TABLE IV. Suppressive Effect of Viscosin on Mice Infected with 10000 ID_{50} of Influenza A Virus.

Viscosin	Mice sacrificed after ... days	D/T	I/T	Avg score*
0	2	1/12	4/12	.6
5 mg SC × 1	2	0/11	4/11	.4
2	2	0/12	1/12	.1
0	3	4/12	12/12	2.3
5 mg SC × 1	3	3/12	11/12	2
3	3	0/12	8/12	.7
0	4	11/12	12/12	3.9
5 mg SC × 1	4	8/12	12/12	3.3
4	4	8/12	12/12	3.2

SC = subcut. × 1 = 1 inj. immediately after infection. × 3 = 3 daily inj. beginning on day of infection. D/T = No. of mice dead/total. I/T = No. of mice infected/total.

* Score: 1 = 5-25% lung tissue consolidated; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

cosin was found to be strikingly specific, not only among the bacteria(1,4) but also among the viruses. The most outstanding characteristic of viscosin observed was its marked protective effect in eggs subsequently infected with infectious bronchitis virus. It is of interest in this connection that subinhibitory amounts of interfering material (heated virus) in the viral inoculum enhanced the suppressive effect of inhibitory amounts of viscosin injected one hour after infection in embryonated eggs. The suppressive effect of viscosin on the progress of infection with influenza A virus in mice was detectable but minimal, and interpretation of the results obtained is difficult. While repeated demonstration of a slight suppressive effect cannot be disregarded, little can be learned from the data save that the effect is minimal. Taken as a whole, the data obtained indicate that viscosin shows little promise of any immediate practical application.

Summary. Viscosin was found to exert a marked protective effect in embryonated eggs subsequently infected with infectious bronchitis virus. A slight but detectable suppressive effect on the progress of infection in mice infected with influenza A virus was demonstrated.

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Adrenal Medullary Stimulating Action of Tetraethylammonium.* (19072)

CLEMENT A. STONE,† GEORGE ENTWISLE, AND E. R. LOEW.

From the Department of Physiology, Boston University School of Medicine, Boston.

The primary action of the tetraethylammonium (TEA) ion is ganglionic blockade. This action results in transitory decreases in blood pressure in most species when the intravenous doses are less than 10 mg/kg(1). Administration to animals of larger amounts of the drug, however, frequently produces rises of blood pressures, although the quantity required to elicit a pressor response appears rather variable(1). In addition, pressor responses have been reported in some human subjects given therapeutic amounts(2), and can seemingly be obtained routinely in patients with pheochromocytoma(3). This hypertensive action of TEA has never been adequately explained. It has been attributed to a direct peripheral constricting effect(4), and more recently, to the release of nor-epinephrine primarily from the liver and to a lesser extent from the adrenals(5). No attempt has been made to explain the pressor action on the basis of ganglionic and/or

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adrenal medullary stimulating properties. Indeed, the possession of ganglionic stimulating properties has been denied(1). Nonetheless, the fact that the ion can cause release of pressor principles from adrenal medullary tumors(3) suggests that the drug may possess an adrenal stimulating property of sufficient intensity to account for the pressor activity observed under the conditions specified. This concept is supported by the fact that pressor effects of the drug are more often obtained, and with smaller doses, in spinal animals(1). Furthermore, weak adrenal medullary stimulating action would not be surprising in view of the close relation between TEA and tetramethylammonium, a well known ganglionic stimulant.

The experiments presented here were performed in an effort to explain more fully the mechanism of the pressor response to TEA, and to gain some insight relating to the rationale for using the drug in diagnosis of pheochromocytoma. A preliminary report of these experiments has been published previously(6).

