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ESTIMATION OF ADRENALINE-*NOR*ADRENALINE MIXTURES

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Biological methods for the estimation of adrenaline and *nor*adrenaline in solutions or extracts have recently been described by Gaddum, Peart, and Vogt (1949), Bülbring (1949), and Gaddum and Lembeck (1949). Gaddum and his colleagues have used the rat uterus method of de Jalon, and also the rat colon, the former being more sensitive to adrenaline and the latter more sensitive to *nor*adrenaline. Bülbring used the rat uterus and the rabbit intestine, and also the nictitating membranes of the cat. The isolated tissue methods suffer from the disadvantage that they take a long time ; thus a determination of the adrenaline present in a solution by the rat uterus method may occupy a worker the whole of one day if he uses three or four strips of muscle. The nictitating membrane method in which the ratio of the contraction of the denervated membrane to the normally innervated membrane is determined (Bülbring and Burn, 1949) has the disadvantage that the accuracy is low when the proportion of adrenaline is greater than 50 per cent, for the ratio changes very little as the adrenaline percentage increases above this figure.

In this paper we describe a modification of the nictitating membrane method which has a much greater accuracy and which does not require a cat with a denervated nictitating membrane. The method depends on determining the ratio of the contraction of one normally innervated nictitating membrane to the height of the blood pressure rise.

METHOD

Preparation of cat.—We have carried out our experiments in the spinal cat prepared by Dale's method, but it may be equally satisfactory to use cats anaesthetized with chloralose. We prefer the spinal cat partly because we think that in some cats chloralose diminishes the contraction of the nictitating membrane and partly because the spinal cat is a preparation much used in this laboratory and very quickly made. If chloralose is used the cervical sympathetic chain should be cut.

In preparing the spinal cat (for details of method see Burn, Finney, and Goodwin, 1950) the cat is anaesthetized with ether, but the carotid arteries are not ligated as they are ordinarily. The muscles are detached from the spine of the second cervical vertebra, below which the spinal cord is exposed. The cord is cleanly cut through and the medulla and brain are destroyed by a rod passed through the foramen magnum. The hole is plugged by modelling clay. Under artificial respiration the cat will last for many hours if kept warm.

The cat's head is fixed rigidly and the right nictitating membrane is attached by a silk thread to an isotonic lever so that the contractions are suitably magnified. The blood pressure is recorded from the left carotid artery by a mercury manometer. Injections are made into a femoral vein.

The membrane to blood pressure ratio.—The method depends on the fact that, whereas an injection of adrenaline causes not only a rise of blood pressure but also a contraction of the nictitating membrane, an injection of *nor*adrenaline produces a rise of blood pressure but has very little effect on the nictitating membrane, as shown in Fig. 1. When a mixture of adrenaline and *nor*adrenaline is injected the contraction of the membrane therefore depends on the amount of adrenaline present. We have determined the proportion of adrenaline in such a mixture by calculating the ratio of the height of the contraction of the nictitating membrane to the height of the blood pressure rise.

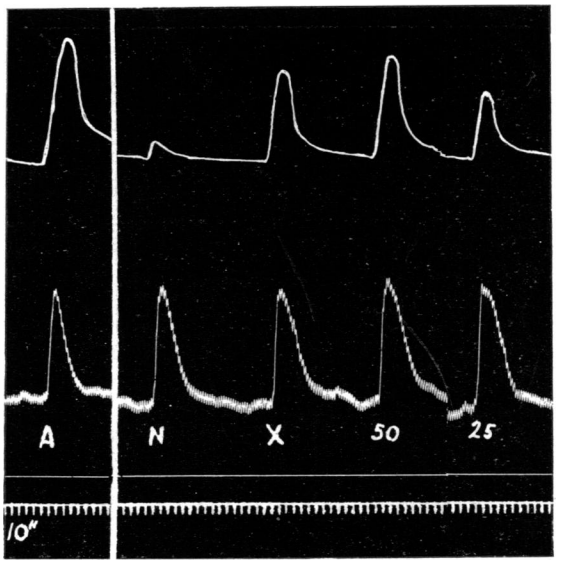

FIG. 1.—Spinal cat. Upper record: contractions of normal nictitating membrane. Lower record: blood pressure. At A, injection of 10 μg. *l*-adrenaline. At N, 10 μg. *nor*adrenaline. At X, 1 c.c. unknown. At 50, 1 c.c. containing 5 μg. adrenaline and 5 μg. *nor*adrenaline. At 25, 1 c.c. containing 2.5 μg. adrenaline and 7.5 μg. *nor*adrenaline. Note that the effect of X on the membrane lies between the effect of 25 and 50.

