The Effects of d-*Tubocurarine and Its Commercial Vehicles on Cardiac Function*

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d-Tubocurarine has been reported to have a depressant effect on cardiac tissues, an effect reversed by high calcium concentrations. Results of this study showed that the depressant effect (20 to 50 per cent of control tension) could be accounted for by the benzyl alcohol or 4-chloro-3-methyl cresol used as preservatives in commercial preparations of *d*-tubocurarine. The effects of these compounds were reversed by increasing the calcium chloride content of the Ringer's solution 25 to 100 per cent (3.0 to 4.8 mM). The findings of previous workers may have reflected the effects of these two substances rather than a depressant effect of the *d*-tubocurarine. (Key words: *d*-Tubocurarine; Cardiac function.)

IT HAS BEEN REPORTED that *d*-tubocurarine has a cardiac depressant effect[1,3] which can be reversed by high calcium concentrations.[4] Some investigators believe this depressant effect is the result of histamine release by *d*-tubocurarine.[1,2] Others claim a direct cardiac action for the drug.[3,5] In our studies we have been interested in involvement of calcium ion in vascular and cardiac muscle function. We have used reserpine to modify vascular calcium content and, we believe, availability to the contractile apparatus.[6,7] We thus became interested in the possibility of a curare–reserpine–calcium interaction at various muscle sites. In one study[8] we found that reserpine decreased the apparent potency of *d*-tubocurarine at the skeletal neuromuscular junction. We, therefore, decided to study this relationship in the heart. However, in the initial phase of the study we were unable to demonstrate any cardiac depressant activity with *d*-tubocurarine, nor any calcium–curare relationship in the isolated rabbit atrial preparation. Further work to resolve this difference between our results and those of the previous workers was needed. The results of this study are the subject of the present report. The cardiac depressant effect which had been attributed to *d*-tubocurarine resulted from the preservatives used in the injection preparations of the drug, and *d*-tubocurarine, *per se*, had none.

Methods

EXPERIMENTAL PROCEDURES

Albino New Zealand rabbits weighing approximately 1 kg were used in the study. Each animal was sacrificed by a sharp blow to the head, and the heart was excised immediately. The hearts were placed in oxygenated Ringer's solution and the atria removed. Atria were suspended in organ baths (50 ml) in Ringer's solution (composition: NaCl, 154 mM; KCl, 5.4 mM; $CaCl_2$, 2.4 mM; $NaHCO_3$, 6 mM; dextrose 11 mM; distilled water to 1 liter). Tension measurements were made with "E and M" myographs and recording equipment (Physiograph) after adjusting the diastolic tension of the atria to 1 gram. Three preparations were used in these studies: the spontaneously-beating right atrium, spontaneously-beating left and right atria, and the electrically-driven left atrium. Left atria were stimulated at a frequency of 1/sec with supramaximal square-wave stimulation 3 msec in duration. Recording of contractile amplitude and rate was begun immediately after the atria were mounted. A 30-minute equilibration period preceded the final adjustment to 1-gram tension, and a second 30-minute equilibration period followed prior to the addition of drugs or calcium. After the equilibration period, *d*-

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tubocurarine was added to the bath in the concentrations indicated (see "Results"). After sufficient time had elapse for any changes due to the d-tubocurarine to occur, calcium chloride was added. Changes in contractile amplitude and rate were used to assess the effects of the drugs. One series of rabbits was pretreated 24 hours before the experiment with 3.5 mg/kg reserpine. All solutions of *d*-tubocuararine were tested for paralytic activity in unanesthetized rabbits before use in the *in vitro* experiments.

Drugs

The commercial drugs used were the injectable preparations of *d*-tubocurarine: Abbott's *tubocurarine chloride*, containing 3 mg *d*-tubocurarine chloride, 1 mg sodium metabisulfite, 9 mg benzyl alcohol and 1 ml of water made isotonic with NaCl; Burroughs Wellcome's *Tubarine*, containing 3 mg *d*-tubocurarine chloride, 1 mg p-chloro-m-cresol (4-chloro-3-methyl-phenol) and 1 mg potassium metabisulfite in 1 ml of water made isotonic with NaCl; Squibb's *tubocurarine chloride*, containing 3 mg *d*-tubocurarine chloride, 9 mg benzyl alcohol and 1 mg sodium bisulfite in 1 ml of water made isotonic with NaCl. Also used were the pure crystalline *d*-tubocurarine chlorides obtained from Abbott, Burroughs Wellcome, Squibb, and Nutritional Biochemicals. Other agents used were reagent-grade benzyl alcohol and 4-chloro-3-methyl-phenol. Drug vehicles used were: Abbott 3386, containing 9 mg benzyl alcohol, 1 mg sodium metabisulfite and 4.6 mg NaCl in 1 ml of water; Ab-