Method. A preparation first described by Feldberg and Minz(7) seemed suitable for the study of the effect of TEA upon the adrenal medulla. Two to 4 kg cats were etherized, the trachea and jugular vein cannulated, and the cats were then given anesthetic doses of either dial and urethane or pentobarbital, intravenously, or were used in the spinal condition. Evisceration was performed, the renal artery and vein tied close to the hilus of the kidney, and then the inferior vena cava and abdominal aorta were tied just below the origin of the renal artery. Finally, the stump of the coeliac artery was cannulated for retrograde intra-arterial injection into the abdominal aorta. Carotid blood pressure was measured by means of a mercury manometer. Splanchnicotomy or coeliac ganglionectomy was performed on all preparations. The procedure described above drastically restricted the circulation and, if the carotid blood pressure was low (70-100 mm Hg), the preparation served as a sensitive indicator for released pressor principles from the adrenal gland. The sensitivity of this preparation to injected epinephrine appeared to vary inversely with the blood pressure. It was therefore necessary to prevent a high blood pressure due to an overloaded circulatory system in the upper half of the body. This was accomplished by tying the inferior vena cava 1-2 minutes prior to ligation of the abdominal aorta, thus allowing some pooling of blood in the lower extremities. In addition, apparent non-sensitivity of the vacular system to epinephrine was overcome in some cats by using, in addition to blood pressure responses, contractions of the nictitating membrane, made sensitive by removal of the superior cervical sympathetic ganglion 14 to 16 days prior to the experiment. The contractions of the nictitating membrane were recorded on the kymograph by means of an isotonic lever magnifying the responses 12-14 times. The doses of TEA employed are reported herein as total doses and varied from 0.25 to 4 mg. The concentration of drug in saline was adjusted so that any given dose was administered via the arterial cannula in a volume of not more than 0.1 ml, and followed by a saline wash of 0.2 ml over a period of approximately 5 seconds. As a control, frequent injections of saline (0.3 ml) were found to have little or no effect. Comparisons of the effects of TEA administered intravenously with those given intra-arterially were made by a catheter placed in the jugular vein.

The role of the adrenals in the pressor response to retrograde administration of TEA in the coeliac artery was assessed by adrenalectomy after appropriate control responses had been obtained. The adrenalectomy was performed by mass ligation and excision of the whole gland. Test responses to intra-arterial TEA were obtained within 30 minutes of the completion of the adrenalectomy at a time when the blood pressure attained control levels.

Results and discussion. Pressor responses obtainable with relatively large intravenous doses of TEA in intact animals have been reported to vary considerably with respect to

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TABLE I. Effect of Intra-arterial (Abdominal Aorta) Tetraethylammonium on Blood Pressure of Cats.

Preparation	Dose TEA, mg	No. of cats (and injections)		Pressor response, mm Hg ± s
Spinal	4	13	(42)	51 ± 27
Non-spinal	4	6	(22)	41 ± 48

magnitude and even to appearance of a pressor phase(1,5). More consistent results were obtained by Page(5) in spinal animals and in animals with various parts of the autonomic nervous system resected.

A summary of the pressor responses obtained in the present experiments when TEA was administered via the coeliac artery appears in Table I. The results are based on responses obtained with 4 mg, although smaller doses were frequently employed. It is to be noted that much greater variation occurs in non-spinal animals, as evidenced by the large standard deviation. A greater variation in non-spinal animals is not surprising, since concomitant depression of autonomic ganglia would oppose the effect of any released pressor principle. Thus the first dose of TEA administered into the coeliac artery in a non-spinal cat almost always caused a depression of blood pressure, but, at the same time, a contraction of the nictitating membrane. Subsequent doses were almost always pressor, possibly due to the fact that significant ganglionic blockade had occurred following the first dose. Elimination of all the first depressor doses in the data obtained in non-spinal cats in Table I results in a significant reduction in the standard deviation. This value, 55.3 ± 32.6, is more nearly comparable to the values obtained in spinal animals.

In addition to variation in the pressor responses between cats, there was considerable variation to the same dose in a given animal, although this intra-individual variation decreased with repeated administration. This has been reported to occur when TEA is given intravenously to human subjects(2) and to other species(5,8). Variation in the pressor responses to TEA in these experiments in any given spinal animal cannot be explained by various degrees of ganglionic blockade, but may depend on small changes in rate of administration and varying degrees of peripheral sensitization to released epinephrine(9).

It is difficult to compare the doses employed in these experiments with those required to produce pressor responses when the drug is given intravenously. The animals used in these experiments had undergone a drastic reduction in circulatory capacity, and for that reason the doses are reported on a total dose basis. But it is reasonable to assume that a dose of 4 mg intra-arterially in this preparation compares with a 1-3 mg/kg intravenous dose in an intact animal. Such doses usually produce only depressor responses of short duration(1).

The pressor effects of TEA as given in the manner described could conceivably be due to one or more of the following: 1) a direct peripheral vascular stimulating action and/or 2) ganglionic stimulating action involving (a) only the adrenal gland or (b) the adrenal gland as well as other autonomic ganglia. Evidence that a peripheral constricting action plays only a minor role, if any, and that a humoral agent was involved was obtained by comparing the responses of nictitating membrane and blood pressure following intra-arterial (coeliac) and intravenous (jugular) injections of the same dose of TEA. These results are presented in Table II. It is seen that responses of the nictitating membrane and pressor response occur only upon intra-arterial injection. If a direct peripheral constricting action were involved in the pressor response, one would expect greater effects upon intravenous injection than were actually obtained.

