The Metabolism and Elimination of d-Tubocurarine-H³

Ellis N. Cohen, M.D., H. Winslow Brewer, Ph.D.,† Dana Smith, B.S.‡*

Metabolism and elimination of *d*-tubocurarine-H³ were studied in 31 dogs. Combined renal and hepatic elimination accounted for over 85 per cent of the injected dose within 24 hours. In the absence of renal function (ligation of renal pedicles), the liver greatly increased its capacity to transport *d*-tubocurarine into the bile. A search for metabolites of *d*-tubocurarine in urine and bile indicated that no metabolites were present in urine, but that approximately 1 per cent of the injected tritium appeared in the bile in alternate molecular form.

THE AVAILABILITY of a purified preparation of *d*-tubocurarine-H³ has provided the opportunity for additional studies on the distribution and fate of this compound. Although it has been shown that the kidney plays an important role in the elimination of *d*-tubocurarine,[1-4] recovery in the urine of only a fraction of the injected dose led many investigators to speculate that the compound was metabolized within the body. The present study was designed to evaluate routes of elimination and to search for possible metabolites.

Method

d-Tubocurarine chloride (500 mg.), glacial acetic acid (2 ml.), platinum catalyst (50 mg.), and tritiated water (50 ml.) were heated for 12 hours at 100° C. The tritiated water was distilled off, the residue taken up in methanol, and then evaporated to dryness to effect removal of most of the easily exchangeable tritium.* Further purification was achieved by partitioning the crude tritiated mixture between ethylene dichloride and potassium iodide-glycine buffer.[5] Evaporation of the ethylene dichloride phase afforded a pale yellow residue which was dissolved in water and heated at 37° C. until constant activity was reached.† Activity of the final solution

* Professor of Anesthesia, Stanford University School of Medicine.

† Research Associate, Stanford University School of Medicine.

‡ Senior Technician, Stanford University School of Medicine.

Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California.

Accepted for publication July 22, 1966. This work supported grant GM 12527-02, National Institute General Medical Sciences, National Institutes of Health.

* Exchange tritiation of the *d*-tubocurarine performed by New England Nuclear Corporation, Boston.

† Total activity corresponds to tritiation of 0.007 per cent the available hydrogen atoms: Since one mole of *d*-tubocurarine chloride ($C_{38}H_{44}N_2O_6Cl_2$, M.W. 695.67) contains 6.02×10^{23} molecules,

$$\text{Number hydrogen atoms/mg.} = \frac{6.02 \times 10^{23} \times 44}{695.7 \times 10^3} = 3.8 \times 10^{19}$$

$$\text{Specific activity of fully tritiated } d\text{-tubocurarine-H}^3 \text{ (curies/mg.)} = \frac{\text{number of radioactive atoms} \times 0.693}{\text{half life of tritium (sec.)} \times \text{curie (dps./sec.)}}$$

$$= \frac{3.8 \times 10^{19} \times 0.693}{3.9 \times 10^8 \times 3.7 \times 10^{10}} = 2.1$$

$$\text{Per cent tritiation} = \frac{125}{2.1 \times 10^6} \times 100 = 0.007 \text{ per cent.}$$

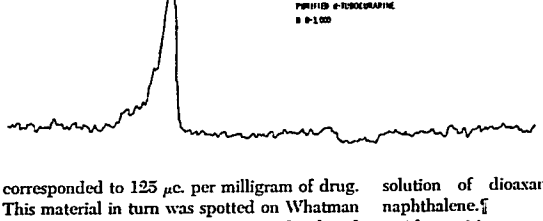

FIG. 1. Radiochromatogram of purified *d*-tubocurarine-H³, scale range 0–3,000. Paper developed with saturated aqueous butyl alcohol, acetic acid 10:1.

corresponded to 125 μc. per milligram of drug. This material in turn was spotted on Whatman no. 1 paper, and the papers were developed with two different solvent systems. The radioactivity was confined to one spot and corresponded in R_f to a specimen of authentic *d*-tubocurarine chloride (fig. 1).

