molar concentration. However, this large polypeptide has a molecular weight of 5600; the concentrations used would be meaningful only if there is just one histamine-releasing site on each molecule.

The proportion of histamine released was dependent on the concentration of 48/80 but independent of the concentration of mast cells (and thus, the amount of available cellular histamine). The number of molecules of histamine released by one molecule of 48/80 thus would be proportional to the amount of cellular histamine. With 4×10^{-7}M 48/80, 58% of the intracellular histamine was released or about 0.8 μg of base (7.2×10^{-3} μmoles) from 1 ml of reference cell suspension. Thus, 1 molecule of 48/80 would release up to 18 molecules of histamine, and might have released more from more concentrated suspensions.

Summary. Release of histamine from suspensions of rat peritoneal mast cells under various conditions has been measured directly by a microchemical technic. Compound 48/80 released histamine in a concentration as low as 2×10^{-7}M and was the most potent substance investigated. Several other substances released histamine; the effects of compound 1935L, protamine, n-octylamine and stilbamidine were evaluated at several concentrations and compared with those of 48/80. Stilbamidine was considerably less potent than the others. As expected, certain physical changes released histamine, and low temperatures prevented 48/80 histamine release. Qualitative variations in response of these mast cells to different releasers suggested that mechanisms of histamine release vary considerably among the chemical agents now being investigated.

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Acute Effects of Guanethidine on Myocardial Contractility and Catecholamine Levels.*† (26303)

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Recent pharmacological reports describe guanethidine, [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate, as a potent antihypertensive agent(1,2). An immediate period of adrenergic-like stimulation—manifested by hypertension, increased cardiac output, and positive chronotropism—exists after the agent is acutely administered to dogs(3). The objects of this study were to determine the initial changes in heart contractility and in plasma and tissue catechola-

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mine concentrations produced by guanethidine.

Methods. Studies were done on acutely prepared, bilaterally vagotomized, open-chest dogs under pentobarbital anesthesia. Heart contractile force (isometric systolic tension) (IST) and arterial blood pressure were measured respectively with a strain gauge arch (4,5,6) and a Statham transducer. Strain analyzers and direct writing oscillographs were used to record these parameters.

Such studies were grouped in 4 series. In series 1, cardiovascular responses and plasma catecholamine levels were observed following acute injections of guanethidine§; in series 2, circulatory responses were similarly observed after various autonomic blocking effects had been produced; in series 3, tissue analyses of catecholamine content were made after administration of guanethidine for 2 days. In a 4th series with isolated rabbit hearts, inotropic and chronotropic responses to guanethidine were determined and the perfusates were analyzed for catecholamines following guanethidine.

In series 1, five animals were injected intravenously with guanethidine 5 mg/kg. Successive doses were given to 2 of these animals. Venous and arterial blood samples from the 3 other dogs were drawn during control and cardiovascular stimulation and analyzed for catecholamines(7). Three acutely prepared animals with intact vagi were administered guanethidine.

In series 2, dogs were treated as follows before receiving guanethidine: 2 received hexamethonium 10 mg/kg; epidural spaces of 2 dogs were irrigated with tetracaine 0.1% (8); the heart of one animal was denervated by extirpating the stellate through the fifth thoracic ganglia; 2 received phentolamine 0.5 mg/kg; 2 were atropinized 0.25 mg/kg; and 4 were injected intramuscularly with reserpine 0.1 mg/kg 48 and 24 hr prior to being prepared for IST and blood pressure recordings. In one animal the responsiveness of the adrenal glands to guanethidine and KCl(9) was compared by injecting each agent through a catheter into the thoracic aorta. Arterial blood samples withdrawn prior to and during cardiovascular stimulation after these intra-aortic injections were analyzed fluorimetrically for catecholamines(7).

In series 3, seven dogs administered guanethidine 5 mg/kg on two successive days were sacrificed on the third day with pentobarbital. Samples of left ventricular wall from all and aorta from 5 animals were analyzed fluorimetrically for catecholamines(10). An equal number of heart and aorta samples from 7 control dogs were analyzed similarly.

