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THE QUANTITATIVE ASSAY OF VASOPRESSIN

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There are three animal preparations which may be used for the biological assay of pituitary pressor potency: the spinal cat (Dale and Laidlaw, 1912; Hogben, Schlapp, and Macdonald, 1924), the anaesthetized dog (Hamilton and Rowe, 1916; Kamm, Aldrich, Grotte, Rowe, and Bugbee, 1928), and the anaesthetized rat (Landgrebe, Macaulay, and Waring, 1946); of these, the rat preparation is the most economical, the most sensitive, and the most reliable.

In a previous report (Dekanski, 1951) dealing with the preparation and qualitative assay of a pressor fraction from normal urine, N: N-dibenzyl-β-chloroethylamine (" Dibenamine ") was injected into the rat preparation in order to inhibit the action of certain pressor substances known to be present in fresh urine. Dibenamine was found to have a potent and prolonged action in abolishing the usual pressor effects of adrenaline, noradrenaline, hydroxytyramine, nicotine, and piperidine. On the other hand, the rise in blood pressure produced by pitressin or by the urinary pressor fraction was not abolished. The results of these experiments indicated that a quantitative assay of the pressor potency of pituitary extracts might be readily obtained by means of a comparatively simple rat's blood pressure preparation into which dibenamine had been injected.

In the rat preparation described by Landgrebe *et al.* (1946) for pressor assays of pituitary extracts, the vagi and associated sympathetics are severed and the posterior cord pithed. Neither is, in fact, necessary if the rat preparation is treated with dibenamine. Subcutaneous urethane anaesthesia was found to be as efficient as that by " Dial " followed by urethane both injected intraperitoneally. The modified rat's blood pressure method, appears to be more satisfactory and more specific than any other method for the assay of pressor activity of pituitary preparations.

METHODS

Technique.—A male albino rat weighing about 300 g. is anaesthetized with urethane (175 mg. per 100 g. body weight) injected subcutaneously. After 45 to 60 min. the rat is tied to the operating table by its hind legs, the front legs not being secured. The trachea is cannulated with a short glass or polythene tube of about 2.5 mm. external diameter, and one carotid artery dissected ready for cannulation. The femoral vein close to the inguinal ligament is cannulated with a special polythene cannula. The abdominal muscles are retracted to expose the inguinal ligament. The superficial pudendal vein is retracted to one side and the femoral vein dissected towards the inguinal ligament from the corresponding artery. The other deep branch reaching the femoral vein has to be found and tied off, otherwise bleeding may occur during cannulation. A short polythene cannula

is tied into the femoral vein by two ligatures and joined by a short piece of rubber tubing to a 1 c.c. burette with an attached thistle funnel containing warm normal saline. Finally, the cannula is fixed firmly to the thigh. The burette is attached to the operating table to avoid accidental disconnection. Heparin (200 u. per 100 g. body weight) is injected through the venous cannula and washed in with saline. The carotid cannula is tied in and connected with a special mercury manometer (Crawford and Outschoorn, 1951) of about 2 to 3 mm. internal diameter by a column of normal saline. Both the venous and the carotid cannulae are of 1 mm. external diameter. The central and peripheral nervous system, including both vagi and associated sympathetics, is left intact. No artificial respiration is necessary. All solutions, filtered if necessary and warmed to body temperature, are injected through the venous cannula by means of a 1 c.c. tuberculin syringe and are washed in with 0.2 c.c. normal saline from the burette. Care should be taken that no air is injected, as the smallest air bubble may be fatal to the animal.

At first the preparation does not maintain a constant basal pressure, but 20–30 min. after the intravenous injections of dibenamine the blood pressure establishes itself on a new basal level of about 50 mm. Hg and remains constant for at least 8 hours. Dibenamine, 100 μg. per 100 g. body weight per dose, is injected twice at an interval of 10 min. Twenty minutes after the first injection of dibenamine the volume effect due to the injection of 0.3–0.5 c.c. normal saline should be significantly reduced; if not, a third dose of dibenamine is usually sufficient to minimize or even to abolish the blood pressure response to the injection of saline, and to produce a constant basal pressure.