Thirty-one mongrel dogs, average weight 17.2 kg. were anesthetized with intravenous pentobarbital sodium 30 mg./kg. Following endotracheal intubation, the lungs were ventilated with a fixed volume Harvard respirator. An intraarterial catheter was placed in the femoral artery for measurements of blood pressure and for sampling; the left femoral vein was cannulated for administration of drugs and fluids. Under fluoroscopic control a radiopaque catheter was passed through the right femoral vein and positioned at the orifice of the right renal vein. A second catheter was passed through the jugular vein and directed into either the right or left hepatic vein. The position of both renal and hepatic catheters was confirmed by injection of radiopaque dye. The cystic duct was exposed and ligated, and the common bile duct cannulated and exteriorized. An indwelling catheter in the bladder provided for continuous collection of urine. In 16 animals, the renal pedicles were ligated bilaterally. Following the intravenous injection of *d*-tubocurarine 0.3 mg./kg.,‡ samples of blood, urine, and bile were collected and analyzed for tritium.§ Samples of blood 0.1 ml., urine 0.1 ml., and bile 0.05 ml. were placed in plastic vials containing a 10 ml. solution of dioxane, PPO, POPOP, and naphthalene.¶

After tritium counts had been obtained, quenching factors were determined for each specimen of urine and bile by adding known amounts of radioactivity to the samples. In the case of blood (plasma), quenching factors were determined to be less than ±5 per cent, and these were disregarded. Weighed specimens of liver tissue (50–100 mg.) were dissolved in hyamine solution# (50° C. for 4–6 hours) and treated in similar fashion to the biological fluids.

Preliminary experiments were designed to determine if any unaccounted loss of tritium occurred during the study. Possible sources of tritium loss included an exchange of *d*-tubocurarine-H³ with the general water pool, as well as loss of *d*-tubocurarine-H³ into the gut. Transfer of tritium into the general water pool was investigated by trapping exhaled air and collecting the water of exhalation. Loss of *d*-tubocurarine-H³ into the gastrointestinal tract was examined by a wash of gastric and intestinal contents. In each instance only negligible amounts of the injected tritium were recovered.

Quantitative aspects of the renal elimination of *d*-tubocurarine were calculated in terms of its renal plasma clearance.[6]

$$C = \frac{UV}{P}$$

C = plasma clearance (ml./min.), U = urine concentration of *d*-tubocurarine (μg./ml.), V = urine flow (ml./min.), P = plasma concentration of *d*-tubocurarine (μg./ml.).

‡ Injection consisted of approximately 10 per cent *d*-tubocurarine-H³ (specific activity 125 μc./mg.).

§ Packard Tricarb, Model no. 3003, Downers Grove, Massachusetts.

¶ PPO-2,5-diphenyloxazole—7 g. POPOP-1,4-bis-2-(5-phenyloxazolyl)-benzene—50 mg. p-Dioxane—1,000 ml.

p-(iisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium hydroxide—1 molar solution in methanol.

The extraction ratio of *d*-tubocurarine (E) is given by the ratio A-V/A where A and V represent concentrations of the drug in renal arterial and renal venous plasma. Renal plasma flows were calculated by the formula:

$$RPF = \frac{C}{E}$$

RPF = renal plasma flow (ml./min./kg.), C = plasma clearance (ml./min./kg.), E = extraction ratio of *d*-tubocurarine.

No adjustment is necessary for drug found in red blood cells since *d*-tubocurarine is entirely confined to the plasma.[1]

The relative contribution of the kidney to the total elimination of *d*-tubocurarine was studied by ligating both renal pedicles prior to the intravenous injection of *d*-tubocurarine. Plasma concentrations of *d*-tubocurarine-H³ were determined at prescribed intervals, and compared to those of the control dogs.

The elimination of *d*-tubocurarine-H³ by the liver was studied in 13 dogs. In 6 of these animals hepatic A-V gradients were determined following placement of a catheter into the right or left hepatic vein. Concentrations of plasma *d*-tubocurarine taken from this catheter were then compared with plasma samples taken from the femoral artery or vein. Total bile outflow was collected through the exteriorized common bile duct and calculations of hepatic plasma flow rates were made using the Fick principle.

$$HPF = \frac{BV}{G}$$

HPF = hepatic plasma flow (ml./min./kg.), B = biliary concentration of *d*-tubocurarine (μg./ml.), V = bile flow (ml./min./kg.), G = arterial-venous gradient for *d*-tubocurarine (μg./ml.).

Saturation of the liver transport system for *d*-tubocurarine was studied in 4 dogs simultaneously given *d*-tubocurarine (0.3 mg./kg.) and procaine amide ethobromide** (5 mg./kg.). Renal pedicles were ligated in these animals to prevent urinary elimination of drugs. In 3 additional animals, *d*-tubocurarine was given alone, but at a higher dosage (0.75 mg./kg.). Hypotension was carefully avoided through slow intravenous injection of the *d*-tubocurarine.

