# A METHOD FOR THE ESTIMATION OF ADRENALINE AND NORADRENALINE IN URINE.*

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Received November 26, 1957

A method for the estimation of sympathin (adrenaline plus noradrenaline) in urine is described. The catecholamines are adsorbed on a cation exchange resin, Amberlite IRC–50, and eluted with acid. The sympathin in the eluate is estimated by a fluorimetric method, in which adrenaline and noradrenaline yield the same intensity of fluorescence so that sympathin estimates are uninfluenced by the proportions of the two amines. Estimates of the adrenaline and noradrenaline are obtained after separation of the amines in the urine extract by paper chromatography. A mean recovery of 82 per cent (±6 per cent S.D. (51)) was obtained when adrenaline or noradrenaline (2–5000 μg.) was added to a urine sample analysed for sympathin content. Estimation of the separated amines from mixtures of adrenaline or noradrenaline (2–15 μg. of either amine) showed a mean recovery of 51 per cent (±9 per cent S.D. (10)). Experimental evidence is presented of the validity of applying appropriate correction factors to analytical results, the correction factors being determined by recovery experiments run concurrently with each series of estimations.
The specificity of the method is discussed briefly.

MOST methods for the estimation of adrenaline and noradrenaline in urine involve adsorption of the amines on aluminium hydroxide[1], or on aluminium oxide[2]. After elution, the catecholamines are estimated either by bioassay[1] or by fluorimetry[2]. Attempts to use Lund's[3,4] fluorimetric method of assay following adsorption of the amines on aluminium oxide or hydroxide lead to unsatisfactory results in our hands.

Better results were obtained using the cation exchange resin, Amberlite IRC–50, as adsorbent, and this paper describes a method for the estimation of adrenaline and noradrenaline in urine utilising this material. After elution, the sympathin is estimated by a modification of Lund's fluorimetric method. The term, "sympathin", is used to indicate a mixture of adrenaline and noradrenaline irrespective of the proportions of the two amines in the mixture. Separate estimations of the two amines in the eluates are made by the fluorimetric technique after paper chromatographic separation[5].

The method has been used to study the urinary sympathin excretion of normal rats and of rats subjected to various experimental procedures. This study was undertaken primarily with a view to demonstrating that changes in the urinary sympathin excretion would provide an indication of alteration in the level of sympathico-adrenal discharge. The results of this investigation will be reported elsewhere.

The method has also been used to study the urinary sympathin excretion of normal male infants and of infants suffering from pink disease[6].

* Much of the work reported in this paper formed part of a Ph.D. thesis submitted by W. L. in May 1955, at Edinburgh University.

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## Reagents

*Amberlite resin IRC–50 (H)*, analytical grade (The Rohm and Haas Co., Philadelphia, U.S.A., obtained from The British Drug Houses, Ltd.).

*L-Ascorbic acid* (The British Drug Houses, Ltd.).

(−)-*Adrenaline* (synthetic, Burroughs Wellcome and Co.). A stock solution, 1·00 mg./ml., was prepared by suspending the base in 0·01N HCl, dissolving it by the addition of a slight excess of concentrated HCl and diluting to volume with 0·01N HCl. The solution was stored at 5°.

(−)-*Nordrenaline*-(+)-*bitartrate monohydrate* ("Levophed", Bayer Products, Ltd.). A stock solution containing 1·00 mg. base/ml. was prepared in 0·01N HCl. The solution was stored at 5°.

*Manganese dioxide* (technical powder). 250 g. was treated twice for 0·5 hours and overnight with a litre 12 per cent (v/v) acetic acid with intermittent shaking. The acid was removed by decantation and washing with water until the wash fluid was above pH 4 (Universal Indicator Paper). The $MnO_2$ was air-dried, reduced to a powder and finally heated, with intermittent stirring, in an evaporating basin over a Meker burner for 2–3 hours. This intense heating was found necessary since, in its absence, the $MnO_2$ frequently gave rise to troublesome dark brown colloidal suspensions in the course of the amine determinations.

*Ethanol.* Absolute ethanol refluxed for 4 hours with NaOH (5 g./l.) and twice distilled.

*Other reagents* were of A.R. quality.

*De-ionised water* was used throughout.

## Methods

*pH Determinations* were made with a glass electrode unless otherwise stated.

