TABLE II. Effect of Intravenous Administration of 4.5 mg Dromoran[R] per kg to the Maternal Circulation on Respiratory Rates of the Mother and Fetuses.

4.5 mg/kg	Control	15 min	30 min	60 min	90 min	2 hr	3 hr	4 hr
Maternal	150	15	20	19	31	45	59	85
Fetus: 1	24	0	0	0	0	0	14	10
2	30	4	6	6	4.5	14	18	15
3	26	0	0	0	0	2	2	7
4	31	0	0	0	0	5	5	0
5	65	2.5	0	0	0	2	1.5	10
Mean fetal rates	35	1.3	1.2	1.2	.9	4.6	8.1	8.4

passes without difficulty across the placental barrier and produces a decrease in the rate of respiratory movements of the fetuses. This passage across the placental barrier might be expected in view of the general principle stated by Needham(8) that the placental barrier is freely passed by practically all substances of low molecular weight. Thus in obstetrical analgesia one can assume that the agent will pass the placental barrier and the best analgesic will be one which will have high analgesic potency in the mother and a minimal effect on fetal respiration activity. The final evaluation of an analgesic must therefore be made under clinical conditions where the analgesic property can be evaluated in the patient. The doses of Dromoran® used in these experiments were much higher than would be used for human patients and there is no doubt but what satisfactory analgesia in humans can be obtained with lower doses. The question of whether these doses depress the respiratory function of the baby can be answered by clinical observation only with difficulty. On the basis of the parallelism shown in the rabbit experiments between the response of the maternal respiration rate and the rate of fetal respiratory movement it would appear safe to assume that if, in the human mother, respiratory function was depressed there would almost certainly be a corresponding effect on the respiratory function of the baby. Thus an obstetrician might make use of changes in the maternal respiration rate as an index to the respiratory function of the baby.

1. Lee, R. E., and Hasegawa, A. K., *J. Pharm. and Exp. Therap.*, 1950, v98, 20.

2. Theye, R. A., Stoelting, V. K. and Graf, J. P., *J. Pharm. and Exp. Therap.*, 1950, v98, 31.

3. Zager, L. L., Sawtelle, W. W., Gross, E. G., Nagyfy, S. F., and Tidrick, R. T., *J. Lab. and Clin. Med.*, 1949, v34, 1530.

4. Snyder, F., *Obstetric Analgesia and Anesthesia*, W. B. Saunders, 1949.

5. Snyder, F., and Rosenfeld, M., *Am. J. Physiol.*, 1937, v119, 153.

6. Bonar, B. E. and Blumenfeld, C. M., *Surg. Gyn. and Obs.*, 1938, v66, 179.

7. Snyder, F., *Bull. Johns Hopkins*, 1934, v54, 1.

8. Needham, J., *Chemical Embryology*, Macmillan, Sec. 21, p1512, 1931.

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Dibenamine Blockade as a Method of Distinguishing Between Inotropic Actions of Epinephrine and Digitalis.*† (19229)

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A differentiation of the inotropic effects of epinephrine and digitalis has direct application in the characterization of such drugs as the veratrine alkaloids. These alkaloids produce cardiac actions which resemble, in some particulars, both those of epinephrine and

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digitalis(1). The present report describes conditions under which Dibenamine blocks about 70% of the inotropic action of epinephrine without significantly affecting the inotropic response of digitalis. The pharmacology of Dibenamine has been comprehensively reviewed by Nickerson(2).

Methods and Procedure. The contractile force of a segment of the right ventricle was determined with strain gauge equipment attached to modified Cushny levers in open-chest dog preparations. These preparations routinely included the use of barbital-pentobarbital anesthesia and bilateral cervical vago-sympathectomy. Twenty-eight such experiments were conducted. Additionally, 2 experiments were conducted in intact-chest preparations using a more compact type of strain gauge equipment stitched to the anterior aspect of the right ventricle on the previous day. The conditions and limitations of these methods have been previously described(3,4) and their use in characterizing various drug groups has been described recently(5,6). Electrocardiographic recordings of standard lead II were made in most experiments with a direct writing EPL Cardiotron. N,N-dibenzyl-b-chloroethylamine (Dibenamine) in the form of its hydrochloride salt was administered in doses of 20 or 40 mg/kg by intravenous infusion over a 20 minute period. Fresh 1% solutions in distilled water were prepared each day. Freshly diluted epinephrine solutions were made every 20 to 30 minutes from the stock 1:1000 solution of the hydrochloride (Adrenalin, Parke-Davis). Test doses of epinephrine were 3 γ/kg in the open-chest preparations and 2 γ/kg in the intact-chest preparations. Digitalis was administered in the form of the diluted tincture by intravenous infusion in doses of 0.75 cat unit/kg over intervals of 30 minutes. Similarly, in 2 experiments, a potent extract of erythrophleum alkaloids was used in doses of 0.5 cat unit/kg.

