq. 7)}$$

The red blood cell/plasma distribution coefficient is then defined as:

$$D = [A_{RBC}]/[A_P{}^u] \quad \text{(Eq. 8)}$$

Upon rearrangement of Eq. 7:

$$D = \{[A_B]/[A_P{}^u](1 - H) - [A_P{}^b]/[A_P{}^u] - 1\}\,(1 - H)/H \quad \text{(Eq. 9)}$$

If the fraction, f, of drug bound to plasma proteins is known:

$$[A_P{}^b] = [A_P]f \quad \text{(Eq. 10)}$$

and:

$$[A_P{}^u] = [A_P]\,(1 - f) \quad \text{(Eq. 11)}$$

where $[A_P]$ is the total plasma concentration of drug, and Eq. 9 becomes:

$$D = \{[A_B]/[A_P]\,(1 - f)\,(1 - H) - f/(1 - f) - 1\} \times (1 - H)/H \quad \text{(Eq. 12)}$$

[16] Renografin.

$$n[A_P{}^u] + [P] \overset{K}{\rightleftarrows} [A_nP]$$

Scheme II

and the concentration of unbound drug in the plasma can be obtained by a rearrangement of this equation and is:

$$[A_P{}^u] = (1 - f)[A_P] = [A_B] \Big/ \left(DH + \frac{1 - H}{1 - f}\right) \quad \text{(Eq. 13)}$$

The fraction of drug bound to a plasma protein may not be invariant. It may be a function of the unbound concentration of drug (12, 13), *i.e.*:

$$f = 1/(1 + [A_P{}^u]/n[P] + 1/nK[P]) \quad \text{(Eq. 14)}$$

where K is the association constant for the binding of a molecule of drug to one of the equivalent n binding sites of the protein molecule in accordance with Scheme II, where $[P]$ is the concentration of protein.

At low drug concentrations or when the association constant K is small, the second term in the denominator of Eq. 14 becomes negligible and:

$$f \sim 1/(1 + 1/nK[P]) = [A_P{}^b]/[A_P] \quad \text{(Eq. 15)}$$

may be taken as constant for a given protein concentration and is independent of drug concentration.

Distribution of Drug among Blood Components and Fractions of Drug in Blood in Each—The amount of drug in the blood that is in the red blood cells may be calculated on the basis of Eqs. 5, 8, and 11:

$$A_{RBC} = V_{RBC}[A_{RBC}] = HV_{B_{true}}[A_{RBC}] = HDV_{B_{true}}[A_P{}^u] = HDV_{B_{true}}(1 - f)[A_P] \quad \text{(Eq. 16)}$$

The amount of drug in the blood that is bound to plasma protein and in the plasma may be calculated on the basis of Eqs. 6 and 10:

$$A_P{}^b = V_{P_{true}}[A_P{}^b] = (1 - H)V_{B_{true}}[A_P{}^b] = (1 - H)fV_{B_{true}}[A_P] \quad \text{(Eq. 17)}$$

The amount of drug in the blood that is unbound to plasma protein and in the plasma may be calculated on the basis of Eqs. 6 and 11:

$$A_P{}^u = V_{P_{true}}[A_P{}^u] = (1 - H)V_{B_{true}}[A_P{}^u] = (1 - H)(1 - f)V_{B_{true}}[A_P] \quad \text{(Eq. 18)}$$

When Eqs. 1 and 16–18 are considered:

$$A_B = V_{B_{true}}[A_P]\{HD(1 - f) + (1 - H)\} \quad \text{(Eq. 19)}$$

Division of Eqs. 16–18, respectively, by Eq. 19 gives the fraction of drug in the blood that is in the red blood cells as:

$$\gamma_{RBC} = A_{RBC}/A_B = HD(1 - f)/\{HD(1 - f) + (1 - H)\} \quad \text{(Eq. 20)}$$

and the fraction of drug in the blood that is bound to plasma protein and in the plasma as:

$$\gamma_P{}^b = A_P{}^b/A_B = (1 - H)f/\{HD(1 - f) + (1 - H)\} \quad \text{(Eq. 21)}$$

and the fraction of drug in the blood that is unbound to plasma protein and in the plasma as:

$$\gamma_P{}^u = A_P{}^u/A_B = (1 - H)(1 - f)/\{HD(1 - f) + (1 - H)\} \quad \text{(Eq. 22)}$$

Also, the total fraction of drug in the blood that is in the plasma is:

$$\gamma_P = \gamma_P{}^u + \gamma_P{}^b = (1 - H)/\{HD(1 - f) + (1 - H)\} \quad \text{(Eq. 23)}$$

Relations and Conversions among Variously Calculated Apparent Volumes of Distribution of the Central Compartment—Apparent volumes of distribution may be considered as the operational parameters that relate the measured concentrations of drug in the obtainable fluids of the body to the amounts in the individual compartments of the pharmacokinetic multicompartmental model that describe quantitatively the disposition of the drug in the body (12–16).

However, the numerical estimates of these apparent volumes of distribution for these several compartments can vary widely for the same multicompartmental model, dependent on whether the time course of free or total drug concentration is used and on whether blood or plasma is analyzed. Thus, the relations among these several estimates must be delineated to permit the calculation of one from the other. Only then will there be a common basis to compare the pharmacokinetic parameters of the same and different drugs within and among species.

If identification of apparent volumes of distribution with true volumes of body fluids or tissues is to be attempted, the criteria for the choice of the method of calculation of apparent volumes of distribution from pharmacokinetic data must be consistent with the physiological and physicochemical realities of drug disposition in the body.

The concentrations of a drug, A, are measured in aliquots of the fluid of the central compartment and are generally obtained as concentrations in blood, $[A_B]$, or in plasma, $[A_P]$. The apparent volumes of distribution referenced to blood, V_B, or to plasma, V_P, can be estimated from the extrapolation to zero time of the plot of drug concentration in blood or plasma against time on the premise of instantaneous mixing of the intravenously administered drug in the fluids of the central compartment (12, 13).

Thus, the apparent volume of distribution of the central compartment referenced to the total drug concentration in the blood is:

$$V_B = A_0/[A_B]_0 \quad \text{(Eq. 24)}$$

and the apparent volume of distribution of the central compartment referenced to the total drug concentration in the plasma is:

$$V_P = \{A_0 - (A_{RBC})_0\}/[A_P]_0 \quad \text{(Eq. 25)}$$

where A_0 and $(A_{RBC})_0$ are the intravenously administered dose and amount in the red blood cells, respectively; $[A_B]_0$ and $[A_P]_0$ are the total drug concentrations in blood and plasma, respectively, at zero time.

Significance of Variously Calculated Apparent Volumes of Distribution of the Central Compartment—These are fictitious volumes, since they may include the volumes of associated fluids where the drug concentration is in instantaneous equilibration with that of blood or plasma, *i.e.*:

$$V_B = V_{B_{true}} + V_{B_z} \quad \text{(Eq. 26)}$$

where $V_{B_{true}}$ is the true blood volume, and V_{B_z} is the apparent volume of this instantaneously equilibrated fluid or tissue on the presumption that its drug concentration is *exactly* the same as that of the blood. This apparent volume, V_{B_z}, may be termed the *associated* (with the central compartment) apparent volume of distribution *referenced* to the total drug concentration in the blood. Similarly:

$$V_P = V_{P_{true}} + V_{P_z} \quad \text{(Eq. 27)}$$

where $V_{P_{true}}$ is the true plasma volume, and V_{P_z} is the volume of this instantaneously equilibrated fluid or tissue on the presumption that its drug concentration is *exactly* the same as that of the plasma. This apparent volume, V_{P_z}, may be termed the *associated* (with the central compartment) apparent volume of distribution *referenced* to the total drug concentration in the plasma.

If none of the drug is sequestered by, or partitioned into, the red blood cells, all of the drug is assayed in the plasma and a simple relation exists between the respective apparent volumes of distribution. When Eqs. 6, 24, and 25 are considered:

$$V_P = V_{P_s} = A_0/[A_P]_0 = A_0 \Big/ \left(\frac{A_0}{V_{P_{true}}}\right) = A_0 \Big/ \frac{A_0}{V_{B_{true}}(1 - H)} = A_0 \Big/ \frac{[A_B]_0}{1 - H} = (1 - H)V_B \quad \text{(Eq. 28)}$$

and it follows from Eqs. 26–28 that:

$$V_{P_{s_z}} = V_{P_z} = V_{B_z}(1 - H) \quad \text{(Eq. 29)}$$

and the associated apparent volume of distribution referenced to the total drug concentration in plasma is less than the associated apparent volume of distribution referenced to the total drug concentration in blood.

These relations of Eqs. 26–29 are based on the presumption that the concentration in V_{P_x} is the same as that in plasma, $V_{P_{true}}$. The apparent volume V_{P_x} cannot be true plasma; it must be a fluid or tissue readily accessible to drug which is freely diffusible from the plasma water and would be unbound to plasma proteins. If the drug is completely protein bound and not partitioned into the red blood cells, the associated apparent volumes of distribution referenced to total drug concentration in blood and plasma, V_{B_x} and V_{P_x}, are zero and the apparent volumes of distribution, referenced to blood and plasma, V_B and V_P, are the same as the true volumes of blood and plasma, $V_{B_{true}}$ and $V_{P_{true}}$, respectively.

Equations for Calculation of Apparent Volumes of Distribution of the Central Compartment Referenced to Unbound Drug in Plasma from Apparent Volumes of Distribution Referenced to Total Drug Concentration in Blood and Plasma—The apparent volumes of distribution that have the highest probability of physiological significance are those calculated on the premise that only unbound drug in the true plasma volume (*i.e.*, diffusible drug in the plasma water) equilibrates with the other volumes of distribution of the various compartments of the multicompartmental model. The apparent volume of distribution in the central compartment referenced to unbound drug in the plasma water can be formulated as the amount of drug administrated intravenously less the amounts of drug partitioned into red blood cells and bound to plasma proteins at zero time divided by the zero time concentration of unbound drug in the plasma:

$$V_P{}^u = \{A_0 - (A_{RBC})_0 - (A_P{}^b)_0\}/[A_P{}^u]_0 \quad \text{(Eq. 30)}$$

In terms of the respective true volumes of red blood cells and plasma:

$$V_P{}^u = (A_0 - V_{RBC}[A_{RBC}]_0 - V_{P_{true}}[A_P{}^b]_0)/[A_P{}^u]_0 \quad \text{(Eq. 31)}$$

Substitution of values for V_{RBC}, $V_{P_{true}}$, $[A_{RBC}]_0$, $[A_P{}^b]_0$, and $[A_P{}^u]_0$ obtained from Eqs. 5, 6, 8, 10, and 11, respectively, into Eq. 31, with subsequent rearrangement, gives:

$$V_P{}^u = A_0/[A_P]_0\,(1 - f) - HDV_{B_{true}} - (1 - H)V_{B_{true}}f/(1 - f) \quad \text{(Eq. 32)}$$

in terms of the measured total concentration of the drug in plasma at zero time.

The substitution of Eq. 13 into Eq. 32 permits the apparent volume of distribution of the central compartment referenced to unbound drug concentration in the plasma to be expressed in terms of the measured total concentration of the drug in blood at zero time:

$$V_P{}^u = A_0\left(DH + \frac{1 - H}{1 - f}\right)\Big/[A_B]_0 - HDV_{B_{true}} - (1 - H)V_{B_{true}}f/(1 - f) \quad \text{(Eq. 33)}$$

On consideration of Eqs. 6 and 24, this apparent volume of distribution can be expressed in terms of the apparent volume of distribution referenced to the total concentration of drug in blood:

$$V_P{}^u = V_B\left(DH + \frac{1 - H}{1 - f}\right) - HV_{B_{true}}D - (1 - H)V_{B_{true}}f/(1 - f)$$
$$= V_B\left(DH + \frac{1 - H}{1 - f}\right) - \frac{H}{1 - H}V_{P_{true}}D - V_{P_{true}}f/(1 - f) = V_{P_{true}} + V_{P_x}{}^u \quad \text{(Eq. 34)}$$

On consideration of Eqs. 6 and 26, the associated apparent volume of distribution referenced to the unbound drug concentration in plasma can be defined in terms of the associated apparent volume of distribution referenced to the total drug concentration in blood:

$$V_{P_x}{}^u = V_{B_x}\left(DH + \frac{1 - H}{1 - f}\right) \quad \text{(Eq. 35)}$$

When the apparent volume of distribution of the central compartment is referenced to total concentration of drug in the plasma, it is expressed as the quotient of the amount of drug in plasma less the amount in the red blood cells and the total plasma concentration at zero time.

Thus, when Eqs. 25 and 31 are considered, a process similar to the development of Eq. 34 permits the expression of $V_P{}^u$ in terms of the apparent volume of distribution referenced to the total concentration of drug in plasma:

$$V_P{}^u = (V_P - V_{P_{true}}f)/(1 - f) = V_{P_{true}} + V_{P_x}{}^u \quad \text{(Eq. 36)}$$

On consideration of Eq. 27:

$$V_{P_x}{}^u = V_{P_x}/(1 - f) \quad \text{(Eq. 37)}$$

It follows from Eqs. 35 and 37 that:

$$V_{P_x} = V_{B_x}[DH(1 - f) + 1 - H] \quad \text{(Eq. 38)}$$

Pseudoapparent Volumes of Distribution of the Central Compartment and Calculation of Variously Referenced Apparent Volumes—Pseudoapparent volumes of distribution of the central compartment referenced to the total drug concentration in the plasma or to the unbound drug concentration in the plasma have been calculated as:

$$V_{P_s} = A_0/[A_P]_0 \quad \text{(Eq. 39)}$$

and:

$$V_{P_s}{}^u = A_0/[A_P]_0(1 - f) = V_{P_s}/(1 - f) \quad \text{(Eq. 40)}$$

without considering the fact that the numerator of the quotient in Eq. 25 should be lessened by the amount of drug in the red blood cells, $(A_{RBC})_0$.

Thus, consideration of Eqs. 11, 39, and 40 for the pertinent values in Eq. 32 permits the apparent volume of distribution of the central compartment referenced to unbound drug concentration in the plasma to be expressed in terms of these unrealistically calculated pseudoapparent volumes of distribution referenced to total drug concentration (or unbound drug concentration) in plasma:

$$V_P{}^u = V_{P_s}/(1 - f) - HV_{P_{true}}D/(1 - H) - V_{P_{true}}f/(1 - f) = V_{P_{true}} + V_{P_x}{}^u \quad \text{(Eq. 41)}$$

Other pertinent conversions can be obtained. On consideration of Eq. 27:

$$V_{P_x}{}^u = V_{P_{sx}}/(1 - f) - HDV_{P_{true}}/(1 - H) \quad \text{(Eq. 42)}$$

It also follows, on consideration of Eqs. 37 and 42, that:

$$V_{P_x} = V_{P_{sx}} - HDV_{P_{true}}(1 - f)/(1 - H) \quad \text{(Eq. 43)}$$

and:

$$V_P = V_{P_s} - HDV_{P_{true}}(1 - f)/(1 - H) \quad \text{(Eq. 44)}$$

It can be shown from Eqs. 35 and 42 that:

$$V_{B_x} = \frac{V_{P_{sx}} - HDV_{B_{true}}(1 - f)}{DH(1 - f) + 1 - H} \quad \text{(Eq. 45)}$$

so that, on cognizance of Eqs. 6, 23, 26, and 27:

$$V_B = V_{P_s}/[DH(1 - f) + 1 - H] = \gamma_P V_{P_s}/(1 - H) \quad \text{(Eq. 46)}$$

Relations among Variously Referenced Apparent Volumes of Distribution in the Absence of Protein Binding and/or Red Blood Cell Partition—If there is no partitioning into red blood cells, $D = 0$, $V_{P_s} = V_P$, $\gamma_P = 1$, $\gamma_{RBC} = 0$, $\gamma_P{}^b = f$, $\gamma_P{}^u = 1 - f$, and Eqs. 28, 29, 36, and 37 hold.

If there is no protein binding, $f = 0$, $V_P{}^u = V_P$, $V_{P_x}{}^u = V_{P_x}$, $V_{P_s}{}^u = V_{P_s}$, $\gamma_P = \gamma_P{}^u$, $\gamma_P{}^b = 0$ and, from Eq. 34:

$$V_P = V_B(DH + 1 - H) - HV_{B_{true}}D = V_B(DH + 1 - H) - HV_{P_{true}}D/(1 - H) \quad \text{(Eq. 47)}$$

and from Eqs. 35 and 38:

$$V_{P_x} = V_{B_x}(DH + 1 - H) \quad \text{(Eq. 48)}$$

and from Eqs. 41 and 44:

$$V_P = V_{P_s} - HDV_{P_{true}}/(1 - H) \quad \text{(Eq. 49)}$$

and from Eqs. 42 and 43:

$$V_{P_z} = V_{P_{sz}} - HDV_{P_{true}}/(1 - H) \quad \text{(Eq. 50)}$$

and from Eq. 45:

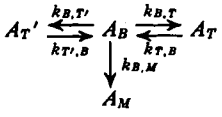
$$V_{B_z} = \frac{V_{P_{sz}} - HDV_{B_{true}}}{DH + 1 - H} \quad \text{(Eq. 51)}$$

and from Eq. 46:

$$V_B = V_{P_s}/(DH + 1 - H) = \gamma_P V_{P_s}/(1 - H) \quad \text{(Eq. 52)}$$

If there is no partitioning of drug into the red blood cells and no binding to plasma proteins, $D = 0, f = 0$, $V_P{}^u = V_P = V_{P_s}$, $V_{P_z}{}^u = V_{P_z} = V_{P_{sz}}$, $\gamma_P = \gamma_P{}^u = 1$, and Eqs. 28 and 29 hold.

