RESEARCH PAPERS

THE STABILITY OF ADRENALINE AND NORADRENALINE IN HUMAN URINE

BY MONICA MANN

From the Department of Pharmacology, School of Pharmacy, University of London

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IT is a well-known fact that adrenaline is very readily oxidised in aqueous solution, forming a pink-coloured substance, adrenochrome. However, Euler and Hellner[1] reported that adrenaline is remarkably stable in urine. They showed that the adrenaline present in human urine is not destroyed when stored at room temperature and pH 3 to 4 for 48 hours.

Since the detection of adrenaline and noradrenaline in urine is of value in the diagnosis of phæochromocytomas and since a considerable volume of the urine used for this test is that which has been stored overnight in the bladder, at a neutral or possibly slightly alkaline *p*H, before it is collected and acidified, it was thought to be of some interest to investigate the stability of adrenaline and noradrenaline in urine at an alkaline *p*H and at body temperature. It was also hoped to determine the nature of the substance or substances inhibiting the oxidation.

EXPERIMENTAL PROCEDURE

Urine samples were collected from different individuals and *l*-adrenaline or *l*-noradrenaline added to give a concentration of 10 μg/ml. The *p*H was then adjusted to 7·5 to 8·0 with sodium hydroxide and the samples incubated at 37° C. for 5 hours, unless otherwise stated. They were then either assayed immediately or acidified with concentrated hydrochloric acid and stored in the refrigerator until the next day. No allowance was made for the naturally occurring amines in the urine since Euler[1] has shown that they are present in a concentration of about 0·04 μg./ml. This is negligible in comparison with the added amount of 10 μg./ml.

Assay methods

Initially all the urine samples were extracted by the method of Euler and Luft[2] and subsequently assayed on the cat blood pressure. Later it was found that direct estimations of the unextracted urine could be made on the isolated intestine of the rabbit, using oxygenated Tyrode Ringer solution in a 60-ml. bath. Sometimes it was also possible to perform a direct assay on the cat blood pressure. Care was taken in all instances, to set up adequate control urine samples and for all solutions to be neutralised before assaying.

RESULTS

Urine samples incubated under the above conditions showed complete protection of the adrenaline and noradrenaline added. Aqueous solutions

of the two amines, under the same conditions, showed complete loss of activity (Fig. 1). There must therefore be some substance or substances present in urine which prevent the oxidation of the amines.

The urinary constituent which seemed most likely to prevent this oxidation is ascorbic acid. Therefore the ascorbic acid content of each

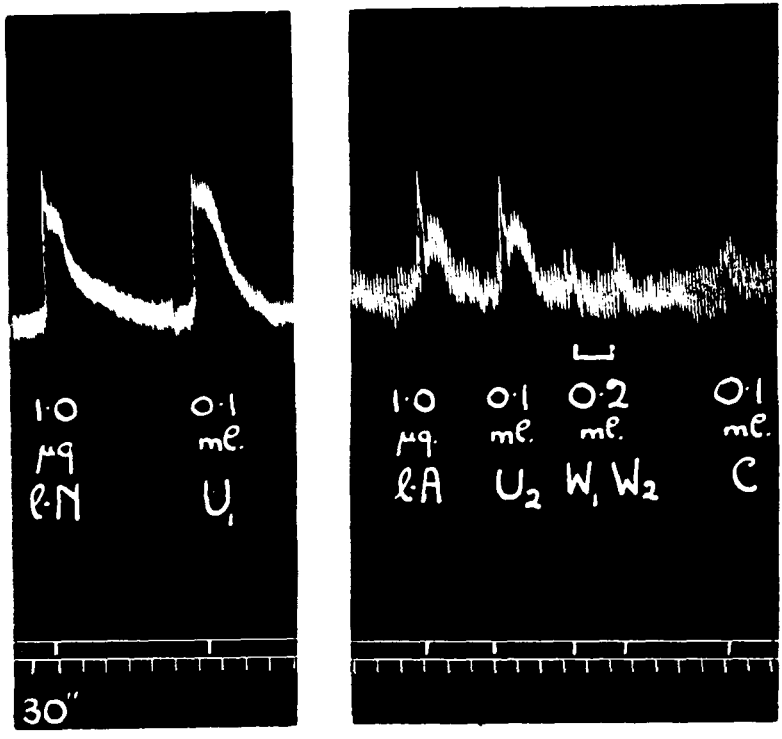

FIG. 1. Cat blood pressure. Chloralose anæsthesia.