In 8 experiments, digitalis was administered after Dibenamine had abolished the major portion of the inotropic response to epinephrine. In 4 other experiments, digitalis was administered after Dibenamine under similar conditions of dose and time interval but without such tests of epinephrine blockade. Another series of 14 experiments was designed to determine the conditions of the Dibenamine-produced blockade of epinephrine inotropic responses. These included determinations of the influence of post-Dibenamine epinephrine hypotension; marked reductions in body temperature; responses to multiple dose injections of epinephrine following Dibenamine; and effects of repeated epinephrine injections over intervals of 4 to 5 hours without Dibenamine. These 14 experiments were conducted in open-chest preparations with controlled body temperatures. In the case of multiple dose injections of epinephrine following Dibenamine, 2 additional experiments were conducted in intact-chest preparations. In 5 of these experiments with epinephrine, the contractile force and blood pressures were recorded simultaneously with a Brush BL-902A oscillograph. Pressures from the carotid artery were recorded in these instances by means of a Statham transducer amplified through a Brush Analyzer Model BL-310. Arterial hypotension was counteracted by means of a ligature placed around the thoracic aorta and drawn out through a glass tube in the posterior chest wall. Body temperatures were maintained at approximately constant level by means of electrical heat pads. In all cases, body temperatures were determined by mercury bulb thermometers inserted about 18 cm beyond the anal orifice to about the level of the sigmoid colon. In 2 special experiments, in which the body temperatures were deliberately altered, reduction was accomplished by placing crushed ice on the body surface; temperatures were subsequently raised by means of electrical heat pads.

Results. The positive inotropic action produced by epinephrine was substantially reduced by the prior administration of Dibenamine. The experiment shown in Fig. 1 demonstrates that the administration of 3 γ/kg of epinephrine 37 minutes after completing the Dibenamine administration resulted in a reduction of the inotropic response by about 85%. Reversal of the hypertensive response to epinephrine was obtained within 21 minutes after Dibenamine administration. In 20 other experiments, approximately similar degrees of

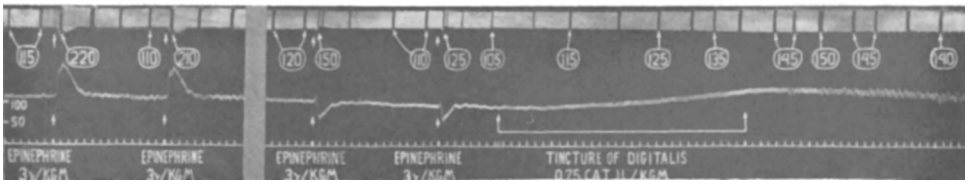

FIG. 1. Myocardiograph and arterial pressure tracing. Upper tracing obtained with Cushny levers typically attached to the anterior aspect of the right ventricle in the open chest, vagotomized dog preparation under barbiturate anesthesia. Encircled figures represent contractile force in g determined by means of strain gauge equipment attached to Cushny levers. Drugs administered intravenously. Time marker in min. Dibenamine (20 mg/kg) administered between first and second sections. Fifteen min elapsed between completion of Dibenamine infusion and beginning of the second section of tracing.