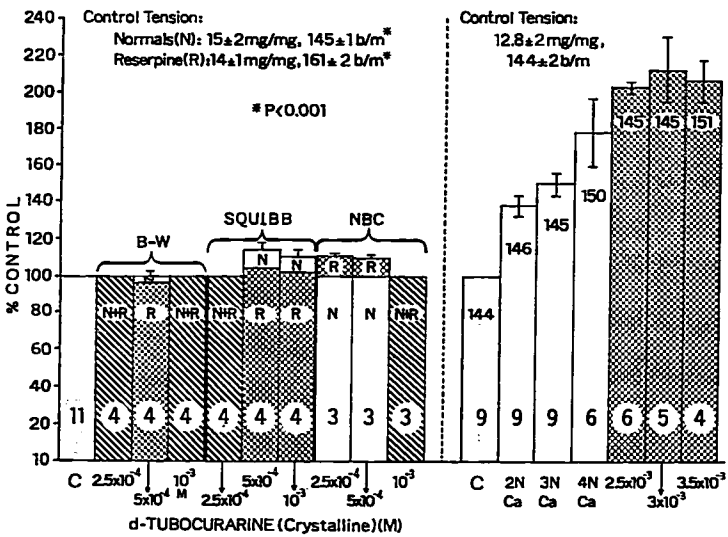

FIG. 1. Effects of *d*-tubocurarine on contractile tension of unstimulated rabbit left and right atria. Left side of figure: C, control; N + R, normal and reserpine-treated atria superimposed; R, reserpine-treated atria; N, normal atria; B-W, Burroughs Wellcome; NBC, Nutritional Biochemicals. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of *d*-tubocurarine. Right side of figure: Shaded bar, control; clear bars after addition of calcium; cross-hatched bar, after both high calcium concentration (4 N) and *d*-tubocurarine. Ordinate, contractile tension as per cent of control; abcissa, concentration of calcium added as multiples of normal (N = 2.4 mM), a molar concentration of *d*-tubocurarine. Numbers in lower parts of bars represent the numbers of atria tested, those in upper parts (right side) represent heart rates.

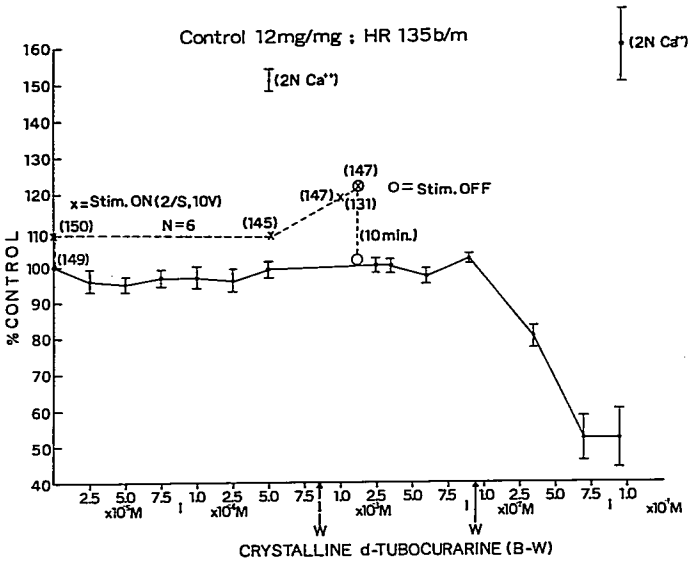

FIG. 2. Effects of *d*-tubocurarine on the stimulated and unstimulated rabbit left and right atrial preparation. Solid line, unstimulated; dashed line, stimulated. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of *d*-tubocurarine (Burroughs Wellcome). The result of calcium addition is shown by two points off the curve. The preparation was washed at W. Vertical bars, standard errors of the means. Six atrial preparations were tested.