Similar comparisons were made with acetyl-

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TABLE II. Comparisons of Effects of Tetraethylammonium Given Intravenously (Jugular) and Intra-arterially (Abdominal Aorta) in the Cat.

Drug	Dose	Number of cats (and comparisons)	Blood pressure, mm Hg ± s		"P"* value	Nictitating membrane,† mm ± s	
			Intra-art.	Intraven.		Intra-art.	Intraven.
TEA (chloride)	4 mg	13 (18)	+48 ± 21	+7 ± 7	<.01	20 ± 12	2 ± 2
Acetylcholine (chloride)	5-10 γ	7 (9)	+27 ± 14	+3 ± 5	<.01		
Histamine (Base)	0.1-2 γ	10 (13)	+22 ± 8	+8 ± 13	<.01		

* Calculated by the "t" test of paired variates. Significantly different if "p" is 0.05 or less.
† Responses are average of 10 comparisons in 6 non-spinal cats.

choline chloride and histamine base; and the results with these agents agree qualitatively with those obtained with TEA (Table II). These results suggested that a pressor substance, probably originating from the adrenals was involved. If such were the case, adrenalectomy should abolish the response, and in addition, furnish additional evidence as to the role of a direct peripheral constricting action in the response.

The participation of the adrenals was proved by adrenalectomy after appropriate control responses had been obtained. It can be seen from the data in Table III that the responses to 4 mg TEA intra-arterially were almost completely abolished following adrenalectomy in each of 6 cats. In addition, the intravenous administration of the adrenergic blocking drugs, piperidinomethyl-3-benzodioxane (933F) and N-ethyl-N-[2-chloroethyl]-9-fluorenamine · HCl (SY-21) in doses which blocked pressor responses to epinephrine likewise abolished the pressor responses to TEA. Blockade of the pressor response to intravenous TEA in intact dogs and cats with adrenergic blocking drugs has been reported (5).

The effects observed after adrenalectomy were slight compared to the control responses. Thus a direct peripheral constricting action of TEA seemed not to be involved, but the released pressor principles from the adrenal gland appeared to account for the entire pressor effect.

Page (5) has recently published evidence which indicates that TEA pressor effects are mediated largely through nor-epinephrine re-

TABLE III. Effect of Adrenalectomy on Pressor Responses to Intra-arterial Tetraethylammonium in Cats.

Exp.	Blood pressure response (mm Hg)			
	Controls		After adrenalectomy	
	TEA (4 mg)	Saline (.3 cc)	TEA (4 mg)	Saline (.3 cc)
1	+ 42		+13	0
	+ 43	- 9	+19	
2	+ 94		0	
	+ 88	—	- 9	—
3	+102		+ 8	
	+104	+10	+14	0
4	+102		+18	
	+ 94	0	+ 8	0
5	+ 62	0	+ 4	
	+ 62		+ 2	0
6	+ 36		0	
	+ 36	0	0	0
Mean ± s	72 ± 33		6.5 ± 7.5	

leased from the liver. These conclusions were based on the facts that pressor responses to TEA were never reversed, but only blocked, by adrenergic blocking agents, and that adrenalectomy only reduced the response, while hepatectomy completely abolished the response. The results of our experiment indicate clearly that the source of the pressor principle was the adrenal gland; the liver could not be involved in these animals since evisceration and ligation of the coeliac artery removed all effective blood flow to that organ. The results reported at this time neither indicate the nature of the humoral agent responsible nor the reason for the discrepancy between these experiments and those of Page, although it is possible that only the adrenal component was dealt with in these experiments.

Summary. The injection of .25 to 4 mg of TEA into the blood supplying the adrenal gland of cats with restricted circulation caused definite pressor effects and contraction of the nictitating membrane. The same amounts injected into the jugular vein were without effect. Since adrenalectomy or administration of adrenergic blocking agents abolished the responses, it is concluded that the effects observed were probably due to epinephrine and/or nor-epinephrine released from the adrenal medulla. It is suggested that this simulated nicotinic action is responsible for the pressor effects of TEA that are observed in man and animals under the conditions discussed.

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