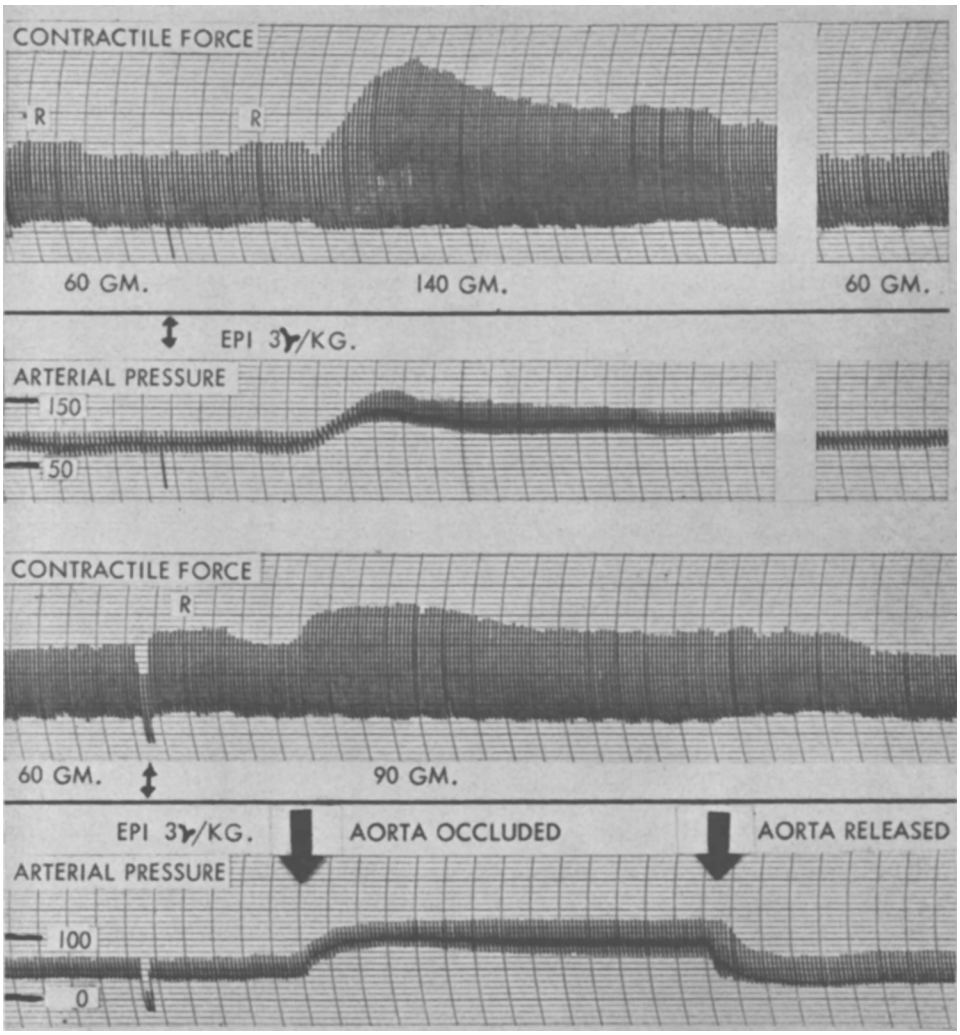

FIG. 2. Synchronous oscillograph recordings of contractile force and arterial pressure. In upper pair of tracings the injection of epinephrine increased contractile force by 133%. The lower pair of tracings were obtained from the same preparation 107 min after Dibenamine had been infused in a dose of 40 mg/kg. In this case the same dose of epinephrine increased contractile force by only 50%. The letter R designates intervals of inspiratory movements which, at times, have a limited but recognizable effect on contractile force; calculation of contractile force is based on intervals between such respiratory movements.

epinephrine inotropic blockade were obtained. The average reduction of the inotropic response for all these 21 experiments was about 70% of the control response. The maximum reduction (about 90%) was obtained in 6 of the 21 experiments. During each individual experiment, the blockade reached its maximum in 35 to 110 minutes and then did not change in any important degree. Blockade was approximately as complete with doses of 20 mg/kg as with doses of 40 mg/kg Dibenamine. Inotropic responses to multiple dose injections of epinephrine after Dibenamine were studied in 1 open-chest and in 2 closed-chest experiments. In these cases, the epinephrine inotropic response to single test doses of 2 or 3 γ/kg was reduced by about 75% after Dibenamine. When multiple test doses (up to 8 single doses) were given after Dibenamine, the positive inotropic response was usually less than that obtained by one single test dose in the control period. In all of these calculations, the inotropic response was evaluated in terms of the percent change from the immediately preceding control period.

Effect of repeated epinephrine injections without Dibenamine administration. In 2 experiments, with stabilized body temperatures but without Dibenamine administration, 10 injections of 3 γ/kg of epinephrine over intervals of 4 to 5 hours did not result in substantial differences in the quantitative inotropic responses. These time intervals under parallel conditions were longer than the intervals over which the experiments were conducted with Dibenamine.

General effects of Dibenamine. Characteristically under these conditions, Dibenamine produced lowered body temperature, hypotension, tachycardia and, in about half of the instances, a substantial increase in contractile force. The question naturally arises as to whether the blockade just described is secondary to some of these changes or whether it is a local, specific cardiac action. The following described observations demonstrate that the effect is direct and specific. At the same time, they serve to estimate the influence of these different variables on the final quantitative values.