bott 3841, containing 1 mg sodium metabisulfite and 6.8 mg NaCl in 1 ml of water; Burroughs Wellcome placebo (injection), containing 1 mg potassium metabisulfite and 1 mg methylparaben (P-hydroxybenzoic acid methylester) per ml of water made isotonic with NaCl. All drugs and calcium chloride were made up in concentrated stock solution for use, or taken directly from original vials. Delivery of drugs or calcium to the organ bath was in as small a volume as possible, depending upon the maximum concentration of the drug that could be put in solution. In most instances the volume did not exceed 0.1 ml. Periods of 15 to 30 minutes were allowed between drug or calcium additions to the organ baths. All baths were oxygenated with a mixture of 95 per cent O_2 and 5 per cent CO_2. Temperature was maintained at 37 ± 0.2 C, and *p*H was maintained at 7.4 ± 0.5. At times, small quantities of HCl or NaOH had to be added to the baths to adjust *p*H.

Results

EFFECTS OF CRYSTALLINE *d*-TUBOCURARINE ON ATRIA FROM NORMAL AND RESERPINE-TREATED RABBITS

The results obtained when isolated, unstimulated atria from control and reserpine-treated (3.5 mg/kg 24 hours prior to the experiment) rabbits were subjected to crystalline *d*-tubocurarine from 2.5×10^{-4} M to 10^{-3} M are depicted by the bar graph on the left in figure 1. There were no significant changes in contractile tension in either control or reserpine-treated atria when *d*-tubocurarine was added. When the calcium content of the Ringer's solution was raised to four times normal (9.6 mM

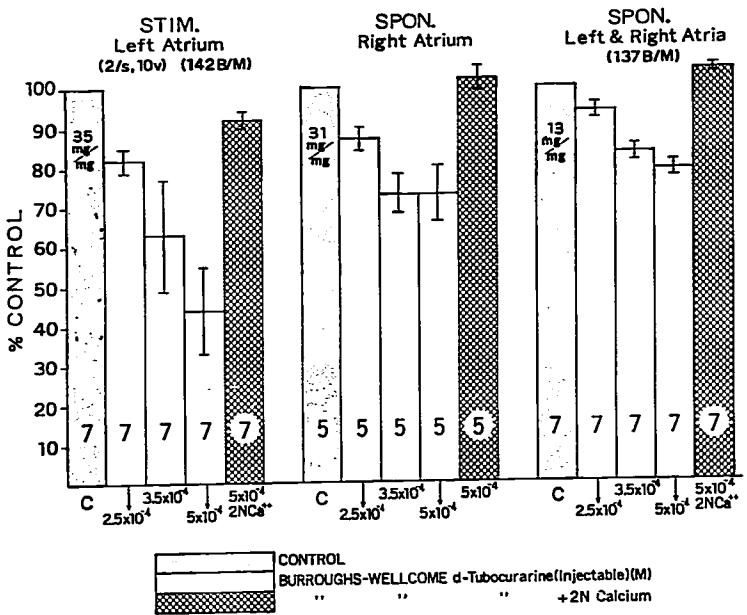

FIG. 3. Depressant effect of injectable *d*-tubocurarine preparations and reversal by the addition of calcium. Control tension is given in the first bar for each preparation. Heart rates are shown at the top. The rate of the spontaneous right atrium was not significantly different from that of spontaneous left–right atria. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of *d*-tubocurarine. Vertical bars, standard errors of the means.

final concentration) there was a significant increase in contractile tension of normal atria. *d*-Tubocurarine ($2.5–3.5 \times 10^{-3}$ M) had no significant effect on the tension of these atria in the presence of high calcium concentrations (bar graph on the right in figure 1). In figure 2 we show the results of an experiment to test the effect of stimulation on the left and right atrial preparation treated with *d*-tubocurarine (2.5×10^{-5}–10^{-1} M). (Previous investigators reporting curare depression used the stimulated left atrial preparation.[4]) Stimulation at 2/sec (10 v) increased contractile tension about 10 per cent; however, *d*-tubocuraraine had no depressant effect on either preparation up to 10^{-3} M. From 10^{-2} M *d*-tubocurarine to 10^{-1} M, a progressive decline in tension was observed. The addition of two times normal calcium (final concentration three times normal, 7.2 mM, $CaCl_2$) increased the tensions of both stimulated and unstimulated atrial preparations (41 and 52 per cent, respectively) in the presence of 5×10^{-3} M tubocurarine. The addition of 2 N calcium in the presence of 10^{-1} M *d*-tubocurarine increased atrial tension from 52 per cent to 160 per cent of control.