A search for metabolites of *d*-tubocurarine in the urine and in bile was made through radioactive scanning and further attempts were made to identify specific metabolites present. Since all the radioactivity present in bile and urine could be removed by extraction with ethylene dichloride at an alkaline *p*H in the presence of potassium iodide,†† a preliminary extraction and concentration of these materials was carried out. The extracts were taken to dryness and the residue dissolved in 0.01 *N* HCl. Acid hydrolysis (1 *N* HCl at 80° C. for 3 hours), and enzymatic hydrolysis[7] with β-glucuronidase ‡‡ were subsequently carried out. Additional samples were spotted on Whatman no. 1 paper for separation by descending paper chromatography or by electrophoresis. In the absence of any visual evidence of metabolites, radioscan of the chromatography strips was carried out searching for areas of H³ activity.§§ Two solvent systems were used to develop these chromatograms.¶¶

Plasma protein binding of *d*-tubocurarine was evaluated with *in-vitro* experiments; 100 μg. *d*-tubocurarine-H³ were added to 5 ml. aliquots of human plasma suspended within a dialysis membrane (polyvinyl plastic). The

** Supplied by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

†† Potassium iodide–glycine buffer: prepared by combining 6 ml. 0.1 *M* glycine buffer with 4 ml. 0.1 *N* NaOH and 12.8 g. KI. The glycine buffer is prepared from 7.505 g. glycine, 5.85 g. NaCl, and diluted to 1,000 ml. with distilled water.

‡‡ Worthington Biochemical Corp. Freehold, New Jersey.

§§ Vanguard Autoscanner, Model no. S00, La Grange, Illinois.

¶¶ 1. ethyl acetate— 80
ethyl alcohol— 20
water— 10
conc. ammonia— 1
2. saturated aqueous butyl alcohol— 10
glacial acetic acid— 1
Chromatograms sprayed with bromphenol blue solution:
bromphenol blue— 5 mg.
citric acid— 200 mg.
water— 100 ml.

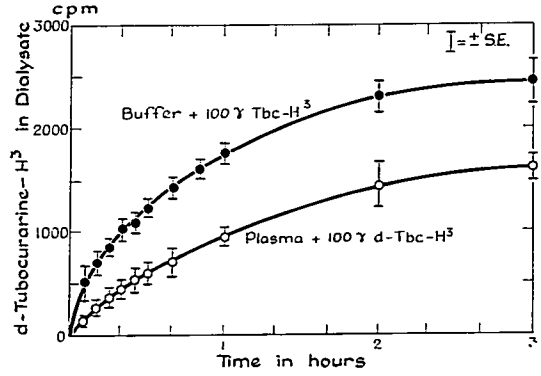

FIG. 2. Protein binding of *d*-tubocurarine-H³, 100 μg. *d*-tubocurarine-H³ added to 5 ml. plasma and dialyzed against 40 ml. buffer *p*H 7.4. Buffer solution, 5 ml. used in dialysis bag in control studies.

increase in tritium count (cpm.) in the surrounding 30 ml. of buffer solution## was monitored for a 3-hour period. The resultant activity was then compared to a control dialysis of *d*-tubocurarine-H³ in buffer. All solutions were maintained at *p*H 7.4 and at 37° C. (fig. 2).

Sorensons buffer—J. P. Remington, Practice of Pharmacy, Mack Publishing Co., Easton, Pa., 1961.

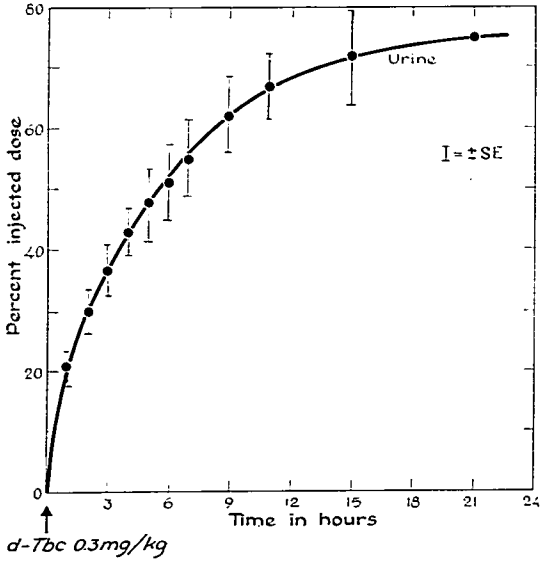

FIG. 3. Recovery of *d*-tubocurarine-H³ in urine.