*Preparation of columns of ion exchange resin.* The Amberlite resin IRC–50 (H) was converted to the sodium form by treatment overnight with excess 8 per cent (w/v) NaOH. Excess alkali was removed by decantation and repeated washings with water until the wash fluid was about neutral (indicator paper). The resin was then stored for at least 2 days under 0·2M sodium phosphate buffer, pH 6·5 with 2 or 3 changes of the fluid in the interval.

A suspension of the treated resin in the same buffer solution was poured into a tapered 11 mm. internal diameter glass tube plugged with glass wool, to give a resin column of 21 cm. (for treatment of 50–100 ml. of urine) or 11 cm. (for treatment of 10–20 ml. of urine). The outflow from the column was controlled by a small screw clip on a short length of narrow bore rubber tubing fitted to the taper of the tube and carrying a short capillary jet at the lower end. The resin was then buffered to about pH 7 by passage of 300 ml. of 0·2N sodium phosphate buffer, pH 6·5, at a flow rate not exceeding 2 ml./min. This was most conveniently carried out by placing the buffer solution in a mercury levelling bulb set about 100 cm. above the column and connected by polythene tubing (2 mm. internal diam.) to a capillary stop-cock inserted through a rubber stopper fitting tightly into the top of the resin-containing tube. The

flow rate, once adjusted by means of the stop-cock, showed negligible alteration throughout the passage of the buffer solution. The prepared column was left overnight if necessary. Immediately before use, 50 ml. of 0·02M sodium phosphate buffer, pH 7, was passed through the column (the pH of the last 10 ml. of the effluent should not exceed 7·5) followed by 25 ml. water.

*Adsorption of urinary sympathin.* Twenty-four hour specimens of urine were collected in vessels containing 2N $H_2SO_4$, 0·5 ml. for rat urine, or 5 ml. for human urine to preserve the excreted sympathin[1]. To 10 ml. of rat urine or 50–100 ml. of human urine was added 1 mg. of ascorbic acid/ml. as antoxidant and 2 volumes of water. The mixture was adjusted to approximately pH 6·5 by the slow drop-wise addition, with constant stirring, of 2N NaOH and finally to pH 7 with 0·5N NaOH. The sample, followed by 25 ml. of 0·02M sodium phosphate buffer, pH 7, and then by 25 ml. of water, was passed through a resin column of suitable size at 1·0–1·5 ml./minute (larger column) or 0·5–0·7 ml./minute (smaller column).

*Elution of adsorbed sympathin.* The adsorbed sympathin was eluted by passage of 2N $H_2SO_4$ at 0·3–0·5 ml./minute. The eluate was collected in a measuring cylinder containing 2 drops of the acid to acidify the neutral effluent issuing initially from the column and so stabilise any sympathin therein. Elution was continued until the effluent was strongly acidic (tested with short narrow strips of Universal indicator paper). At least 13 ml. or 6 ml. of the acid were required for the larger and smaller columns respectively. The flow was stopped and the resin left in contact with the acid for at least 0·5 hour. The elution was then continued at the same rate as before using 0·01N $H_2SO_4$ until, in all, 40 ml. of eluate (larger column) or 20 ml. of eluate (smaller column) had been collected. Omission of the stationary phase in the elution resulted in decreased recoveries.

The eluate, usually about pH 1·5, was adjusted to pH 3·5 by the slow dropwise addition, with constant stirring, of 2N NaOH.

It has been found possible for one individual to deal with six samples simultaneously during the adsorption and elution stages of the method.

*Preparation of the eluate for the estimation of sympathin.* If the concentration of sympathin in the eluate was known to exceed 0·5 $\mu$g./ml., the estimation by the fluorimetric method described below could be made using portions of the eluate without further treatment. With lower amounts of sympathin, concentration of the eluate was necessary and this required the removal of at least part of the dissolved salts by the addition of 2 volumes of an ethanol : acetone (1 : 1 v/v) mixture. After standing overnight at 5°, the mixture was filtered or centrifuged and the precipitated salt washed twice with 5 ml. of ethanol : acetone mixture, the washings being added to the main bulk of the solution. The solution was evaporated just to dryness *in vacuo* at less than 35° (external temperature). Care was taken to avoid continued heating of any dried-out residue on the sides of the flask as this resulted in a loss of sympathin, a finding which has also been commented on by Goldenberg and others[7].

The addition of 4 volumes of ethanol : acetone (1 : 1 v/v) mixture to the eluate instead of 2 volumes produced a more complete removal of the dissolved salts. This was necessary when it was desired to make a biological assay in parallel with fluorimetry or a paper chromatographic separation of the adrenaline and noradrenaline prior to their separate estimation.