Conversions among Microscopic Pharmacokinetic Rate Constants Based on Various Expressions of Drug Concentrations in Blood Components—The differential equation for the loss of drug from the central compartment of the linear three-compartment body model, Scheme III, may be expressed in terms of the amount of drug



Scheme III

in the blood and associated fluids, A_B, and in terms of the amounts of drug in the tissue compartments, A_T and $A_{T'}$:

$$-dA_B/dt = (k_{B,T} + k_{B,T'} + k_{B,M})A_B - k_{T,B}A_T - k_{T',B}A_{T'} \quad \text{(Eq. 53)}$$

It may be expressed in terms of the amounts of drug in the tissues, A_T and $A_{T'}$, and unbound drug, $A_P{}^u$, in the plasma and associated fluids, equilibrated with drug bound to protein and equilibrated with drug partitioned into red blood cells:

$$-\frac{dA_B}{dt} = (k_{P_u,T} + k_{P_u,T'} + k_{P_u,M})A_P{}^u - k_{T,P_u}T - k_{T',P_u}T' \quad \text{(Eq. 54)}$$

When the value for $A_P{}^u$ in Eq. 22 is substituted into Eq. 54:

$$-\frac{dA_B}{dt} = (\gamma_P{}^u k_{P_u,T} + \gamma_P{}^u k_{P_u,T'} + \gamma_P{}^u k_{P_u,M})A_B - k_{T,P_u}T - k_{T',P_u}T' \quad \text{(Eq. 55)}$$

Comparison of Eqs. 53 and 55 clearly shows that $k_{B,T} = \gamma_P{}^u k_{P_u,T}$, $k_{B,T'} = \gamma_P{}^u k_{P_u,T'}$, $k_{B,M} = \gamma_P{}^u k_{P_u,M}$, $k_{T,P_u} = k_{T,B}$, and $k_{T',P_u} = k_{T',B}$.

Estimations of Apparent Volumes of Distribution of Observed Tissue Compartments Referenced to Unbound Drug Concentration in Plasma from Variously Calculated Apparent Volumes of Distribution and Microscopic Pharmacokinetic Rate Constants—The apparent volume of distribution of a tissue compartment in a pharmacokinetic model such as Scheme III should be referenced to the unbound drug concentration in the plasma of the central compartment on the realistic premise that potential equilibration of drug between the central compartment and this tissue is only effected by unbound drug in the plasma water.

On the assumption of the possibility of an eventual equilibration or partition between unbound drug concentration in plasma water and drug concentration in a tissue (*e.g.*, as an ultimate consequence of the steady state resulting from a continuous zero-order infusion):

$$[A_T]_{eq}K = [A_P{}^u]_{eq} = A_P{}^u/V_P{}^u = A_T/KV_{T_P}{}^u \quad \text{(Eq. 56)}$$

where $V_P{}^u$ and $V_{T_P}{}^u$ are the apparent volumes of distribution referenced to the unbound drug concentration in plasma on the presumption that the partition coefficient, K, for the equilibration is unity (12, 13). If the tissue is truly homogeneous and an actual or operational K exists that is not equal to unity, V_T becomes the apparent volume of distribution of the tissue referenced to total drug concentration in that tissue.

The kinetic conditions for this potential steady-state equilibration are:

$$dA_T/dt = dA_P{}^u/dt = 0; k_{P_u,T}A_P{}^u = k_{T,P_u}A_T \quad \text{(Eq. 57)}$$

so that, from Eqs. 56 and 57 on the usually taken premise that $K = 1$, the apparent volume of distribution of the tissue compartment referenced to the unbound drug concentration in the plasma may be defined in terms of the apparent volume of distribution of the central compartment referenced to the unbound drug concentration in plasma and the microscopic pharmacokinetic constants for rate dependence on the amount of unbound drug in the plasma and its associated fluids (12, 13):

$$V_{T_P}{}^u = (k_{P_u,T}/k_{T,P_u})V_P{}^u \quad \text{(Eq. 58)}$$

This relation is valid even when a steady state does not exist between the tissue and plasma compartments.

Thus, as a consequence of the identity between Eqs. 53 and 55, the apparent volume of distribution of the tissue, T, can be related to the microscopic rate constants determined on the postulation of rate dependence on the total amount of drug in the blood as:

$$V_{T_P}{}^u = (k_{B,T}/\gamma_P{}^u k_{T,B})V_P{}^u \quad \text{(Eq. 59)}$$

Similarly:

$$V_{T'_P}{}^u = (k_{B,T'}/\gamma_P{}^u k_{T',B})V_P{}^u \quad \text{(Eq. 60)}$$

where $\gamma_P{}^u$ is defined in Eq. 22.

The apparent volumes of distribution of the tissues, T and T', referenced to the unbound drug concentration in plasma can be calculated from the variously calculated apparent volumes of distribution such as V_B, V_P, and V_{P_s} by appropriate substitutions of the values of $V_P{}^u$ obtained from Eqs. 34, 36, and 41, respectively, into Eqs. 59 and 60.

It can be shown similarly that the apparent volumes of distribution of the tissues, T and T', can be related to the microscopic rate constants determined on the postulation of rate dependence on the total amount of drug in the plasma:

$$V_{T_P}{}^u = (\gamma_P{}^u k_{P,T}/\gamma_P k_{T,P})V_P{}^u \quad \text{(Eq. 61)}$$

and:

$$V_{T'_P}{}^u = (\gamma_P{}^u k_{P,T'}/\gamma_P k_{T',P})V_P{}^u \quad \text{(Eq. 62)}$$

where $\gamma_P{}^u$ and γ_P are defined in Eqs. 22 and 23; and since $\gamma_P{}^u/\gamma_P = 1 - f$, then $k_{P,T} = (1 - f)k_{P_u,T}$, $k_{P,T'} = (1 - f)k_{P_u,T'}$, $k_{P,M} = (1 - f)k_{P_u,M}$, $k_{T,P} = k_{T,P_u}$, and $k_{T',P} = k_{T',P_u}$.

Pharmacokinetic Modeling—At the conclusion of all pharmacokinetic experiments, blood and urine samples were assayed for trichloroacetic acid, trichloroethanol, and its glucuronide. A defined procedure was followed to prepare a model that would be consistent with the observed data and to derive the parameters that quantifiably described the system.

The study of the pharmacokinetics of 30 mg./kg. of trichloroethanol in Dog D (20 kg.) will be used to illustrate the analysis and fitting procedures.

Graphical Analysis of Trichloroethanol Data—A semilogarithmic plot of trichloroethanol concentration in whole blood, given as the trichloroethanol–chlorobutanol peak height ratio per milliliter, is shown in Fig. 2. A peak height ratio per milliliter of 1.0 is equivalent to 8.4 mcg. trichloroethanol/ml.

The concentration of trichloroethanol in blood, $[TCE_B]$, as a function of time can be described by a sum of exponentials (17):

$$[TCE_B] = [A_B] = \sum_{i=1}^{n} [A_B]_i e^{-k_i t} = [A_B]_1 e^{-k_1 t} + [A_B]_2 e^{-k_2 t} + [A_B]_3 e^{-k_3 t} \quad \text{(Eq. 63)}$$

The methods by which the logarithmic plot of such data against time may be analyzed to yield its component $[A_B]_i$ (intercept) and k_i (slope) values were described in detail by Riggs (12).

The sum of the $[A_B]_i$ values, $\Sigma[A_B]_i$, is the time zero concentration, $[A_B]_0$, in the central compartment on intravenous administra-

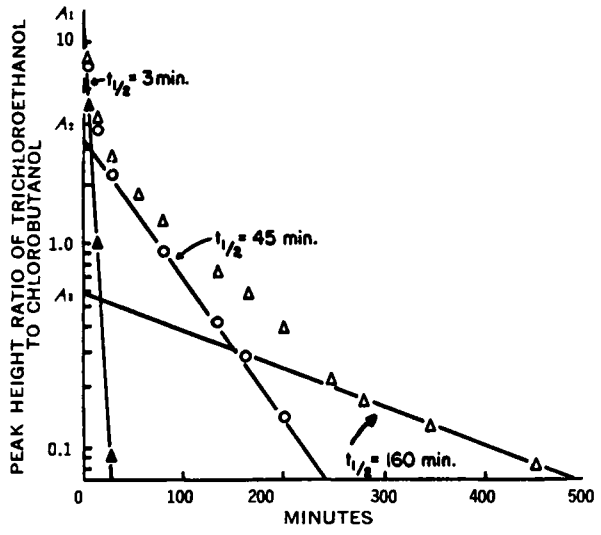


Figure 2—*Feathering of the semilogarithmic plot against time of the peak height ratios (△) of trichloroethanol to the internal standard chlorobutanol derived from the GC assay of 5 μl. of a 2-ml. ether extract of 1 ml. of acidified dog blood after the intravenous administration of 30 mg./kg. of trichloroethanol to the 20-kg. Dog D. A peak height ratio of 1.00 is equivalent to 8.4 mg. of trichloroethanol/ml. of whole blood. The three distinct phases obtained by feathering (the solid triangles, circles, and terminal open triangles) are labeled respectively with their apparent half-lives.*

tion and may be obtained from the calibration curve of peak height ratios *versus* concentrations (7). The trichloroethanol concentration in whole blood can be converted to the concentration of unbound trichloroethanol in plasma (in milligrams per milliliter) by Eq. 13. The apparent volume of distribution, V_B, referenced to the total concentration of trichloroethanol in the blood can be estimated from the dose (in milligrams) by Eq. 24.

The $\Sigma[A_B]_i$ or $[A_B]_0$ in terms of peak height ratio was estimated from the data of Fig. 2 extrapolated to zero time; it was 17.1 and corresponded to an initial whole blood concentration of 0.142 mg. of trichloroethanol/ml. or 0.0237 as percent of dose per milliliter of blood. The separate loading factors for the three exponentials that described the data of Fig. 2 were 13.0, 3.5, and 0.6 for $[A_B]_1$, $[A_B]_2$, and $[A_B]_3$, respectively. Insertion of the $[A_B]_0$ value into Eq. 13 and use of a red blood cell/plasma distribution coefficient D value of 2.1 with a hematocrit, H, of 0.45 (18) and a fraction, $f = 0.35$, of drug bound to plasma proteins (see *Results*) gave an initial concentration of unbound trichloroethanol in plasma, $[A_P{}^u]$, of 0.080 mg./ml.

The fractional amounts of drug in the blood that are in the red blood cells, in the plasma, unbound to protein and in the plasma, and bound to protein and in the plasma are: $\gamma_{RBC} = 0.527$ (Eq. 20), $\gamma_P = 0.475$ (Eq. 23), $\gamma_{P^u} = 0.310$ (Eq. 22), and $\gamma_{P^b} = 0.165$ (Eq. 21), respectively. When each of these fractions is multiplied by the initial milligrams of trichloroethanol per milliliter of blood, 0.142 mg./ml., the amount of drug in each blood component that is initially in 1 ml. of blood is: $(A_{RBC})_0/V_B = 0.075$ mg. for red blood cells, $(A_P)_0/V_{B_{true}} = 0.067$, $(A_P{}^u)_0/V_{B_{true}} = 0.044$, and $(A_P{}^b)_0/V_{B_{true}} = 0.023$. Thus, the respective concentrations are: $[A_{RBC}]_0 = (A_{RBC})_0/HV_{B_{true}} = 167$ mg./ml., $[A_P]_0 = (A_P)/(1 - H)V_{B_{true}} = 122$ mg./ml., $[A_P{}^u] = (A_P{}^u)_0/(1 - H)V_{B_{true}} = 0.080$ mg./ml., and $[A_P{}^b]_0/(1 - H)V_{B_{true}} = 0.043$ mg./ml.

On the premise that $V_{B_{true}} = 2.0$ l. and $V_{P_{true}} = 1.10$ l. for Dog C (20 kg.), where 100 and 55 ml./kg. are given in the literature (18) for dog blood volumes and plasma volumes, respectively, the various apparent volumes of the central compartment can be calculated. The pseudoapparent volume of distribution referenced to total drug concentration in plasma is $V_{P_s} = 4.88$ l. (Eqs. 39 and 46), the apparent volume of distribution referenced to total drug concentration in plasma is $V_P = 3.77$ l. (Eq. 44), the apparent volume of distribution referenced to the concentration of drug not bound to protein in the plasma is $V_P{}^u = 5.10$ l. (Eqs. 34 and 36), and the apparent volume of distribution referenced to drug concentration in blood is $V_B = 4.22$ l. (Eqs. 24 and 46).

The semilogarithmic plot of trichloroethanol concentration in blood *versus* time was resolved by the appropriate "feathering" techniques into three distinct exponential segments. The first exponential, which describes a rapid decline of trichloroethanol concentration in blood to 30% of the calculated initial value within 15 min. after drug administration, may be largely assigned to the diffusion of trichloroethanol from the initial volume of distribution ascribed to the central compartment into a larger volume of a readily available equilibrating body fluid. The low molecular weight of trichloroethanol and its extensive solubility in nonaqueous solvents (9) suggest that such rapid and extensive distribution might be expected.

The sum of the antilogarithms of the extrapolated zero-time intercepts (Fig. 2) assignable to the second and third exponentials of the polyexponential fit of the data (Eq. 63) was $[A_B]_2$ and $[A_B]_3 = 4.1$ in terms of peak height ratio per milliliter. This serves as a preliminary estimate of the assay value of trichloroethanol in blood that would have been observed after the ready equilibration with tissues if no significant drug elimination had occurred in the initial time interval. Insertion of the corresponding whole blood concentration (0.0346 mg./ml.) into Eq. 13 yields a concentration of free trichloroethanol in the plasma that is not bound to proteins of 0.0193 mg./ml. The estimated sum of the apparent volumes of distribution of the central compartment and that of the readily perfused tissues referenced to the total concentration of drug in the blood can be estimated from:

$$V_{D_B} = V_B + V_{T_B} = \text{dose}/([A_B]_2 + [A_B]_3) = A_0/(\Sigma[A_B]_i - [A_B]_1) \quad \text{(Eq. 64)}$$

and was 17.3 l. for a 600-mg. dose where the divisor of the last two terms is the concentration of trichloroethanol in the equilibrated fluids after the ready equilibration on the assumption of insignificant drug excretion or metabolism during the time interval of the equilibration. Subtraction of the initial apparent volume of distribution of the central compartment referenced to total concentration in the blood, $V_B = 4.2$ l., from this value provides an estimated volume of 13.1 l. for the tissue fluids, V_{T_B}, referenced similarly.

It can be argued also (Eq. 30) that if the curves of unbound drug concentration $[A_P{}^u]$ in the plasma against time are analyzed similarly:

$$V_{D_P{}^u} = V_P{}^u + V_{T_P{}^u} = \frac{\text{dose} - (\text{amounts in red blood cells and bound to plasma protein})}{[A_P{}^u]_2 + [A_P{}^u]_3} \quad \text{(Eq. 65)}$$

The estimated sum of the apparent volumes of distribution of the central compartment and of the readily perfused tissues referenced to unbound drug concentration in the plasma, $V_{D_P{}^u}$, can be calculated from the sum of the apparent volumes of distribution, V_{D_B}, referenced to total drug concentration in the blood by an equation derived on the same premises as, and similar to, Eq. 34:

$$V_{D_P{}^u} = V_{D_B}\left(DH + \frac{1 - H}{1 - f}\right) - HDV_{B_{true}} - (1 - H)V_{B_{true}}f/(1 - f) = 28.7 \text{ l.} \quad \text{(Eq. 66)}$$

Subtraction of the initial apparent volume of distribution of the central compartment referenced to unbound drug concentration in the plasma, $V_P{}^u = 5.1$ l., provides an estimated volume, $V_{T_P{}^u}$, of 23.6 l. for the tissue fluids referenced similarly.

More refined estimates of the volumes of distribution were obtained when the data were analyzed with the analog and digital computers, since the value of $[A_B]_0 = \Sigma[A_B]_i$ may be best obtained by computer extrapolation to zero time.

The loss of trichloroethanol from blood during the time period of the second exponential was postulated to be predominantly due to metabolic conversion of trichloroethanol to its glucuronide by hepatic microsomal uridine 5′-diphosphate-glucuronyltransferase. This was verified by the fact that the maximal rates of appearance of the glucuronide in blood and urine occurred in this interval. The rate constant of the second exponential of Eq. 63, k_2, is not equal to $k_{B,M}$, the metabolic rate constant in Scheme III. On the assumption of a predominantly two-compartment body model with rapid

equilibration (13), where k_3 is small or almost zero, k_2 is coincident with k_e (19, 20), the overall disposition rate constant for trichloroethanol where this latter constant can be expressed either in terms of the microscopic rate constants referenced to the total amount of drug in the central compartment of blood and associated fluids or, on consideration of Eq. 22 and the coincidence of Eqs. 53 and 55, in terms of the microscopic rate constants referenced to the amount of unbound drug in the central compartment of plasma and associated fluids:

$$k_2 = k_e = \frac{k_{B,M}}{1 + k_{B,T}/k_{T,B}} = \frac{k_{P_u,M}}{1/\gamma_P{}^u + k_{P_u,T}/k_{T,P_u}} = \frac{k_{P_u,M}}{1 + HD/(1 - H) + f/(1 - f) + k_{P_u,T}/k_{T,P_u}} \quad \text{(Eq. 67)}$$

and where $k_{B,T}$ or $k_{P_u,T}$ and $k_{T,B}$ or k_{T,P_u} are the respective rate constants for diffusion into and out of the rapidly equilibrating fluid compartment responsible for the first exponential of Eq. 63 that characterizes the initial loss of drug from the central compartment referenced to amounts of drug in blood and associated fluids or plasma and associated fluids, respectively.