U_1 Urine containing noradrenaline 10 μg./ml.
U_2 Urine containing adrenaline 10 μg./ml.
W_1 Water containing noradrenaline 10 μg./ml.
W_2 Water containing adrenaline 10 μg./ml.
C Control urine. All 5 samples incubated at 37° C. for 5 hours.

urine sample was estimated immediately before incubation, using 2:6-dichlorophenolindophenol reagent, and the degree of protection of adrenaline and noradrenaline compared with the protection in aqueous solutions of ascorbic acid. As can be seen from Table I, 1 mg. per cent. of ascorbic acid was never adequate to cause any protection of adrenaline or noradrenaline in water and 2 mg. per cent. only caused a certain degree of protection. However, urine containing 1 mg. per cent. of ascorbic acid caused complete protection in 6 out of 8 samples and even 0·25 mg. per cent. caused some protection (Table II). Thus, although in a few urine samples the protection could be accounted for by the amount of

ascorbic acid present, in the majority of samples there was still complete protection of the amines although the ascorbic acid content was below the amount required to protect an aqueous solution of adrenaline or noradrenaline (Fig. 2). In 2 cases the urine samples were incubated

TABLE I

PROTECTION OF *l*-ADRENALINE AND *l*-NORADRENALINE IN WATER BY ASCORBIC ACID

Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours
1·0	0
1·0	0
1·0	0
1·0	0
1·0	0
2·0	50
2·0	80
2·0	10
2·0	40
2·0	5

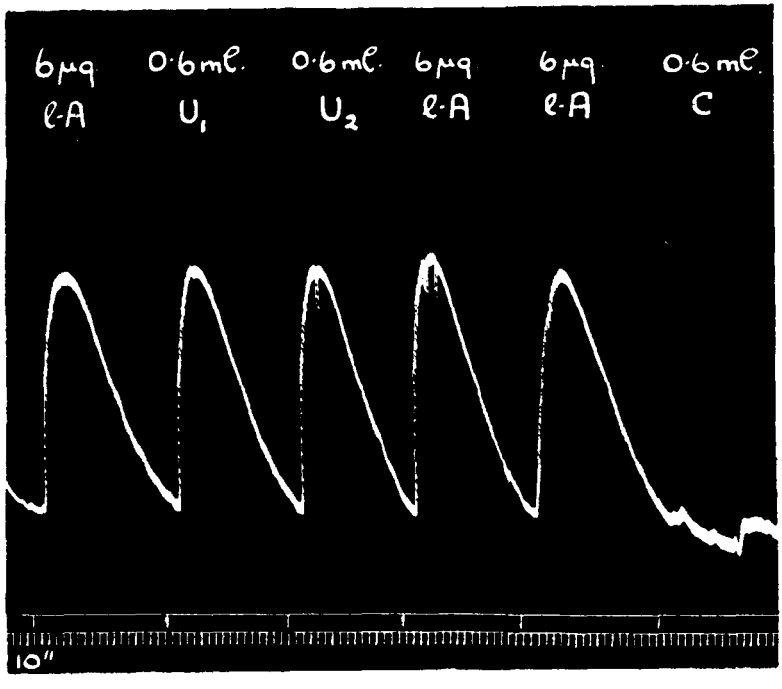

FIG. 2. Cat blood pressure. Chloralose anæsthesia.

C Control urine.
U_1 Urine containing adrenaline 10 μg./ml. and ascorbic acid 0·5 mg. per cent.
U_2 Same urine as U_1, containing adrenaline 10 μg./ml., but ascorbic acid added to give a concentration of 5·0 mg. per cent.

All 3 samples incubated at 37° C. for 5 hours.

TABLE II

PROTECTION OF *l*-ADRENALINE AND *l*-NORADRENALINE IN URINE BY ASCORBIC ACID

Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours	Ascorbic acid before incubation mg. per cent.	Percentage protection in 5 hours
0·5	100	0·5	100
1·0	100	0·4	100
0·6	100	1·0	100
0·5	100	0·3	25
0·4	10	1·0	100
0·5	100	0·5	100
2·5	100	1·0	70
0·8	100	2·8	100
0·75	100	0·5	100
0·25	25	0·8	100
1·0	100	1·5	100
1·3	100	1·0	100
1·0	30	1·2	100
1·2	100	1·0	100

TABLE III

PROTECTION OF ADRENALINE BY ASCORBIC ACID ON PROLONGED INCUBATION

	Ascorbic acid before incubation mg. per cent.	Adrenaline present after—		
		5 hours per cent.	18 hours per cent.	24 hours per cent.
Urine I ..	0·75	100	50	12·5
Urine II ..	1·0	100	15	10
Water ..	1·5	0	—	—