Effect of body temperature on the Dibenamine blockade of epinephrine inotropic responses. The effect of a marked decrease and subsequent increase in body temperature on the quantitative inotropic response produced by epinephrine was examined in 2 experiments without Dibenamine administration. Frequent injections of 3 γ/kg were given as the temperature was altered. A reduction of the body temperature to 30°C from a control of 37°C did not appreciably reduce the quantitative inotropic response to epinephrine. This reduction of temperature did not significantly change the contractile force of the heart during the interval between the injections. Similarly, increasing the temperature from 30°C to 38°C did not appreciably influence the inotropic response to epinephrine or the control level of contractile force. On the other hand, the chronotropic response to epinephrine was markedly altered under these same conditions. As the temperature fell, the peak rates produced by epinephrine were drastically reduced (from 200/min. to 110/min.). The control rates were also reduced but were less markedly affected (from 155/min. to 100/min.). When the body temperature was raised from 30°C to 38°C, opposite effects of approximately the same magnitude were observed. The results of these experiments, in which the body temperature was markedly altered, are considered to demonstrate the relative stability of the contractile force measurements over this subnormal range. Other studies(7) have demonstrated markedly increased contractile force during advance stages of hyperpyrexia. In 3 open-chest experiments, in which the body temperature was maintained by heat pads within 0.5°C of the control (38°C), the inotropic responses to 3 γ/kg of epinephrine were blocked to the same degree as was produced in the other epinephrine-Dibenamine experiments without temperature control. In all of these 3 experiments the contractile force during the control intervals was increased moderately and the heart rate markedly (up to 240/min.) following Dibenamine administration. When body temperatures were not artificially maintained, heart rate increases were less pronounced and the chronotropic responses to epinephrine were considerably diminished. These experiments,

in which the body temperature was either altered or kept constant demonstrated that the blockade of the inotropic response of epinephrine by Dibenamine was not directly related to or dependent upon the level of the body temperature.

Circulatory changes produced by Dibenamine. In about half of 26 experiments, the administration of Dibenamine produced an increase in contractile force of approximately 55%; in 10 experiments there was no important change and in 2 there was reduction of contractile force by about 25%. In those experiments with contractile force increase, the greatest portion of the effect was established by the time of completion of the Dibenamine infusion; it was persistent and was not related to changes in blood pressure or heart size. This change was associated with an average increase in heart rate of about 35 beats per minute and usually a slow, progressive fall in blood pressure. Blood pressure usually stabilized at about 70-80 mm Hg but in some cases reached levels of 40 mm Hg. Electrocardiographic changes were usually limited to a sinus tachycardia and moderate S-T segment depression with variable T-wave changes. In those experiments without important contractile force changes due to Dibenamine, the increment in heart rate and the decrease in blood pressure were less pronounced. The blockade of epinephrine inotropic response was not related to these variabilities in blood pressure, heart rate or contractile force levels.

Possible influence of epinephrine produced hypotension on inotropic blockade. In the immediately preceding experiments, it was observed that Dibenamine-produced blockade of epinephrine inotropic responses occurred as fully at high blood pressure levels as at lower levels. It is well known that in the absence of Dibenamine the epinephrine inotropic response is readily elicited at levels of profound hypotension or in instances of complete circulatory failure. Nevertheless, there is reason to consider the possibility that the immediate hypotension produced by epinephrine following Dibenamine may have an influence on the quantitative inotropic response. The fact that such hypotension is not obtained with digitalis would suggest that this might be the basis for the distinction between their inotropic blockade characteristics. The experimental observations of this study, however, fully demonstrate that this immediate hypotensive phase is of little consequence in the production of epinephrine inotropic blockade. For instance, it was seen that the maximal contractile force increments typically occurred before the full hypotensive action was developed. In 5 experiments with synchronous oscillograph recordings of arterial pressure and contractile force, it was determined that the maximal contractile force response to epinephrine typically developed before the pressure had fallen more than 25% of its full hypotensive phase. In several other experiments, arterial pressure was maintained at or above control levels by tensing a ligature previously placed around the thoracic aorta; in such cases there was no significant difference in the degree of inotropic blockade as compared with similar experiments in which hypotension developed in the usual way (Fig. 2). Further, in some instances, in which blood pressure had reached levels of 40 mm Hg there was no hypotensive phase in the epinephrine response and in such cases there was the usual grade of blockade of inotropic responses.