Results

Results of these studies are discussed under the headings of renal elimination, hepatic elimination, and metabolism.

Renal Elimination. The cumulative appearance of *d*-tubocurarine-H³ in the urine was studied in 11 dogs for periods as long as 24 hours. At the end of 3 hours, 35.8 per cent (±8.3) of the injected dose appeared as H³ in the urine. By 24 hours, 74.7 per cent (±9.1) of the drug could be recovered in the urine (fig. 3). In 7 dogs mean plasma clear-

TABLE 1. Calculation of Renal Plasma Clearance Following the Intravenous Injection of *d*-Tubocurarine (0.3 mg./kg.)

Hour	Urine Volume (ml./hr.) (11 dogs)	*d*-tbc Urine (μg./min./kg.) (11 dogs)	*d*-tbc Plasma (μg./ml.) (7 dogs)	Plasma Clearance* (ml./min./kg.)
2	39	0.512±0.042	0.178±0.016	2.87±0.35
3	32	0.367±0.043	0.123±0.016	2.98±0.52
4	28	0.237±0.025	0.095±0.013	2.49±0.43
5	43	0.200±0.030	0.076±0.013	2.63±0.60

* Standard error of the ratio calculated according to Kendall, M. G., and Stuart, A.: Standard error of a ratio, *In*: Advanced Theory of Statistics, Vol. 1. London, Charles Griffin Co., 1958, p. 236.

TABLE 2. Calculations of Renal Plasma Flow Following the Intravenous Injection of *d*-Tubocurarine (0.3 mg./kg.)

Hour	*d*-tbc Urine (μg./min./kg.) (11 dogs)	*d*-tbc Fem. Art. (μg./ml.) (7 dogs)	*d*-tbc Renal Vn. (μg./ml.) (7 dogs)	$\bar{A}$-V Grad. (μg./ml.) (7 dogs)	Renal Plasma Flow* (ml./min./kg.)
2	0.512 ± 0.042	0.178 ± 0.016	0.136 ± 0.013	0.042 ± 0.006	12.2 ± 2.0
3	0.367 ± 0.043	0.123 ± 0.016	0.098 ± 0.013	0.025 ± 0.005	14.7 ± 3.4
4	0.237 ± 0.025	0.095 ± 0.013	0.080 ± 0.011	0.015 ± 0.004	15.8 ± 4.5
5	0.200 ± 0.030	0.076 ± 0.013	0.061 ± 0.009	0.015 ± 0.004	13.3 ± 4.1

* Standard error of the ratio calculated according to Kendall, M. G., and Stuart, A.: Standard error of a ratio, *In*: Advanced Theory of Statistics, Vol. 1. London, Charles Griffin Co., 1958, p. 236.

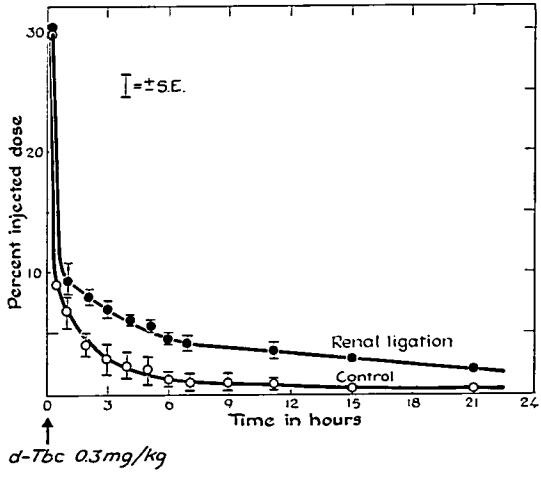

FIG. 4. Plasma concentration of *d*-tubocurarine-H³ in control dogs and in those with bilateral renal ligation.

