Michaelis[8] or the same buffer containing 2.5% of normal yolk from fresh chicken eggs. To each mixture were added 1.5 ml of anaesthetic ether. The two phases were then shaken together vigorously 30 times. The aqueous layer was drawn off, freed of ether with vacuum and centrifuged at 4000 r.p.m. for one hour to sediment the rickettsiae. The supernatants were tested for antigen content by the complement fixation test[9] using 4 units of homologous convalescent guinea pig serum. The results of the tests with five species of rickettsiae are shown in the table.

The readings in the table show that in the presence of 2.5% yolk higher titers were obtained with *R. prowazeki, R. mooseri, R. akari,* and *R. rickettsii.* With *R. burneti* no soluble antigen could be detected.

When normal yolk was diluted 10 times with 0.85% sodium chloride solution and extracted with 2 volumes of ether, it was found that the water soluble constituents in the aqueous layer had the same degree of activity in releasing soluble antigen from rickettsiae as the original yolk.

Although it will be noted that some antigen was released without the addition of yolk, such release may have been due to the amount of yolk remaining with the preparation of rickettsiae, since the more carefully purified Breinl and Wilmington suspensions showed little release except when yolk was added. It has been noted that the increase in titer obtained by adding yolk is variable unless the rickettsiae are carefully washed. Yolk sacs are known to carry more or less yolk after harvest and it is evident that the amount of adherent yolk can effect the complement fixation titer of an ether extracted antigen. For consistent results it has been found advantageous to add 5 or 10% normal egg yolk to all method I and method II antigens[11] for use in complement fixation tests.

In the preparation of Rocky Mountain spotted fever antigens by method I or II,[11] the addition of yolk is especially valuable[12] since the growth of *R. rickettsii* in yolk sacs is poor, and antigen release is frequently not marked.

Summary. The addition of as little as 2.5% egg yolk to suspensions of yolk sacs infected with the rickettsiae of epidemic and endemic typhus fever, Rocky Mountain spotted fever, and rickettsialpox (*R. prowazeki, R. mooseri, R. rickettsii,* and *R. akari*) prior to treatment with ether has resulted in antigens with enhanced titers as measured by the complement fixation test.

8 Michaelis, L., *Biochem. Z.*, 1931, **234**, 139.

9 Bengtson, I. A., *Pub. Health Rep.*, 1944, **59**, 402.

10 Shepard, C. C., and Topping, N. H., *J. Immunol.*, 1947, **55**, 97.

11 Topping, N. H., and Shepard, C. C., *Pub. Health Rep.*, 1946, **61**, 701.

12 Unpublished experiments.

16013

Renal Hemodynamic Effects of Adrenaline and "Isuprel": Potentiation of Effects of Both Drugs by Tetraethylammonium.

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Vasodepressor drugs have been prepared which are built on the chemical nucleus of sympatheticomimetic compounds. The pharmacological properties of one of these, the N-isopropyl homologue of adrenaline, called "Isuprel" (1-(3[1],4[1]-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride) have been described by Lands and his colleagues.[1] The suggestion has been made that the activity of

1 Lands, A. M., Nash, V. L., McCarthy, H. M., Grainger, H. R., and Dertinger, B. L., *J. Pharm. Exp. Therap.*, 1947, **89**, 110.

TABLE I.
Effects on Renal Function of Adrenaline, Tetraethylammonium, and "Isuprel."

Dog	Per.	C_{PAH} cc per min	C_{CR} cc per min	FF	R	B P mm Hg	Pulse	Duration min
4-9	1-2	208	62.6	0.30	0.27	149	110	20
14 kg			Adrenaline 0.1 cc 1/1000 i.v.					
	3-4	198	63.7	0.32	0.29	152	130	20
			Et_4N 2 cc 10% i.v.					
	5-6	254	69	0.27	0.18	129	135	20
			Adrenaline 0.1 cc 1/1000 i.v.					
	7	163	51	0.31	0.39	161	100	10
	8	197	60	0.30	0.22	128	112	10
6-3	1-2	178	46	0.26	0.24	141	98	20
18 kg			Adrenaline 0.1 cc 1/1000 i.v.					
	3-4	130	47.4	0.36	0.31	140	100	20
			Et_4N 2 cc 10% i.v.					
	5-6	145	40.4	0.28	0.27	134	130	20
			Adrenaline 0.1 cc 1/1000 i.v.					
	7-8	111	32.5	0.29	0.46	158	84	18
4-9	1-2	149	56	0.37	0.32	149	110	20
14 kg			"Isuprel" 0.2 cc 1/1000 i.v.					
	3	144	58	0.40	0.29	150	145	10
	4	175	58	0.33	0.20	123	150	31
	5	169	40	0.24	0.29	147	120	20
			Et_4N 2 cc 10% i.v.					
	6	170	53	0.31	0.28	147	140	30
	7	129	49	0.38	0.33	140	130	20
			"Isuprel" 0.2 cc 1/1000 i.v.					
	8	68	24	0.35	0.17	83	148	15
	9	278	46	0.31	0.10	86	176	20
	10	251	46	0.34	0.19	107	172	12
3-7	1-3	218	74	0.34	0.21	135	55	30
12 kg			"Isuprel" 0.1 cc 1/1000					
	4-5	202	61	0.30	0.19	122	80	20
			Et_4N 2 cc 10% i.v.					
	6-7	236	58	0.25	0.14	114	128	30
			"Isuprel" 0.1 cc 1/1000 i.v.					
	8	223	63	0.28	0.10	93	140	20
	9	231	68	0.29	0.20	136	112	20