The dry residue was dissolved in 3 ml. of water for the estimation of the sympathin content by fluorimetry or in 3 ml. of saline for biological assay.

*Paper chromatographic separation of adrenaline and noradrenaline in urine extracts.* When individual estimates of the adrenaline and noradrenaline were required, preliminary separation of the two amines in the evaporated desalted eluate from the ion exchange column was carried out by paper chromatography. The technique was that of Crawford and Outschoorn[5] with modifications to permit fluorimetric assay of the separated amines.

Just before use, a Whatman No. 1 filter sheet "for chromatography" was washed by descending chromatography with 0·01N HCl for at least 12 hours and dried at room temperature. The sympathin in the evaporated desalted eluate was transferred to the paper with acid-ethanol as described by Crawford and Outschoorn[5]. Preliminary treatment of the paper with ascorbic acid was unnecessary and undesirable since it interfered with the fluorimetry. The chromatogram was developed with a mixture of phenol, distilled from zinc powder, and 0·1N HCl (15 ml./100 g. of phenol), in an atmosphere of nitrogen and not of sulphur dioxide as originally described. Evaporation of the 0·4 per cent $NaH_2PO_4 \cdot 2H_2O$ eluates of the paper strips carrying the separate amines was not required when the fluorimetric method of assay was employed. When biological assays were to be carried out in parallel with fluorimetry, the paper strips were eluted with 0·01N HCl and the eluates evaporated just to dryness *in vacuo* at 35° (external temperature) to remove HCl and traces of phenol. The residues were taken up in saline for assay.

*Fluorimetric estimation of sympathin.* The method was modified from that of Lund[3,4]. The intensity of fluorescence was measured with a Farrand Fluorometer, Model A (Farrand Optical Co. Inc., New York) using a Corning 5860 filter, which transmits the 365 m$\mu$ line of the mercury vapour lamp, as the primary filter and an Ilford gelatin filter No. 625 (Ilford Ltd., London) transmitting between 510 and 590 m$\mu$ as the secondary filter.

A measured sample, less than 1 ml. and of pH not less than 3·5, of the solution to be assayed was diluted to 4·00 ml. in a 15 ml. centrifuge tube with 0·4 per cent $NaH_2PO_4 \cdot 2H_2O$ and 1·00 ml. of 1 per cent $Na_2HPO_4$, $2H_2O$ added. The pH of the mixture should be about 6·5 (indicator paper). Fifty mg. of $MnO_2$ were added, the tube closed with a clean rubber stopper and the mixture shaken moderately vigorously for 30–35 seconds. The supernatant obtained after centrifuging for 30–35 seconds at 3500 rev./minute was filtered immediately through a double thickness of 4·25 cm. diameter Whatman No. 41 filter paper. One ml. of the filtrate was pipetted without delay into each of two cuvettes. To one cuvette was then added 0·02 ml. of 0·5 per cent ascorbic acid (prepared

fresh daily and kept in a tightly stoppered test-tube). Immediately after inversion of the cuvette twice to mix the solution, 0·20 ml. NaOH (20 g. in 100 ml. of water, stored in a tightly stoppered polythene bottle) was added and the cuvette inverted six times to mix the solution. The fluorescence intensity was measured every half minute from the time of addition of the alkali and the maximum reading recorded. To the other cuvette was added 0·20 ml. of the NaOH solution and the liquid mixed by inversion of the cuvette six times. Twenty minutes later, 0·02 ml. of 0·5 per cent ascorbic acid was added, the solution mixed by inversion of the cuvette twice and the fluorescence intensity measured. This measured the fluorescence of the solution which, unlike that derived from adrenaline and noradrenaline, was stable in alkali in the absence of a reducing agent. It served as the "blank" for the sympathin fluorescence measured in the presence of ascorbic acid.

The presence of substances potentiating or diminishing the fluorescence derived from the sympathin was detected by repeating the estimation on another sample of the test solution to which a known quantity, usually 0·20 $\mu$g. of adrenaline or noradrenaline had been added. Potentiation was never encountered from a urine extract and only rarely was there detected material which caused some diminution of the expected additional fluorescence intensity. A correction factor based on the measurement of the fluorescence of the added amine could be applied in such cases but such corrected estimates were always regarded with dubiety.

Before each set of estimations the fluorimeter was calibrated using suitable amounts of a 1 $\mu$g./ml. solution of adrenaline or noradrenaline prepared fresh daily in 0·4 per cent $NaH_2PO_4 \cdot 2H_2O$ from the stock 1 mg./ml. solution.

