enates from normal or tolerant mice after receiving an L.D.$_{50}$ dose of thiopental.

4. It is concluded that the observed *in vitro* depression of brain respiration by barbiturates cannot be the sole mechanism responsible for barbiturate hypnosis.

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A Method for Quantitative Estimation of Small Amounts of D-Tubocurarine Chloride in Plasma.* (17909)

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(Introduced by H. C. Hodge)
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To date no chemical method for the determination of D-tubocurarine in plasma has been reported. A method by which two micrograms of D-tubocurarine chloride per ml of plasma can be estimated has been developed and is presented along with data on the plasma levels associated with paralysis in the rabbit. The principle of the procedure is that employed by Brodie and Udenfriend (1) for many organic bases, that is, extraction of the alkaloid into an organic solvent and subsequent complex formation with methyl orange. D-tubocurarine cannot be extracted from alkaline solution into organic solvents. It may, however, be extracted from a potassium iodide solution at pH 10. Other salts were unsatisfactory because they caused the formation of emulsions between the plasma and solvent, gave high blanks, or failed to result in complete recovery.

Reagents. Ethylene dichloride. Because this solvent usually contains impurities which react with methyl orange, it was purified in one of 2 ways: (a) by treating with activated charcoal, Norite, or (b) by washing once with one-fifth volume of N hydrochloric acid and then several times with water. The purified solvent was stored in a glass-stoppered bottle. *Methyl Orange.* One gram of the sodium salt of methyl orange was dissolved in 500 ml of water and the resulting solution was shaken several times with equal volumes of purified ethylene dichloride to remove soluble organic impurities. The free acid of methyl orange was then precipitated by the addition of 2 ml of concentrated sulfuric acid, filtered on a Buchner funnel, washed several times with water and dried *in vacuo*. *Acid-alcohol.* Two ml of concentrated sulfuric acid were dissolved in 100 ml of absolute ethanol. *Potassium-iodide-glycine buffer.* A buffer solution of about pH 10 was prepared by mixing 6.0 ml of 0.1 M glycine solution containing sodium chloride (7.505 g glycine and 5.85 g sodium chloride per liter) with 4 ml of 0.1 N sodium hydroxide. To 10 ml of this buffer, 12.8 g of reagent grade potassium iodide were added and the resulting solution was kept in a brown bottle to protect it from the light. The buffer is very stable but the buffer-potassium-iodide solution is not and the mixture should be prepared daily. *Citric acid buffer.* A citric acid buffer solution, pH 5.0 was prepared by mixing 10.3 ml of 0.2 M dibasic sodium phosphate with 9.7 ml of 0.1 M citric acid.

Procedure. Three ml of plasma containing 5 to 60 micrograms of D-tubocurarine chloride are pipetted into a 60 ml glass-stoppered bottle containing 10 ml of ethylene dichloride. One-half ml of the potassium iodide-glycine buffer solution is added and the mixture is shaken for 5 minutes, decanted into a test tube, centrifuged and the aqueous

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1. Brodie, B. B., and Udenfriend, S., *J. Biol. Chem.*, 1945, v158, 705.

phase removed by aspiration. The ethylene dichloride phase is transferred to a glass-stoppered test tube, three drops of the citric acid buffer and a few crystals of methyl orange are added. The test tube with contents is shaken for 2 minutes, centrifuged and the excess dye solution is completely removed by aspiration with a finely drawn glass tube. Five ml of the ethylene dichloride containing the dye complex is transferred to a colorimeter tube and 0.5 ml of the sulfuric acid-ethanol solution is added. The optical density of the pink-colored solution is measured in a Klett-Summerson photoelectric colorimeter using a No. 540 filter with the zero adjusted using ethylene dichloride. In plasma and urine there are ethylene dichloride soluble substances which react with methyl orange. The blank obtained with one ml plasma is equivalent to about 0.06 μg of D-tubocurarine; that obtained with urine was so much higher that further investigation of urine samples was not attempted. Recoveries of 5 to 60 μg D-tubocurarine chloride added to plasma were satisfactory (Table I).

Standard curve. A stock solution containing 100 mg of crystalline D-tubocurarine chloride in 100 ml of water was kept in the refrigerator and dilute solutions prepared as needed. A standard curve was prepared by extracting various amounts of D-tubocurarine chloride, 5 to 60 μg, from water in the same manner as for the plasma determination. A reagent blank was tested by the same procedure. A linear relationship between the concentration of alkaloid and optical density was obtained. A net density reading of 60 was obtained on the Klett-Summerson photoelectric colorimeter for a concentration of 1 μg of D-tubocurarine chloride per ml of ethylene dichloride.