Dog number and body weight are indicated in the left hand column. Per. = intervals of urine collection. C_{PAH} = plasma clearance of *p*-aminohippurate. C_{CR} = plasma clearance of creatinine. FF = filtration fraction (C_{CR}/C_{PAH}). R = renal resistance as sum of R_A and R_E calculated by the method of Lamport. B.P. = average of arterial pressure during the time of the observation. Pulse = average of pulse rate during time of observation. Duration of the observation is indicated in minutes.

this and similar compounds resembles that posited for sympathin I, since many of its effects contrast with those of adrenaline and with those credited to sympathin E. Since adrenaline is a renal vasoconstrictor, it seemed likely that 'Isuprel' might be a renal vasodilator. One of the purposes of this report is to describe the effect of injection of "Isuprel" on the renal circulation.

Another aspect rests on the observation of Page and Taylor[2] that the effect on arterial pressure of adrenaline and other pressor drugs is increased by pre-treatment with tetraethylammonium (Et_4N). We demonstrate below that potentiation of the action of adrenaline by pre-treatment with Et_4N is reflected in renal circulation as well as arterial pressure and (b) that the depressor, cardiac accelerator and renal vasodilator properties of "Isuprel" are similarly increased.

Procedures. Six experiments were done, 3 each with adrenaline and 'Isuprel', on trained conscious dogs. In these renal plasma clearances of p-aminohippurate and creatinine

2 Page, I. H., and Taylor, R. D., *Science*, 1947, **105**, 622.

were determined. The plasma clearance of p-aminohippurate is taken as equivalent to renal plasma flow and the plasma clearance of creatinine as equivalent to the rate of glomerular filtration. Femoral arterial pressure was measured from a mercury manometer and inlying needle. Each experiment consisted of (a) 2 or 3 control periods of clearance measurement; (b) observations after intravenous infusion of small doses of either adrenalin or "Isuprel"; (c) observations after infusion of Et_4N and (d) observations after a second injection of either of the 2 sympatheticomimetics. One experiment was done in a dog anesthetized with pentobarbital. In this only "Isuprel" and Et_4N were injected.

Results. Observations in 2 experiments on conscious dogs in which adrenaline and Et_4N were injected and in 2 in which "Isuprel" and Et_4N were tested are summarized in Table

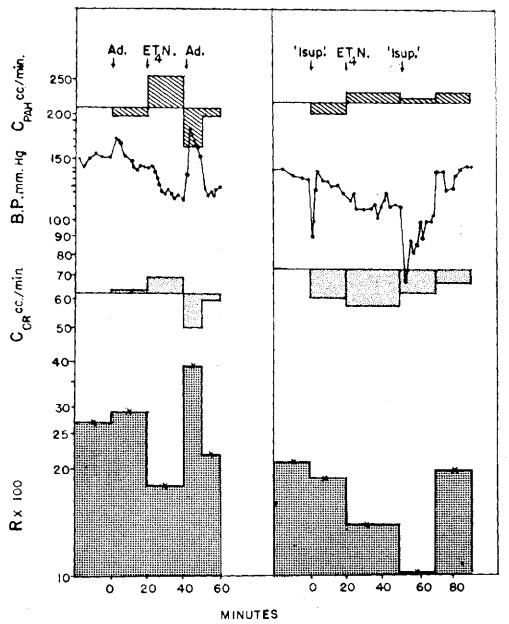

FIG. 1.

Graphic summary of 2 experiments. C_{PAH} = renal plasma clearance of *p*-aminohippurate. C_Cr = plasma or creatinine clearance. B. P. mm Hg = arterial pressure and is shown from repeated observations of femoral arterial pressure. R = renal resistance, and, for convenience, is shown times 100. The experiment on the left was done on dog No. 4-9, body weight 14 kg, and corresponds to the first experiment in Table I. The experiment on the right was done on dog No. 3-7 and corresponds to the last experiment in Table I.

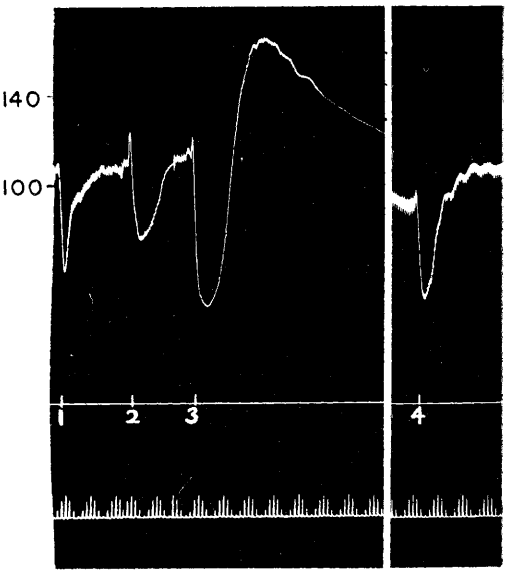

FIG. 2.

