NORADRENALINE IN INFUSION SOLUTIONS

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INTRODUCTION

It is generally known that the activity of adrenaline and noradrenaline is affected by oxidation, alkalinity, light, high temperature and the presence of heavy metal ions (West, 1952, von Euler, 1956).

Häggendal and Johnsson (1967) showed that noradrenaline was stable in isotonic glucose (5.5%), sodium chloride (0.9%) and sodium bicarbonate (1.4%) solutions for up to 4 hours at $22\pm1°C$. These workers carried out their observations on sterile solutions to which noradrenaline was added, and their conditions were similar to those used clinically for "drop" infusions with a flow rate of 250 ml. per hour.

No such work has been carried out in the tropics. It was therefore considered desirable to carry out an investigation on similar lines to see whether the findings of Häggendal and Johnsson (1967) would hold in this region. A short report on preliminary findings has already been presented (Ogle, 1968). Further work on the stability of noradrenaline in infusion solutions commonly used clinically in Singapore is reported in this paper.

METHODS

A. Fluorometric Estimation of Noradrenaline

A modification of the method described by Joyce, Gallwas and McCallum (1965) was adopted for the fluorometric estimation of noradrenaline. The sodium form of Amberlite IRC-50 (Analytical Grade—B.D.H.) was used instead of alumina to adsorb the noradrenaline. The resin was prepared as follows. After washing a number of times with distilled water (glass-distilled), 5 volumes of 6N HCl were added and the suspension stirred for 30 minutes. The acid was poured off and the resin washed by decantation with 8×5 volumes of distilled water. It was converted into the sodium form by adding 3 volumes of distilled water followed by 2 volumes of 10N NaOH, which were slowly added with constant stirring over a period of 15 minutes. The mixture was allowed to settle, the supernatant discarded and the resin washed thoroughly with 8×5 volumes of distilled water. After the final wash, the resin was suspended in an equal volume of distilled water and the pH of the suspension adjusted to 6.5 with concentrated phosphoric acid; it was then ready for use.

The prepared resin was placed in a chromatographic tube (8 cm. high with an internal diameter of 0.7 cm.) and washed by passing 25 ml. of distilled water (pH 6.5) through it. The samples (2 ml.) had their pH first adjusted to 6.5 before being applied to the column. The flow rate through the resin was 2 ml. per minute. After the application of the sample, the column was washed with 50 ml. distilled water (pH 6.5) initially at a similar rate, but quickening to 3 ml. per minute for the last 25 ml. 1N H_2SO_4 at a flow rate of 3 ml. per minute was used to elute the noradrenaline, the first 25 ml. of the eluate was collected for estimation.

All pH measurements were carried out with a glass electrode and a pH meter (Beckman 'Expandomatic') at a room temperature of 23°C.

B. Noradrenaline

The noradrenaline used in this study was l-noradrenaline bitartrate, prepared for injection by the Government Pharmaceutical Laboratories and Stores, Singapore. Each ampoule contained per ml: 4 mgm. noradrenaline bitartrate (expressed as salt), 1 mgm. sodium metabisulphite and 8 mgm. sodium chloride in distilled water.

C. Infusion Solutions

The solutions examined were physiological saline (0.9% NaCl), dextrose (5%), dextrose (5%) in physiological saline, prepared according to BP requirements and sodium bicarbonate (4.2%). All solutions were prepared by the Government Pharmaceutical Laboratories and Stores, Singapore.

D. Procedure

1 ml. of the noradrenaline solution was injected into the infusion solution (540 ml.) under test. Immediately after mixing, a 2 ml. sample was taken for the estimation of the initial concentration; this was designated the 'O' hour value. After taking the sample for the determination of the O hour value, a disposable infusion apparatus ('Sangofix', B. Braun. Melsungen) was then connected to the solution bottle. The

drip rate was so adjusted for the solution to last 4 hours (flow rate of 134 ml. per hour, approximately 40-42 drops per minute). At the end of the 2nd and 4th hour, samples were again taken for estimations. Their values were expressed as percentages of the O hour value. The noradrenaline content of samples was determined immediately after each collection. As far as possible, all conditions were made to simulate those in clinical practice.