Volumes of solution are chosen for injection which give similar rises in blood pressure, and it would probably be best to confine comparisons to those effects in which the blood pressure rise is exactly the same. Since this is not practicable the ratio of the nictitating membrane contraction to the blood pressure rise is determined in order to correct for small differences in blood pressure rise. The relative effects of *nor*adrenaline (N) and adrenaline (A) on the blood pressure of spinal cats were found by Bülbring (1949) to vary greatly, the N/A ratio in 15 experiments being 0.615–2.0, and it might be argued that this variation must affect the result of the calculation of the ratio of the nictitating membrane contraction to the blood pressure rise. However, our procedure appears to be justified in practice by its accuracy, which we have determined.

RESULTS

Solutions of *l*-adrenaline and *l-nor*adrenaline were prepared each containing 10 μg./ml., and from these three mixtures were made containing 25 per cent, 50 per cent, and 75 per cent adrenaline. The five solutions were then injected into different spinal cats, using amounts of each which produced similar effects on the blood pressure. Thus in one of the earliest experiments the results shown in Table I were obtained in five consecutive injections. The figures for the ratio have been plotted as ordinates against the percentage of *l*-adrenaline as abscissae in curve A (Fig. 2).

TABLE I

Substance	Amount injected	Rise of blood pressure	Contraction of membrane	Ratio × 1,000
*l-nor*adrenaline	7.5 μg.	116 ml.	4 mm.	34
75% N and 25% A	0.75 ml.*	114 ,,	6 ,,	53
50% N and 50% A	0.75 ml.*	113 ,,	9 ,,	80
25% N and 75% A	0.75 ml.*	108 ,,	12 ,,	111
l-adrenaline	7.5 μg.	110 ,,	16 ,,	145

* Containing 7.5 μg. of mixture.

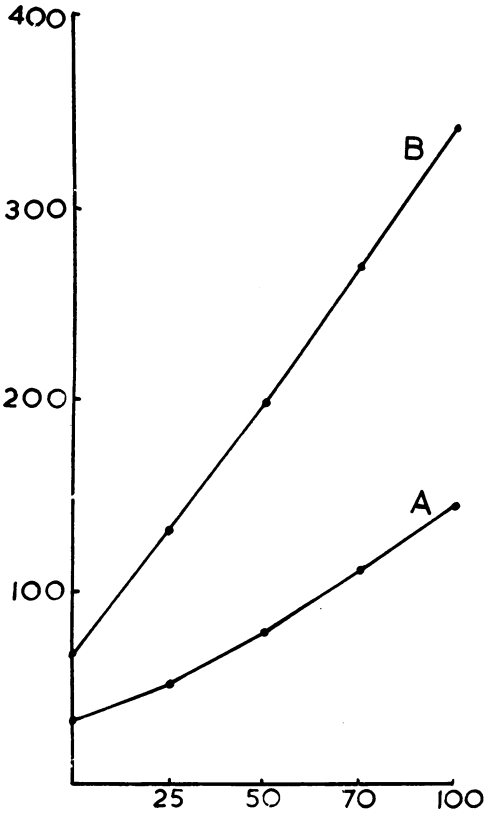

FIG. 2.—Abscissae: percentage of adrenaline in a mixture with *nor*adrenaline. Thus 25 means 25 per cent adrenaline and 75 per cent *nor*adrenaline. Ordinates: Ratio (× 1,000) of contraction of normal nictitating membrane to blood pressure rise. (A) shows relation obtained in an experiment in which carotids were clamped during the preparation. (B) shows relation in a later experiment in which the carotid artery was left open.

In this and in other earlier experiments the left carotid artery was ligated and the right carotid artery was closed by an artery clip during the period of making the spinal preparation; the nictitating membrane was therefore without a blood supply for some time and the contractions when the artery was released were poor. In later experiments much better contractions of the membrane were obtained by leaving the carotid artery open. Thus curve B (Fig. 2) was drawn from the mean results in another experiment in which several observations were made of the ratio for each solution, as shown in Table II. The relation in Fig. 2 is nearly but not quite linear, both curves having a slight upward concavity, due to the very slight activity possessed by *nor*adrenaline.

