tions in the distribution of plasma protein and increases of fibrinogen. Such patients may experience a general humoral stimulation in which clotting factors participate.

Summary. A new formula expressing the dilution curve of plasma prothrombin is presented. Normal standards and examples of change in experimental clinical conditions are given.

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Inhibitory Effect of Dibenamine on Vasoconstrictor Substances.

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Recently Nickerson and Goodman[1] reported the results of the study of a new adrenolytic and sympathicolytic drug, dibenzyl-β-chloroethyl amine hydrochloride (dibenamine). They found that after the administration of this agent to dogs and cats, intravenous epinephrine in high doses causes a reversal of blood pressure (a fall of 20 to 40 mm Hg). In addition to the blocking and reversing of the excitatory adrenergic response, dibenamine had the ability to prevent epinephrine-induced cardiac irregularities in dogs sensitized by cyclopropane.[2] Most of the studies on dibenamine reported above deal with responses of heart rate, blood pressure, nictitating membrane, pupillary reactions.

Because of the possible clinical usefulness of dibenamine in angiospastic conditions associated with peripheral vascular diseases, causalgic states or hypertension,† an investigation of vasomotor responses to this agent, as expressed in terms of peripheral blood flow, seemed of interest. The present report deals with a study of the inhibitory effect of dibenamine on vasoconstrictor substances.

Methods. All the experiments reported in this investigation were carried out in the Laewen-Trendelenburg (L-T) preparation in frogs *(Rana pipiens)*. This preparation is a sensitive indicator in testing vascular responses, and offers a simple tool for the analysis of vasomotor agents and their antagonists. The perfusate used was a solution containing 0.65% NaCl and 0.02% KCl. Two Mariotte bottles were connected by a Y-piece with the perfusion system, *i.e.* the rubber tubing leading to the aortic cannula. One bottle contained the plain perfusion solution, the other, added to the latter, dibenamine.‡ This substance was used at various concentrations. Epinephrine hydrochloride, nicotine base (Eastman Kodak) and hypertensin (angiotonin)§ were used in testing the vasoconstrictor-blocking ability of dibenamine. The injections of these vasopressor substances were made through the rubber tubing into the glass cannula inserted into the abdominal aorta of the frog. Their vasomotor activity was evaluated in terms of the output variations of drops per minute.

Results. The injection of a small amount of epinephrine (1/10-1 γ), nicotine (1-10 γ) or hypertensin (0.05-0.5 cc) in the L-T preparation induces a marked vasoconstriction as

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1 Nickerson, M., and Goodman, L. S., *Fed. Proc.*, 1946, **5**, 194.

2 Nickerson, M., Smith, S. M., and Goodman, L. S., *Fed. Proc.*, 1946, **5**, 195.

† Clinical investigation of the action of dibenamine in such conditions is now in progress.

‡ Dibenamine was supplied by Givaudan-Delawanna, Inc., Delawanna, N.J., through the courtesy of Dr. W. Gump.

§ Hypertensin (angiotonin) was prepared from the interaction of Renin (Smaco) and sheep serum according to the method described by Sapirstein *et al.*[3]

3 Sapirstein, L. A., Reed, R. K., and Southard, F. D., Jr., *J. Lab. and Clin. Med.*, 1944, **29**, 633.

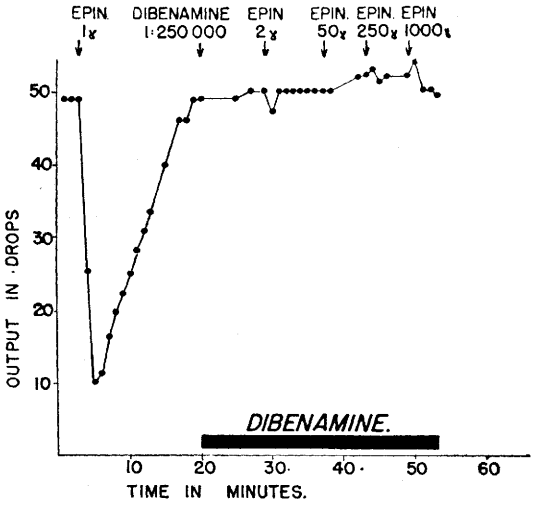

FIG. 1.

Inhibitory effect of dibenamine on the vasoconstrictor action of epinephrine. (The bar indicates the time of perfusion with dibenamine).

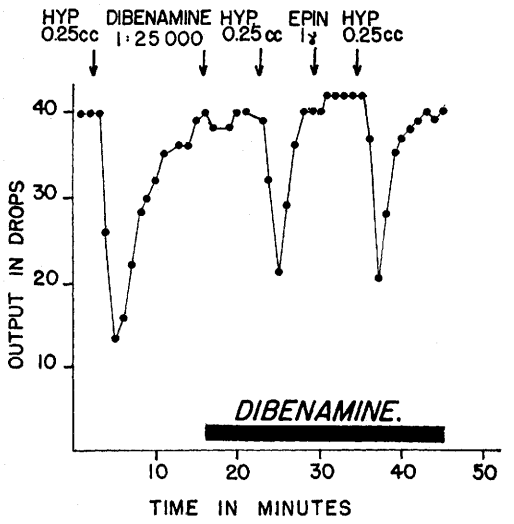

FIG. 2.

Effect of dibenamine on hypertensin. (The bar indicates the time of perfusion with dibenamine).

expressed by the reduction of the output (50-80%) and the duration of this effect ranging from 10 to 30 minutes or more, *i.e.* before the output returns to its initial level. Each individual preparation was first tested for its sensitivity to epinephrine as well as to the other pressor substances used in this investigation.