The ambient room temperature at which the drips were operated was 29-31°C. The experiments were conducted in a room mainly illuminated by fluorescent light. A light meter (Model 3, AVO Limited) was used to measure the actual intensity of illumination falling on the drip bottle. This was found to be 18 foot candles per square foot. The intensity of illumination was quite constant over the experimental period.

RESULTS

Recovery rates of noradrenaline

Recovery tests were carried out to ascertain the reliability of the extraction procedure. Noradrenaline solutions in concentrations of 1, 2, 3 and 4 μg (expressed as base) per ml. were used since the concentrations of noradrenaline in the infusion solutions were in this range.

The method, together with its modifications was quite consistent. Table I shows the percentage recoveries of various amounts of noradrenaline. The volume in which these amounts were contained was 2 ml., similar to that of samples taken from solutions under test. The over-all recovery rates ranged from 87-102% and the mean was 93%.

TABLE I

RECOVERY RATES OF KNOWN AMOUNTS OF NORADRENALINE

Observation Number	Percentage Recovery of Noradrenaline			
	8 μg	6 μg	4 μg	2 μg
1	87	87	89	98
2	91	98	94	92
3	89	94	87	99
4	91	87	88	100
5	100	98	94	92
6	94	89	89	89
7	89	88	101	98
8	102	100	96	87
9	102	87	89	92
10	94	96	93	90
11	102	93	94	90
12	89	101	91	—
13	95	89	89	—
14	87	88	—	—
15	91	—	—	—
16	91	—	—	—
Mean	93	93	92	93
± Standard error	± 1.34	± 1.41	± 1.10	± 1.38
Range	87-102	87-100	88-101	87-100

The values of noradrenaline in μg are expressed as base.

Noradrenaline activity in infusion solution

(a) *Physiological saline*

The noradrenaline activity at the end of the 2nd hour was 75% of the O hour value and at the 4th hour only 44% remained (Table II). These changes, when compared with the O hour value were significant ($P < 0.001$ for both). The initial pH of 7.6 fell to 5.9 on addition of 1.0 ml. noradrenaline bitartrate solution (Table III) and fell further by the 4th hour.

(b) *Dextrose 5%*

There was no significant fall in activity by the 2nd or the 4th hour (Table II). The initial pH of 3.8 (Table III) fell slightly to 3.7 on addition of noradrenaline.

(c) *Dextrose 5% in Physiological Saline*

There was also no significant fall in activity over the 4 hours (Table II). The initial pH of 3.9 (Table III) fell to 3.8 on addition of noradrenaline.

(d) *Sodium Bicarbonate 4.2%*

Activity remaining after 2 hours was 76% of the O hour value and by the 4th hour was 64% (Table II). These falls were significant ($P < 0.01$ for the 2nd and $P < 0.001$ for the 4th hour). The pH (8.1) of the solutions after the addition of noradrenaline, unlike physiological saline, did not differ from the initial value, nor was there any change over the 4 hours (Table III).

(e) *Acidified Physiological Saline*

Physiological saline solutions were acidified with 1N HCl to a pH of 3.6. Noradrenaline was found to be stable in these acidified solutions (Table II). The initial pH was not appreciably lowered by the addition of noradrenaline (Table III).

TABLE II

THE ACTIVITY OF NORADRENALINE IN RELATION TO TIME

	0 Hour	2nd Hour	4th Hour
Physiological Saline (7)	100	75 ± 1.1**	44 ± 3.9**++
Dextrose 5% (4)	100	96 ± 3.7	102 ± 2.9
Dextrose 5% in Physiological Saline (4)	100	98 ± 1.7	97 ± 2.3
Sodium Bicarbonate 4.2% (4)	100	76 ± 3.5*	64 ± 2.8**+
Acidified Physiological Saline (4)	100	99 ± 5.4	101 ± 1.6

The values shown are the mean percentages ± their standard errors.
The figures in parenthesis are the number of observations made.
* = the significance where a mean differs from its control value (100%).
\+ = the significance where a mean value at the 4th hour differs from that at the 2nd.
One = $P < 0.01$, two = $P < 0.001$.