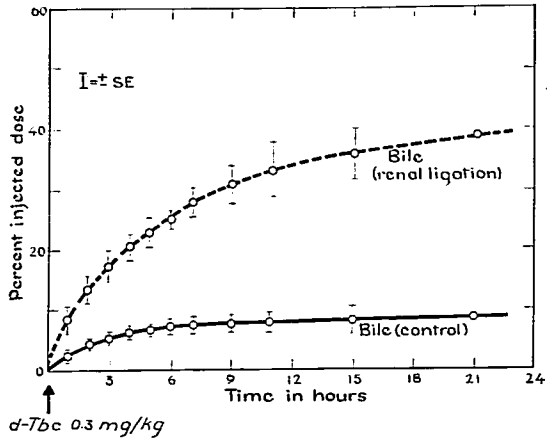

FIG. 5. Recovery of *d*-tubocurarine-H³ in the bile in control dogs and in those with bilateral renal ligation.

ance ratios were calculated at 2.74 ml./minute/kg. ranging from 2.49 to 2.98 ml./minute/kg. (table 1). From the second through the fifth hours of sampling, femoral artery and vein concentrations of *d*-tubocurarine-H³ were identical, indicating equilibration. Mean extraction ratios for *d*-tubocurarine were 0.249 (range 0.188–0.309). Renal plasma flows averaged 13.9 ml./minute/kg. (range 12.2–15.8 ml./minute/kg.) (table 2).

As previously reported,[4] the initial distribution of *d*-tubocurarine in plasma is uninfluenced by renal factors. After 30 minutes, however, there was a slower decline in plasma concentration in the renal ligated animals, and higher plasma of *d*-tubocurarine were maintained. Nonetheless, plasma levels continued to decline in the "nephrectomized" animals indicating an alternative route of elimination or metabolism (fig. 4).

Hepatic Elimination. The elimination of *d*-tubocurarine-H³ by the liver was studied in 12 animals for periods up to 24 hours. In the control animals, 5.9 per cent (±1.8) of the injected dose appeared as H³ in the bile within 3 hours, while in 4 animals with ligated renal pedicles 15.6 per cent (±3.6) was recovered in the bile in a similar time interval. Within 24 hours biliary elimination totalled 11.3 per cent in the control group, versus 38.9 per cent in the dogs with ligated renal pedicles (fig. 5). Hepatic plasma flows averaged 2.82 ml./min-

TABLE 3. Calculations of Hepatic Plasma Flow Following the Intravenous Injection of *d*-Tubocurarine (0.3 mg./kg.)

Hour	Bile Volume (ml./hr.) (6 dogs)	*d*-tbc Bile (μg./min./kg.) (6 dogs)	*d*-tbc Fem. Art. (μg./ml.) (11 dogs)	*d*-tbc Hepatic Vn. (μg./ml.) (5 dogs)	$\overset{\Delta}{\text{A-V}}$ Grad. (μg./ml.) (5 dogs)	Hepatic Plasma Flow* (ml./min./kg.)
2	7.9	0.089 ± 0.007	0.178 ± 0.016	0.141 ± 0.017	0.032 ± 0.009	2.78 ± 0.82
3	7.4	0.053 ± 0.008	0.123 ± 0.016	0.109 ± 0.020	0.015 ± 0.005	3.53 ± 1.29
4	7.2	0.038 ± 0.004	0.095 ± 0.013	0.076 ± 0.011	0.013 ± 0.002	2.92 ± 0.54
5	6.8	0.029 ± 0.005	0.076 ± 0.013	0.058 ± 0.010	0.014 ± 0.004	2.07 ± 0.69

* Standard error of the ratio calculated according to Kendall, M. G., and Stuart, A.: Standard error of a ratio, *In*: Advanced Theory of Statistics, Vol. 1. London, Charles Griffin Co., 1958, p. 236.

FIG. 6. Descending paper chromatogram: urine extract (a); authentic *d*-tubocurarine (b).

ute/kg. with a range from 2.07 to 3.53 ml./minute/kg. (table 3).

Metabolism. Although in a 24 hour period over 85 per cent of the injected *d*-tubocurarine-H³ could be recovered as tritium activity in the urine and bile, we had no assurance that this activity represented unchanged *d*-tubocurarine-H³. In chromatograms developed from two solvent systems, spots appeared on the paper which corresponded in R_f to that of authentic *d*-tubocurarine. No additional spots were visually noted (fig. 6). Radioactive scan of these strips served to confirm the presence of a single radioactive peak in the case of urine (fig. 7). Since the presence of a glucuronide ester might go unnoticed if the R_f value were the same as that of *d*-tubocurarine, the urine was subjected both to acid hydrolysis (1 *N* HCl at 80° C. for 3 hours) and to enzymatic hydrolysis with β-glucuronidase. In both instances, the optical densities of the solutions remained unchanged (determined spectrophotometrically at 280.5 mμ).