During this preparatory period a rough estimate of the potency of some unknown solution may be obtained, by matching it with two dose-levels, about 6 mu. and 10 mu. of the International Pituitary Posterior Lobe Standard (100 mu./c.c. normal saline, freshly made each time). It was found (Landgrebe *et al.*, 1946) that even a large dose of the unknown solution can be injected without affecting the response to the succeeding smaller doses.

In each assay four doses are used, two of the standard and two of the unknowns, the ratio of high to low dose being the same (usually 3 to 2) for both the standard and the unknown. Each dose is injected every 6 to 10 min. once in each group of four, and there are 6 groups in all.

Drugs used:

Subcutaneously: Urethane (B.D.H.) 25 g. per 100 c.c. solution in distilled water—0.7 c.c. per 100 g. body weight.

Intravenously: Heparin B.P. (Boots) sterile, 200 u. per 100 g. body weight dissolved in 1 to 2 c.c. normal saline.

Dibenamine HCl or N: N-dibenzyl-β-chloroethylamine hydrochloride (Smith, Kline, and French)—1 mg./c.c. solution in faintly acid saline prepared by dissolving 5 mg. dibenamine in 0.1 c.c. 95 per cent ethanol made acid (approx. 0.05 N) with conc. H_2SO_4 and then diluted up to 5 c.c. with normal saline.

Various commercial pituitary preparations.

Results and Discussion

Fig. 1 demonstrates the blood pressure responses to increasing doses of pituitary standard. Fig. 2, which has been constructed from Fig. 1, shows the log dose-response regression line relating response to log dose of pituitary. A straight log dose-response regression line could always be obtained by the method used. The preparation treated with dibenamine maintains a constant basal pressure and readily discriminates between doses, differing by not more than 15–20 per cent. The

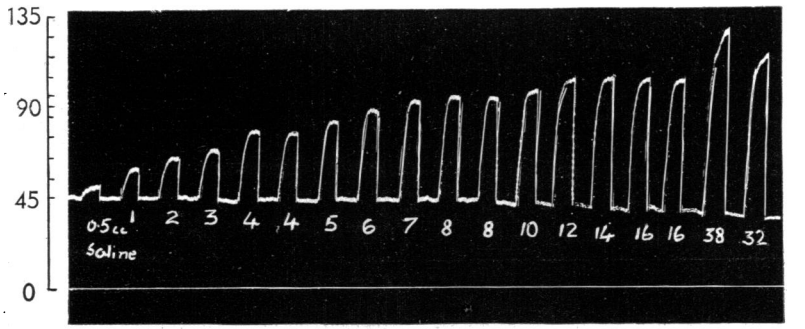

FIG. 1.—Rat's blood pressure responses to different doses of pituitary standard ranging from 1 to 38 milliunits (100 mu./c.c.). From these results a log dose-response graph has been constructed in Fig. 2. Weight of rat 270 g.

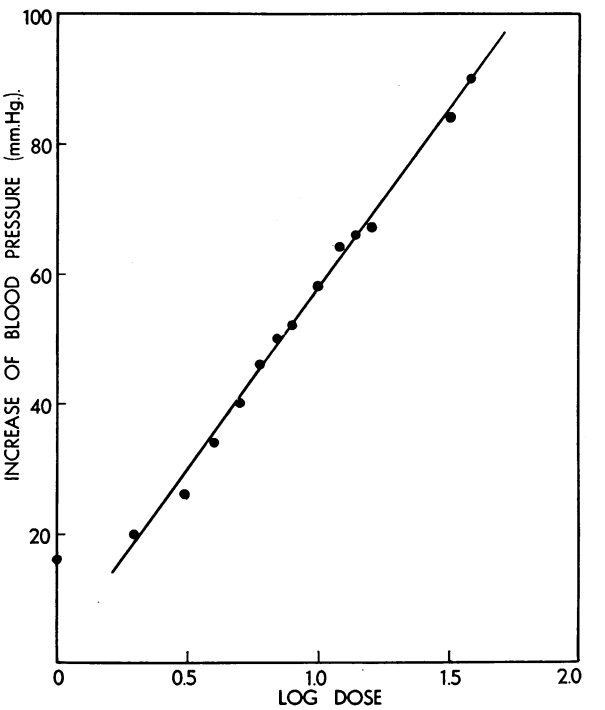

FIG. 2.—Log dose-response regression line relating response to log dose of pituitary standard, based on results shown in Fig. 1.

injections of small doses (4 to 12 milliunits) can be made every 6 to 10 min. without tolerance developing, and the responses are satisfactory for about 40 injections.