As a stable fluorescing standard for the adjustment and continuous check of the sensitivity setting of the instrument during a series of estimations quinidine sulphate (15 $\mu$g. per cent) in 0·1N $H_2SO_4$ was used.

*Estimation of "total" and "free" sympathin in urine.* Others[1] have shown that sympathin is excreted in the urine partly in the free state and partly in a biologically inactive form from which the amines could be released by boiling the urine at a pH of about 2 for 20 minutes. Estimation of the sympathin in untreated urine gives a measure of the "free" sympathin while estimation after acid hydrolysis of the urine gives a measure of the "total" sympathin (but see discussion). To assay the "total" sympathin, a portion (10 ml. rat urine; 50–100 ml. human urine) of the urine specimen was adjusted to pH 1·8 with 2N $H_2SO_4$ and heated in a briskly boiling water bath for 20 minutes, the pH being maintained throughout close to 2 (indicator paper) by the addition of acid. After cooling, the urine was analysed as described above.

*Recovery experiments.* Estimates of the recovery of sympathin from urine were made. At first, the difference between the sympathin content of a urine sample and the content of a like sample from the same urine to which a known amount of adrenaline and/or noradrenaline had been added was determined. Later, the following method was used. A suitable sample of urine was adjusted to pH 10 with 2N NaOH and

maintained at this pH (indicator paper) during gentle boiling for 20 minutes to destroy the sympathin present. After cooling, the sample was adjusted with 2N $H_2SO_4$ to pH 4 for "free" sympathin estimation or to pH 1·8 for "total" sympathin estimation. A known amount of adrenaline and/or noradrenaline, about equal to that expected to be

TABLE I

COMPARISON OF THE FLUORESCENCE INTENSITIES DERIVED FROM ADRENALINE AND NORADRENALINE. REAGENT BLANK DEDUCTED

| Amount of amine μg. | Fluorescence intensity. Galvanometer reading | |
|---|---|---|
| | Adrenaline | Noradrenaline |
| 1·00 | 82 | 82 |
| 1·00 | 78 | 82 |
| 1·00 | 81 | — |
| 0·75 | 61 | 64 |
| 0·75 | 60 | 65 |
| 0·50 | 39 | 41·5 |
| 0·50 | 41·5 | 40·5 |
| 0·25 | 20·5 | 22·5 |
| 0·25 | 22 | 25 |

present in the experimental urine samples, was added and the sample analysed concurrently with the experimental urine samples. The results for the latter were then corrected by the appropriate factor derived from the recovery estimate.

Both methods gave similar results.

## RESULTS

### *Fluorimetric Estimation of Adrenaline and Noradrenaline in pure Solution*

Preliminary experiments showed a linear relation to exist between fluorescence intensity (galvanometer reading) and the amount of amine present with quantities in the range examined (0–1 μg.).

TABLE II

ADRENALINE AND NORADRENALINE MIXTURES. COMPARISON OF THE FLUORESCENCE INTENSITIES WITH THOSE EXPECTED FROM THE SUM OF THE INTENSITIES FROM THE TWO AMINES. REAGENT BLANKS DEDUCTED

| Amount of amine in mixture | | Fluorescence intensity Galvanometer reading | |
|---|---|---|---|
| Adrenaline μg. | Noradrenaline μg. | Observed | Expected |
| 0·50 | — | 42·5 | — |
| — | 0·50 | 44 | — |
| 0·25 | 0·25 | 44 | 43 |
| 0·25 | 0·50 | 67 | 65 |
| 0·50 | 0·25 | 67 | 64 |

*Accuracy of single estimates.* Calibration curves were constructed from the fluorescence intensity reading for 1·00 μg. and for 0·20 μg. of either amine. With various quantities in the range 0·10–1·00 μg. eighteen single estimates for each amine showed a standard deviation of ±8 per cent for adrenaline and ±6 per cent for noradrenaline from the expected value

on reference to the appropriate (1·00 μg.) calibration curve. Ten single estimates for each amine in the range 0·05–0·20 μg. showed a standard deviation of ± 13 per cent for adrenaline and ± 12 per cent for noradrenaline from the expected value on reference to the appropriate (0·20 μg.) calibration curve. As might be expected, the scatter of replicate estimates was greatest for smallest amounts.

*Similar fluorescence intensities from equal amounts of adrenaline and noradrenaline.* (Table I.)