In the case of bile extract, an additional peak of radioactivity was discovered by radioscan of the paper chromatogram. This material had an R_f ratio of 2.0 compared to that of authentic *d*-tubocurarine-H³ (fig. 8). Quantitatively, this second peak accounted for 1–10 per cent of the H³ activity present in the bile. Efforts were made to isolate this metabolite for identification. Appropriate sections of the paper chromatogram were cut off and extracted. The eluted material was then reacted with methyl iodide and respotted on a descending paper chromatogram. Radio-scanning indicated that this reaction had converted a portion of the non-curare radioactivity to *d*-tubocurarine.

Discussion

The role of the kidney in the elimination of *d*-tubocurarine has been previously demonstrated in a number of investigations.[1-4] In most of these studies, only a fraction of the injected dose has been recovered in the urine. In our experiments we were able to recover 75 per cent of the drug in the urine within 24

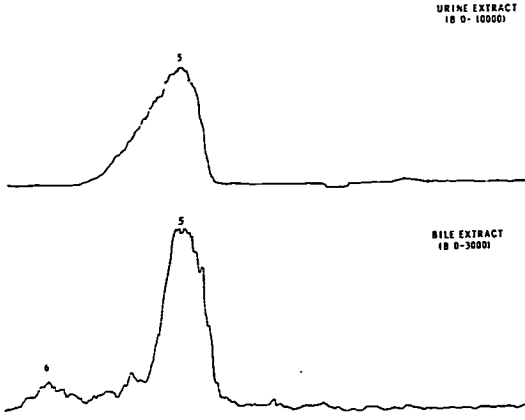

FIG. 7. Radiochromatogram developed from urine extract, range 0–10,000.

FIG. 8. Radiochromatogram developed from bile extract, range 0–3,000 (bilateral renal ligation). Peak 5 represents *d*-tubocurarine-H³; peak 6 represents metabolite-II³.

hours in unchanged form. The efficiency of the kidney in eliminating *d*-tubocurarine can be shown in terms of renal plasma clearance. These figures averaged 2.74 ml./kg./minute and approached the clearance rate for urea. Thus the elimination of *d*-tubocurarine can be explained in terms of glomerular filtration without evoking mechanisms of tubular secretion.[9] Lack of tubular reabsorption is suggested by the physical-chemical properties of *d*-tubocurarine which include a low lipid solubility coefficient[10] and a high ionization constant.[11]

In the intact animal, 85 per cent of the total injected dose of *d*-tubocurarine-H³ can be recovered within 24 hours through combined urinary and biliary elimination (figs. 3 and 5). An additional 1–2 per cent was recovered in the gut (biliary stream diverted). No *d*-tubocurarine was recovered in the water of exhalation. The specific contributions of the kidney and the liver can be separately evaluated in the animal with ligated renal pedicles. In these animals, the plasma concentration of *d*-tubocurarine continued to decline, although at a slower rate than in the intact animal (fig. 4). This would indicate metabolism or the presence of an alternative route of elimination. Evidence for the latter is presented, and in the "nephrectomized" animal the liver was able to eliminate a greatly increased amount of *d*-tubocurarine (fig. 5). Since only 40 per cent of the total injected dose could be recovered in the bile within 24 hours, we cannot rule out the possibility of prolonged storage accompanied by a slow process of metabolism.

The ratio of *d*-tubocurarine-H³ concentration in the bile to that in plasma is 40:1. This would suggest that an active transport system may be involved. Schanker *et al.*[8] have demonstrated that the liver possesses such an active transport system for certain quarternary ammonium ions. These authors were able to demonstrate competition between

TABLE 4. Elimination of *d*-Tubocurarine-H³ in the Bile

Dogs Studied	*d*-tbc Injected (mg./kg.)	PAEB Injected (mg./kg.)	% *d*-tbc-H³ Recovered (cumulative-5 hrs.)
4	0.3	—	22.5 ± 2.6
4	0.3	5.0	14.6 ± 3.1
3	0.75	—	16.3 ± 3.7

The control group may be compared with a group given procaine amide ethobromide (PAEB), and with a group given 2½ times the paralyzing dose of *d*-tubocurarine. The renal pedicles were bilaterally ligated in all animals.

drugs for this system. We were also able to show a similar competition with *d*-tubocurarine for this transport process by procaine amide ethobromide (PAEB). There also appears to be a saturation level in the ability of the liver to secrete *d*-tubocurarine from blood into bile (as evidenced by lesser proportionate recovery with 0.75 mg. *d*-tubocurarine/kg. than with 0.3 mg./kg. (table 4).