Effect of Dibenamine on the inotropic action of digitalis. Typical increments in contractile force were obtained with digitalis in 8 experiments after the demonstrated blockade by Dibenamine of about 75% of the epinephrine inotropic response. The experiment shown in Fig. 1 demonstrates that, following blockade of about 85% of the contractile force response to epinephrine, the inotropic response to digitalis was 43% of the control level. As compared with a previous characterization study with the digitalis glycosides(3), this represented no important reduction of the usual inotropic response to digitalis. The absence of any important digitalis inotropic blockade in all of the 8 experiments was demonstrated by a quantitative comparison with the previous study with measured inotropic responses to digitalis. Such quantitative comparisons include recognition of the previously demonstrated variation in responses obtained at varying control levels. The characteristic

rise in blood pressure ordinarily seen with digitalis also occurred in these experiments. In 4 of the 8 experiments, in which the blood pressure was above 90 mm Hg before digitalis administration, the average increase in arterial pressure was 41 mm Hg as compared with 43 mm Hg average increase obtained in the previous digitalis study. The average increase in blood pressure for the other 4 experiments was only 10 mm Hg; in these latter experiments, the blood pressure before digitalis administration was about 55 mm Hg. The failure to obtain a typical rise in blood pressure in these 4 experiments was probably related to special features of the hypotension produced by Dibenamine. The associated electrocardiographic changes seen in the digitalis-Dibenamine experiments were typical of those obtained following digitalis administration. Typical inotropic increments were also produced by the erythrophleum alkaloids in 2 experiments in which there was demonstrated blockade of the epinephrine inotropic response. The erythrophleum alkaloids had previously been shown to produce characteristic digitalis-like effects on the mammalian heart (8,9).

In 2 experiments, without demonstrated epinephrine inotropic blockade but following similar Dibenamine administration and time intervals, digitalis produced typical contractile force increments.

In 2 additional experiments, in which an extreme hypotension (30 mm Hg or less) developed after Dibenamine, the subsequent administration of digitalis was followed by progressive depression of contractile force and blood pressure with termination of the experiment. These depressant effects were clearly related to the generally deteriorated condition of the preparations and they illustrate a limitation in the application of these procedures.

Discussion. Previous reports, based chiefly on experiments with heart-lung and papillary muscle preparations, have shown that Dibenamine under such conditions does not alter the inotropic effect produced by epinephrine (10,11). The present experiments, on the contrary, involve the complete organism, large doses of Dibenamine and relatively considerable intervals of time. Under such conditions a substantial degree of blockade is demonstrable.

Summary and conclusions. In open-chest, vagotomized dogs, Dibenamine has been shown to be effective in producing a substantial blockade of the inotropic effects of epinephrine while leaving the inotropic effects of digitalis relatively unaffected. This inotropic blockade of epinephrine by Dibenamine is not the result of secondary cardiovascular changes.

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1. Cotten, M. deV., and Walton, R. P., *Arch. internat. pharmacodyn.*, 1951, v87, 473.

2. Nickerson, M., *J. Pharm. Exp. Therap.*, 1949, v95, 27.

3. Walton, R. P., Leary, J. S., and Jones, H. P., *J. Pharm. Exp. Therap.*, 1950, v98, 346.

4. Walton, R. P., Cotten, M. deV., Brill, H. H., and Gazes, P. C., *Am. J. Physiol.*, 1950, v161, 489.

5. Walton, R. P., and Gazes, P. C., *South. Med. J.*, 1951, v44, 418.

6. Marsh, D. F., and Herring, D. A., *J. Pharm. Exp. Therap.*, 1951, v103, 178.

7. Walton, R. P., Goldberg, L. I., Gazes, P. C., Leary, J. S., Hanna, C. B., and Prystowsky, R. P., *Am. J. Physiol.*, in press.

8. Maling, H. M., and Krayer, O., *J. Pharm. Exp. Therap.*, 1946, v86, 66.

9. Goldberg, L. I., and Walton, R. P., *Fed. Proc.*, 1951, v10, 299; *Arch. internat. pharmacodyn.*, in press.

10. Hunt, C. C., *J. Pharm. Exp. Therap.*, 1949, v95, 177.

11. Acheson, G. H., Farah, A., and French, G. N., *J. Pharm. Exp. Therap.*, 1949, v97, 455.

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