With doses of 15 to 100 milliunits the blood pressure takes at least 15 min. to return to the basic level and the response usually becomes erratic.

The results of a pressor assay of the pituitary are given in Fig. 3, from which Tables I and II and Fig. 4 have been constructed. Fig. 4 represents the simple

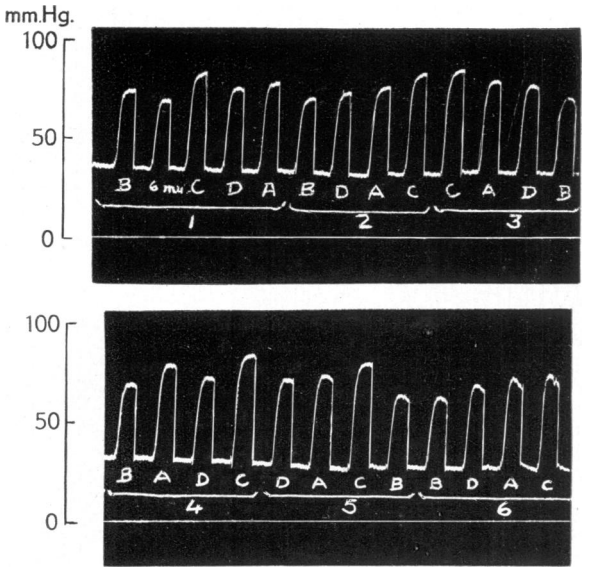

FIG. 3.—The pressor assay of pituitary standard. The record shows 24 responses of a rat blood pressure. The increases of blood pressure are responses to four different doses of pituitary standard A, B, C, and D, each of which was injected once in each group of four. There are six groups in all. A=10 mu., B=7 mu., C=12.5 mu., and D=8.75 mu. A and B were treated as "standard." C and D were treated as "unknown." D : C =B: A=7: 10. Estimates of unknown : standard = 1.251. True value unknown: standard $=\frac{8.75}{7}=1.250$. A dose was injected intravenously every 8 min. and washed in with 0.2 c.c. normal saline. Weight of rat 300 g. Total dose of dibenamine 900 μg. Total experimental time about six hours.

TABLE I

INCREASE OF BLOOD PRESSURE IN MM. HG=Y

	Concentration mu./c.c.	Dose c.c.	Dose mu.	Log dose =x	1	2	3	4	5	6	Sum	Mean
A	100	0.10	10	1.000	46	45	47	48	47	46	279	46.5
B	100	0.07	7	$\bar{1}$.8451	40	38	39	38	38	38	231	38.5
C	125	0.10			50	50	52	54	54	50	310	51.7
D	125	0.07			44	43	45	43	44	42	261	43.5
Group totals					180	176	183	183	183	176	1,081	

TABLE II

Source of variation	Sum of squares	Degrees of freedom	Variance	F	P
1. Groups	15	5	3	2.37	>0.05
2. Standard and unknown ..	155	1	155	118.4	<0.001
3. Regression	392	1	392	309.4	<0.001
4. Deviation from parallelism ..	<1	1	<1		
5. Residual error	19	15	1.267		
Total	581	23			

$$S_M=\sqrt{\frac{4\times1.267}{24\times52.2}\left(\frac{0.09742}{0.15492}+1\right)}=\sqrt{0.00010814}=0.0104$$

For 15 degrees of freedom t=2.13 (P=0.05). The fiducial limits for M= $\pm S_M \times t = \pm 0.0104 \times 2.13 = 0.0222$.

Since M=$\bar{1}$.9026, fiducial limits are $\bar{1}$.9248 and $\bar{1}$.8804. The fiducial limits for R=119.0 and 131.7 mu./c.c.