Although calculations for renal plasma flow (13.9 ml./minute/kg.) are within normal range for the anesthetized dog,[6, 12] similar calculations for hepatic plasma flow (2.82 ml./minute/kg.) were lower than the expected range.[12, 14] Two factors introduce inaccuracy in the calculations of liver plasma flow rates. Nordenström *et al.*[15] have shown that during normal respiration an admixture of blood occurs between the inferior vena cava and the hepatic veins, and that true hepatic vein sampling is not obtained. A second error in calculation relates to storage of *d*-tubocurarine within the liver.[4] Delay in the prompt passage of *d*-tubocurarine-H³ from the liver into the bile would yield lower calculations of rate for hepatic plasma flow. For the above reasons, calculations of hepatic plasma flow as presented in table 3 are subject to interpretation.

Metabolism of *d*-tubocurarine in the body has been suggested by several authors, as influenced by their ability to recover only a fraction of the injected dose in the urine.[1-3] Marsh suggested that biotransformation might occur either by N-demethylation with formation of tertiary basic compounds or by aromatic ring oxidation to quinone-like structures. We were unable to verify such biotransformation or a significant degree of metabolism for *d*-tubocurarine. A small amount of *N*-dealkylation may take place since about 1 per cent of the total injected dose is converted into non-curare form(s), a portion of which may be reconverted to *d*-tubocurarine by treatment with methyl iodide.

Summary

A study of the metabolism of *d*-tubocurarine-H³ in 31 dogs indicates that this drug does not undergo significant degrees of biotransformation. The kidneys normally provide the major avenue of elimination, with the liver contributing an alternative route. In the absence of renal function, the ability of the liver to eliminate *d*-tubocurarine via bile is greatly increased.

References

1. Mahfouz, M.: The fate of tubocurarine in the body, Brit. J. Pharmacol. 4: 295, 1949.
2. Marsh, D. F.: Distribution, metabolism, and excretion of d-tubocurarine chloride and related compounds in man and other animals, J. Pharmacol. 105: 299, 1952.
3. Kalow, W.: Urinary excretion of d-tubocurarine in man, J. Pharmacol. 109: 74, 1953.
4. Cohen, E. N., Corbascio, A., and Fleischli, G.: The distribution and fate of d-tubocurarine, J. Pharmacol. 147: 120, 1965.
5. Cohen, E. N.: Quantitative determination of d-tubocurarine in body tissue and fluids, J. Lab. Clin. Med. 62: 979, 1963.
6. Smith, H. W.: Measurement of the rate of glomerular filtration, *In:* Principles of Renal Physiology. New York, Oxford University Press, 1956, p. 27.
7. Fishman, W. H., Springer, B., and Brunetti, R.: Application of an improved glucuronidase assay method to the study of human blood β-glucuronidase, J. Biol. Chem. 173: 449, 1948.
8. Schanker, L. S., and Solomon, H. M.: Active transport of quarternary ammonium compounds into bile, Amer. J. Physiol. 204: 829, 1963.
9. Cohen, E. N.: Renal elimination of d-tubocurarine, Invest. Urol. 4: 69, 1966.
10. Cohen, E. N.: Blood brain barrier to d-tubocurarine, J. Pharmacol. 141: 356, 1963.
11. Albert, A.: Ionization, *In:* Selective Toxicity. New York, John Wiley and Sons, Inc., 1965, p. 208.
12. Conn, H. L., Jr., and Markley, K.: Simultaneous comparison of renal blood flow as measured by the Fick principle and by the bubble flow meter, Amer. J. Physiol. 160: 547, 1950.
13. Ketterer, S. C., Wiegand, W. T., and Rapaport, E.: Hepatic uptake and biliary excretion of indocyanine green and its use in estimation of hepatic blood flow in dogs, Amer. J. Physiol. 199: 481, 1960.
14. Restrapo, J. E., Warren, W. D., Nolan, S. P., and Mudler, W. H.: Radioactive gold technique for the estimation of liver blood flow, Surgery 48: 748, 1960.
15. Nordenström, B., and Norhagen, A.: Effect of respiration on venous return to the heart, Amer. J. Roentgenol. 95: 655, 1965.