Effects of Injectable *d*-Tubocurarine on the Contractile Tension of Rabbit Atria

A depressant effect of Burroughs Wellcome injectable *d*-tubocurarine on contractile tension was found in the stimulated left atrial, spontaneous right atrial, and spontaneous left and right atrial preparations (fig. 3). There

was a direct relationship between the depression observed and the concentration of drug used for each preparation. The stimulated left atrial preparation was depressed far more than either of the spontaneous preparations. In the latter there was no further significant depression at concentration of *d*-tubocurarine above 3.5×10^{-4} M. In each case when the calcium content of Ringer's solution was increased by twice the normal concentration (final concentration three times normal, 7.2 mM $CaCl_2$) the depression was completely reversed. In figure 4 the depressant effect of Abbott's injectable *d*-tubocurarine on contractile tension is shown. These results are similar to those obtained with the Burroughs Wellcome product. Similar experiments with Squibb's injectable *d*-tubocurarine produced similar results. In figure 4 results obtained with the two vehicles for *d*-tubocurarine, Abbott 3386 and 3841, are illustrated. Vehicle 3386 had a depressant effect similar to that of the injectable tubocurarine, while 3841 did not. The depression resulting from 3386 was reversed by calcium. The depression by 3386 obtained upon the addition of 1.0 ml to the bath was not significantly different from that obtained with 8×10^{-5} M *d*-tubocurarine (final bath concentration upon the addition of 1 ml of the injectable drug to the bath). The vehicle used for Abbott's injectable tubocurarine chloride in these experiments was identical to 3386.

Effects of Benzyl Alcohol and 4-Chloro-3-Methyl Phenol on Atrial Tension

The depressant effects of Squibb's and Abbott's *d*-tubocurarine on atrial tension resulted from the benzyl alcohol contained in their vehicles (fig. 5). Benzyl alcohol had the depressant effect previously accredited to the *d*-tubo-

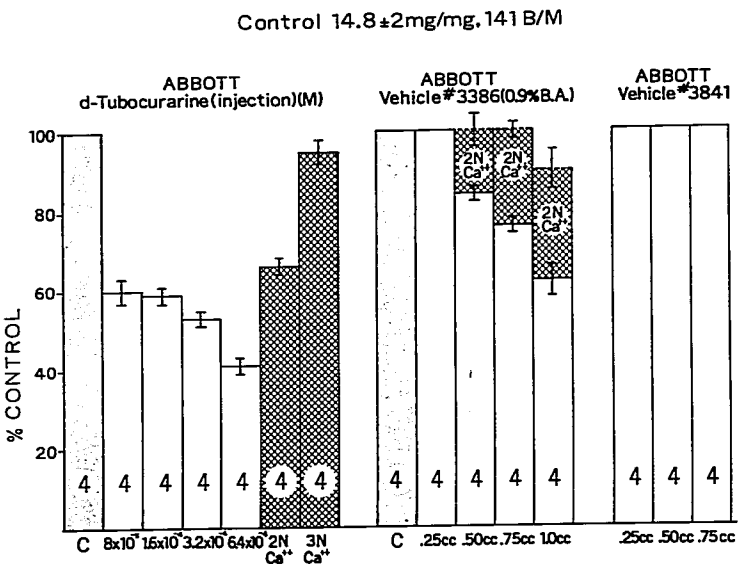

FIG. 4. Depressant effects of Abbott's injectable *d*-tubocurarine and vehicles 3386 and 3841 and calcium reversal. Control tension and rate at top of figure. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of *d*-tubocurarine, calcium concentration as multiple of normal (N = 2.4 mM), and volume of vehicle delivered. Numbers at lower parts of bars, numbers of atria tested; verticle bars, standard errors of the means.