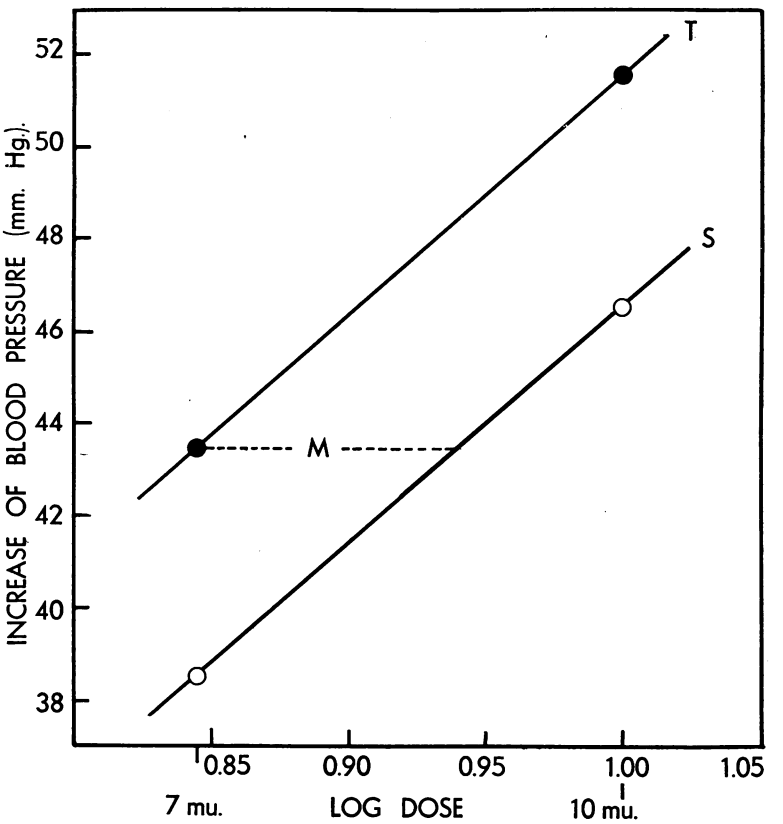

FIG. 4.—Ordinates: mean increase of blood pressure in mm. Hg. Abscissae: Log_{10} dose in milliunits of pituitary standard. White circles are the mean responses for high and low doses of standard, black circles are the mean responses for high and low doses of unknown. S and T are the regression lines relating response to log dose of standard and unknown. The lines were drawn by eye. Graphically $M = \log (0.943 - 0.845) = 0.098$.

$$\therefore \frac{\text{unknown}}{\text{standard}} = \text{antilog } 0.098 = 1.253.$$

graphical measurement of M, the log of the ratio of unknown to standard, and Table II the calculation of the potency ratio and error, by the methods of calculation and analysis of variance given by Schild (1942) and Holton (1948). By calculation the true value of M was found to be $\bar{1}.9026$. Hence R=0.7991 and the potency per c.c.=125.1 mu. The slope b=52.2, and the index of precision $\lambda = Sy/b = 0.0215$.

The method has been found to give satisfactory results in the routine assays of the pressor activity of various commercial pituitary preparations. From 30 such assays the mean λ with standard error was found to be 0.042 ± 0.023.

These results seem to prove that the rat preparation treated with dibenamine is a simple, sensitive, and specific method for the quantitative assay of pressor potency both in pituitary extracts and in commercial pituitary preparations. The rat does not react to the pituitary oxytocic factor in this way. The pressor response

is not interfered with by the possible presence of small amounts of other pressor substances nor by that of small amounts of histamine (Landgrebe *et al.*, 1946) which is a common contaminant of the pituitary extracts.

SUMMARY

1. A four-point procedure was applied to the pressor assay of posterior pituitary extract and various pituitary commercial preparations.

2. A simple rat's blood pressure preparation treated with dibenamine was found to be the most sensitive and the most specific test preparation.

3. The mean index of precision with standard error (30 values of λ) was found to be 0.042 ± 0.023.

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