Performance of test.—When determining the proportions of adrenaline and of *nor*adrenaline present in a mixture it is not necessary to construct a curve like B in Fig. 2. It is sufficient to determine by inspection between which known mixtures the unknown mixture lies, and then to obtain several records of the effects of these known mixtures and of the unknown mixture. Thus in Fig. 1 it will be seen that the effect of the unknown is slightly less than that of a 50:50

TABLE II

Adrenaline	Ratio (× 1,000) of membrane contraction to B.P. rise 75 A/25 N	50 A/50 N	25 A/75 N	*Nor*adrenaline
344	258	212	148	95
	297	203	141	
		184	155	
	258		122	
		193	110	46
			159	
		218		
		193	112	63
			114	
Mean 344	271	200	133	68

mixture and much greater than that of a mixture of 25 A/75 N. This information was obtained as a result of only five injections, and at once indicated that the proportion of adrenaline was about 45 per cent.

In making the estimation more certain, the course adopted depends on the preparation. In some spinal cats the effect of injecting a given volume of a known mixture varies greatly as the experiment proceeds. It is then essential to compare the effect of each injection of the unknown with the adjacent injections of known mixtures only and to do this several times. Several results are then obtained for the percentage of adrenaline and *nor*adrenaline in the unknown, and the mean result is taken as the correct value. In other cats, however, the effect of a given volume of a known mixture does not vary much, and then the result can be obtained by comparing the mean ratio for the unknown with the mean ratios for the known mixtures.

Accuracy and applicability of the method.—We have determined the accuracy of the method by preparing ten mixtures of adrenaline and *nor*adrenaline. Five of these were examined by D. E. H. and five by R. H. O. P., neither of whom knew what the actual composition of any mixture was. The results are given in Table III,

TABLE III

ESTIMATES OF KNOWN MIXTURES AS UNKNOWN

Figures are adrenaline percentage

Exp.	First	Second	Third	Fourth	Fifth	Mean	Actual
1	56	66	61	—	—	61	65
2	87	60	65	—	—	71	70
3	30	22	24	—	—	25	25
4	75	56	69	75	—	69	66
5	42	57	52	59	45	51	50
6	83	88	85	—	—	85	85
7	27.5	42	26	47.5	58	40	40
8	49.8	50.8	49.2	54.2	—	51	50
9	60	57.5	59	63	53	59	65
10	8	31	42	—	—	27	30

which also shows the scatter of the different estimates in each experiment. Since R.H.O.P. had no previous experience of work with this preparation, the agreement obtained between the estimated and the actual composition is satisfactory.

The method is applicable when the total amount of adrenaline and *nor*adrenaline together is not less than 10 μg. This when present in 1 ml. would allow one injection of unknown. If 50 μg. is available, the accuracy of the result should be high.

In comparing adrenal gland extracts, the method might give a figure for the percentage of adrenaline present which is too great, since such extracts have a depressor constituent which may reduce the rise of blood pressure, and so increase the value of the ratio. However, in comparing two similar extracts, an estimate of the difference in the percentage of adrenaline present should still be possible. In comparing extracts we exclude the suprarenal glands from the circulation.

SUMMARY

1. A rapid biological method for the estimation of the percentage of adrenaline and *nor*adrenaline in a mixture is described.

2. The contractions of a normal nictitating membrane and the blood pressure are recorded in a spinal cat. Probably a cat anaesthetized with chloralose could also be used.

3. The ratio of the height of the membrane contraction to the rise in blood pressure bears an almost linear relation to the percentage of adrenaline present, provided that the solutions injected cause about the same rise of blood pressure.

4. The method is applicable to total amounts of adrenaline and *nor*adrenaline not less than 10–30 μg. in 1–3 ml.

5. The method has a high degree of accuracy.

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REFERENCES

Bülbring, E. (1949). *Brit. J. Pharmacol.*, **4**, 234.
Bülbring, E., and Burn, J. H. (1949). *Brit. J. Pharmacol.*, **4**, 202.
Burn, J. H., Finney, D. J., and Goodwin, L. G. (1950). *Biological Standardization*, 2nd Edition. London: Oxford University Press.
Gaddum, J. H., and Lembeck, F. (1949). *Brit. J. Pharmacol.*, **4**, 401.
Gaddum, J. H., Peart, W. S., and Vogt, M. (1949). *J. Physiol.*, **108**, 467.