THE BIOASSAY OF EPINEPHRINE-PROCAINE MIXTURES.*

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For the production of local anæsthesia in dental practice, a commonly employed epinephrine-procaine mixture contains twenty milligrams of procaine and 0.04 milligram of epinephrine per cc., or per tablet. The ratio of procaine to epinephrine is 500 to 1. The chemical determination of this small quantity of epinephrine in the mixture is quite complicated. Our attention was directed to the possibility of physiologically assaying a number of these samples by the method outlined in U. S. Pharmacopœia X for the bioassay of epinephrine solution.

A series of increasing doses of standard epinephrine were administered intravenously to anæsthetized dogs to determine the sensitivity of the test animals. Efforts were made to inject doses which would produce a rise of about thirty millimeters in blood pressure. Results obtained were then plotted and the dose determined which would produce that increase. The epinephrine-procaine solutions, prepared by mixing standard epinephrine with procaine hydrochloride, were then injected, giving the same quantities of epinephrine as had been given of standard. The increases in blood pressure were plotted in the same manner and the dose producing exactly thirty millimeters' increase determined. Then a final series of injections of standard epinephrine were made to determine any potentiation of pressor response following procaine, and the results plotted to determine the dose producing a thirty-millimeter increase.

The U. S. Pharmacopœia specifies that a suitable quantity of epinephrine produces a rise of thirty to sixty millimeters in blood pressure. In these investigations, many of the test doses gave increases of about thirty millimeters so that figure was selected solely as a matter of convenience. By using a constant degree of response to epinephrine, the great variability of different dogs to the action of epinephrine may be overcome, at least in part. The same quantity of epinephrine injected into the same dog produces essentially the same increase in blood pressure. However, when the same quantity of epinephrine was injected into each of a series of dogs, some animals gave ten times as large an increase in blood pressure as others.

Hatcher (1) shows a tracing in which procaine has greatly potentiated and prolonged the action of epinephrine. Apparently the mixture contained five hundred parts of procaine to one part of epinephrine. Tainter (4) has not obtained evidence of definite potentiation following procaine in his work.

In our experiments, mixtures were made in which the procaine:epinephrine ratio varied from 50:1 up to 1000:1. The detailed results of individual injections

TABLE I.—MICROGRAMS OF EPINEPHRINE REQUIRED TO PRODUCE RISE OF 30 MILLIMETERS IN BLOOD PRESSURE OF ANÆSTHETIZED DOGS.

		Ratio of Procaine to Epinephrine Injected.							
Chart no.	Epinephrine.	50.	100.	125.	250.	375.	500.	750.	1000.
169	20	32	—	22	12	15	15	—	—
		30	—	15	20	17	16	—	—
170	88	—	88	—	62	—	45	45	45
		—	62	—	50	—	45	45	45
171	32	—	—	—	—	—	32	—	—
		—	—	—	—	—	32	—	—

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have been consolidated in Table I, which shows the quantity of epinephrine required to produce an increase of thirty millimeters in blood pressure. The values given for epinephrine alone refer to the U. S. Pharmacopœia standard epinephrine. Between each of the series of procaine:epinephrine mixtures, a series of injections of the standard were repeated. The quantity calculated to produce a thirty-millimeter rise in this standard series is given on the following line of the table. For example, in Chart No. 169, it was found that twenty micrograms of standard epinephrine were required to produce an increase of thirty millimeters in blood pressure. When a solution containing fifty parts of procaine to one part of epinephrine was injected, thirty-two micrograms were required to produce this same response. After the injection of this 50:1 series, it required thirty micrograms of standard epinephrine to produce the desired increase. This particular proportion of procaine:epinephrine decreased the pressor response to epinephrine in the same solution and in a subsequent series of injections. This depression of response was not noted in any other instance and is being further investigated. The next injection was made with a solution containing one hundred and twenty-five parts of procaine to one part of epinephrine; twenty-two micrograms were required to produce the desired degree of response. Following this, only fifteen micrograms of standard epinephrine were needed to produce the same effect. This mixture had potentiated the action of epinephrine 20/15 or 1.33 times. In order to make direct comparisons of effects without regard to the differences in susceptibility of different dogs, ratios of potency for the series are given in Table II. It is noticed that there

TABLE II.—RATIOS OF ACTIVITY OF EPINEPHRINE BEFORE AND AFTER PROCAINE.

		Ratio of Procaine to Epinephrine Injected.							
Chart no.	Epinephrine.	50.	100.	125.	250.	375.	500.	750.	1000.
169	1.0	0.67	—	1.0	1.67	1.33	1.33	—	—
		0.67	—	1.33	1.0	1.25	1.25	—	—
170	1.0	—	1.0	—	1.5	—	2.0	2.0	2.0
		—	1.33	—	1.75	—	2.0	2.0	2.0
171	1.0	—	—	—	—	—	1.0	—	—
		—	—	—	—	—	1.0	—	—

is a tendency for an increase in ratio with increase in the quantity of procaine in the mixture. The absolute value for the increase differs in different animals but tends to approach two.

In Chart No. 171, results are recorded with the 500:1 mixture alone. Here it is evident that the same degree of response is produced, in other words the presence of procaine did not affect the pressor response of a simultaneous or a subsequent injection of epinephrine. This series differed from the others in that a smaller number of injections were made, and accordingly the test animals received a smaller total quantity of procaine.

In a further series of experiments, it was found that the potentiating effect of procaine tended to disappear after about an hour. This is receiving further consideration.

A number of commercial procaine-epinephrine tablets, containing five hundred parts of procaine to one part of epinephrine, were assayed. Within the limits of experimental error the results agreed with the quantities known to be present. It is considered advisable to give a series of preliminary injections of standard epi-

nephrine. A series of injections of procaine:epinephrine mixture are then injected and the results compared.

Procaine is aminobenzoyl-diethyl-amino-ethanol. A similar potentiation of pressor action of epinephrine has been obtained with phenylpropanolamine (2), with cocaine (methyl-benzoyl-ecgonine) (3) and with a number of other local anæsthetics of unrelated chemical structure (3). No particular chemical nucleus has been found essential in producing this pressor potentiation.

CONCLUSIONS.

1. By comparing the increases in blood pressure produced by a series of injections of procaine-epinephrine mixture with those produced by a previous series of injections of standard epinephrine it is possible to assay these mixtures by the method outlined in U. S. Pharmacopœia X for epinephrine. The results fall within the limits of experimental error.

2. A number of successive injections of solutions containing between one hundred parts and one thousand parts of procaine to one part of epinephrine potentiate the pressor response of epinephrine in the same solution and in standard epinephrine injected within a period of one hour. After that time the potentiating action seems to disappear.

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AMINO ALCOHOLS. 3.—THE POTENTIATION OF THE PRESSOR ACTION OF EPINEPHRINE BY ARYLPROPANOLAMINES.*

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The bioassay of ephedrine has been complicated by the findings (1, 5 and 6) that successive intravenous injections of the same quantity to dogs or cats give progressively smaller rises in blood pressure. To determine the pressor activity of ephedrine and its related homologues, Chen used the method first developed by Elliott (3) for the assay of epinephrine. Decerebrate cats are injected intravenously with varying doses of a standard solution of epinephrine, after which a single injection of ephedrine or related drug is given intravenously. The increase in blood pressure produced by this single injection is compared with the rises in the epinephrine series and the relative potency determined.

In the authors' investigations of amino alcohols (4) of the ephedrine and epinephrine type, we were interested in determining the effect of a single dose of ephedrine and its homologues upon the subsequent pressor effects of epinephrine itself. Experiments were conducted upon medium-sized dogs, anæsthetized with

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