This latter equation can also be derived from consideration of a general equation when the rate of loss of drug from a complex of rapidly equilibrating compartments is defined as being proportional to the amount of drug in only one of those compartments, *e.g.*:

$$-d(A_B + A_T)/dt = d(A_{RBC} + A_P{}^b + A_P{}^u + A_T)/dt = k_{P_u,M}A_P{}^u \quad \text{(Eq. 68)}$$

When the amount of drug in any one of the components or compartments of this rapidly equilibrating complex can be defined as being in equilibrium with the amount of drug in the rate-determining compartment, *i.e.*, $A_i = K_iA_P{}^u$, where K_i is the respective equilibrium constant for the ith compartment, Eq. 68 can be expressed as:

$$-d(K_{RBC/P_u}A_P{}^u + K_{P_b/P_u}A_P{}^u + A_P{}^u + K_{T/P_u}A_P{}^u)/dt = -dA_P{}^u/dt(1 + K_{RBC/P_u} + K^b_{P_b/P_u} + K_{T/P_u}) = k_{P_u,M}A_P{}^u \quad \text{(Eq. 69)}$$

On consideration of Eqs. 20 and 22:

$$K_{RBC/P_u} = \gamma_{RBC}/\gamma_P{}^u = HD/(1 - H) \quad \text{(Eq. 70)}$$

On consideration of Eqs. 21 and 22:

$$K_{P_b/P_u} = \gamma_P{}^b/\gamma_P{}^u = f/(1 - f) \quad \text{(Eq. 71)}$$

On consideration of Eq. 57:

$$K_{T/P_u} = k_{P_u,T}/k_{T,P_u} \quad \text{(Eq. 72)}$$

Substitution of Eqs. 70–72 into Eq. 69 with subsequent rearrangement reaffirms that Eq. 67 properly represents the overall disposition rate constant for the overall loss of drug from the complex of rapidly equilibrating compartments in terms of microscopic rates and equilibrium constants.

If a third compartment can be discriminated for trichloroethanol distribution in the body and the rate of return from this compartment is extremely slow, the observed k_e may be estimated (19) as:

$$k_e = \frac{k_{B,M} + k_{B,T'}}{1 + (k_{B,T}/k_{T,B})} = \frac{k_{P_u,M} + k_{P_u,T'}}{1/\gamma_P{}^u + k_{P_u,T}/k_{T,P_u}} \quad \text{(Eq. 73)}$$

where $k_{B,T'}$ and $k_{P_u,T'}$ are the rate constants for transfer of drug into this "deep" compartment from the central compartment referenced to the total drug and drug not bound to plasma, respectively.

When such a deep compartment, T', exists and when the return of drug to the central compartment becomes significant, Eq. 73 is inadequate for describing the rate-determining rate constant for the disposition of the drug in the body, and the disposition rate begins to mirror the effects of drug return from the deep compartment (19). The presence of the third exponential demonstrated this deep compartment return, and its k_3 value gave a preliminary estimate of $k_{T',B} = k_{T',P_u}$, the rate constant for transfer of drug from the deep compartment into the central compartment.

To describe mathematically the distribution and metabolism of trichloroethanol, it was necessary to obtain the values of the microscopic rate constants that describe the system of Eq. 63 and the apparent volumes of distribution of the equilibrating compartments.

Initial estimates of $k_{B,T}$, $k_{T,B}$, and $k_{B,T'} + k_{B,M}$ were obtained from the parameters of the first two exponentials that characterize the time course of trichloroethanol in the blood as demonstrated in Fig. 2 ($[A_B]_1$, $[A_B]_2$, k_1, and k_2) by their insertion into the equations of Skinner *et al.* (21) as simplified by Riggs (12). The rate constant estimates, in units of reciprocal minutes (min.$^{-1}$), were: $k_{B,T}$ = 0.128, $k_{T,B} = k_{T,P_u}$ = 0.060, and $k_{B,M} + k_{B,T'}$ = 0.058 so that $k_{P_u,T}$ = 0.413 and $k_{P_u,M} + k_{P_u,T'}$ = 0.187. The k_e estimated from these values by use of Eq. 67 was 0.0185 min.$^{-1}$, which was in good agreement with the value of 0.015 min.$^{-1}$ obtained from 2.303 times the slope of the log A_B plot against time during the period characterized by the second exponential in Fig. 2.

The rate constant for trichloroethanol return from the deep tissue was obtained from 2.303 times the slope for the period characterized by the third exponential in Fig. 2 and was $k_{T',B} \sim k_3$ = 0.004 min.$^{-1}$. The fitting of the data with the analog and/or digital computer was then attempted with utilization of these preliminary estimates of the microscopic pharmacokinetic constants.

Analysis of Trichloroethanol Data by Analog Computation[17]—The bases and details of analog computer programming have been described in several excellent texts and brochures (22–24). Applications to fitting the data to complex pharmacokinetic models have also been given (17, 25–29).

The peak height ratio at any time, PHR_t, of trichloroethanol to chlorobutanol per milliliter of whole blood was plotted against time. The ordinate was scaled from 0 to 100%, where 100% of the dose in the blood was taken as the peak height ratio extrapolated to zero time, *i.e.*, $[A_B]_0 = \Sigma[A_0]_i$, and equated to 10 v., so that the amount of trichloroethanol in the blood at any time could be expressed as the percent of the initial dose that appeared in the blood:

$$\%\ TCE_t = \frac{PHR_t}{[A_B]_0} \times 100 \quad \text{(Eq. 74)}$$

on the assumption of instantaneous homogeneity in the central compartment of the intravenously administered drug. The amount of drug lost in the interval t = 0–30 min. (the time period of the first exponential) was fitted by adjusting the ratio $k_{B,T}/k_{T,B}$ while the rate of its initial disappearance was controlled by the magnitude of these constants (26). A preliminary fit of the remaining data was attempted by holding $k_{T',B}$ at 0.004 min.$^{-1}$ and adjusting the magnitudes of $k_{B,T'}$ and $k_{B,M}$ while their sum remained at 0.058 min.$^{-1}$. Minor adjustments in the rate constant values, to account for the interaction of the two curve segments, were then made to achieve a "best" fit of the data.

The time axis potentiometer was set to generate a time function to drive the X–Y recorder such that 2.54 cm. (1 in.) = 1 v. = 40 sec. of machine time. The data were plotted on a 38.1-cm. (15-in.) abscissa with a scale of 2.54 cm. (1 in.) = 40 min. real time. This choice of time scales permitted the values of rate constants to be read directly from the adjusted rate constant potentiometers. A different choice of settings would have necessitated conversions of potentiometer readings to their corresponding rate constant values.

The analog computer fit of the trichloroethanol blood data for Dog D, dosed at 30 mg./kg. and a total dose of 600 mg., is given in Fig. 3.

Analysis of Trichloroethanol Glucuronide Data by Analog Computation—The total amount of free trichloroethanol excreted in the urine was less than 1% of the administered dose. No assayable trichloroacetic acid was found in blood or urine for the intravenous administration of a 30-mg./kg. dose in Dog D. These facts substantiate the basic pharmacokinetic model for trichloroethanol as given in Scheme III and demonstrate that its only significant pathway for removal from the body in the dog is *via* the route of metabolism to its glucuronide. The analog computer model of Scheme III generated amounts of glucuronide with time in terms of a percent of the total dose of trichloroethanol intravenously administered.

[17] The analog computer used was a model TR-10, Electronic Associates, Long Branch, N. J. A recording X–Y plotter, Moseley model 202, manufactured by Hewlett-Packard/Moseley Division, Pasadena, Calif., was used.

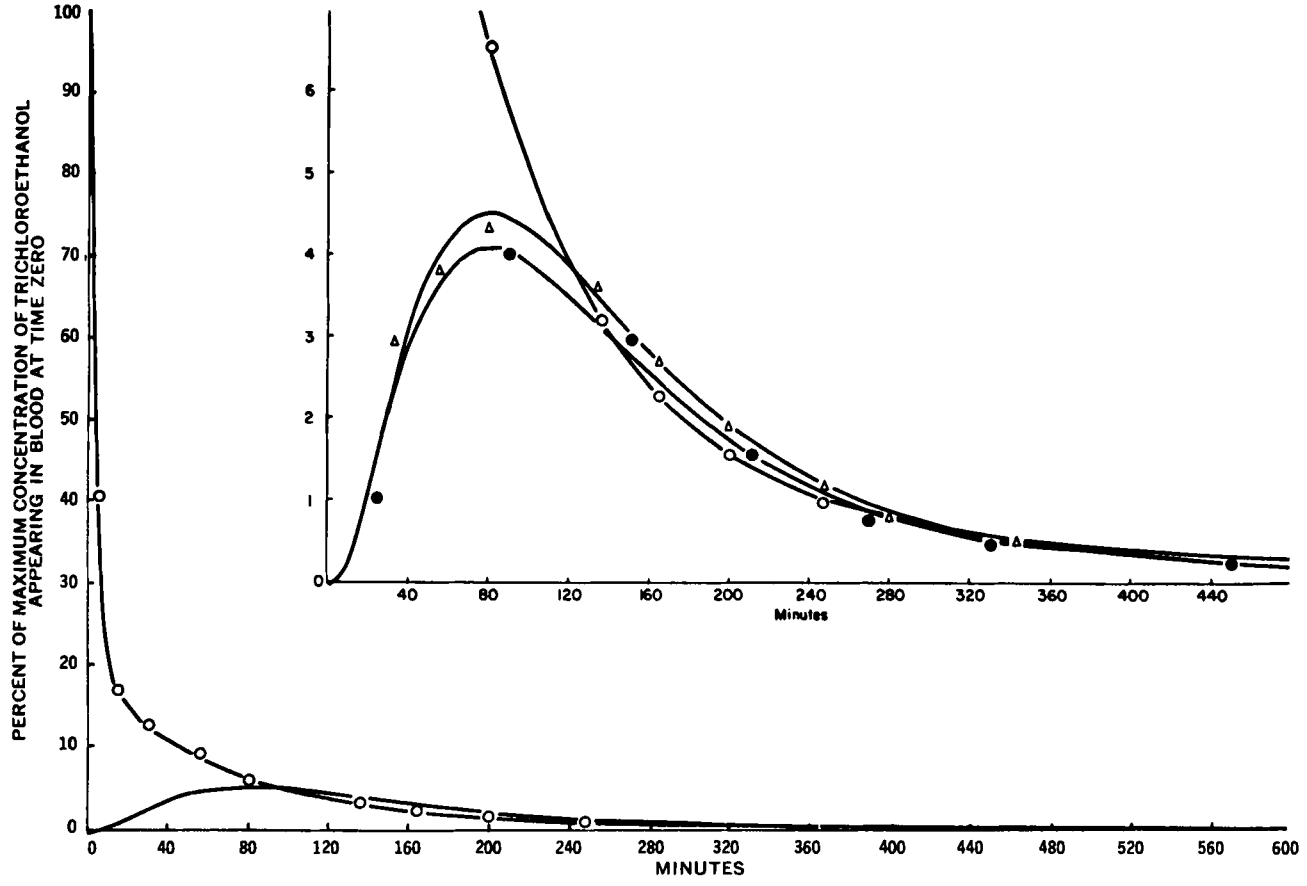


Figure 3—*Analog computer fit of blood and urine data for a dose of 30 mg. trichloroethanol (TCE) per kilogram in the 20-kg. Dog D. The full-scale portion shows the computer fit of the data for trichloroethanol as the percent of the maximum concentration that appears in the blood at zero time* (○), *and the solid line is the predicted trichloroethanol glucuronide in the blood with time. The inset curves are expanded scale plots of the data for trichloroethanol* (○) *and its glucuronide* (△) *with time. The dark circles* (●) *are the urinary excretion rates of trichloroethanol glucuronide (percent dose per minute), which are plotted on a 10-fold smaller scale (0–1%/min.) than that represented on the inset ordinate. The trichloroethanol data were fitted by the program of Scheme V to the specified model by the pertinent microscopic rate constants in Tables I and V, and the computer-generated trichloroethanol glucuronide levels were fitted to the glucuronide data* (△), *which had been multiplied by a factor that represented the ratio of the apparent volumes of distribution of the glucuronide to the trichloroethanol in the central compartment.*

The assayed peak height ratios of trichloroethanol glucuronide to chlorobutanol per milliliter of whole blood were estimated as a percent of the total dose of trichloroethanol administered per volume of whole blood by means of an estimate of the volume of whole blood in the dog on the basis of the preliminary assumption that the glucuronide was only distributed in the central compartment, which was blood. The equation used for this preliminary estimate was:

$$\text{estimated \% of total dose of trichloroethanol as glucuronide in whole blood} = (\text{peak height ratio of glucuronide/chlorobutanol per ml. whole blood}) \times \frac{(8.4 \times 10^{-3}\ \text{mg./peak height ratio})\ (20\ \text{kg.})\ (94\ \text{ml./kg.}) \times 100}{600\ \text{mg.}} \quad \text{(Eq. 75)}$$

where 8.4×10^{-3} mg./peak height ratio is the conversion factor to transform peak height ratios to milligrams of trichloroethanol. The 94-ml./kg. factor has been reported (18) as the whole blood volume of the dog, and 600 mg. is the dose of trichloroethanol administered to Dog D (20 kg.).

The maximum estimated glucuronide in the blood was only 4.2% of the dose. Thus the scale used in the plots of the trichloroethanol was 10-fold, and the estimated percent values as the glucuronide were plotted on the same paper in the expanded range of 0–10% (Fig. 3). This was a reasonable procedure, since the recorder used had a switch that permitted an instantaneous 10-fold amplification of the glucuronide amount in blood generated by the analog computer from an appropriate fitting of the trichloroethanol data. It must be realized that this percent of the dose of trichloroethanol as glucuronide in blood (Eq. 75) was only an estimate of the real situation since the true equilibrated distribution volume, V_{D_B}, of the glucuronide referenced to the total concentration of glucuronide in the blood was not known and only was estimated as being equivalent to the volume of whole blood in the dog. The relationship between the estimated and true values as glucuronide is:

$$\frac{\text{true \% of trichloroethanol dose as glucuronide}}{\text{estimated \% of trichloroethanol dose as glucuronide}} = \frac{[\text{TCE-G}_B] \times V_{D_B}^{\text{true}}}{[\text{TCE-G}_B] \times V_{D_B}^{\text{est}}} = \frac{V_{D_B}^{\text{true}}}{V_{D_B}^{\text{est}}} = \alpha \quad \text{(Eq. 76)}$$

where $[\text{TCE-G}_B]$ is the experimentally observed concentration of glucuronide in whole blood.

The factor α can be estimated from the setting of a variable potentiometer used to multiply the trichloroethanol glucuronide amount in the dog (in terms of percent of trichloroethanol dose as glucuronide) generated from the analog computer fit of the trichloroethanol data in the blood, so that the time course of actual values of glucuronide plotted was fitted when plotted as the estimated values obtained from Eq. 75. This determined α factor would permit a true estimate of the apparent volume of distribution of glucuronide referenced to its total concentration in the blood from a rearrangement of Eq. 75:

$$V_{D_B}^{\text{true}} = \alpha V_{D_B}^{\text{est}} \quad \text{(Eq. 77)}$$

where $V_{D_B}^{\text{est}}$ was taken as 94 ml./kg. (Eq. 76).

The rates of urinary excretion of trichloroethanol glucuronide, $d(\text{TCE-G}_U)/dt$, were determined from the experimental analyses of

total glucuronide in the urine excreted per unit of time in accordance with:

$$\text{urinary excretion rate of glucuronide (\% of dose/min.)} = \frac{100 \times \text{glucuronide excreted in mg. trichloroethanol}}{\text{(dose in mg.) (time interval of excretion in min.)}} \quad \text{(Eq. 78)}$$

These values were plotted on the same graph used for the plotting of estimated glucuronide in the blood as percent of administered trichloroethanol, except that the ordinate was magnified 10-fold to give a range of 0–1% of total trichloroethanol dose excreted per minute (Fig. 3). Since (13):

$$d(\text{TCE-G}_U)/dt = k_e' [\text{TCE-G}_B] \quad \text{(Eq. 79)}$$

the proper multiplication of the amounts of glucuronide in blood as a function of time by a constant k_e' should fit the plotted rates of urinary excretion as a function of time. A selector switch on the recorder permitted instantaneous 10-fold amplification of the generated rate data to conform with the plotted scale. Good fits of both the blood and urine data of glucuronide were necessary before a set of parameters could be said to describe the fate of glucuronide formed from trichloroethanol.