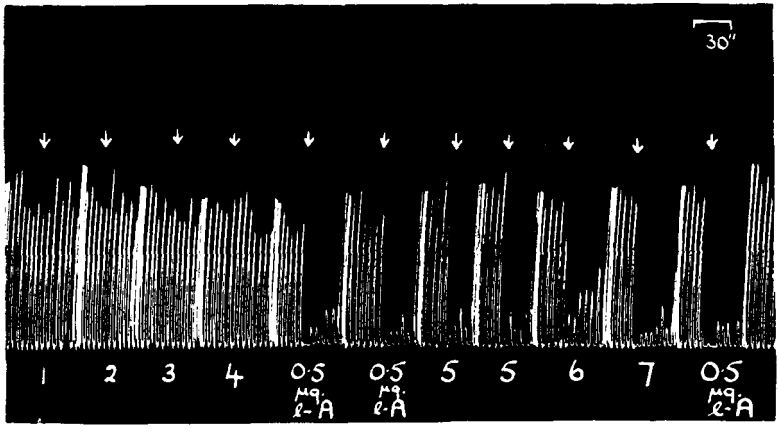

FIG. 3. Isolated intestine of the rabbit.

1. 0·2 ml. control "synthetic urine."
2. 1·0 ml. of water containing adrenaline 10 µg./ml.—no ascorbic acid.
3. 0·5 ml. of water containing adrenaline 10 µg./ml. and ascorbic acid 0·5 mg. per cent.
4. 1·0 ml. No. 3.
5. 0·05 ml "Synthetic urine" containing adrenaline 10 µg./ml. and ascorbic acid 0·5 mg. per cent.
6. 0·05 ml. "Synthetic urine" containing adrenaline 10 µg./ml.—no ascorbic acid.
7. 0·1 ml. No. 6.

All solutions incubated at 37° C. for 5 hours.

with adrenaline for 24 hours and the amount of adrenaline present was estimated at certain times during this period. The results were as shown in Table III.

It was therefore evident that although ascorbic acid plays some part in the stability of the amines there must be some other constituent or constituents in urine which cause this protection. The simplest way of tackling the problem was thought to be to make up a "synthetic urine" of the composition shown in Table IV, both with and without ascorbic acid and to see whether or no this protected the amines. If so, the substances causing such a protection could be determined by a process of elimination. It was found that "synthetic urine" of the composition given and containing 0·5 mg. per cent of ascorbic acid caused, when incubated at 37° C. for 5 hours, complete protection of the amines while without ascorbic acid there was a partial protection. With water, under the same conditions, there was complete destruction of adrenaline and noradrenaline (Fig. 3).

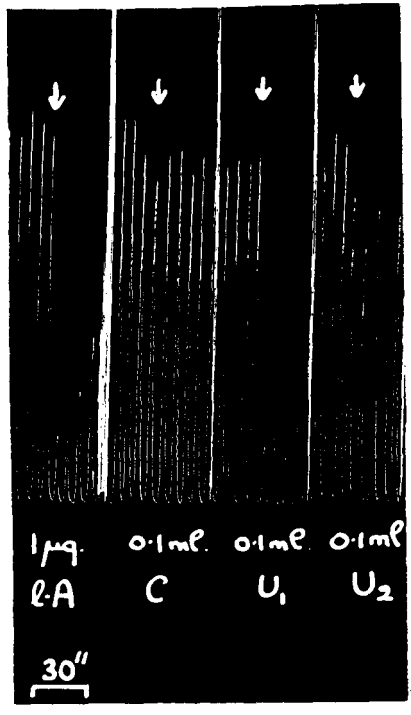

FIG. 4. Isolated intestine of the rabbit.

C Control urine.
U_1 Urine containing adrenaline 10 µg./ml. and ascorbic acid 0·25 mg. per cent.
U_2 Dephosphated urine containing adrenaline 10 µg./ml. and ascorbic acid 0·25 mg. per cent.

All urine samples incubated at 37° C. for 5 hours.

By a process of elimination it was found that only the ascorbic acid and the phosphate present caused any appreciable protection although uric acid had a little effect in delaying the destruction of the amines. When the phosphate was present in amounts approximating to those in normal urine there was protection of the amines with an ascorbic acid content as low as 0·5 mg. per cent. Confirmation of these results was given by taking urine samples of low ascorbic acid content and removing the phosphates with magnesia mixture. The dephosphated urine was then incubated in the usual way. Such urine did not cause protection of the adrenaline, whereas the same urine but containing phosphates showed complete protection (Fig. 4). Dephosphated urine, but containing more ascorbic acid, still protected the added adrenaline (Fig. 5).