In series 4, ten isolated perfused rabbit hearts were injected with guanethidine 0.25-1.0 mg. Six hearts received successive doses. In addition 7 groups of 3 hearts were perfused. Perfusate samples were taken prior to and after injecting guanethidine 0.7 mg. Like samples from a group were pooled and analyzed for catecholamines(7). Isotonic contractions were recorded on a kymograph with a heart lever.

Results. Fig. 1 illustrates the typical cardiovascular response to guanethidine in

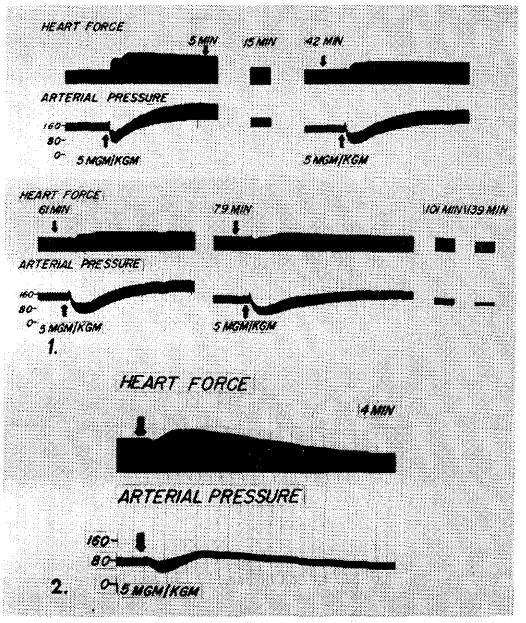

FIG. 1. Effects of repetitive doses of guanethidine on cardiovascular system.

FIG. 2. Effects of guanethidine after reserpine.*

§ Supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J., through the courtesy of Dr. Edgar A. Jack.

* 0.1 mg/kg reserpine I.M. 48 and 24 hr pre-exp.

TABLE I. Tissue Content of Catecholamines in Normal and Guanethidine Treated Dogs.

No.	Exp.	Tissue		Norepinephrine (μg/g)	Epinephrine (μg/g)
10	5	Aorta	Control	.89	.08
			After guan.*	.14 $P < .01$	.03 $P > .10$
14	7	Ventricle	Control	.51	.08
			After guan.*	.07 $P < .01$	.02 $P < .05$

* 5 mg/kg guanethidine IV 48 and 24 hr before analysis.

acutely prepared vagotomized dogs. With the 5 dogs of this series, hypotension averaging 34% (S.D. ± 7.9%) below control mean blood pressure persisted 48 sec (S.D. ± 5.9 sec). Mean blood pressure then rose to 85% (S.D. ± 24%) above control level. IST (heart force) increased abruptly. The initial positive inotropism decreased or reached a plateau before a more intense positive inotropism of longer duration developed. At most, 35 sec intervened between the two peaks. In 2 of 5 dogs, the primary and secondary peaks were fused. The latter peak averaged 121% (S.D. ± 36%) above the control. Blood pressure and inotropic responses persisted 40-60 min.

Of the animals repetitively injected, both displayed diminution in duration as well as in intensity of pressor and inotropic responses. Successive doses caused the hypotensive period to become more pronounced. Blood pressure leveled off below control after each dose. Following the fourth dose, IST was substantially below initial control.

Pressor and inotropic responses of animals with intact vagi were qualitatively similar but less intense and shorter in duration than those of vagotomized animals.

In dogs with preganglionic tetracaine blockade, pressor and inotropic responses elicited by guanethidine were qualitatively the same as in animals without blockade. The blockade, however, by removing sympathetic influences, reduced both pressure and IST to about half the previous values. Under these relatively depressed conditions, the percentage responses to guanethidine were considerably increased although maximal levels of recorded responses did not exceed those obtained under conditions without blockade. Essentially the same relations were observed in 2 animals receiving hexamethonium and in one subjected to cardiac denervation. That is, guanethidine markedly increased both pressure and IST as judged by percentage change. In other experiments, atropinization did not affect the responses to guanethidine in acutely prepared vagotomized animals. Phentolamine totally blocked pressor response in one animal and limited the response to 17% above control in another; inotropic response in both these instances was the same as in dogs which had not received this adrenergic blocking agent.