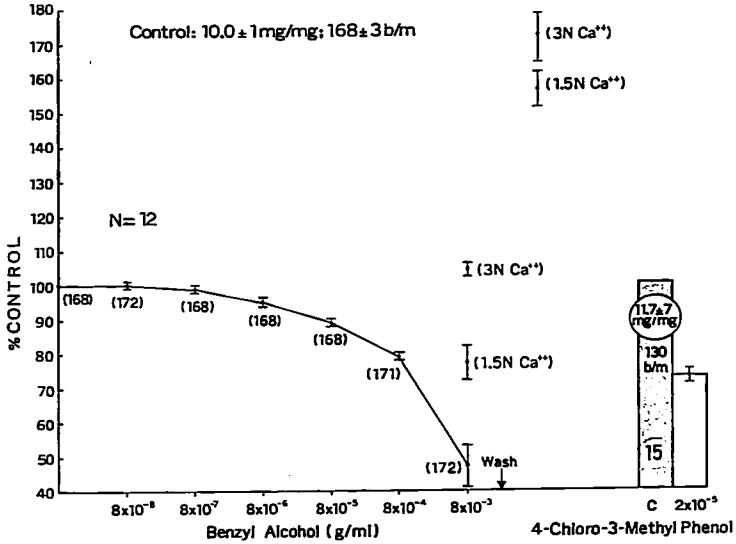

FIG. 5. Depressant effects of benzyl alcohol and 4-chloro-3-methyl phenol on the unstimulated left and right rabbit atrial preparation. Reversal by the addition of calcium is shown by isolated points in the figure both before and after washing (at "wash" arrow). Control rate and tension for atria tested with benzyl alcohol are given at the top, for those tested with the phenol, rate and tension are shown in control bar. Numbers in parentheses under the curve indicate heart rate. N, number of atria. Number in lower part of shaded bar, number of atria. Ordinate, contractile tension as per cent of control; abcissa, concentrations of benzyl alcohol and phenol in g/ml.

curarine. The active preservative in the Burroughs Wellcome product is 4-chloro-3-methyl phenol. When tested in 15 preparations, it reduced the tension of the spontaneous right and left atrial preparation 30 ± 5 per cent at 2×10^{-5} g/ml. The addition of calcium to as much as two times normal reversed both the benzyl alcohol and the phenol depressions.

EFFECTS OF ADDITION OF BENZYL ALCOHOL TO ATRIAL PREPARATIONS AFTER ADDITION OF CALCIUM

Experiments were done to determine the effects of pretreatment of the isolated atria with high calcium concentrations prior to the addition of benzyl alcohol, and benzyl alcohol plus *d*-tubocurarine. Results of these experiments are presented in table 1. At 1.25 normal calcium (final concentration), 10^{-7} to 10^{-4} g/ml benzyl alcohol reversed slightly the positive inotropic effect of the calcium. At 1.5 and 2.0 normal calcium, benzyl alcohol did not overcome the calcium response. At 2.5 normal calcium, the addition of the alcohol resulted in arrhythmias in all instances, so that the experiments were terminated. Addition of 1 mg of *d*-tubocurarine with each mg of benzyl alcohol did not affect these results. In these experiments the increase in calcium caused an increase in heart rate as well as an inotropic response. At 1.25 normal calcium, 10^{-3} g/ml benzyl alcohol brought the rate back to control from an increase of 25 per cent above control. At 1.5 normal calcium, benzyl alcohol only decreased the rate toward normal, about 10 per cent. At higher calcium concen-

TABLE 1. Effects of Benzyl Alcohol on Atrial Preparations after the Addition of Calcium (Control Tension 16 ± 1 mg/mg; Control Rate 137 ± 2 beats/min)

Number of Atria Tested	Calcium Concentration Times Normal (2.4 mM)	Change after Calcium		Change after Calcium + Benzyl Alcohol*	
		Tension (Per Cent)	Rate (Per Cent)	Tension (Per Cent)	Rate (Per Cent)
7	1.25	+45 ± 1	+25	−55 ± 1†	−25†
4	1.50	+85 ± 3	+25	−50 ± 10	−10
4	2.0	+120 ± 5	+26	−30 ± 8	0
4	2.50	+240 ± 6	+22	Arrhythmias	0

* Atria were subjected in each instance to 10^{-7} to 10^{-4} g/ml benzyl alcohol, with the same result obtained at all concentrations. This amount of benzyl alcohol is equivalent to 1.1×10^{-6} to 1.1×10^{-3}M *d*-tubocurarine. Results obtained with benzyl alcohol plus crystalline *d*-tubocurarine (1 mg for each mg benzyl alcohol) after calcium were not significantly different from those obtained without the *d*-tubocurarine.

† Percent reduction in tension and rate from increased level caused by calcium addition, *i.e.*, benzyl alcohol reduced the rate to control level when added after 1.25 N calcium chloride.

trations benzyl alcohol had no effect. The decrease in rate observed after the addition of the benzyl alcohol to the high-calcium-concentration solution could be restored to the initial high level (25 per cent above control) by the further addition of 0.5 normal calcium (1.2 mM).