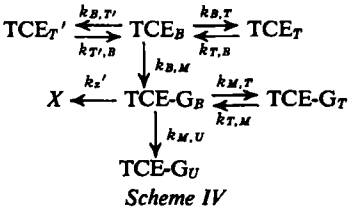
The simplest model for fitting the glucuronide data in blood with the rate constants generated from the fit of trichloroethanol data in blood assumed that the glucuronide appearance in blood reflected trichloroethanol loss as in Scheme III, *i.e.*:

$$d(\text{TCE-G}_B)/dt = k_{B,M}(\text{TCE}_B) \quad \text{(Eq. 80)}$$

If and only if the apparent volumes of distribution of the central compartment referenced to concentrations in blood for both drug, $(V_B)_{TCE}$, and metabolite, $(V_B)_{TCE\text{-}G}$, were the same would this equation be applicable to concentrations. In general:

$$d[\text{TCE-G}_B]/dt = \{(V_B)_{TCE}/(V_B)_{TCE}\} k_{B,M}[\text{TCE}_B] \quad \text{(Eq. 81)}$$

An appropriate expanded model might be as shown in Scheme IV,



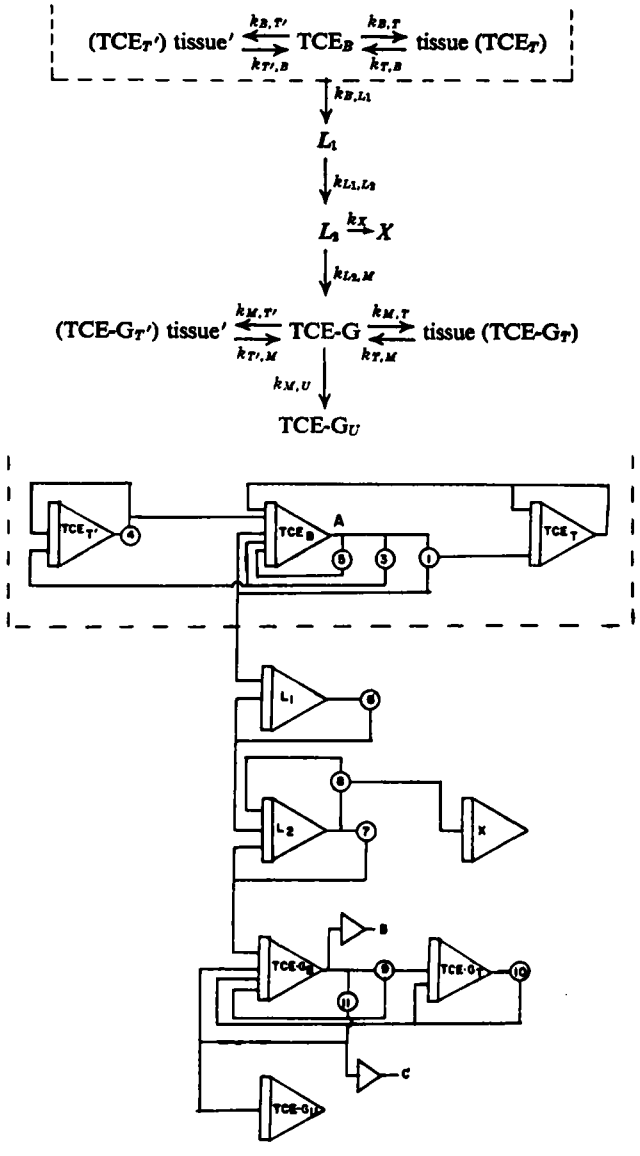
Scheme IV

where the k_e' of Eq. 79 would be defined (13) by the microscopic constants in a manner analogous to Eq. 73 where the glucuronide is rapidly equilibrated with a tissue, TCE-G$_T$, as:

$$k_e' = \frac{k_{M,U} + k_z'}{1 + k_{M,T}/k_{T,M}} \quad \text{(Eq. 82)}$$

where k_z is inserted as a microscopic rate constant for a possible elimination route that occurred simultaneously with the urinary elimination of glucuronide from the body. The urinary excretion rates of glucuronide were always proportional to its concentration in the blood in accordance with Eq. 79. The fact that an intravenous dose of glucuronide was, for all practical purposes, completely excreted into the urine permitted the decision that $k_z' = 0$. The initial estimates of $k_{M,T}$, $k_{T,M}$, and $k_{M,U}$ were obtained from a separate analysis of the pharmacokinetics of the intravenously administered dose of glucuronide.

However, the model of Scheme IV could not be fitted to the glucuronide data plotted as values derived from Eq. 75 against time (Fig. 3). No combination of α values and distribution and elimination rate constants could fit the observed data for glucuronide in blood and urine. The major inconsistencies observed were that the analog computer curves generated from the fits to the trichloroethanol data gave rapid early blood levels, whereas the experimental glucuronide data demonstrated an induction period, a slower rate of glucuronide appearance in the blood, and a broader maximum. It was, therefore, necessary to postulate an additional compartment, L_1, in the model, between trichloroethanol in the blood, TCE$_B$, and the glucuronide in the blood, TCE-G$_B$ (Scheme V), which could serve to accumulate the glucuronide and release it slowly into blood. Although the presence of this compartment improved the fit of calculated to observed data, the lag time required to fit the rate of appearance of glucuronide in blood was still not accommodated. To fit the lag in appearance of glucuronide blood levels while continuing to assume concentration-dependent or first-order processes, it was necessary to insert another compartment, L_2, in the model (Scheme V).



Scheme V—The multicompartmental model and corresponding analog computer program required for the analysis and fitting of pharmacokinetic data. The portions enclosed in dashed lines were used to evaluate trichloroethanol data as analyzed in blood, TCE$_B$, alone while the entire model and program were used to fit trichloroethanol glucuronide data as analyzed in blood, TCE-G$_B$. The potentiometers in the program and their corresponding rate constants in the model were: potentiometer 1, $k_{B,T}$; 2, $k_{T,B}$; 3, $k_{B,T'}$; 4, $k_{T',B}$; 5, k_{B,L_1}; 6, k_{L_1,L_2}; 7, $k_{L_2,M}$; 8, k_x; 9, $k_{M,T}$; 10, $k_{T,M}$; and 11, $k_{M,U}$. Analog computer plots of calculated data were obtained from points A for trichloroethanol in blood, TCE$_B$; B for trichloroethanol glucuronide in blood, TCE-G$_B$; and C for the urinary excretion rate of trichloroethanol glucuronide, d(TCE-G$_U$)/dt.

The presence of compartments L_1 and L_2 (in series between TCE$_B$ and TCE-G$_B$), coupled with an additional compartment (X) to account for the fraction of the dose unaccounted for in urine, allowed for accurate fitting of the glucuronide data in blood and urine in accordance with Scheme V. The analog computer program for this model is also given in the scheme. The portions of the model and program enclosed in dashed lines were used in the preliminary

Table I—Values of Pharmacokinetic Parameters[a] in Dogs Obtained from Computer Fitting of Blood and Urine Data after Intravenous Administration of Trichloroethanol

Dose, mg./kg.	$[A_B]_0$[b]	V_B[c]	V_{P^u}[d]	$V_{T_{P^u}}$[e]	$V_{T'_{P^u}}$[f]	$k_{B,T}$	$k_{T,B}$	$k_{B,T'}$	$k_{T',B}$	k_{B,L_1}	k_{L_1,L_2}	$k_{L_1,M}$	k_e
							Dog A (16 kg.)						
10	0.034	2.94	3.27	14.6	18	0.155 (0.023)	0.112 (0.009)	0.022 (0.003)	0.013 (0.0004)	0.075 (0.008)	0.184	0.018	0.002
50				14.2	22	0.172 (0.049)	0.127 (0.069)	0.039 (0.012)	0.019 (0.001)	0.061 (0.014)	0.160	0.021	0.002
							Dog B (18.6 kg.)						
10	0.030	3.33	3.68	21.6	6	0.069	0.038	0.005	0.009	0.061	0.140	0.016	0.002
25				21.6	6	0.069 (0.012)	0.038 (0.008)	0.005 (0.009)	0.009 (0.001)	0.061 (0.007)	0.140	0.016	0.002
100				19.8	5	0.065 (0.019)	0.039 (0.008)	0.005 (0.006)	0.010 (0.003)	0.039 (0.006)	0.013	0.023	0.001
							Dog C (10 kg.)						
10	0.044	2.27	2.83	13.9	31	0.122 (0.093)	0.080 (0.021)	0.009 (0.004)	0.003 (0.001)	0.070 (0.030)	0.208	0.023	0.002
50				15.7	36	0.186	0.108	0.007	0.002	0.084	0.140	0.019	0.001
100				11.6	36	0.165	0.130	0.007	0.002	0.047	0.025	0.014	0.001
176				16.1	27	0.186	0.106	0.005	0.002	0.033	0.015	0.013	0.001
							Dog D (18 kg.)						
17	0.031	3.22	3.54	22.2	35	0.124 (0.002)	0.064 (0.009)	0.013 (0.002)	0.004 (0.0007)	0.059 (0.001)	0.030 (0.010)	0.022 (0.004)	0.008 (0.003)
50				20.0	19	0.147 (0.020)	0.084 (0.012)	0.009 (0.001)	0.005 (0.0006)	0.045 (0.001)	0.030	0.022	0.008
100				19.6	19	0.161 (0.040)	0.094 (0.011)	0.007 (0.001)	0.004 (0.0003)	0.038 (0.005)	0.010	0.019	0.006
150				19.6	21	0.161 (0.040)	0.094 (0.011)	0.004 (0.0004)	0.002 (0.0007)	0.034 (0.0008)	0.010	0.019	0.006
25[g]				19.2	21	0.158 (0.007)	0.039 (0.006)	0.010 (0.009)	0.005 (0.0009)	0.068 (0.001)	0.032	0.022	0.008
30[g]				28.7	28	0.151 (0.026)	0.060 (0.012)	0.008 (0.003)	0.003 (0.007)	0.050 (0.008)	0.040	0.019	0.006
							Dog E (10 kg.)						
60	0.042	2.38	3.03	10.4	20	0.119	0.112	0.013	0.007	0.063	—	—	—
60[h]				10.4	20	0.119	0.112	0.013	0.007	0.063	—	—	—
60				12.5	19	0.202	0.158	0.021	0.012	0.075	—	—	—
60[i]				11.0	15	0.129	0.115	0.026	0.018	0.035	—	—	—

[a] The microscopic rate constants are defined in Scheme V and are given in minutes^{-1}. The values of $k_{M,T}$, $k_{T,M}$, $k_{M,T'}$, $k_{T',M}$, and $k_{M,U}$ were 0.133, 0.190, 0.008, 0.017, and 0.069 min.$^{-1}$, respectively, and were obtained from the separate studies on trichloroethanol glucuronide pharmacokinetics. The numbers in parentheses are standard deviations of the stated parameters obtained by digital computer fitting with the SAAM program. [b] The computer-fitted zero time concentration of trichloroethanol in blood, $[A_B]_0$, in terms of percent of dose per milliliter of whole blood. The $[A_B]_0$ values were reasonably coincident for all doses in a given dog (Fig. 8). [c] The apparent volume of distribution of the central compartment referenced to total concentration in blood is $V_B = 100\%/[A_B]_0$ in liters. [d] The apparent volume of distribution referenced to the concentration of trichloroethanol in the plasma unbound to protein is $V_{P^u} = V_B\,(1.792) - 1.24\,V_{B_{true}}$ after Eq. 34, where $H = 0.45$, $D = 2.1$, $f = 0.35$, and $V_{B_{true}} = 0.1 \times$ weight of dog in kilograms (16). [e] The apparent volume of distribution referenced to the concentration of unbound trichloroethanol in the plasma of the rapidly equilibrating compartment, T, in liters, where $V_{T_{P^u}} = V_{P^u} - (k_{B,T}/\gamma_{P^u} k_{T,B})$. [f] The apparent volume of distribution referenced to the concentration of unbound trichloroethanol in the plasma of the slowly equilibrating compartment, T', in liters, where $V_{T'_{P^u}} = V_{P^u} - (k_{B,T'}/\gamma_{P^u} k_{T',B})$. [g] The dog weights in these studies were 20 kg. [h] Trichloroethanol, 60 mg./kg. or 600 mg., was administered initially. At 250 min. later, 100 mg. of sulfobromophthalein (10 mg./kg.) was administered concurrently with an additional dose of 600 mg. of trichloroethanol (60 mg./kg.) and an intravenous drip of 0.6 mg. sulfobromophthalein/min. was started and continued until 490 min. [i] Trichloroethanol, 60 mg./kg. or 600 mg., was administered initially. At 320 min. later, 500 mg. of sulfobromophthalein (50 mg./kg.) was administered concurrently with an additional dose of 600 mg. of trichloroethanol (60 mg./kg.) and an intravenous drip of 3 mg. sulfobromophthalein/min. was infused until 500 min.

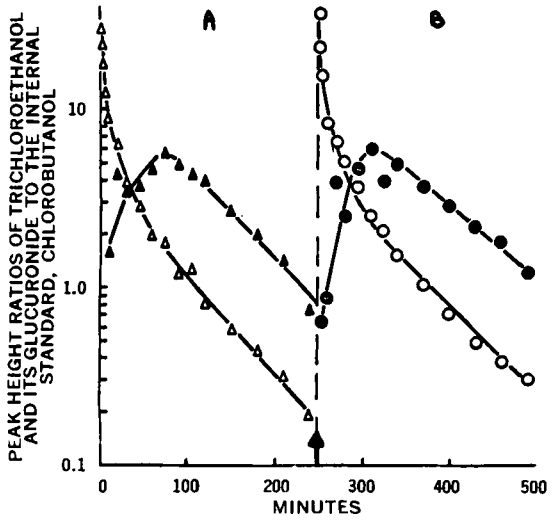


Figure 4—*Semilogarithmic plots of the peak height ratios of trichloroethanol (△,○) and trichloroethanol glucuronide (▲,●) as representative of blood levels with time in the absence and presence of sulfobromophthalein for the 10-kg. Dog E. Segment A is a consequence of dosing 60 mg./kg. of trichloroethanol at zero minute. Segment B is a consequence of dosing 60 mg./kg. of trichloroethanol and 10 mg./kg. of sulfobromophthalein at 250 min. A peak height ratio of 1.0 is equivalent to 8.4 mcg. trichloroethanol or 18.3 mcg. trichloroethanol glucuronide/ml. of whole blood.*

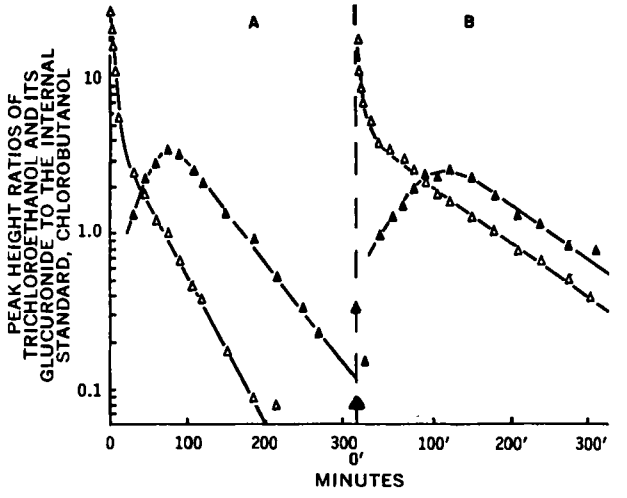


Figure 5—*Semilogarithmic plots of the peak height ratios of trichloroethanol (△) and trichloroethanol glucuronide (▲) as representative of their respective blood levels with time in the absence and presence of sulfobromophthalein for the 10-kg. Dog E. Segment A is a consequence of dosing 60 mg./kg. of trichloroethanol at zero minute. Segment B is a consequence of dosing 60 mg./kg. of trichloroethanol and 50 mg./kg. of sulfobromophthalein at 320 or 0' min. A peak height ratio of 1.0 is equivalent to 8.4 mcg. trichloroethanol or 18.3 mcg. trichloroethanol glucuronide/ml. whole blood.*

fit of the trichloroethanol data in the blood, where k_{B,L_1} is the rate constant given previously as $k_{B,M}$ (Schemes III and IV).

The placement in the multicompartmental model of k_X, a rate constant for elimination of the missing drug fraction, was arbitrary since the route of elimination was unknown. The urinary recovery, for all practical purposes, of all of an intravenous dose of the glucuronide indicated that the TCE-G_B compartment would not be a logical place to remove a fraction of the dose with a rate constant, k_x. Since postulation of this elimination route from the trichloroethanol in the blood, TCE_B, would alter those parameters previously obtained for the loss of trichloroethanol, it was decided that the missing fraction of drug exited from compartment L_2. The fact that an amount of the trichloroethanol dose is secreted into bile as the glucuronide made this choice consistent with physiological reality.

The ratio, $k_X/k_{L_2,M}$, could be estimated (13) from:

$$\frac{k_X}{k_{L_2,M}} = \frac{100 - (\%\ \text{TCE-G}_U)_\infty}{(\%\ \text{TCE-G}_U)_\infty} \qquad \text{(Eq. 83)}$$

where $(\%\ \text{TCE-G}_U)_\infty$ is the total percent of the trichloroethanol dose excreted in the urine.

Analysis of Data by Digital Computation—The major disadvantage of the analog computer in data analysis is that the goodness of fit must be evaluated visually. Statistical determination of the agreement between calculated and observed points would demand a nonlinear regression analysis for every fit. Fortunately, programs are available for the digital computer that include such statistical analysis along with methods to evaluate pharmacokinetic parameters. The SAAM (Simulation, Analysis, and Modeling) program of Berman and Weiss (30) is such a program and was used on a digital computer[18] to fit the pharmacokinetic data.

SAAM is a general-purpose computer program designed to fit physical or mathematical models to data by adjusting the parameter values of the model until a "best" fit is obtained. Any set of mathematical equations (differential, integral, or algebraic) or functions may serve as a model provided an analytical or numerical procedure exists for its solution.

Since the model for trichloroethanol pharmacokinetics (Scheme III) assumed first-order transfers among all compartments, the equations describing the system were linear differential equations with constant coefficients. The equations are solved by the SAAM program with the fourth-order approximative technique of Runge-Kutta (31).

Data to be analyzed were punched out on IBM cards. The data for trichloroethanol in blood were given in terms of percent of total dose, where 100% was the extrapolated zero-time blood concentration (Eq. 74).

Estimates of the rate constants needed to fit the data (lambdas in SAAM program terminology) were those obtained with the preliminary analog computer data fit (Scheme V). These rate constants required iterative adjustment to obtain a least-squares fit of the data. A variable proportionality factor, kappa, was also included within the program to adjust the data in the case of a poor estimate for the initial volume of distribution. If the initial rate constant (lambda) estimates did not provide a good statistical fit of the data, the SAAM program increased or decreased the data by a constant factor, kappa (hence decreasing or increasing the volume of distribution), while simultaneously adjusting the lambda values until the best statistical fit was achieved.

To specify that a parameter be subject to iterative adjustment, upper and lower limits were assigned to each kappa and lambda estimate in the model. Lower limits for lambdas were zero (0.0) while upper limits were twice the value of the initial estimate. Upper and lower limits for kappas were 0.5 and 2.0, twice and one-half the initially estimated volume of distribution, respectively.

A routine printout was produced for every problem deck submitted to SAAM and included: a listing of the problem deck with each card printed as reformatted by SAAM, a computer language reorganization of the information in the program deck, parameter values and a table of the initial solution values (zero iteration), and results for each iteration associated with the fitting of the data and final results including parameter values and the corresponding solution table for the best fit with estimated standard errors and correlation coefficients. An optional arithmetic plot of calculated and observed data could be requested and was used in all trichloroethanol pharmacokinetic analyses.