Effect on arterial pressure of injection of "Isuprel" and Et_4N in an anesthetized dog, of 10.7 kg body weight. At 1 there was injected 0.2 cc of 1/20,000 "Isuprel." At 2 the animal was given intravenously 10 mg of tetraethylammonium chloride per kg body weight. At 3 the dose of "Isuprel" was repeated. At 4, 30 minutes after injection of tetraethylammonium, the same dose of "Isuprel" was again injected.

I. The course of one experiment of each type is shown graphically in Fig. 1. Fig. 2 demonstrates the effect on arterial pressure of injections of "Isuprel" Et_4N in the anesthetized dog.

Adrenaline. The first injection of adrenaline caused a slight, transient increase of arterial pressure with some increase in renal vascular resistance as calculated by the method of Lamport.[3] Injection of Et_4N slightly decreased renal resistance and arterial pressure in 2 of the 3 experiments. A second injection of adrenaline 20 minutes after injection of Et_4N resulted in increases of arterial pressure and renal vascular resistance which were much greater than the responses to the first injection.

"Isuprel." The first injection caused transient hypotension and tachycardia and a modest decrease in renal resistance. Injection of Et_4N caused again slightly decreased ar-

[3] Lamport, H., *J. Clin. Invest.*, 1943, **22**, 461.

terial pressure and renal resistance in 2 of 3 experiments. A second injection of "Isuprel" resulted in hypotension, tachycardia and decreased renal resistance which were greater in extent and duration than the effects observed before injection of Et_4N. In the anesthetized dog, the vasodepressor effect of "Isuprel" is shown to be increased by injection of Et_4N. The augmentation is demonstrable at 5 but not at 30 minutes after injection of Et_4N. The reactive pressor effect of "Isuprel" after injection of Et_4N was observed in other experiments on anesthetized dogs.

Comment and Summary. The increased pressor responsiveness to adrenaline caused by injection of Et_4N is shown to be associated with an increase in renal vasoconstriction. The depressor response to injection of "Isuprel," the N-isopropyl homologue of adrenaline, is shown to be associated with renal vasodilation. The depressor, cardioaccelerator and renal vasodilator effects of injection of "Isuprel" are augmented by prior injection of tetraethylammonium. On the assumption that the effects of tetraethylammonium are due to blocking of autonomic ganglia,[4] we conclude that inhibition of these ganglia increases vascular responsiveness to typical vasoconstrictor and vasodilator sympatheticomimetics.

We acknowledge the donation of "Isuprel" hydrochloride by Dr. Earl Burbidge, Frederick Stearns Co., Inc.; of tetraethylammonium chloride ("Eatmon") by Dr. E. C. Vonder Heide, Parke, Davis and Co., Inc.; of *p*-aminohippurate by Drs. Karl Beyer and W. Boger, Sharp and Dohme, Inc., and the skillful assistance of William West.

[4] Acheson, G. H., and Moe, G. K., *J. Pharm. Exp. Therap.*, 1945, **84**, 189.

16014 P

Pharmacology of Dibenzyl-β-Chloroethylamine Hydrochloride (Dibenamine).*

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Recently Nickerson, Goodman and Smith,[1,2,3] Raab and Humphreys,[4] Acheson and co-workers,[5] Haimovici[6] reported their experiments with dibenzyl-β-chloroethylamine hydrochloride (dibenamine).

We wished to investigate further the pharmacology of dibenamine and the mechanism of transmission of sympathetic nerve impulses.

Methods. The experiments have been carried out on dogs under chloralosane anesthesia. The blood pressure was registered from a femoral artery. In some experiments the peripheral vasomotor reactions were recorded by means of the 3-manometers-method.[7]

Results. I. *Effects of Dibenamine on blood pressure and heart rate.* Dibenamine, from 1 to 5 mg/kg, does not induce a noticeable change in blood pressure, nor any variations of heart rate. Doses of 10 to 15 mg/kg produce a slight and transient hypotension, but no direct change of heart rate. Subsequent injections of dibenamine do not produce the some hypotension. A primary, slight increase

* This investigation was made with the assistance of grants from the Ella Sachs Plotz Foundation, New York.

[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.

[3] Nickerson, M., and Goodman, L. S., *J. Pharm. Exp. Therap.*, 1947, **89**, 167.

[4] Raab, W., and Humphreys, R. J., *J. Pharm. Exp. Therap.*, 1946, **88**, 268.

[5] Acheson, G. H., and co-workers, *Fed. Proc.*, 1947, **6**, 305.

[6] Haimovici, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 486.

[7] Nolf, P., *Bull. Acad. Roy. Belg.*, 1902, p. 895.