There was also the possibility that the stability of ascorbic acid was not the same in urine as in water. Consequently both urine and water were incubated at *p*H 7·5 to 8·0 at 37° C. for 5 hours with different amounts of ascorbic acid added. The ascorbic acid content was deter-

TABLE IV

"SYNTHETIC URINE"

	per cent.
Urea	3·0
Uric acid	0·05
Creatinine hydrochloride	0·1
Sodium chloride	1·5
Potassium chloride	0·25
Sodium acid phosphate	0·25
Sodium sulphate	0·2
Magnesium sulphate	0·01
Calcium chloride	0·01

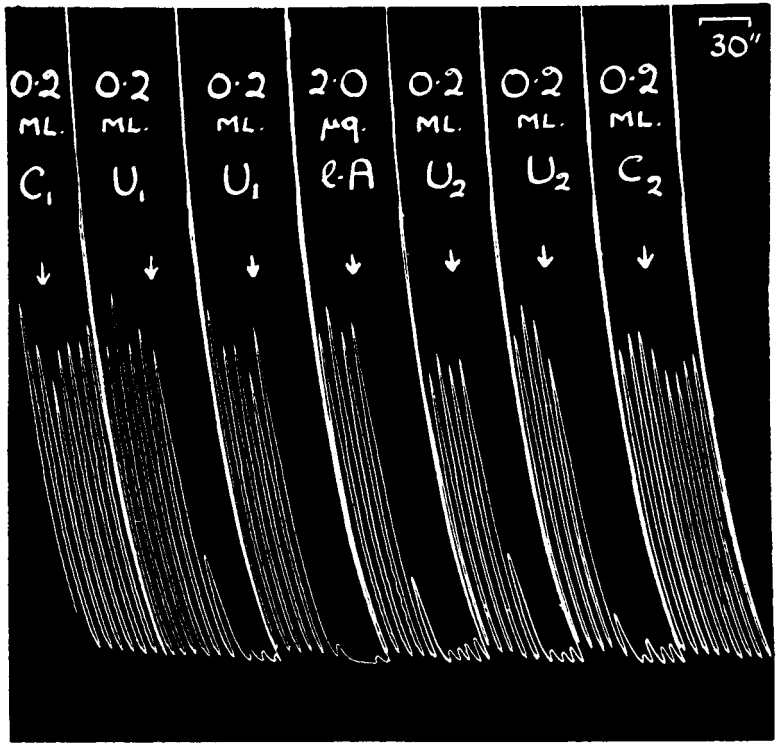

FIG. 5. Isolated intestine of the rabbit.

C_1 Control dephosphated urine.
U_1 Dephosphated urine containing adrenaline 10 µg./ml. and ascorbic acid 2·25 mg. per cent.
C_2 Control urine.
U_2 Urine containing adrenaline 10 µg. ml. and ascorbic acid 2·25 mg. per cent.
All urine samples incubated at 37° C. for 5 hours.

mined both immediately before and after incubation. It was found that with an ascorbic acid content of 0·5, 1·0 or 2·0 mg. per cent. in water there was no detectable amount (i.e. less than 0·05 mg. per cent.) left after incubation whereas with urine there was only a percentage loss. With 5 mg. per cent. there was a percentage loss in both urine and water, this being greater in water. Therefore ascorbic acid is considerably more stable in urine than in water and this partly accounts for the adrenaline stability.

Discussion

It is of some interest that adrenaline and noradrenaline are so stable in human urine. Although this can be due entirely to the ascorbic acid if it is present in a sufficient quantity, in most urine samples the protection is due to both the ascorbic acid and the phosphates present. Although each may be present in a subthreshold amount to prevent oxidation over the time interval studied, together they cause a complete protection.

It is also of interest that urine can be assayed for sympathomimetic activity directly, i.e. without previous extraction—using the isolated intestine of the rabbit. In only 1 or 2 instances did the control urine have any effect on the intestine, in the volumes used. This saves having to perform the time-consuming extraction method and is very useful in the detection of adrenaline and noradrenaline in the urine of suspected cases of phæochromocytoma. In cases where a tumour is present there is such a high concentration of amines that a satisfactory direct assay can readily be performed.

Summary

1. Both adrenaline and noradrenaline are considerably more stable in urine than in water, even at body temperature and an alkaline *p*H.

2. This stability is due to the ascorbic acid and the phosphates present in urine. It is also partly due to the greater stability of ascorbic acid in urine than in water.

3. Dephosphated urine of low ascorbic acid content does not prevent the oxidation of the amines.

4. Adrenaline and noradrenaline can be estimated in urine without previous extraction, using the isolated intestine of the rabbit.

References

1. Euler, von and Hellner, *Acta Physiol. scand.* 1951, **22**, 161.
2. Euler, von and Luft, *Acta endocr., Copenhagen.* 1949, **3**, 323.