The final parameter values obtained with the SAAM program were $k_{B,T} = 0.1506$, $k_{T,B} = 0.0602$, $k_{B,T'} = 0.0082$, $k_{T',B} = 0.0030$, and $k_{B,L_1} = 0.0505$ for the 30-mg./kg. dose given Dog D. A kappa value of 1.30 was required to fit the data. This kappa served to raise the calculated initial trichloroethanol concentration from 0.142 mg. trichloroethanol/ml. of blood (or 0.0237 as percent of total dose per milliliter of blood) to 0.185 mg./ml. blood (or 0.031 as percent of total dose per milliliter blood) and hence changed the calculated apparent volume of distribution, V_B, referenced to total concentration in the blood (Eq. 24) from 4.24 to 3.26 l. Thus, the

[18] IBM model 360/50.

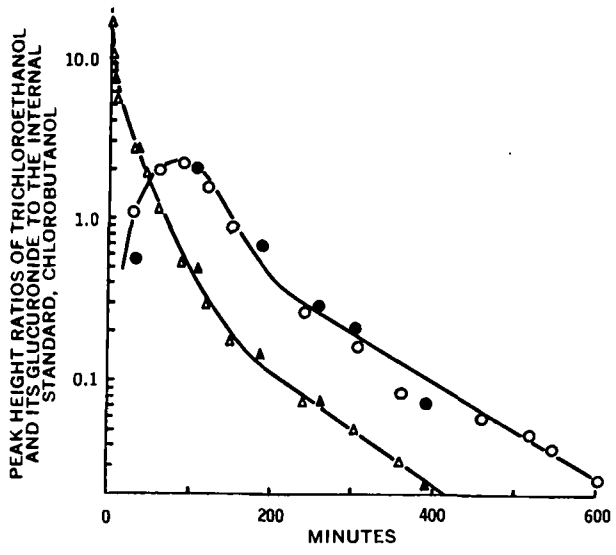


Figure 6—*Semilogarithmic plots of the peak height ratios to the internal standard chlorobutanol of trichloroethanol and trichloroethanol glucuronide blood concentrations with time for a 50-mg./kg. trichloroethanol dose in the absence and presence of pentobarbital anesthesia. The open circles and triangles represent the trichloroethanol and trichloroethanol glucronide blood concentrations for the first dose of trichloroethanol given to the dog in the absence of pentobarbital anesthesia. The solid circles and triangles are the trichloroethanol and trichloroethanol glucuronide data for an identical dose of trichloroethanol given 7 days later to the dog anesthetized with 30 mg./kg. pentobarbital. A peak height ratio of 1.0 is equivalent to 8.4 mcg. trichloroethanol or 18.3 mcg. trichloroethanol glucuronide/ml. whole blood.*

apparent volume of distribution referenced to the concentration of unbound trichloroethanol in the plasma, V_P^u, is 3.37 l. (Eq. 34).

It also follows that the apparent volumes of distribution of the tissue compartments referenced to the unbound drug concentration in plasma are $V_{T_P}^u$ = 26.1 l. from Eq. 59 and $V_{T'_P}^u$ = 29.7 l. from Eq. 60. These are in decided contrast to the apparent volumes of distribution of the tissue compartments if they were referenced to the total drug concentration in blood, since V_{T_B} = 8.1 l. and $V_{T'_B}$ = 8.8 l.

RESULTS AND DISCUSSION

Sensitivity and Reliability of Analytical Methods—A typical chromatogram for a 5-μl. aliquot of the ether extract of a solution of trichloroethanol and its glucuronide, chloral hydrate, and trichloroacetic acid is given in Fig. 1. The dashed line over the trichloroethanol chromatographic peak was the peak observed after 48 hr. of hydrolysis with β-glucuronidase when the glucuronide was present in the sample. It includes free and conjugated trichloroethanol.

The trichloroethanol produced by the β-glucuronidase- (1 mg.) catalyzed hydrolysis of its glucuronide in urine was monitored gas chromatographically as a function of time. A plot of the logarithm of the difference between the peak height ratio (trichloroethanol to chlorobutanol) at infinite time and the peak height ratio at a time, t, against that time was linear and demonstrated a first-order hydrolysis of the glucuronide. The half-life of the solvolysis of glucuronide under the conditions specified in this paper was obtained from the slope of this plot and was 8 hr., so that 98% hydrolysis would be effected after 48 hr. of incubation at 37°.

Concentrations as low as 0.5 mcg. of trichloroethanol, its glucuronide, or chloral hydrate (3.3, 1.54, or 3.0 μmoles, respectively) in 3.0 ml. of water, urine, or blood were assayable. The sensitivity for trichloroacetic acid was 5 mcg. (31 μmoles) in 3.0 ml. Linear calibration curves were obtained for all compounds over a 20-fold concentration range (7). Assay values (trichloroethanol–chlorobutanol peak height ratios) from the trichloroethanol "spiked" blood of four different dogs showed a maximum deviation of less than 3%. Deviation of 2–4.8% resulted between refrigerated blood samples stored up to 168 hr. and calibration standards.

***In Vivo* Inhibition of Trichloroethanol Glucuronidation by Sulfobromophthalein**—The intravenous administration of 10 mg./kg. of sulfobromophthalein concurrently with a 60-mg./kg. dose of trichloroethanol and subsequent infusion of 0.6 mg./min. sulfobromophthalein did not result in any alteration in the pharmacokinetic profile of trichloroethanol and its glucuronide in Dog E when the data were compared with those obtained for an equal dose of trichloroethanol with no sulfobromophthalein administered 4 hr. previously (Fig. 4 and Table I). When the experiment was repeated and the dose of sulfobromophthalein was increased in Dog E to 50 mg./kg., with a subsequent infusion of 3 mg./min. of sulfobromophthalein, a significant decrease in the rate of loss of trichloroethanol from the blood was observed (Fig. 5 and Table I).

Possible Interaction of Pentobarbital Anesthesia with Trichloroethanol Pharmacokinetics—The use of pentobarbital anesthesia in the pharmacokinetic studies of trichloroethanol and its metabolites in the dog was necessitated by the animal's hyperexcitability and difficulty in handling without initial sedation prior to drug administration. However, studies were made in the same animal (Dog E) with the same trichloroethanol doses (50 mg./kg.) with and without pentobarbital anesthesia. The plotted data (Fig. 6) demonstrated no apparent difference in the time course of trichloroethanol or its glucuronide in blood, a valid indication that pentobarbital anesthesia had no significant effect on the pharmacokinetics of trichloroethanol and its glucuronide.

Hepatic Storage of Trichloroethanol and/or Its Glucuronide—The portal and hepatic veins were sampled with time and assayed for trichloroethanol and its glucuronide in the dog that was surgically modified (11) so that the vena cava was occluded and the vena caval flow did not dilute the hepatic output. The trichloroethanol extracted by the liver per milliliter of blood, $[TCE]_L$/ml., was determined from the subtraction of the trichloroethanol concentration in the hepatic vein blood from the concentration in the portal vein blood. The trichloroethanol glucuronide per milliliter of blood, $[TCE\text{-}G]_L$/ml., released by the liver was determined from the subtraction of the glucuronide concentration (as trichloroethanol) in the portal vein blood from the concentration in the hepatic vein blood.

The difference in these two values, $[TCE]_L$/ml. − $[TCE\text{-}G]_L$/ml., represents the storage of trichloroethanol and/or its glucuronide in the liver in terms of concentration per milliliter of blood. When this difference is multiplied by an estimate of the hepatic blood flow, 600 ml./min. for a 15-kg. dog (32), the net amount of trichloroethanol and/or its glucuronide extracted per minute from the hepatic blood is estimated. A plot of such data against time (Fig. 7) indicates that significant accumulation of trichloroethanol and/or its glucuronide in the liver is terminated within an hour of drug administration; *i.e.*, the liver may be considered saturated with respect to the drug and/or its metabolites. When blood hepatic and portal vein samples were analyzed at 90 min., the process was reversed. The amount of glucuronide released by the liver exceeded the trichloroethanol extracted or transformed by the liver so that it can be concluded that the accumulated liver storage of trichloroethanol and/or its glucuronide was being depleted.

The insert of Fig. 7 plots the area under the estimated curve of Fig. 7 against time and thus represents the cumulative amount of trichloroethanol and/or its glucuronide retained by the liver with time. Since the initial rates of drug and/or metabolite retention were estimated from a presumed linear relation with time, the maximum amount of drug and/or metabolite retention in the insert of Fig. 7 is undoubtedly underestimated.

Trichloroethanol Distribution in Dogs—The fractions of trichloroethanol and its glucuronide bound to protein were based on the equilibrium dialysis experiments with dog plasma and calculated from:

$$\text{fraction bound} = \frac{\text{concentration of drug inside dialysis bag} - \text{concentration outside}}{\text{concentration of drug inside dialysis bag}} \quad \text{(Eq. 84)}$$

The values obtained (Table II) were in agreement with those reported by Marshall and Owens (5) for trichloroethanol binding by reconstituted lyophilized human plasma. Control experiments (dialysis in the absence of plasma) gave no evidence that trichloroethanol or its glucuronide was bound by the dialysis sac. Approxi-

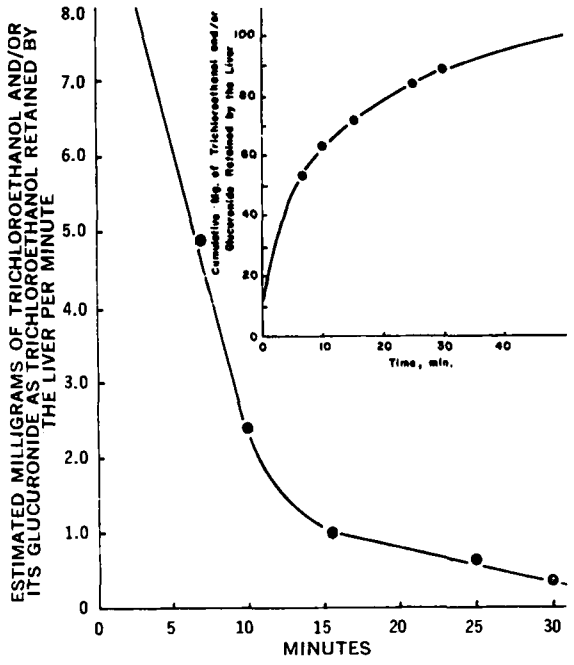


Figure 7—*Estimated amount of trichloroethanol as itself or as its glucuronide that is stored in the liver per minute after injection of a 50-mg./kg. dose of trichloroethanol in the hepatic portal vein in a dog that was surgically modified so that the vena cava was occluded and the vena caval flow did not dilute the hepatic output. The differences in the simultaneously assayed concentrations in milligrams per milliliter of trichloroethanol plus its glucuronide of the hepatic and portal vein bloods were multiplied by an estimated hepatic blood flow of 600 ml./min. to estimate the milligrams of trichloroethanol and/or its glucuronide per minute accumulated in the liver. At 90 min., the glucuronide released by the liver exceeded the trichloroethanol extracted or transformed by the liver. The inset is the cumulative curve for the milligrams of drug and/or its metabolite retained by the liver with time.*

metely 35% of the trichloroethanol found in plasma was bound to protein, and this percent was independent of the plasma concentration of trichloroethanol (Table II). Fifty-three percent of the total trichloroethanol in the entire blood was instantaneously distributed into red blood cells; 16% was bound to plasma proteins and 31% was free (Eqs. 20–22).

The partition coefficient for the ratio of drug concentration in the red blood cells to the drug concentration in plasma unbound to protein, D (Eq. 8), was calculated from Eq. 12 and was taken as 2.1 (Table III). The determined hematocrit, H, was 0.45.

Table II—Data for Estimation of Binding of Trichloroethanol and Its Glucuronide to Plasma Proteins

Compound	Micrograms per Milliliter[a]	Equilibrated Concentration inside Sac[b]	Equilibrated Concentration outside Sac[a]	Percent Bound[d]
Trichloroethanol	5	0.89	0.56	37.4[e]
	50	9.5	5.8	38.7[e]
	500	106.7	70.6	33.8[d]
Trichloroethanol glucuronide	9	0.67	0.4	36.2
	45	2.9	2.2	25.3

[a] Concentrations in 10.0 ml. of pH 7.4 phosphate buffer dialyzed against 2 ml. of dog plasma inside the dialysis sac. [b] Given in terms of trichloroethanol–chlorobutanol peak height ratio, $(TCE/CB)_t$, per milliliter of plasma. [c] Given in terms of trichloroethanol–chlorobutanol peak height ratio, $(TCE/CB)_0$, per milliliter of pH 7.4 phosphate buffer. [d] Percent bound = $100\ [(TCE/CB)_t - (TCE/CB)_c]/(TCE/CB)_t$. [e] The percent errors for total trichloroethanol in the plasma–buffer dialyses *versus* the buffer–buffer controls were 6, 6.7, and 9.4%.

Table III—Red Blood Cell–Plasma Partition Coefficients (D) for Trichloroethanol and Its Glucuronide

Compound	Micrograms per Milliliter	Concentration[a] Whole Blood	Concentration[a] Plasma	D[b]
Trichloroethanol	0.294	0.03	0.02	2.1
	2.94	0.32	0.28	2.0
	47.7	4.68	3.30	2.9[c]
	26.7	3.18	2.17	3.1[c]
	34.0	4.03	3.29	2.3
Trichloroethanol glucuronide	29.1	1.87	3.57	—[d]

[a] Given in terms of trichloroethanol–chlorobutanol peak height ratios per milliliter. [b] Calculated from Eq. 12 for the ratio of concentration of drug in the red blood cells to the concentration of unbound drug in plasma as defined in Eq. 8. [c] Blood samples from a dog that had received 50 mg./kg. of trichloroethanol and 50 mg./kg. of sulfobromophthalein. [d] A value of −0.07 was obtained for the trichloroethanol glucuronide.

The initial apparent volumes of distribution of trichloroethanol referenced to the concentration in whole blood, V_B, and to the unbound concentration in plasma, $V_P{}^u$, were reasonably consistent among the animals studied at all doses (Tables I and IV). The apparent volumes of distribution of the central compartment referenced to the concentration of unbound drug in plasma were approximately equivalent to the extracellular body water of the dog, *i.e.*, 200–350 ml./kg. (18). Thus, it may be assumed that the diffusion of trichloroethanol into the extracellular body water occurred during its first cycles through the circulatory system and the kinetics of its distribution could not be readily observed.

Table IV shows that the apparent volume of distribution of the central compartment which was equated to extracellular water was significantly larger for small dogs (10 kg.) compared to larger dogs (16–20 kg.) on a milliliter per kilogram basis.

The compartment, T (Scheme V), that readily equilibrated with the central compartment had an apparent volume of distribution referenced to the concentration of unbound drug in plasma, $V_{T_P}{}^u$, of 4–6 times the apparent volume of the central compartment similarly referenced (Table IV). Since the total body water is only about 2.5 times that of the extracellular body water (500–750 ml./kg.) in the dog (18), it is implied that trichloroethanol in the central compartment readily equilibrates with lipoidal tissues as well as total body water. The presence of a deep compartment, T', of relatively large capacity in most instances (Table IV) implicates a

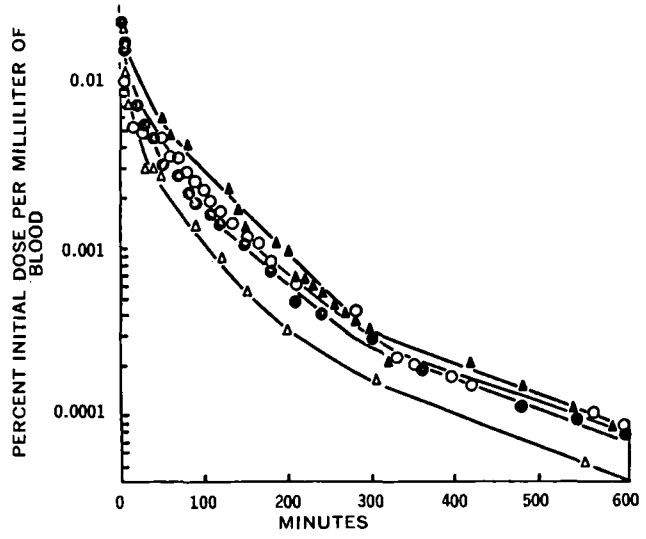


Figure 8—*Semilogarithmic plot of trichloroethanol blood concentrations (as percent initial dose per milligrams whole blood) with time for doses of 17 (△), 50 (●), 100 (○), and 150 (▲) mg. trichloroethanol/kg. in Dog D. The data points were calculated by dividing the trichloroethanol concentration (milligrams per milliliter) by the dose (milligrams) and multiplying by 100.*

Table IV—Range of Apparent Volumes of Distributions for Trichloroethanol (ml./kg.) for All Doses in Dogs

	Dog				
	A	B	C	D	E
Weight, kg.:	16	18.6	10	18	10
V_B	184	180	227	180	240
V_{P^u}	204	198	283	197	303
$V_{T_{P^u}}$	900 ± 10	1130 ± 50	1430 ± 200	1200 ± 100	1100 ± 100
$V_{T'_{P^u}}$	1250 ± 120	310 ± 20	3300 ± 400	1300 ± 300	1800 ± 200

slowly equilibrating, nonvascularized depot in the body which is also most probably lipoidal.