TABLE I.
Recovery of D-Tubocurarine Chloride from Plasma.

D-tubocurarine chloride added, μg	D-tubocurarine chloride found, μg	Recovery %
5	5.0	100
	5.0	100
10	10.0	100
	10.3	103
20	20.4	102
	20.0	100
30	30.3	101
	30.3	101
40	38.4	96
	40.0	100
50	50.0	100
	48.5	97
60	58.3	97
	58.3	97

Plasma level of paralytic dose of tubocurarine in rabbits and its pharmacological effect. Plasma levels of D-tubocurarine were correlated with the dose required to produce "head drop" in rabbits. The criterion for the "head drop" dose was the relaxation of the neck muscles so that the vertebrae could easily be felt; at this time the animal was unable to lift its head or move its legs and the respiration was slowed. On recovery, first the respiration returned to normal, followed by a resumption of tone in the neck muscles and then in the extremities. The animal appeared normal about ten minutes after the injection was stopped.

Both crystalline D-tubocurarine chloride and a partially purified preparation, Intocostrin, were used. Each preparation was made up in an isotonic solution so that each ml had a "potency equivalent to 20 units of standard curare by Rabbit-Crossover Test". For all experiments each solution was diluted ten times with saline so that each ml contained two units (0.30 mg per ml) and the rate of injection was maintained at 0.2 ml every 45 seconds. A control blood sample was taken by cardiac puncture before starting the infusion. Data from these experiments are shown in Table II.

Approximately equal amounts of D-tubocurarine chloride and Intocostrin were required to produce paralysis. It is interesting to note with each of these preparations that the plasma level was critical and constant, although no constant dose was required to produce "head drop". Thus, rabbit No. 3 was paralyzed with 0.221 mg per kg, and rabbit No. 4 required nearly twice as much, *viz.*, 0.408 mg per kg; nevertheless the plasma level in each case was 1.6 mg per liter.

Summary. Small amounts of D-tubocurarine can be measured in plasma by extraction with ethylene dichloride and reaction with

TABLE II.
Plasma Concentrations of D-Tubocurarine Found at Time of Rabbit Head-drop After Administration of D-Tubocurarine Chloride or Intocostrin.

	D-Tubocurarine chloride		Intocostrin	
Animal No.	Amt. inj. mg/kg	Plasma level at time of head drop mg/liter	Amt. inj. mg/kg	Plasma level at time of head drop mg/liter
5	.385	2.1	—	—
10	.264	2.2	—	—
6	.252	1.8	—	—
4	—	—	.408	1.6
3	—	—	.221	1.6
5	—	—	.333	1.5

methyl orange. The plasma level for paralysis in rabbits is about 1.5 to 2.2 mg per liter.

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Experimental Production of Sterile Closed Intestinal Loops. (17910)

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The purpose of this paper is (1) to report a method for the production of sterile closed intestinal loops, and (2) to report some subsequent observations upon these loops.

Methods. Several combinations of phthalylsulfathiazole and dihydrostreptomycin were used. However, the simplest and most reliable method consisted of the following. Healthy adult dogs were given, prior to surgery, 6.0 g of phthalylsulfathiazole each 12 hours for 3 days and 0.5 g of dihydrostreptomycin each 12 hours for 2 days before surgery. The animal was fasted for 24 hours and anaesthesized with pentobarbital and ether. All operative procedures were performed with strict aseptic technic. The ligatures and sutures were 000 silk. The abdomen was entered through a midline incision beginning at the xyphoid process and extending inferiorly for 4 inches. Both jejunal and ileal closed loops were made. The segment of either jejunum or ileum was chosen with a good blood supply that would not be embarrassed when brought through the abdominal wall and transplanted subcutaneously. Therefore the length of the loop and its distance from either the ligament of Treitz or the ileocecal valve varied in different dogs; the details are listed in Table I. Having determined the level of the loop and its length, the gut was transected in two places between hemostats. The loop was brought up through the abdominal incision, the continuity of the bowel reestablished by the aseptic anastomosis technic, and the linea alba was closed in a manner which would not interfere with the mesenteric pedicle supplying blood to the loop. The clamps were removed from the ends of the loop, and the loop irrigated five times with 200 cc of 1:1000 aqueous zephiran chloride followed by similar irrigations with sterile water. The ends of the loop were next closed by inverting with a purse string suture followed by interrupted inverting sutures. The closed loop was transplanted subcutaneously by undermining the adjacent skin edges. The skin was then closed by a running suture. Seven

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