*Summation of the fluorescence intensities of adrenaline and noradrenaline in a mixture.* Table II illustrates that a mixture of the two amines yielded

TABLE III

RECOVERY OF ADRENALINE AND NORADRENALINE ADDED TO URINE

| Experiment No. and source of urine | Amine added | Amount added μg. | Recovery per cent |
|---|---|---|---|
| *Hydrolysed urine* | | | |
| 1 (rat) | Adrenaline | 2 | 54 |
| | Noradrenaline | 2 | 67 |
| 2 (rat) | Adrenaline | 2 | 45 |
| | Noradrenaline | 10 | 49 |
| 3 (rat) | Adrenaline | 8 | 41 |
| | Noradrenaline | 8 | 35 |
| 4 (human) | Adrenaline | 10 | 49 |
| | Noradrenaline | 10 | 50 |
| 5 (human) | Adrenaline | 15 | 56 |
| | Noradrenaline | 15 | 62 |
| | Mean recovery | | per cent<br>49 (adrenaline)<br>53 (noradrenaline) |
| *Unhydrolysed urine* | | | |
| 1 (rat) | Adrenaline | 10 | 46·5 |
| | Noradrenaline | 10 | 46 |

a fluorescence the intensity of which was equal, within the limits of experimental error, to the sum of the intensities derived from the individual amines in the mixture.

*Paper chromatography.* Experiments in which mixtures of adrenaline and noradrenaline in quantities varying between 0·25 and 10·0 μg. of either amine were separated by paper chromatography only and then estimated by fluorimetry showed a mean recovery of 92 per cent (±12 per cent S.D. (19).)

*Recovery of Adrenaline and Noradrenaline from Urine*

Adrenaline or noradrenaline was added to 10 ml. samples of normal rat urine, the sympathin of which had previously been destroyed. From acid hydrolysed urine the recovery of adrenaline (4–30 μg.) was 82 per cent ± 6 per cent S.D. (8), and that of noradrenaline (3–8 μg.) was 82 per cent ± 8 per cent S.D. (18). From unhydrolysed urine the recovery of noradrenaline (3–8 μg.) was 81 per cent ± 6 per cent S.D. (16).

When added to 100 ml. samples of normal human urine, the recovery of adrenaline (200–5000 μg.) was 81 per cent ± 3 per cent S.D. (6) and that of noradrenaline (1250–5000 μg.) was 84 per cent ± 1·5 per cent S.D. (3). In all these experiments the urine was unhydrolysed. The

endogenous sympathin was not destroyed in those urine samples to which an amount of amine greater than 1000 μg. was added. In calculating the recovery per cent, the amount of endogenous sympathin, being very small in comparison to that of the added amine, was ignored.

The overall recovery for the 51 experiments referred to above was 82 per cent ±6 per cent (S.D.) for adrenaline and noradrenaline added to urine in quantities in the range 3–5000 μg.

*Separate estimations of adrenaline and noradrenaline in urine. Recovery experiments.* A mixture of adrenaline and noradrenaline was added to 10 ml. of rat urine or to 100 ml. of human urine the sympathin of which

TABLE IV

ESTIMATES OF SYMPATHIN IN URINE EXTRACTS COMPARED WITH THE SUM OF THE SEPARATE ESTIMATES OF ADRENALINE AND NORADRENALINE AFTER PAPER CHROMATOGRAPHIC SEPARATION OF THE SAME EXTRACTS. ESTIMATES CORRECTED BY APPROPRIATE FACTORS DETERMINED BY RECOVERY EXPERIMENTS RUN CONCURRENTLY

The urine specimens were from a male infant suffering from pink disease

| Treatment of urine | Sympathin μg. | Adrenaline μg. | Noradrenaline μg. | Sum. μg. |
|---|---|---|---|---|
| *Specimen* 1 | | | | |
| Hydrolysed .. | 41 | 13·9 | 26·8 | 40·7 |
| Unhydrolysed | 41 | 11·2 | 26·2 | 37·4 |
| *Specimen* 2 | | | | |
| Hydrolysed .. | 21·5 | 4·1 | 15·4 | 19·5 |
| Unhydrolysed | 20 | 3·7 | 13·6 | 16·7 |

had been destroyed. The urine was extracted, in some cases after acid hydrolysis, and the amines separated by submitted paper chromatography. The recoveries, by fluorimetry, are shown in Table III.

