TABLE I.
Pulmonary Edema Caused by *l*-epinephrine and *l-nor*-epinephrine in Guinea Pigs.

Group No.	Dose of drug, mM/kg body wt*	Avg body wt, g	Avg lung wt g/100 g body wt	% change
Controls				
1	0	540	0.55 ± .09	—
l-epinephrine				
2	.0010	585	1.27 ± .14	+131
3	.0015	520	1.72 ± .13	+210
4	.0020	615	1.62 ± .06	+191
l-epinephrine preceded by N-(9-fluorenyl)-N-ethyl-β-chlorethylamine HCl†				
5	.0020	605	0.55 ± .08	0
6	.0050	630	0.56 ± .08	+ 2
l-nor-epinephrine (*l*-arterenol)				
7	.0020	515	0.97 ± .16	+ 76
8	.0030	480	1.13 ± .18	+105
9	.0040	505	1.25 ± .26	+127
l-nor-epinephrine preceded by N-(9-fluorenyl)-N-ethyl-β-chlorethylamine HCl†				
10	.0040	550	0.51 ± .12	— 7
11	.0050	510	0.58 ± .11	+ 5

* 1 mM epinephrine = 183 mg and 1 mM *nor*-epinephrine = 169 mg. † Adrenergic blocking agent known as "SKF-501" (see text).

Summary. 1. As measured by lung weight a lesser degree of pulmonary edema is produced by toxic doses of *l-nor*-epinephrine than smaller but toxic doses of *l*-epinephrine.

2. N-(9-fluorenyl)-N-ethyl-β-chlorethylamine, a β-haloalkylamine adrenergic blocking agent, prevents all symptoms and the development of pulmonary edema due to toxic doses of either *l*-epinephrine or *l-nor*-epinephrine.

Received July 18, 1949. P.S.E.B.M., 1949, **71**.

17294. The Mechanism by Which Dibenamine Blocks Pituitary Activation in the Rabbit and Rat.

CHARLES H. SAWYER, J. E. MARKEE, AND JOHN W. EVERETT.
(Introduced by Duncan C. Hetherington.)
From the Department of Anatomy, Duke University School of Medicine, Durham, N. C.

Markee, Sawyer, and Hollinshead[1,2] presented strongly indicative evidence that hypothalamic control of the release of luteinizing hormone from the rabbit hypophysis is exerted via a neurohumoral mechanism of which an adrenergic mediator is the final component. Ovulation, significant of LH release, was induced by injecting tiny amounts of adrenalin directly into the adenohypophysis.[2] Sawyer *et al.*[3,4] reported confirmation of the adrenergic nature of the secretion stimulus by blocking copulation-induced ovulation with the adrenergic-blocking agent dibenamine (N,N-dibenzyl-β-chloroethylamine).[5] Everett, Sawyer, and Markee[6,7] extended the investigation

1 Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Endocrinology*, 1946, **38**, 345.

2 Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Recent Progr. Hormone Research*, 1948, **2**, 117.

3 Sawyer, C. H., Markee, J. E., and Hollinshead, W. H., *Endocrinology*, 1947, **41**, 395.

4 Sawyer, C. H., Markee, J. E., and Townsend, B. F., *Endocrinology*, 1949, **44**, 18.

5 Nickerson, M., and Goodman, L. S., *Fed. Proc.*, 1948, **7**, 397.

TABLE I.
Effects of Dibenamine, Priscol and 2-Dibenzylaminoethanol on Activation of LH-Release by the Anterior Hypophysis.

Species	Drug	Dose, mg/kg	Time of injection (relative to nervous stimulus)	Ovulated (hypophysis stimulated)	Failed to ovulate (stimulus blocked from reaching hypophysis)	% blocked by drug
Rabbit	Dibenamine*	25-32	<60 sec. after	3	16	84
	Priscol*	40-52	<60 sec. after	9	1	10
	2-dibenzyl-aminoethanol	25-30	<45 sec. after	9	1	10
Rat	Dibenamine†	30	0-6 hr before	8	21	72
	2-dibenzyl-aminoethanol	30	>2 hr before	5	0	0

* Data from Sawyer *et al.*[3]
† Data from Everett *et al.*[7] Six of the 8 ovulating rats were partially "blocked" as evidenced by incomplete or delayed ovulation.

to the rat, finding that, in this spontaneously ovulating species, dibenamine also blocked LH-release.