TABLE III

pH CHANGES IN THE INFUSION SOLUTIONS TESTED

Solutions Tested	Initial pH	pH after the addition of 1 ml. Noradrenaline 0 Hour	2nd Hour	4th Hour
Physiological Saline (7)	7.6 ± 0.10	5.9 ± 0.10	5.6 ± 0.11	5.3 ± 0.10
Dextrose 5% (4)	3.8 ± 0.09	3.7 ± 0.07	3.6 ± 0.09	3.6 ± 0.07
Dextrose 5% in Physiological Saline (4)	3.9 ± 0.03	3.8 ± 0.03	3.8 ± 0.03	3.8 ± 0.03
Sodium Bicarbonate 4.2% (4)	8.1 ± 0.03	8.1 ± 0.03	8.1 ± 0.03	8.1 ± 0.02
Acidified Physiological Saline (4)	3.6 ± 0.03	3.5 ± 0.28	3.4 ± 0.31	3.4 ± 0.31

The values shown are the means ± their standard errors. The figures in parentheses are the number of observations made.

DISCUSSION

The findings indicate that in tropical conditions, at an ambient temperature of 29°-31°C, there is a substantial decrease in the activity of noradrenaline in physiological saline when this is used in a drip form under clinical conditions. This loss of activity could account for the necessity of sometimes having to progressively increase drip rates to maintain the desired effect when physiological saline is used. Instability was also shown when 4.2% sodium bicarbonate solutions were used. Noradrenaline was however stable in solutions of 5% dextrose and 5% dextrose in physiological saline. This observation that stability is least affected in dextrose solutions is in agreement with that of West (1952) who used biological assay methods to assess activity.

The method employed for the extraction of noradrenaline was simple, consistent and the recovery good, ranging from 87-102%. These values compare favourably with those of others who have used ion exchange resins (Kirshner and Goodal, 1957., Bertler, Carlsson and Rosengren, 1958., Häggendal, 1963). However, since the recovery range obtained was from 87-102%, only changes greater than 15% could be relatively measured.

Although the initial pH of physiological saline was reduced to 5.9 by the addition of noradrenaline, this degree of acidity failed to protect the hormone. The suggestion from previous work that stability might be achieved by lowering the initial pH (Ogle, 1968) was confirmed, as shown when the initial pH of physiological saline was reduced to 3.6. The pH of all the solutions, except sodium bicarbonate, tended to progressively decrease during the experimental period. This may be attributable to aeration of the sclution by carbon dioxide in the air replacing fluid lost through the drip. It is not possible to compare closely any of the present data with those of West (1952) as the conditions he used were different. For instance, the aeration in the present experiments, necessary to achieve constant drip flow, has to be taken into account. The decrease in pH was small and possibly not sufficient to contribute significantly to arresting or slowing degeneration. However, the atmospheric oxygen introduced, has to be considered as this is known to cause destruction. It would be interesting to see if aeration with an oxygen-free gas would make a significant difference to the results.

The intensity of illumination measured in this investigation, is similar to that in the wards. Light is known to hasten destruction of noradrenaline. Past workers (West, 1952., Häggendal and Johnsson, 1967) did not state their lighting conditions. This again makes comparisons difficult.

It can be concluded from the experiments reported here that at ambient temperatures of 29°-31°C, noradrenaline is stable only in infusion solutions of low pH. The pH of physiological saline needs to be lowered to the region of 4 before stability can be achieved. These observations differ from those obtained in a temperate environment (Häggendal and Johnsson, 1967).

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