*Agreement between urinary sympathin estimates and the sum of the estimates of adrenaline and noradrenaline.* In view of the variable recoveries of adrenaline and noradrenaline added to urine, which, in addition were surprisingly low for the separated amines, all analyses embodied recovery experiments in parallel. The analytical values were then corrected by a recovery factor so obtained under the conditions of the particular series of analysis. An indication of the validity of this procedure is given in Table IV in which are recorded the results of several estimations of the sympathin and of adrenaline and noradrenaline in specimens of urine from pink disease in a male infant. In these experiments the sympathin was estimated in part of the extract from the ion exchange column while the remainder of the extract was treated by paper chromatography and the separated amines estimated. The figures in the Table refer to the estimated contents of the 24 hour urine specimens and not to the 100 ml. samples analysed. The agreement between the corrected estimates of the separate amines may be considered satisfactory.

## DISCUSSION

The conditions for the production and measurement of the fluorescent derivatives of adrenaline and noradrenaline in the assay of these substances described by Lund[3,4] have been modified so that equal intensities of

fluorescence are obtained from equal amounts of the two amines. Under these conditions, the estimate of the sympathin (adrenaline and noradrenaline) content of a solution is uninfluenced by the proportions of the two amines in the mixture.

The cation exchange resin, Amberlite IRC–50, has been used for the preliminary extraction of urinary sympathin in preference to aluminium hydroxide[8] or aluminium oxide[3]. If adrenaline or noradrenaline is added to the final extract from the oxide, or hydroxide of aluminium there is a considerable diminution of the expected fluorescence. Very rarely was a similar effect seen with extracts from Amberlite IRC–50 but the possible presence of substances modifying the fluorescence was checked in every analysis.

To determine the adrenaline and the noradrenaline in a urine extract, the amines were first separated by paper chromatography. This procedure avoids the interdependence of the estimates of the two amines inherent in such methods as that of Lund[4].

The recovery of adrenaline and noradrenaline added to rat or human urine was about 80 per cent when estimated as sympathin and about 50 per cent when the amines were separately determined. Some variability in the recoveries made it advisable to perform control experiments with each series of analyses and to correct the analytical results accordingly. Experimental evidence in support of this procedure has been presented.

In the estimation of sympathin in urine extracts, it has been assumed that the fluorescence stable in alkali in the absence of ascorbic acid is derived from substances other than adrenaline and noradrenaline and that only these two amines account for the additional fluorescence measured in the presence of ascorbic acid. The good agreement between the sympathin estimates and the sums of the estimates of the adrenaline and noradrenaline after separation by paper chromatography (Table IV) provides some evidence for the validity of this assumption. But the possibility of errors arising from the presence of substances reacting similarly and having similar $R_F$ values to adrenaline and noradrenaline is not thereby excluded. One such substance found in human urine is dopamine, 2-(3′:4′-dihydroxyphenyl)ethylamine[9]. With an $R_F$ of 0·44, this substance would be present in the adrenaline ($R_F$ 0·5) fraction of a paper chromatogram of a urine extract. Dopamine, however, gave rise to a fluorescence only about 0·6 per cent the intensity of that from adrenaline and its presence would lead to a significant error of the adrenaline (or sympathin) estimate only if it constituted more than 95 per cent of the mixture of catechol amines. In an experiment with an extract from normal rat urine, the adrenaline and noradrenaline fractions from a paper chromatogram were each assayed against the appropriate (−)-amine by fluorimetry and by three biological tests, namely the rat's blood pressure[5], the isolated rat's uterus[10], and the perfused isolated rabbit's ear[11]. All four estimates for each amine were in agreement within the limits of accuracy of the methods indicating an absence of gross error in the fluorimetric estimates as a result of the presence of substances reacting similarly to adrenaline and noradrenaline.

The available evidence indicates that the fluorimetric method as described gives a reasonably accurate measure of the sympathin in urine extracts obtained by the Amberlite IRC–50 adsorption technique and of the adrenaline and the noradrenaline after separation by paper chromatography.

In the determination of the "total" sympathin in urine, preliminary hydrolysis at pH 2 has been used to release that portion excreted in conjugated form[1]. Euler and Orwén[12] have found that hydrolysis at pH 0 instead of pH 2 leads to a higher estimate for the catecholamines in human urine. As they point out, however, there is no evidence that all the conjugates are split by this treatment. Thus estimates of urinary sympathin after acid hydrolysis can be considered as those of *total* sympathin only in so far as they refer to certain conditions of hydrolysis.

*Acknowledgements.* The authors wish to express their gratitude to Professor J. H. Gaddum, F.R.S. for his advice and encouragement throughout this work. One of us (W. L.) is indebted to the Government of the Union of Burma for a grant.

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