Dibenamine effectively blocked ovulation in the rabbit only if injected intravenously within 60 seconds post coitum; if delayed a few minutes, ovulation followed in all cases.[3] Since dibenamine does not exert its maximal adrenergic-blockade until at least 1.5 hours after injection, Nickerson[8] recently suggested that the drug may inhibit LH release in the rabbit by a central nervous effect independent of adrenolytic activity. Nickerson suggested further that this possibility be controlled by the use of 2-dibenzylaminoethanol, a dibenamine-hydrolysis product which retains the central excitant properties but lacks the adrenergic-blocking power of dibenamine. The present experiments demonstrate that 2-dibenzylaminoethanol does not prevent activation of LH-release in the rabbit or rat, and that at least certain anti-adrenergic properties of dibenamine are exerted within a matter of seconds after intravenous administration.

Sexually mature female rabbits and rats were employed. The rabbits were mated with a vasectomized buck, and 2-dibenzylaminoethanol* was injected intravenously during the following 45 seconds. The rats, whose vaginal-smear records had each shown two or more regular 4-day cycles, were injected with the drug intravenously at noon on the day of proestrus. Previous work[7] had revealed that 4-day cyclic rats of our colony receive the neurogenic stimulus for the release of LH between 2 and 4 P.M. on the afternoon of proestrus.

The central excitatory effects of 2-dibenzylaminoethanol in rabbits are more dramatic though shorter lived than those induced by dibenamine. The most commonly employed dosage of 2-dibenzylaminoethanol, 25 mg/kg, always induced severe convulsions lasting up to 20 minutes, and this dose level was nearer the LD_{50} than was 30 mg/kg dibenamine. A dosage of 30 mg/kg 2-dibenzylaminoethanol was above the MLD for rats, but in this species the excitatory effects were somewhat less marked than those following a similar dose of dibenamine.

The results of injections of 2-dibenzylaminoethanol, as they pertain to pituitary activation are summarized in Table I and compared with the results of earlier work with dibenamine and priscol (benzyl-imidazoline).

[6] Sawyer, C. H., Everett, J. W., and Markee, J. E., *Endocrinology*, 1949, **44**, 218.

[7] Everett, J. W., Sawyer, C. H., and Markee, J. E., *Endocrinology*, 1949, **44**, 234.

[8] Nickerson, M., *Endocrinology*, 1949, **44**, 287.

* Generously supplied by Dr. Nickerson.

TABLE II.
Relative Potencies of Drugs to Counteract Immediately a 2X-Lethal Dose of Adrenalin in Rabbits.

Drug	Dose, mg/kg	Timing of drug injection in seconds from 0 time		Timing of 1 mg/kg adrenalin inj. in seconds from 0 time		Survived	Died	% protection
		Began	Ended	Began	Ended			
—	—	—	—	0	15	0	10	0
Dibenamine	30	0	45	45	60	5	0	100
Priscol	40	0	45	45	60	1	4	20
2-Dibenzyl-aminoethanol	25	0	45	45	60	0	3	0

It is apparent that, whereas dibenamine is highly effective in blocking pituitary activation in both rabbits and rats, 2-dibenzylaminoethanol, like priscol, is quite ineffective in preventing the neurogenic stimulus from reaching the hypophysis.

A second series of experiments was designed to demonstrate whether dibenamine exerts any of its anti-adrenergic activities within the first minute following intravenous injection. The experiment is outlined and the results summarized in Table II. The rabbits receiving the intravenous adrenalin alone immediately revealed maximally dilated pupils, muscular hypotonia and respiratory embarrassment followed in a few minutes by death. When adrenalin injections were preceded within the minute by dibenamine, the subsequent symptoms were those of dibenamine only—pupillary constriction and spontaneous muscle contractions. Priscol delayed the lethal effect of adrenalin for many hours in 4 animals, and a fifth recovered. However, neither priscol nor 2-dibenzylaminoethanol counteracted the pupillary dilation induced by adrenalin. The 3 rabbits receiving 2-dibenzylaminoethanol and adrenalin expired as rapidly as with adrenalin alone. Although protection against the lethal action of adrenalin is not a highly specific test of adrenolytic activity,[9] the fact that dibenamine-treated rabbits showed none of the symptoms of adrenalin poisoning mentioned above, indicates that at least part of dibenamine's adrenergic-blocking capacity becomes effective almost immediately after injection of high dosages. The results indicate that dibenamine blocks pituitary activation, not by its central excitatory effects but rather by its specific adrenergic-blocking capacity.

Summary. Dibenzylaminoethanol, a non-adrenolytic dibenamine-hydrolysis product which retains the central excitatory action of dibenamine, fails to block the activation of LH release from the rabbit or rat hypophysis. Dibenamine in large doses exerts at least part of its anti-adrenergic properties almost immediately after injection. The evidence indicates that dibenamine blocks pituitary activation by its specific adrenergic-blocking capacity rather than by its central excitatory action.

[9] Nickerson, M., Berghout, J., and Hammerstrom, R. N., *Fed. Proc.*, 1949, **8**, 322.

Received June 21, 1949. P.S.E.B.M., 1949, **71**.