Effect of Dose on Trichloroethanol Pharmacokinetic Parameters in Dogs—Typical semilogarithmic plots of the trichloroethanol per milliliter of whole blood *versus* time for different intravenous doses in a female mongrel dog, Dog D, are given in Fig. 8. The data are plotted as the percent of dose per milliliter of whole blood:

$$\% \text{ dose/ml. whole blood} = \frac{\text{mg. trichloroethanol/ml. whole blood}}{\text{dose in mg.}} \times 100 \quad \text{(Eq. 85)}$$

which normalizes the data for different doses of trichloroethanol to a common scale. If the parameters for drug disposition were independent of dose, the data of Fig. 8 should be superimposable for all doses. This was not so and was typical of the studies in all dogs. Thus, it must be concluded that the pharmacokinetics of trichloroethanol are dose dependent.

Typical semilogarithmic plots for the appearance and subsequent loss of trichloroethanol glucuronide in blood as a function of time after intravenous administration of trichloroethanol are given in Fig. 9 for Dog C. The data are presented as the peak height ratios of trichloroethanol to chlorobutanol per milliliter of whole blood, where a peak height ratio of 1.0 is equal to 19.5 mcg. of the sodium salt of trichloroethanol glucuronide per milliliter of whole blood.

Similar data for Dog D, but given as the percent of the initial dose per milliliter of whole blood *versus* time, are depicted in Fig. 10. The effect of an increasing trichloroethanol dose on the pharmacokinetics of the glucuronide is to delay and broaden the maximum in glucuronide blood level (Figs. 9 and 10). The height of the maximum in terms of percent of the dose is elevated with the increased dose of trichloroethanol (Fig. 10).

The pharmacokinetic parameters of rate constants and volumes of distribution, which describe the distribution and metabolism of trichloroethanol and the appearance, distribution, and urinary elimination of its glucuronide after intravenous administration of different doses of trichloroethanol, are found in Table I for Dogs A–D. Typical analog computer curves generated from the rate constants of Table I are given for widely varied trichloroethanol doses (10 and 176 mg./kg.) in Figs. 11 and 12 for the data of Dog C.

The rate constant, k_{B,L_1} (Scheme V), for the loss of trichloroethanol to the glucuronide decreased as a function of dose in Dogs A–D (Table I and Fig. 13). The dogs formed two groups of similar k_{B,L_1} values, the rate constants for loss of trichloroethanol from the central compartment at an infinitely small trichloroethanol dosage. Curvilinear extrapolation of the data from the two lean dogs, A and C (10 and 16 kg., respectively) gave an estimated k^0_{B,L_1} of 0.086 min.$^{-1}$, while a value of 0.069 min.$^{-1}$ was obtained for the two obese dogs, B and D (18 and 18.6 kg., respectively) (Fig. 13).

The decrease of the rate constant, k_{B,L_1}, for the loss of trichloroethanol to glucuronide with dose is not unique. Similar dose dependency of apparent first-order disappearance of drug from a central compartment was reported for dicumarol (33), ethyl biscoumacetate (34), probenecid (35), and diphenylhydantoin and phenylbutazone (36). As yet, no satisfactory mechanism has been advanced.

A possible explanation is an *in vivo* saturation by a substrate of a metabolic pathway, as described by Levy (37) for salicylate. Probenecid (38) and diphenylhydantoin (39), which also demonstrate dose-dependent pharmacokinetics, also have been reported to be metabolized by glucuronidation. The coumarin derivatives and phenylbutazone have structures that suggest metabolism by the same route. Chloral hydrate (5 m*M*) has been reported to give 50% inhibition of microsomal *O*-aminophenol conjugation (40).

Sulfobromophthalein was shown to be a potent inhibitor of glucuronidation in *in vitro* experiments with rabbit liver homogenate (40). When 10 mg./kg. of this substance was administered simultaneously with 60 mg./kg. of trichloroethanol, no significant change in k_{B,L_1} was observed from the case when sulfobromophthalein was not administered (Dog E, Table I and Fig. 4), even though the sulfobromophthalein dose used was twice that recommended for studies on hepatic function (10). However, when the sulfobromophthalein dose administered simultaneously with 60 mg./kg. of trichloroethanol was increased to 50 mg./kg., a significant 46% decrease in

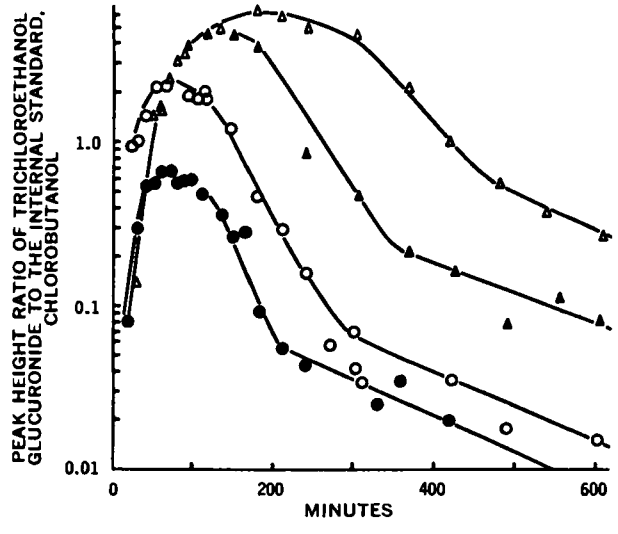


Figure 9—*Semilogarithmic plot of the gas chromatographically obtained peak height ratios for trichloroethanol as trichloroethanol glucuronide to the chlorobutanol internal standard with time for doses of 10 (●), 50 (○), 100 (▲), and 176 (△) mg. trichloroethanol/kg. in Dog C. A peak height ratio, TCE-G/CB, with respect to chlorobutanol (CB) of 1.0 is equivalent to 18.3 mcg. of trichloroethanol glucuronide (or 8.4 mcg. of trichloroethanol)/ml. of whole blood.*

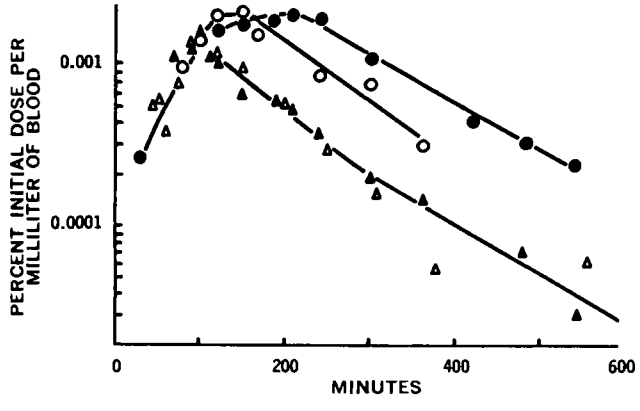


Figure 10—*Semilogarithmic plot of trichloroethanol glucuronide blood levels (as percent initial trichloroethanol dose per milliliter whole blood) with time for doses of 17 (△), 50 (▲), 100 (○), and 150 (●) mg./kg. of trichloroethanol in Dog D. The data points were calculated by converting the glucuronide concentrations to trichloroethanol concentrations (1 mg. glucuronide/ml. = 0.47 mg. trichloroethanol/ml.), dividing by the dose (milligrams), and multiplying by 100.*

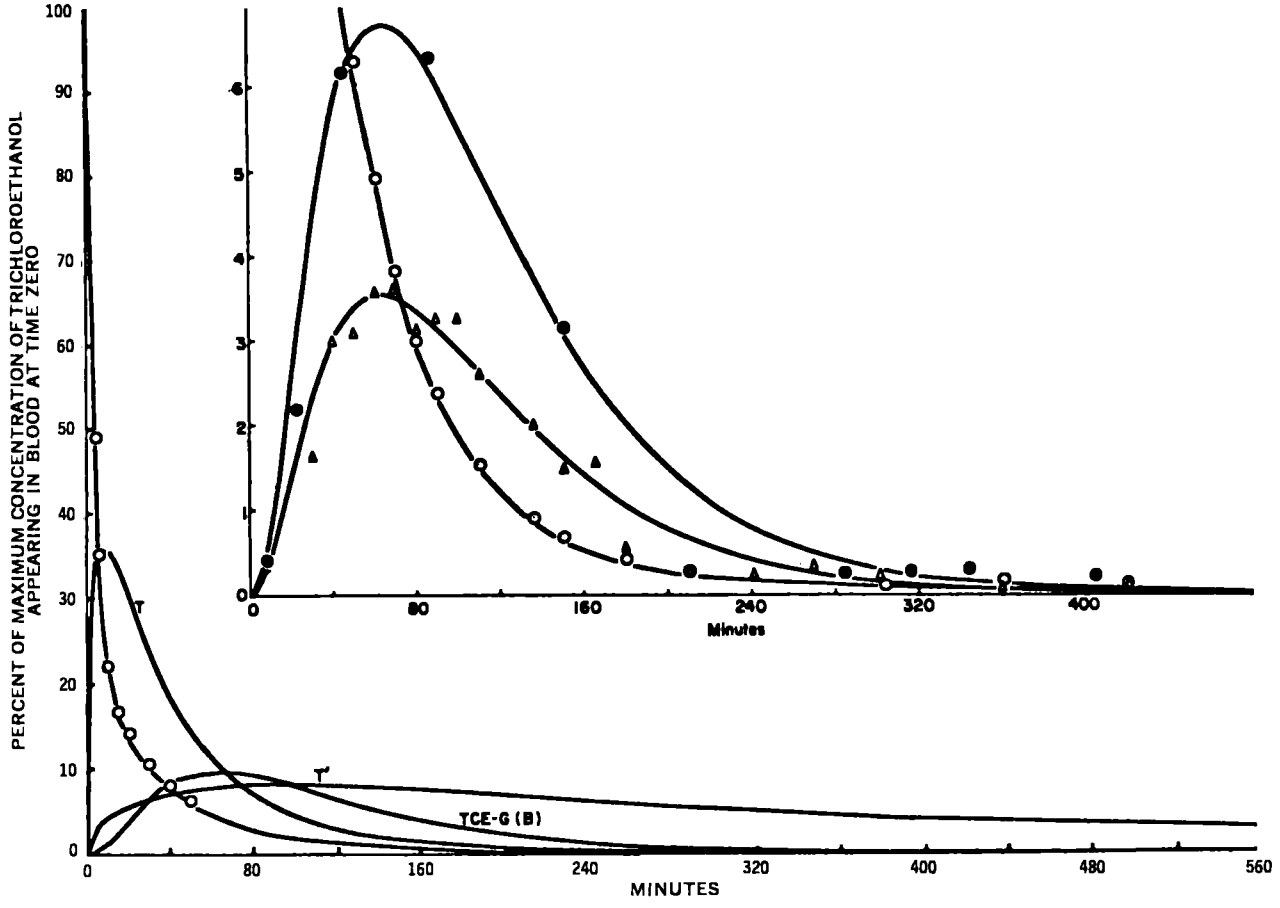
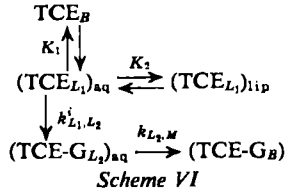


Figure 11—*Analog computer fits of the blood and urine data for a dose of 10 mg. trichloroethanol/kg. in the 10-kg. Dog C. The full-scale portion shows the fit of the data with time for trichloroethanol as the percent of the maximum concentration that appears in the blood at zero time* (○), *as well as the computer curves generated for trichloroethanol in the distribution compartments* T *and* T′ (*Scheme V*). *The remaining curve is for the calculated amount of trichloroethanol glucuronide* (*TCE-G*) *in blood with time. The pertinent microscopic rate constants in Tables I and V were used. The inset curves are expanded scale fittings of the data for trichloroethanol* (○) *and its glucuronide* (△) *in the blood. The latter data were estimated by multiplying the glucuronide blood concentrations by a factor that represented the ratio of the apparent volume of distribution of the glucuronide to that of the trichloroethanol in the central compartment. The solid circles represent the urinary excretion rates of trichloroethanol glucuronide* (*percent dose per minute*) *and are plotted on a 10-fold smaller scale, 0–1.0%/min., than that represented on the inset ordinate.*

$k_{B\cdot L_1}$ was observed (Dog E, Table I and Fig. 5), which indicated a large capacity for glucuronidation. Since sulfobromophthalein is metabolized by conjugation with glutathione and subsequently eliminated by biliary secretion (41), the observed metabolic inhibition of trichloroethanol metabolism was probably not due to a competition for sites on uridine 5′-diphosphate-glucuronyltransferase.

However, these implications of enzyme saturation or substrate inhibition of enzymic action to explain the metabolic rate decrease with increasing dosage are contradicted by the fact that the loss of trichloroethanol from the blood central compartment can be described by first-order kinetic parameters during its entire time course in the blood for any given dosage (Figs. 2, 3, 11, 12, 14, and 15 and Scheme V). It would be anticipated that the rate of trichloroethanol loss would vary from zero-order dependence at high blood levels to first-order dependence on drug concentrations in the central compartment at low blood levels if enzyme saturation or substrate inhibition occurred (37).

Since the reason for metabolic dependence on dose is probably not at the level of the enzymic conjugation, alternative mechanisms may be considered. They could include decreased availability of the uridine 5′-diphosphate-glucuronic acid cofactor with trichloroethanol dose or decreased uptake of trichloroethanol in the biophase of the hepatic cell with dose. This latter could be rationalized by assuming that the lipid–water ratio of trichloroethanol is altered within cells or among liver tissue components by the initial trichloroethanol dose. The fact that significant storage in the liver of trichloroethanol and/or its glucuronide is indicated (Fig. 7) tends to favor this latter explanation.



Scheme VI

The presence of trichloroethanol may stimulate its permeability into a constant volume of liver fat or may cause an increase in the volume of liver lipids. The phenomenon of fatty liver was reported for polychlorinated hydrocarbons (42) and ethanol (43) and might occur for a drug such as trichloroethanol, which possesses the molecular structure of both substances. Such a premise would necessitate the presumption that fat availability is an immediate and persistent consequence of the initial trichloroethanol dose.

An alternative and more probable, although indistinguishable, effect would be the persistent increase in availability of lipid by the action of increased trichloroethanol concentration, which could conceivably lower the surface tension between lipid and aqueous phases or modify the intervening membranic barriers. On the simple assumption that either of these mechanisms is operative, the intermediate steps between the loss of trichloroethanol and the appearance of its glucuronide in the blood in the pharmacokinetic model of Scheme V may be modified to include an instantaneous distribution of trichloroethanol between an aqueous phase $(TCE_{L_1})_{aq}$ and a lipid phase $(TCE_{L_1})_{lip}$ in the liver with an equilibrium constant K_2 (Scheme VI).

If the rate of glucuronide formation in the liver is a function of the trichloroethanol amount in an aqueous biophase:

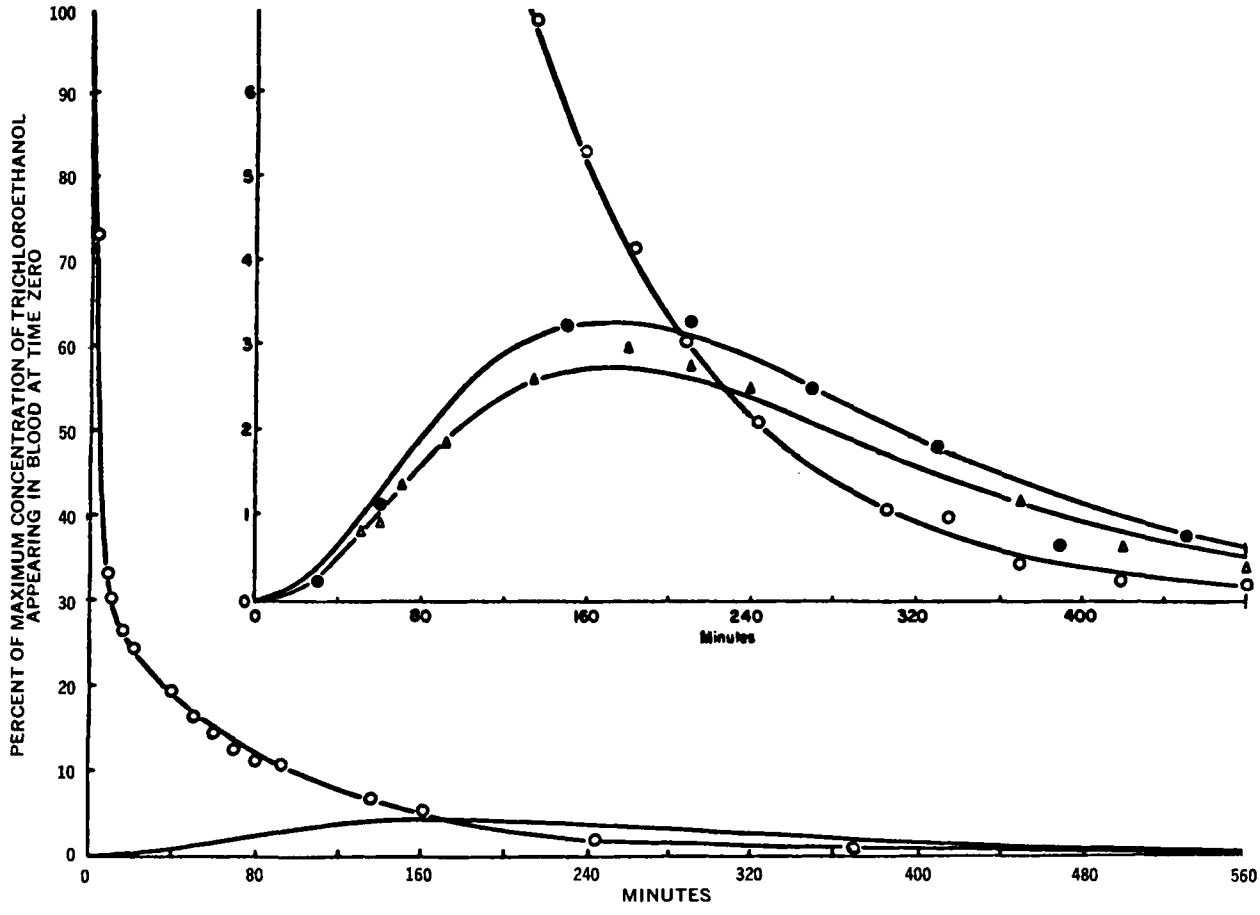


Figure 12—*Analog computer fits of the blood and urine data for a dose of 176 mg. trichloroethanol/kg. in the 10-kg. Dog C. The full-scale portion shows the fit of the data with time for trichloroethanol as the percent of the maximum concentration that appears in the blood at zero time* (○), *as well as the calculated amount of trichloroethanol glucuronide (TCE-G) in blood with time. The pertinent microscopic rate constants in Tables I and V were used. The inset curves are expanded scale fittings of the data for trichloroethanol* (○) *and its glucuronide* (△) *in blood. The latter data were estimated by multiplying the glucuronide blood concentrations by a factor that represented the ratio of the apparent volume of distribution of the glucuronide to that of trichloroethanol in the central compartment. The solid circles represent the urinary excretion rates of trichloroethanol glucuronide (percent dose per minute) and are plotted on a 10-fold smaller scale, 0–1.0%/min., than that represented on the inset ordinate.*

$$d(\text{TCE-G}_{L_1})/dt = -d[(\text{TCE}_{L_1})_{aq} + (\text{TCE}_{L_1})_{lip}]/dt = -d[K_1(\text{TCE}_B) + K_1K_2(\text{TCE}_B)]/dt = k^i_{L_1,L_2}(\text{TCE}_{L_1})_{aq} = k^i_{L_1,L_2}K_1(\text{TCE}_B) \quad \text{(Eq. 86)}$$

It thus follows that:

$$-d(\text{TCE}_B)/dt = \frac{k^i_{L_1,L_2}K_1}{K_1 + K_1K_2}(\text{TCE}_B) = \frac{k^i_{L_1,L_2}}{1 + K_2}(\text{TCE}_B) = k_{B,L_1}(\text{TCE}_B) \quad \text{(Eq. 87)}$$

and the apparent first-order rate constant, k_{B,L_1}, is not an intrinsic constant but a function of the equilibrium, K_2, between the aqueous and fatty phases or its equivalent, the relative volumes of lipid and aqueous phases in the hepatic cell or liver. If either of these factors is a persistent function of the initial trichloroethanol dose so that K_2 increases with dose, the apparent rate constant, k_{B,L_1}, will decrease with dose.

