

# COLORIMETRIC AND BIOLOGICAL ESTIMATION OF ADRENALINE AND NORADRENALINE IN SUPRARENALS OF GUINEA-PIGS

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Adrenaline and noradrenaline can be separately estimated in a mixture either by colorimetric or biological methods. None of the methods is strictly specific, since catechol amines other than adrenaline and noradrenaline, if present, may affect the estimation. It can be shown by paper chromatography, however, whether such other catechol amines, for example hydroxytyramine, are present in any appreciable amount. Only exceptionally does this occur in suprarenal extracts (Goodall, 1951).

The differential colorimetric method described by Euler and Hamberg (1949), based on the formation of iodochromes of adrenaline and noradrenaline, is simple and reasonably accurate when applied to suitably prepared extracts. When adrenaline is present in the mixture in amounts less than 10% of the total catechol content, biological methods give more accurate estimates; whereas small amounts of noradrenaline, less than 10%, will usually be determined more accurately by the colorimetric method.

Recently Shepherd and West (1951) have reported in this journal that the colorimetric method of Euler and Hamberg yields results which differ greatly from those obtained with a biological method (blood pressure and nictitating membrane of the spinal cat). They conclude that the colorimetric method gives erroneous results.

In order to check the basis for this statement, for which we have had no previous evidence, we have estimated colorimetrically and biologically the catechol amines in preparations of guinea-pigs' suprarenals.

## METHODS

Ten preparations of suprarenals (I-X) were made from groups of 5-7 guinea-pigs weighing 300-400 g. each. The animals were killed by a blow on the neck. The right and left suprarenals were prepared separately, weighed, ground with sand, and extracted for about one hour. Preparations were made with acidu-

lated ethanol (I, VII, and IX), with 5% trichloroacetic acid (T.C.A.) (II), and with 10% T.C.A. (III, IV, V, VI, VIII and X). In this way the influence of the extraction method was tested. The volumes of extraction fluids were 20-30 ml.

The ethanol was removed by evaporation *in vacuo* and the lipids extracted with ether. The T.C.A. was removed by extraction 4-5 times with 3-4 volumes of ether. In the second group both preparations (III and IV) became pink during the extraction procedure with the ether, probably owing to some impurity in the ether. Another brand of ether was used for the subsequent preparations which yielded completely colourless extracts. The colorimetric estimations were made according to Euler and Hamberg (1949).

For the biological estimations the cat's blood pressure and the fowl's rectal caecum were used. The extracts were assayed against standards and the results computed according to Euler (1949).

In preparations VII-X the biological activity was estimated on the cat's blood pressure and expressed as adrenaline equivalents. In the Table are also given for comparison the adrenaline equivalents calculated from the colorimetric figures for adrenaline and noradrenaline and the activity ratio found biologically for adrenaline and noradrenaline, as illustrated by the following arbitrary example:

Adrenaline equivalents on the cat's blood pressure:

720  $\mu\text{g.}/\text{g.}$

Activity ratio adr./noradr. on the cat's blood pressure:

0.25

Colorimetric adrenaline figure:

510  $\mu\text{g.}/\text{g.}$

Colorimetric noradrenaline figure:

44  $\mu\text{g.}/\text{g.}$

Calculated adrenaline equivalents:

$$510 + 44 \cdot \frac{1}{0.25} = 686 \mu\text{g.}/\text{g.}$$

## RESULTS

The following conclusions may be drawn from the Table.

1. The figures obtained by the colorimetric method are in good agreement with those found by biological estimation. The greatest difference

TABLE  
ESTIMATIONS OF ADRENALINE AND NORADRENALINE IN SUPRARENALS

Group	Prepara- tions	Extraction	Weight of suprarenals mg.	Colorimetric estimation μg./g.			Biological estimation μg./g.			Remarks
				Adr.	Noradr.	% Adr.	Adr.	Noradr.	% Adr.	
1 (7 animals, 2.36 kg.)	I II	Acid ethanol 5% trichloroacetic acid (T.C.A.)	487	460	27	95	450	+	~90	
			445	530	98	84	550	+	~90	
2 (6 animals, 2.13 kg.)	III IV	10% T.C.A. 10% T.C.A.	431	250	40	86	250	30	89	Pink coloured extracts
			434	87	76	53	60	40	60	
3 (5 animals, 1.97 kg.)	V VI	10% T.C.A. 10% T.C.A.	442	490	71	87	480	80	86	
			438	550	80	87	500	70	87	
4 (5 animals, 2.20 kg.)	VII VIII	Acid ethanol 10% T.C.A.	535	450	62	88	560 found (540 calcul.) 560 found (510 calcul.)		Adrenaline equivalents	
			521	450	44	91				
5 (5 animals, 2.06 kg.)	IX X	Acid ethanol 10% T.C.A.	320	770	44	95	800 found (830 calcul.) 900 found (960 calcul.)		μg./g. on cat's B.P.	White animals
			326	790	120	87				

seen between the results using the two methods for adrenaline estimation in 8 preparations (I, II, V-X) was less than 15%, when exception is made for the deteriorating extract IV.