I. *Effect of dibenamine on epinephrine.* (a) Dibenamine hydrochloride in a concentration of 1:2,500,000 or less inhibits the action of epinephrine only partially. In higher concentrations ranging from 1:1,000,000 to 1:250,000 or more, dibenamine inhibits the vasoconstrictor action of epinephrine completely within 5 to 10 minutes after the onset of the perfusion. The outstanding feature of this effect, as the perfusion with dibenamine progresses (20 to 30 minutes), is a complete inhibition of the response to even large amounts of epinephrine (50-1000 γ) (Fig. 1).

(b) Dibenamine hydrochloride was kept in solution together with epinephrine (100 γ of epinephrine in 50 cc of a solution of 1:250,000 dibenamine) *in vitro* for 30 to 60 minutes. When such epinephrine was tested after that lapse of time, its vasoconstrictor activity was essentially unaltered.

(c) According to the chemical constituents present in the perfusate, dibenamine exhibited a more or less complete inhibitory action on large amounts of epinephrine. The perfusing solution containing 0.65% NaCl and 0.02% KCl is the most suitable vehicle, if dibenamine is to show a complete blockade of large amounts (50-1000 γ) of epinephrine. The bicarbonate-free Tyrode solution is less suitable, for dibenamine does not then inhibit the vasoconstrictor action of large amounts of epinephrine completely. This seems to be due chiefly to the presence of calcium chloride. Indeed, when the perfusate contained 0.035% $CaCl_2$ in addition to 0.65% NaCl and 0.02% KCl, the adrenolytic action of dibenamine was greatly diminished for the large amounts of epinephrine.

II. *Effect of dibenamine on hypertensin (angiotonin).* Amounts of hypertensin ranging from 0.05 cc to 0.5 cc induce a marked and sustained constriction of the vascular bed of the frog's hind legs. 10 to 20 minutes after the onset of the perfusion with dibenamine, in concentrations ranging from 1:250,000 to 1:25,000, the vasoconstrictor action of hypertensin remains unchanged. After 40 to 50 minutes of perfusion its action is altered only slightly (Fig. 2).

III. *Effect of dibenamine on nicotine.* The injection of small amounts of nicotine (1-10 γ) in the L-T preparation elicits 2

major reactions: (a) twitchings of the thigh and leg muscles, (b) marked vasoconstriction.[4] Within 30 to 45 minutes after the onset of the perfusion with dibenamine (1:250,000 to 1:25,000) when the action of large amounts (50-1000 γ) of epinephrine is completely blocked, nicotine still induces notable vasoconstriction and visible twitchings of the muscles.

Discussion. Dibenamine hydrochloride used in concentrations of 1:1,000,000 to 1:25,000 prevents epinephrine-induced vasoconstriction as tested in the Laewen-Trendelenburg preparation. The protective effect afforded by this drug appears to be very potent, for it can block the action of very large amounts of epinephrine (1000 γ). This is practically a total adrenolytic effect. A comparative study with benzyl-imidazoline hydrochloride (priscol),‖ another synthetic autonomic blocking agent,[5] used under the same experimental conditions, exhibits a similar protective action but only for smaller amounts of epinephrine. This is in agreement with the results obtained by other investigators experimenting in intact animals.[2,6] The inhibitory effect of dibenamine on epinephrine does not seem to be due to a direct chemical interaction between the 2 molecules. The *in vitro* experiments reported above seem to support this view. In addition to being adrenolytic, dibenamine exhibits also a marked sympathicolytic action as shown by studies in the intact animal.[1,2] Since dibenamine is an adrenolytic and sympathicolytic agent, its site of action is probably at the neuroeffector cells or between the postganglionic nerve endings and the latter.

Within 30 to 50 minutes after the onset of the perfusion with dibenamine, when the action of large amounts of epinephrine is completely inhibited, small amounts of both hypertensin and nicotine still exhibit a marked vasoconstrictor activity. These facts seem to indicate that dibenamine is either specifically adrenolytic or that these various vasoconstrictor substances do not have the same site of action.

Summary. 1. Dibenzyl - β - chloroethyl amine hydrochloride (dibenamine) inhibits the vasoconstrictor action of epinephrine, as tested in the Laewen-Trendelenburg preparation in the frog. Its adrenolytic action is very potent, for it blocks even large amounts of epinephrine (50-1000 γ). 2. When the action of large amounts of epinephrine is completely inhibited by dibenamine, both hypertensin and nicotine still exhibit a marked vasoconstrictor activity. 3. The peripheral locus of action of dibenamine seems to be at the neuroeffector cells or between the postganglionic nerve endings and the latter.

4 Haimovici, H., and Pick, E. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 234.

‖ Priscol was supplied by Ciba Pharm. Prod., Summit, N. J., through the courtesy of Dr. E. Oppenheimer.

5 Schnetz, H., and Fluch, M., *Z. f. Klin. Med.*, 1940, **137**, 667.

6 Raab, W., and Humphreys, R. J., *J. Pharm. and Exp. Therap.*, 1946, **88**, 268.

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Effect of Thiouracil and Estrogen on Lactogenic Hormone and Weight of Pituitaries of Rats.*

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The weight of the hypophysis increases subsequent to thyroidectomy,[1-4] and this may

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 1042.

1 Hammett, F. S., *Am. J. Anat.*, 1923, **32**, 37.

2 Hammett, F. S., *Endocrinology*, 1926, **10**, 145.

3 Smelser, G. K., *Anat. Rec.*, 1939, **74**, 7.