If K_2 is linearly related to dose, A_0, then:

$$K_2 = K_2^0 + mA_0 \quad \text{(Eq. 88)}$$

where K_2^0 is the intrinsic lipid–aqueous distribution in a liver unchallenged by trichloroethanol.

Substitution of Eq. 88 into Eq. 87 yields:

$$k_{B,L_1} = \frac{k^i_{L_1,L_2}}{1 + K_2^0 + mA_0} \quad \text{(Eq. 89)}$$

and, on taking reciprocals:

$$1/k_{B,L1} = \frac{1 + K_2^0}{k^i_{L_1,L_2}} + \frac{mA_0}{k^i_{L_1,L_2}} \quad \text{(Eq. 90)}$$

A plot of the reciprocal of the apparent first-order rate constant for the loss of trichloroethanol from the blood against trichloro-

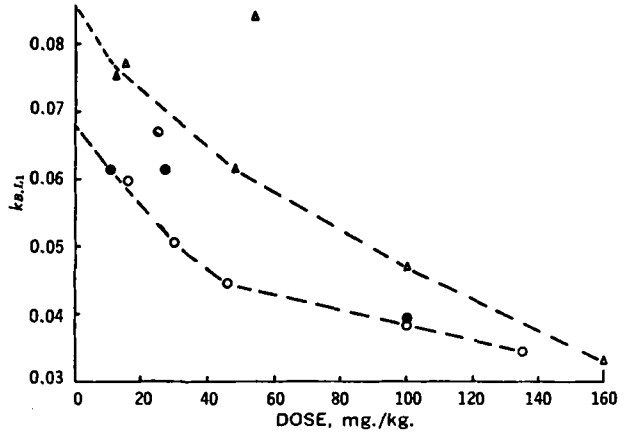


Figure 13—*Plot of the change in the calculated rate constant,* $k_{B,L1}$ *(Scheme V), for the metabolic loss of trichloroethanol with increasing doses of trichloroethanol in Dogs A* (▲), *B* (●), *C* (△), *and D* (○).

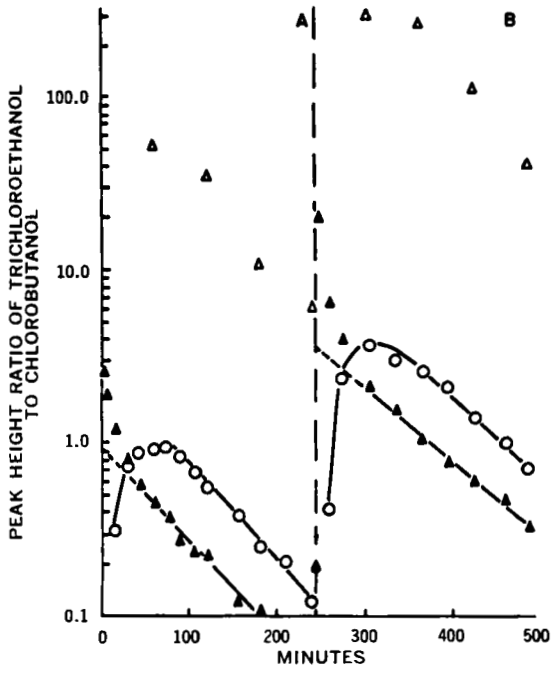


Figure 14—*Semilogarithmic plots of the peak height ratios of trichloroethanol (▲) and trichloroethanol glucuronide (○) from blood and the glucuronide from bile (△) with time for different doses of trichloroethanol. These peak height ratios are referred to the internal standard, chlorobutanol. Segment A is a consequence of dosing 11 mg./kg. of trichloroethanol at zero minute. Segment B is a consequence of dosing 46 mg./kg. of trichloroethanol at 240 min. A peak height ratio of 1.0 is equivalent to 8.4 mcg. of trichloroethanol or 18.3 mcg. of glucuronide/ml. of blood.*

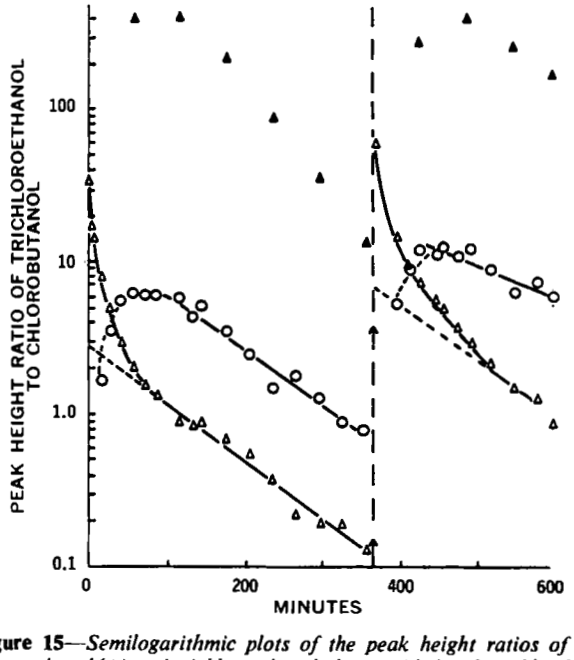


Figure 15—*Semilogarithmic plots of the peak height ratios of trichloroethanol (△) and trichloroethanol glucuronide (○) from blood and the glucuronide from bile (▲) with time for different doses of trichloroethanol. These peak height ratios are referred to the internal standard, chlorobutanol. Segment A is a consequence of dosing 50 mg./kg. of trichloroethanol at zero minute. Segment B is a consequence of dosing 100 mg./kg. of trichloroethanol at 360 min. A peak height ratio of 1.0 is equivalent to 8.4 mcg. of trichloroethanol or 18.3 mcg. of glucuronide/ml. of whole blood.*

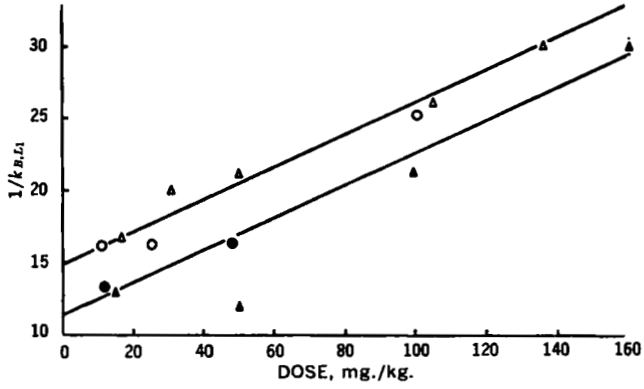


Figure 16—*Plot of the reciprocal of the metabolic rate constant, k_{B,L_1} (Scheme V), for the metabolic loss of trichloroethanol against dose for Dogs A (●), B (○), C (▲), and D (△).*

ethanol dose in accordance with Eq. 90 is given in Fig. 16 and is not inconsistent with the postulated relationship.

Trichloroethanol Glucuronide Distribution and Elimination in the Dog—After immediate equilibration in an initial apparent volume of distribution referenced to the concentration of unbound drug in plasma of 169 ml./kg. (Table V), which is about 3 times the true plasma volume of the dog, the intravenously administered glucuronide rapidly diffused into a compartment (TCE-G_T in Scheme V) of an equivalent apparent volume (Table V). The sum of the two volumes (330 ml./kg.) approximated the 200–380 ml./kg. reported for total extracellular water in dogs (16). The glucuronide did not distribute from plasma into red blood cells. The fraction, f, of glucuronide in plasma bound to protein was taken as 0.35 (Table I).

The urinary elimination of trichloroethanol glucuronide was rapid, with an apparent half-life of 15 min. for the greater portion of drug in the body (Fig. 17). No trichloroethanol or trichloroacetic acid was observed in any assayed blood or urine sample.

The semilogarithmic plot of the decrease in blood concentration of trichloroethanol glucuronide with time (Fig. 17) was triphasic and consistent with the three-component polyexponential expression of Eq. 63. Appropriate feathering, as demonstrated in Fig. 17, permitted preliminary estimates of the parameters of the equation. The cited apparent half-lives in Fig. 17 are inversely related to the k_i constants of Eq. 63. When the logarithm of the amount of unexcreted drug, log $(U_\infty - U)$, obtained from cumulative assays of glucuronide in the urine, was plotted against time (13), similar

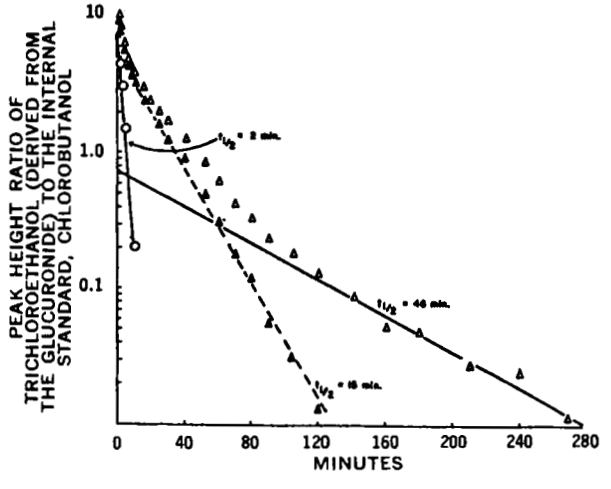


Figure 17—*Semilogarithmic plot of trichloroethanol glucuronide concentrations in blood with time in terms of gas chromatographically obtained peak height ratios of derived trichloroethanol to the internal standard, chlorobutanol, after a dose of 450 mg. (38 mg./kg.) of trichloroethanol glucuronide to Dog C. The original data are represented with open triangles. The three distinct phases obtained by feathering (i.e., the circles, solid triangles, and terminal open triangles) are labeled respectively with their apparent half-lives.*

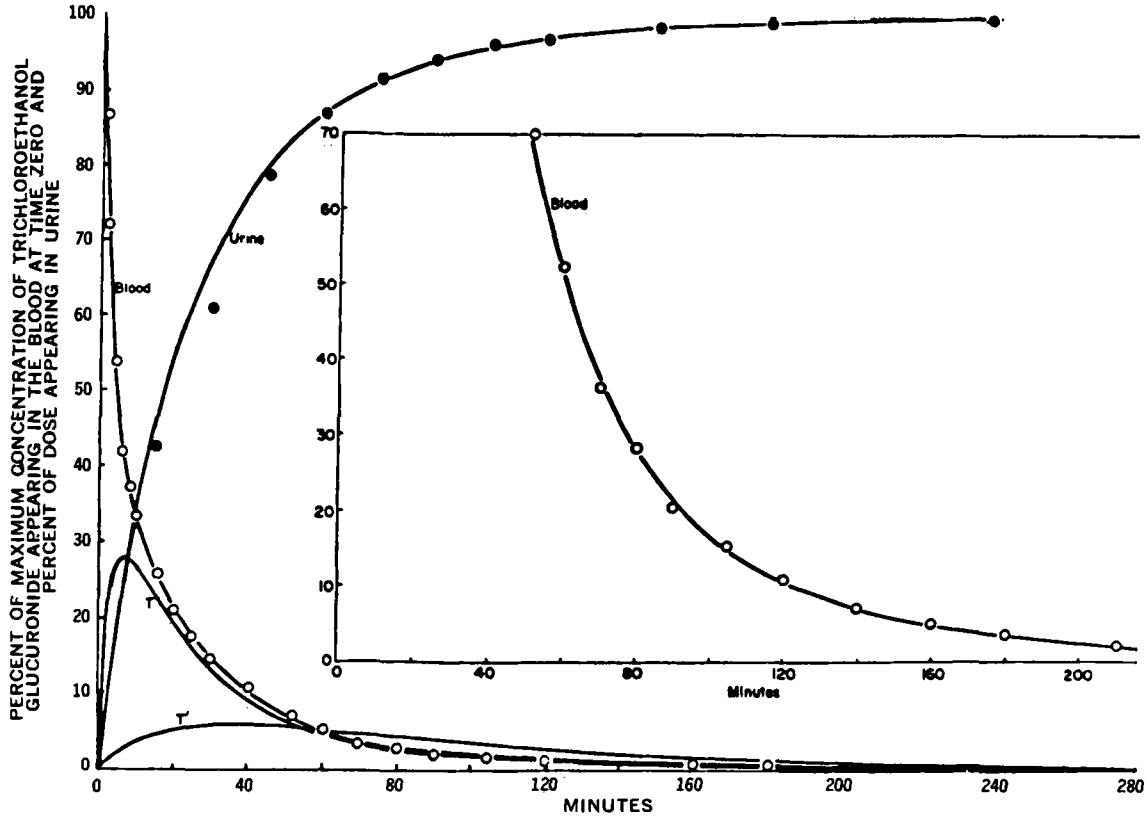


Figure 18—*Analog computer fits of the blood and urine data for trichloroethanol glucuronide in blood and urine after a 39-mg./kg. dose of trichloroethanol glucuronide to the 10-kg. Dog C. The full-scale portion shows the computer fit with time of the data for the glucuronide as the percent of the maximum concentration that appears in the blood at zero time* (○) *and as the percent of the total dose that appears ultimately in the urine* (●). *The curves are also generated for the trichloroethanol glucuronide in the distribution compartments* T *and* T′ *(Scheme V). The inset is an expanded scale fitting of the data for trichloroethanol glucuronide in blood. The microscopic rate constants of Table V were used.*

apparent half-lives for the second and third phases were observed by feathering and were 15 and 45 min., respectively. The first phase of Fig. 17 ($t_{1/2}$ = 2 min.) was not observed in the urinary data. An analog computer fit of trichloroethanol glucuronide data in blood and urine with time in accordance with a model similar to that of Scheme III is given in Fig. 18 (see also Scheme V).

A plot of percent of glucuronide dose excreted in urine per minute against percent of glucuronide dose per milliliter of plasma provided a good linear slope to estimate a clearance of 130 ml./min. This was consistent with the product of $k_{M,U}$ and V_P of 140 ml./min. (13). The $V_P = V_P{}^u(1 - f) + V_{P\text{true}}f$ can be estimated from the $V_P{}^u$ of Table V, where f = 0.30 in accordance with Eq. 36. Since the rena clearance values for inulin (glomerular filtration) for a 12-kg. dog can be calculated to be 51 ±12 ml./min. (44), it is probable that the intravenously administered glucuronide was eliminated by filtration

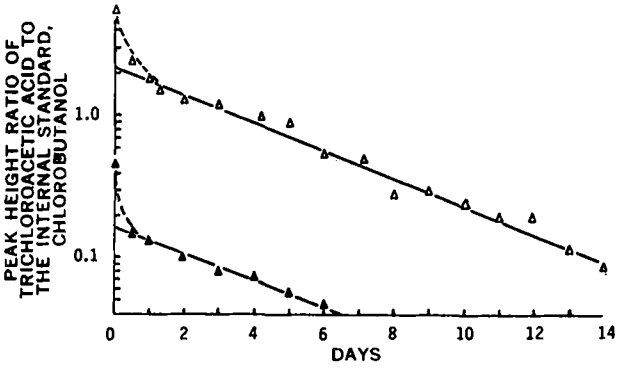


Figure 19—*Semilogarithmic plots of the blood trichloroacetate concentrations with time in terms of the gas chromatographically determined peak height ratios of trichloroacetic acid to the internal standard, chlorobutanol, after doses of 100 mg.* (▲) *and 1.0 g.* (△) *of sodium trichloroacetate to two different dogs. A peak height ratio with respect to chlorobutanol of 1.0 is equivalent to 70 mcg. trichloroacetic acid/ml. of whole blood.*

Table V—Trichloroethanol Glucuronide Pharmacokinetic Parameters[a] of Dog C (12 kg.) Administered 450 mg. of Glucuronide (37.5 mg./kg.)