Pressor and inotropic responses elicited by guanethidine in reserpinized animals averaged 30% and 21% respectively above control (Fig. 2). These responses are significantly less than with acutely prepared vagotomized dogs ($P<0.001$). In contrast to the acutely prepared vagotomized animals, cardiovascular stimulation persisted only 4-10 min following injection. After subsidence of the cardiovascular stimulation, blood pressure and heart force were markedly below control.

Intra-aortic injections of guanethidine 5 mg/kg did not stimulate the adrenals as judged by circulating catecholamine levels. Similar injections of KCl raised epinephrine from 0.72 to 17.85 μg/l of plasma and norepinephrine from 1.41 to 9.74 μg/l. Neither arterial nor venous samples withdrawn during the period of stimulation after the drug's intravenous administration to vagotomized dogs showed a significant change from control.

Table I summarizes the content of catecholamines in ventricles and aortae of control and guanethidine-treated dogs.

A transitory decrease of 22% (S.D. ± 11.3%) in isotonic contractions of isolated rabbit hearts occurred when guanethidine was added to the perfusate. A mean increase

of 17% (S.D. ± 8%) persisted 5-10 min following the depression. A slight positive chronotropism paralleled the period of augmented contractile force. Repetitive doses rendered hearts less responsive to the drug. After guanethidine in 7 groups of 3 hearts, mean concentration of norepinephrine in pooled rabbit heart perfusate increased significantly from 0.17 to 0.94 μg/l of perfusate ($P<0.05$). Epinephrine increased insignificantly from 0.02 to 0.09 μg/l ($P>0.05$).

Discussion. Acute cardiovascular stimulation elicited by guanethidine in dogs with preganglionic blockade suggests the agent acts peripherally to autonomic ganglia and is not dependent upon sympathetic tone to elicit this stimulation. Significantly smaller responses in reserpinized animals, whose catecholamine and 5-hydroxytryptamine levels are diminished, indicates the mechanism may be dependent upon these cardiovascular stimulants. Inhibition of pressor responses to guanethidine by phentolamine indicates the mechanism is dependent upon catecholamines. Maxwell *et al.*(11) suggested that guanethidine depletes the adrenergic transmission agent from the post-ganglionic sympathetic fibers. In the present study, the immediate release of norepinephrine from isolated rabbit hearts as well as depletion of norepinephrine in ventricles and aortae of guanethidine-treated dogs supports this concept. Contemporary studies by Cass *et al.* (12) on depletion of norepinephrine from hearts of cats and rabbits treated with the agent also give a basis to this conception. Depletion of norepinephrine from peripheral sites suggests a reserpine-like action. To equal the degree of cardiovascular stimulation elicited by guanethidine, a large exogenous dose of a catecholamine is required. The fact that circulating catecholamine levels do not increase during the acute cardiovascular stimulation may be due to norepinephrine being released in proximity to end-receptors in quantities large enough to cause gross cardiovascular stimulation but not to discharge into the blood in measurable quantities.

MacIntosh and Paton(13) have shown that numerous organic bases similar to guanethidine release histamine. The initial hypotension seen after injecting guanethidine may be caused by histamine. The transitory nature of the vasodepression could be due to an antagonism of the depression by the pressor effect of the released norepinephrine. As the sources of norepinephrine become depleted after successive doses, the vasodepression is not as greatly antagonized; thus, it becomes more pronounced. Similar enhancement of vasodepression with successive doses of ephedrine have been reported(14). The possibility of a direct depressing action also exists and must be further studied.

Summary. Guanethidine elicits a period of augmented myocardial contractility. Significant decrements in ventricle and aorta norepinephrine content are caused by the drug. With isolated hearts, the agent releases norepinephrine.

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