2. The total catechol figures were consistently much higher per mg. gland in our material than in the material used by Shepherd and West (Fig. 1).

3. There was no consistent difference between the figures, whether acid ethanol or trichloroacetic acid was used for the extraction.

4. Noradrenaline was demonstrated colorimetrically in all extracts. Its presence was also observed in those extracts which were tested biologically.

5. The amount of adrenaline in the gland extracts varied between 84 and 95% of the total catechol amines in the present material.

6. The estimate of biological activity obtained by assay on the cat's blood pressure, and expressed in adrenaline equivalents, showed good agreement with that calculated from the colorimetric estimation of adrenaline and noradrenaline (preparations VII-X).

7. Faulty extraction technique accounts for the low total catechol figures and high noradrenaline percentage in preparations III and IV, due to selective inactivation of adrenaline.

Paper chromatographic analysis of the extracts made with ethanol showed the presence of noradrenaline in addition to adrenaline in amounts which were of the same order of magnitude as those found biologically and colorimetrically.

When trichloroacetic acid extracts were used, a displacement of the noradrenaline and adrenaline spots was observed, both showing higher  $R_F$  values than normally. For this reason extraction with alcohol should be used for paper chromatographic experiments with extracts not subjected to further purification. A displacement of a similar kind has been noted previously after adsorption on alumina from trichloroacetic acid extracts (Euler, 1950).

#### DISCUSSION

Considerable experience of the colorimetric and biological estimations of adrenaline and noradrenaline has been had in this laboratory, yet no indication of consistent discrepancies between the two methods has been found. The results of Shepherd and West differ from ours in three respects, in (1) the total amount of catechol amines; (2) the percentage of noradrenaline; and (3) the difference between colorimetric and biological estimations.

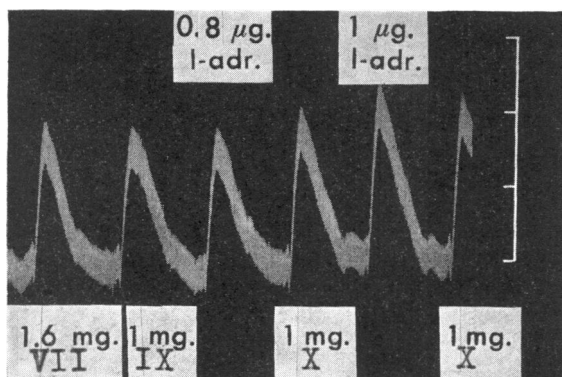


FIG. 1.—Blood pressure, cat, pentobarbitone. I.V. injections of 1.6 mg. VII and 1 mg. IX and X, compared with 0.8  $\mu\text{g.}$  and 1  $\mu\text{g.}$  adrenaline hydrochloride as indicated. Ordinate: 140–200 mm. Hg.

The total amount of catechol amines might differ according to the source of the guinea-pigs; and the possibility cannot be excluded that the guinea-pigs used by Shepherd and West had a much smaller amount of suprarenal medullary catechols than ours. As regards the noradrenaline percentage, this also seems to differ in different groups of animals. Thus one of us (B. H.) found low percentages (less than 5%) of noradrenaline in most of his groups of animals. The reason for these differences is still obscure. However, the good agreement between the colorimetric and biological findings indicate that the stated differences are real.

No support has been obtained for the statement of Shepherd and West to the effect that the colorimetric method of Euler and Hamberg yields erroneous results. We can give no explanation for their observations, but it should be pointed out that low total catechol content and high percentages of noradrenaline may well occur in deteriorating extracts, as illustrated by preparations III and IV in the Table. If the biological testing is done later than the colorimetric it might then easily yield lower figures.

As to the method of biological estimation, Shepherd and West give figures in some experiments of 0 and 2% for the noradrenaline fraction of the total catechols. This surprising accuracy is rather higher than the methods which we have used would permit. In the biological as well as the colorimetric methods the accuracy

usually diminishes sharply as the noradrenaline percentage approaches 10% of the total catechols. However, in some cat blood pressure preparations, even 5–10% noradrenaline mixed with adrenaline may be clearly distinguished from adrenaline alone by the different response pattern.

#### SUMMARY

The adrenaline and noradrenaline content of guinea-pigs' suprarenals was estimated colorimetrically by the method of Euler and Hamberg, and biologically by assay on the cat's blood pressure and the hen's rectal caecum with extracts made up with acid ethanol or trichloroacetic acid. The catechol amine content was generally about 500  $\mu$ g. per g., 5–15% being noradrenaline.

The presence of noradrenaline in the extracts was confirmed by paper chromatography.

The colorimetric method of Euler and Hamberg gave results which tallied closely with those determined biologically.

Extraction with acid ethanol or trichloroacetic acid caused no consistent differences in the catechol amine figures.

#### REFERENCES

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