$[A_B]_0$[b]	0.034
V_B[c]	2.94 l.
$V_P{}^u$[d]	2.03 l.
V_{TP^u}[e]	2.0 l.
$V_{T'P^u}$[f]	1.4 l.
$k_{M,T}$	0.133 (0.013) min.$^{-1}$
$k_{T,M}$	0.190 (0.019) min.$^{-1}$
$k_{M,T'}$	0.008 (0.0006) min.$^{-1}$
$k_{T',M}$	0.017 (0.0007) min.$^{-1}$
$k_{M,U}$	0.069 (0.0006) min.$^{-1}$

[a] The microscopic rate constants are defined in Scheme V for trichloroethanol glucuronide (TCE-G) in the various compartments and are given in minutes^{-1} and are referenced to total amounts in blood. The compartments and transfers for trichloroethanol in Scheme V are to be ignored. The numbers in parentheses are standard deviations of the stated parameters obtained by digital computer fitting with the SAAM program. [b] The computer-fitted zero time concentration of trichloroethanol glucuronide in blood, A_0, in terms of percent of dose per milliliter of whole blood. [c] The apparent volume of distribution of the central compartment referenced to total concentration in blood is V_B = (100%/$[A_B]_0$) in liters. [d] The apparent volume of distribution of the central compartment referenced to the concentration of glucuronide unbound to protein is $V_P{}^u = V_B(2.31) - 0.24V_{B\text{true}}$ after Eq. 34, where H = 0.45, f = 0.30 (Table II), D = 0 (Table III), and $V_{B\text{true}}$ = 0.1 × weight of dog in kg. (16). [e] The apparent volume of distribution referenced to the concentration of unbound glucuronide in the plasma of the rapidly equilibrating compartment, T, in liters, where $V_{TP^u} = V_P{}^u(k_{M,T}/\gamma_P{}^u k_{T,M})$, where $\gamma_P{}^u = 1 - f$ as calculated from Eq. 22. [f] The apparent volume of distribution referenced to the concentration of unbound glucuronide in the plasma of the slowly equilibrating compartment, T', in liters, where $V_{T'P^u} = V_P{}^u(k_{M,T'}/\gamma_P{}^u k_{T',M})$.

Table VI—Renal Clearance of Trichloroethanol Glucuronide at Various Dose Levels of Trichloroethanol

Dog (Weight, kg.)	Trichloroethanol Dose, mg./kg.	Observed Glucuronide Clearance, ml./min.[a]
A	10	139
(16)	50	143
B	10	256
(18.6)	25	256
	100	128[b]
C	10	130
(10)	50	130
	100	60[b]
	176	60[b]
D	17	203
(18)	50	203
	100	101[b]
	150	101[b]

[a] Observed clearance equals percent dose per milliliter plasma/percent dose per minute in urine, as obtained from the slope of plots of glucuronide as percent of trichloroethanol dose excreted into urine per minute plotted against the glucuronide as percent of trichloroethanol dose per milliliter of plasma. [b] At the lower concentrations of glucuronide in plasma, *i.e.*, close to the origin in the plots specified in *Footnote a*, the data follow the straight line drawn through the data for the lower doses of 10–50 mg./kg. of trichloroethanol. The data at the higher concentrations of glucuronide in the plasma fall below this line and become linear at a new and lower slope from which these clearances are estimated.

and tubular secretion at the studied glucuronide dosage of 38 mg./kg.

The glucuronide was distributed into another small compartment of apparent volume, $V_{T'_P}$[u] (Table V). The slow rate of drug return to this compartment was necessary to fit the blood and urine data from 1.5 to 12 hr. after glucuronide dosage (Figs. 17 and 18).

Analyses of the data for glucuronide formed on trichloroethanol dosing in all dogs demanded two compartments between TCE_B and $TCE\text{-}G_B$ (Scheme V) to fit the lag time in glucuronide appearance in the blood and the broad maximum that occurred in the glucuronide blood level–time curves (Figs. 3, 6, 9–12, 14). The rate constant $k_{L_1,M}$ (Table I) from the studies on trichloroethanol administration was remarkably similar to $k_{T',M}$ (Table V) on glucuronide administration. It may be conjectured that the compartment T' for intravenously administered glucuronide represented the hepatic uptake or binding of a small fraction of circulating glucuronide and $k_{T',M}$ was the rate constant for release of this sequestered glucuronide from liver back to blood.

Table VII—Effect of Trichloroethanol Dose on Bile Flow Rate and Biliary Secretion of Trichloroethanol Glucuronide

Dose, mg./kg.	Minutes	Bile Flow, ml./hr.	Percent Dose Excreted in Interval	Total Percent of Dose Excreted as Glucuronide
11.5[a]	60	1.5	0.35	
	120	1.8	0.27	
	180	1.8	0.08	
	240	1.7	0.05	
				0.75
46.0[a]	60	2.5	0.76	
	120	2.1	0.56	
	180	1.8	0.21	
	240	1.7	0.07	
				1.60
50[b]	60	3.8	1.94	
	120	3.8	2.00	
	180	3.0	0.85	
	240	2.4	0.28	
	300	1.7	0.08	
	360	4.3	0.07	
				5.22
100[b]	60	6.7	1.20	
	120	6.2	1.57	
	180	2.0	0.34	
	240	1.7	0.17	
	300	1.0	0.09	
				3.37

[a] Weight of dog was 13.0 kg. [b] Weight of dog was 9.6 kg.

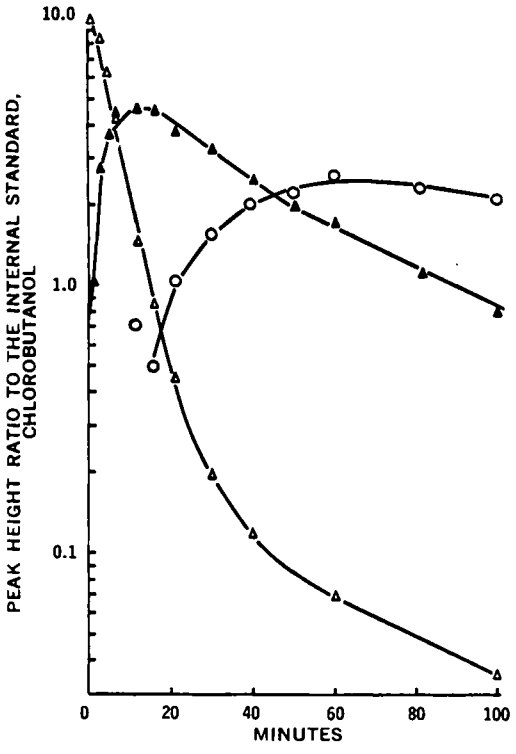


Figure 20—*Semilogarithmic plot of blood concentrations of chloral hydrate (△), trichloroethanol (▲), and trichloroethanol glucuronide (○) in terms of peak height ratio against time for a dose of 500 mg. of chloral hydrate (42 mg./kg.) in Dog C. A peak height ratio to the internal standard, chlorobutanol, of 1.0 is equivalent to 4.3 mcg. chloral hydrate, 8.4 mcg. trichloroethanol, or 18.3 mcg. trichloroethanol glucuronide/ml. whole blood.*

The renal clearances of trichloroethanol glucuronide declined with the higher glucuronide concentration in blood that resulted from the higher doses of trichloroethanol (Table VI). Such clearances were obtained from the slopes of plots of glucuronide as percent of the trichloroethanol dose excreted into the urine per minute against the glucuronide as percent of this dose per milliliter of plasma. These clearances are theoretically the product of the microscopic rate constant for renal excretion of glucuronide, $k_{M,U}$, referenced to total amounts of glucuronide in the plasma and the apparent volume of distribution of the central compartment, V_P, which is also referenced to the drug concentration in the plasma (13) and may be obtained from V_P[u] in Table V by a rearrangement of Eq. 36. The decrease of these clearances with higher blood levels of glucuronide can be assigned to either a lowering of the apparent volume of distribution of the central compartment for glucuronide or a decrease in the $k_{M,U}$ factor for renal excretion of the glucuronide at the higher blood levels. Since the latter is consistent with the claim that a tubular secretory pathway is saturable for carboxylates and other anions (45), it is the preferred explanation.

These decreased glucuronide clearances and the previously discussed lowered rate constant $k_{B,L}$ for the loss of trichloroethanol and glucuronide with increased trichloroethanol dose readily explain the observed retarded time of appearance of maxima in glucuronide blood levels at the higher doses of trichloroethanol (Figs. 9 and 10).

Renal and Fecal Elimination of Trichloroethanol as Its Glucuronide—The excretion of free, unmetabolized trichloroethanol in feces and urine accounted for less than 3% of the administered dose. Essentially, the drug was excreted in the urine as the glucuronide. The average amount of trichloroethanol found in fecal samples collected between 0 and 12 hr. after trichloroethanol administration for 12 experiments was 0.3% of the dose and ranged from 0 to 1.5%. No glucuronide was found in the feces in these experiments. A

mean of 1.5% of the total dose was found in the urine as unconjugated trichloroethanol in 27 experiments. The negligible amounts of fecal elimination of trichloroethanol and its metabolites and urine elimination of trichloroethanol permitted these routes to be ignored in the pharmacokinetic modeling. Any significant amount of trichloroethanol or its glucuronide excreted in the bile must be significantly reabsorbed, with the glucuronide being readily hydrolyzed; *i.e.*, an enterohepatic shunt could be postulated. The highly lipid-soluble trichloroethanol is undoubtedly extensively reabsorbed from the renal tubules and subsequently glucuronidated.

A variable fraction (10–20%) of doses of trichloroethanol could not be accounted for in cumulative urine and fecal assays and most probably was eliminated *via* other routes (*e.g.*, saliva, sweat, and breath), which is to be expected for any low molecular weight alcohol. It is also possible that a small fraction of trichloroacetate was produced and was unavailable for assay because of its tight binding to tissues.

Biliary Secretion of Trichloroethanol and Its Glucuronide—When samples of the bile were assayed from two different dogs administered trichloroethanol, significant biliary concentrations of the glucuronide were obtained. Semilogarithmic plots of the concentration of trichlorethanol and its glucuronide in blood and the glucuronide in bile against time are given in Figs. 14 and 15 for the two experiments.

Maximum biliary concentrations of the glucuronide were 60–85 times the concentrations in whole blood (30–42.5 times the plasma concentration) in the first dog (Fig. 14) for doses of 11.5 and 46.0 mg./kg. However, trichloroethanol concentrations in bile were much less and did not exceed 2.5 times the trichloroethanol blood concentrations so that the total trichloroethanol excreted by this route was not of practical significance.

When the second dog was dosed with 50 mg./kg. of trichloroethanol, the maximum biliary glucuronide concentrations were again greater than 60 times the blood concentration for a 50-mg./kg. dose of trichloroethanol. However, an increase of the trichloroethanol dose to 100 mg./kg. did not increase the biliary concentration of the glucuronide (Fig. 15), which was only 30-fold that of its blood concentration. This apparent inhibition of biliary trichloroethanol glucuronide secretion was concomitant with a slowed loss of trichloroethanol and its glucuronide from the blood (Fig. 15), phenomena which seem to be common to the higher trichloroethanol doses. (See k_{B,L_1} values at various doses in Table I.)

The inhibition by chloral hydrate of the biliary secretion in rats of indocyanine green (a nonmetabolized dye used to evaluate cardiac function) and bilirubin glucuronide was previously reported (40). Doses estimated at 50–100 mg. chloral hydrate/kg. were inhibitory while 10-fold smaller doses had no effect.

Guarino and Schanker (38) reported saturation of biliary secretion of probenecid and its glucuronide at a dose of 0.18 m*M*/kg. The dose of trichloroethanol required for the same effect in these experiments was 0.67 m*M*/kg.

A compilation of the bile flow rates and percentages of the initial dose excreted as the trichloroethanol glucuronide in bile within the specified time intervals is given in Table VII. The data show that bile flow rate increases as a function of increasing trichloroethanol dose. This chloretic effect was previously reported for chloral hydrate (46) and for probenecid (38).

About 5% of the dose was biliary excreted as the glucuronide in the total interval studied. This was consistent with the data of Owens and Marshall (6), from which it was calculated that about 8% of an administered dose was excreted in the bile within 3–4 hr. after intravenous administration of trichloroethanol.

Pharmacokinetics of Trichloroacetic Acid—The apparent disposition half-life for both 0.1- and 1.0-g. doses of trichloroacetic acid as the sodium salt in the dog was 75 hr., which was similar to that in man (47) (Fig. 19). No trichloroacetic acid was found in a pooled 10-day fecal sample. The apparent volume of distribution of 100 and 1000 mg. of sodium trichloroacetate in the central compartment referenced to the concentration in whole blood may be calculated (Eq. 24) from the time zero estimate of blood concentration, $[A_B]_0$ (Fig. 19), as 3.6 l. This value is consistent with the 2.0–3.5 l. (in a 10-kg. dog) or 3.0–5.3 l. (in a 15-kg. dog) of extracellular body water (18), even when a 0.40-l. volume for red blood cells is subtracted. However, since the protein binding of trichloroacetate is in the range of 70–90% (5), the apparent volume of distribution of trichloroacetic acid referenced to unbound drug in the plasma will greatly exceed even the total body water (7.5 l. for a 15-kg. dog). This large value may be the result of the binding of trichloroacetate anions in the readily available tissues and may be the explanation for the comparatively slow elimination of such a highly ionized drug.

The apparent volumes of distribution of 100 and 1000 mg. of sodium trichloroacetate in the total compartments of distribution, *i.e.*, the central compartment plus the more slowly equilibrating tissues, were calculated from the estimates of blood concentrations (Fig. 19) after the linear extrapolation of the terminal points of the semilogarithmic plots of Fig. 13 to zero time and were 7.9 and 6.8 l., respectively, referenced to drug concentration in the blood.

Computer analyses of the data of Fig. 19 in accordance with a model similar to that given in Scheme III (except that $A_{T'} = 0$ and $k_{B,U}$ are substituted for k_B) provided estimates for the microscopic rate constants referenced to blood concentrations for trichloroacetic acid pharmacokinetics, where $k_{B,T} = 0.079$, $k_{T,B} = 0.073$, and $k_{B,U} = 0.22$ hr.$^{-1}$. The overall elimination rate constant, k_e, calculated from Eq. 67 (where $k_{B,U}$ is substituted for the designated $k_{B,M}$) was 0.0105 hr.$^{-1}$ and was consistent with the value obtained from the terminal slopes of the plots of Fig. 19. It was also markedly similar to the value given for man, $k_e = 0.0094$ hr.$^{-1}$ (47).

Pharmacokinetics of Chloral Hydrate—Conversion of chloral hydrate to trichloroethanol in the dog was rapid and quantitative. Blood levels of trichloroethanol (5.5 ± 0.2 mg./ml.) and its glucuronide (36.2 ± 0.2 mg./ml.) 100 min. after intravenous administration of 500 mg. chloral hydrate (42 mg./kg.) were identical (Fig. 20) to those obtained after an equimolar dose of trichloroethanol was given to the same Dog C. The majority (97.5%) of the initial amount of chloral hydrate in blood was lost rapidly, with a half-life of 3 min. ($k_{CH,TCE} = 0.23$ min.$^{-1}$). The semilogarithmic plot was linear for more than five half-lives. The remainder (2.5%) showed a slower decline ($t_{1/2} = 35$ min.).

The appearance of trichloroethanol in the blood was rapid, with a half-life (3 min.) that corresponded to that for loss of chloral hydrate. Maximum trichloroethanol blood levels were observed 12 min. after chloral hydrate administration (Fig. 20). This is consistent with the claim that conversion to trichloroethanol of the chloral hydrate precursor occurs in all body tissues (48). Only a small fraction of the dose shows a deviation from the linear semilogarithmic plot, which can be assigned to a slower return from tissues of equilibration.

The apparent volume of distribution of the 500 mg. of chloral hydrate in the central compartment referenced to concentration in whole blood may be calculated (Eq. 24) from the time zero estimate of blood concentration $[A_B]_0$ (Fig. 20) as 11.5 l., which is very much greater than the values for trichloroethanol (Table I) and its glucuronide (Table V). This undoubtedly implies that chloral hydrate undergoes a more rapid diffusion into, or a more rapid binding with, tissues other than blood during first cycles in the body. No measurable concentrations of trichloroacetic acid were found in blood samples.

The percent of the dose of chloral hydrate excreted in urine as the glucuronide after 2 hr. (46%) was identical to that observed with an equimolar dose of trichloroethanol. In the same period of time, 1.56% of the total dose as unchanged chloral hydrate and 0.27% as trichloroacetic acid were also found in urine.

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GLC Assay for 5-Fluorouracil in Biological Fluids

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Abstract □ A rapid, sensitive GLC method of analysis for 5-fluorouracil was developed to follow the disposition of the drug in patients on 5-fluorouracil therapy. The free drug is removed from aqueous biological samples in a single extraction step, derivatized by silylation, and chromatographed using flame-ionization detection. An internal standard is utilized to quantitate the results. The procedure is rapid, sensitive (0.2 mcg./ml. plasma), specific for the intact 5-fluorouracil molecule, and suitable to support pharmacokinetic studies of 5-fluorouracil in animals and humans.

Keyphrases □ 5-Fluorouracil—GLC analysis in biological fluids □ GLC—analysis, 5-fluorouracil in biological fluids

Detailed pharmacokinetic studies that would provide information useful to the clinician would be desirable to optimize cancer chemotherapy with 5-fluorouracil. The drug is still given empirically by many different dosage schedules, with some recent evidence suggesting that the oral route of administration differs in activity and toxicity from the intravenous route (1). Disposition studies performed previously were limited by the absence of a rapid, sensitive method of analysis capable of determining free 5-fluorouracil in large numbers of biological samples. Studies using radioactively labeled drugs showed wide variability due to extensive metab-