Effects of Burroughs Wellcome Vehicle Containing Neither Benzyl Alcohol Nor 4-Chloro-3-Methyl Phenol

In 20 experiments with the spontaneous left and right atrial preparation this vehicle had no effect on either atrial rate or tension in volumes equivalent to the volumes of the Burroughs Wellcome injectable *d*-tubocurarine, which contained 10^{-9} to 10^{-2} g/ml of 4-chloro-3-methyl phenol.

Except for the experiments in which the calcium concentration was increased before the addition of any other drug no significant changes in heart rate were observed in these studies. When calcium was added first there was usually an increase of about 25 per cent with the initial increase of calcium of one-fourth normal. No further increases were observed with greater amounts of calcium.

Discussion

For many years it was assumed that curare had no cardiac effects.[9,10] The absence of clinical or experimental evidence to the contrary attested to the validity of this assumption. In 1965, based on clinical observations of patients in whom *d*-tubocurarine had reversed various ventricular arrhythmias, including fibrillation, and upon similar results obtained in dogs,[11] Dowdy and her co-workers studied the effects of *d*-tubocurarine in the isolated perfused rabbit heart. They observed a quinidine-like action of *d*-tubocurarine.[3] These authors did point out at the time that the *d*-curarine did not correct atrial fibrillation. In a subsequent study[4] it was reported that *d*-tubocurarine had a depressant action on the contractile tension of isolated left atria of rabbits which could be reversed by high concentrations of calcium. The results of the present study, however, indicate that *d*-tubocurarine has no depressant effect on atrial muscle except at very high concentrations (10^{-2} M), but that the vehicles in most commercial preparations contain substances which do depress the contractile tension of atrial tissue. In addition, this depression caused by the solvent is reversible by high calcium concentrations. The minimum concentration used both *in vivo* and *in vitro*[3,4] in the earlier studies (10^{-4} M), if used in the commercial injectable form (not specified in methods), would be accompanied by 8×10^{-5} M benzyl alcohol or 7.7×10^{-6} M 4-chloro-3-methyl phenol. We have observed that these substances at these concentrations can account for the depressant results obtained.

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Drugs

BARBITURATE INTOXICATION Deep coma resulting from barbiturate overdosage was studied in 50 patients. The length of coma correlated with depth of coma and serum barbiturate levels except in those patients who were drug addicts and had hepatic or renal disease. The complications of pneumonia, bleeding of the gastrointestinal tract, urinary-tract infections, and thrombophlebitis were common; however, all patients survived. Treatment was instituted on a rotation basis with supportive care alone, mannitol diuresis, or peritoneal dialysis. Using length of coma, the slope of disappearance of serum barbiturate, and clearance data as indicators of the effectiveness of treatment, the three treatment forms did not differ. Supportive care alone was associated with less local and systemic morbidity and appeared to be the best method of reducing morbidity and mortality in patients after barbiturate overdosage. (*Hadden, J., and others: Acute Barbiturate Intoxication; Concepts of Management, J.A.M.A. 209: 893 (Aug.) 1969.*)

PROPRANOLOL IN THYROID STORM Although proper preoperative preparation of the thyrotoxic patient and the therapeutic use of radioactive iodine have reduced the incidence of storm, it is still a serious complication. Treatment is still unsatisfactory, however, as evidenced by the mortality figures (20 to 50 per cent). It has been shown recently that most of the cardiovascular and metabolic manifestations of thyrotoxicosis can be controlled effectively by blockade of the beta-adrenergic receptors of the body. Intravenous administration of propranolol was immediately effective in the management of thyrotoxic storm in a patient who did not respond to other modes of therapy. (*Das, G., and Drieger, M.: Treatment of Thyrotoxic Storm with Intravenous Administration of Propranolol, Ann. Intern. Med. 70: 985 (May) 1969.*)

RELAXANTS AND OCULAR PRESSURE Succinylcholine (SCh) increases intraocular pressure in part by contracting extraocular muscles and in part by contracting orbital smooth muscles. If the dose of SCh is large enough to increase arterial pressure, this also contributes to the increase in intraocular pressure. The effects of SCh on extraocular muscles, orbital smooth muscles, and intraocular pressure can be prevented by *d*-tubocurarine, gallamine or hexafluorenium. (*Katz, R. L., and Eakins, K. E.: The Actions of Neuromuscular Blocking Agents on Extraocular Muscle and Intraocular Pressure, Proc. Roy. Soc. Med., 62: 1217 (Dec.) 1969.*)