FACTORS INFLUENCING THE URINARY EXCRETION OF FREE CATECHOLAMINES IN MAN

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SUMMARY

1. The 24 h urinary excretion of noradrenaline and adrenaline is significantly lower in recumbent than ambulant normal subjects.
2. A diurnal variation exists in catecholamine excretion, the lowest values occurring during sleep.
3. Diurnal variation in sodium excretion parallels that in noradrenaline and adrenaline loss but the cycles can be separated by acute alterations in sleep/awake pattern.
4. Diurnal variation is lost during exercise, after myocardial infarction and was absent pre-operatively in a patient with a phaeochromocytoma.
5. Acute alterations in renal blood flow, diuresis, natriuresis and urinary acidification do not alter catecholamine excretion significantly.
6. Urinary catecholamine excretion is related to physical activity and wakefulness; the normal diurnal variation is easily overridden by sustained sympatho-adrenal activity. Alterations in renal function in exercise or after myocardial infarction do not account for the observed increases in catecholamine excretion.

Key words: catecholamines, diurnal variation, renal function, phaeochromocytoma, myocardial infarction.

Investigation of the activity of the sympatho-adrenal system has been limited until recent years by the lack of techniques capable of measuring small quantities of catecholamines in biological fluids. The application of fluorimetry has permitted more precise estimation of catecholamines both in tissues and fluids (Weil-Malherbe & Bone, 1952; Anton & Sayre, 1962). Determination of plasma catecholamines, however, remains difficult and handling of samples is critical for accurate and reproducible results (Carruthers, Taggart, Conway, Bates & Somerville, 1970).

Free noradrenaline and adrenaline in urine come from several sources including adrenal...
medullary tissue and sympathetic nerve endings. Catecholamines released from these sources may be taken up again by active uptake mechanisms (Iversen, 1971), may be excreted as metabolites (vanillyl-mandelic acid, phenylglycols, metanephrines and their glucuronide conjugates) or as free noradrenaline and adrenaline. The measurement of any one of these products in urine can give only partial information about sympatho-adrenal function. Nevertheless, it is generally agreed that the best single indicator of this activity is the measurement of urinary free noradrenaline and adrenaline (Euler, 1964).

Recently, alterations in catecholamine excretion have been demonstrated in human disease states. Chidsey, Braunwald & Morrow (1965) found an increased urinary excretion of noradrenaline in patients with congestive heart failure. Valori, Thomas & Shillingford (1967) investigated catecholamine excretion after myocardial infarction, confirming the original findings of Nuzum & Birchoff (1953) of high excretion rates in many patients in the first few days. It has been suggested that the incidence of ventricular arrhythmias in the post-infarction period may be associated with a high concentration of circulating noradrenaline (Jewitt, Mercer, Reid, Valori, Thomas & Shillingford, 1969).

The significance of any alteration in urinary catecholamine excretion induced by disease or drugs must be assessed from a knowledge of the normal in differing physiological situations. Several studies have been published recording normal urinary output of catecholamines and the effects of posture, exercise and diurnal changes (Euler, Hellner-Bjorkman & Orwén, 1955; Kärki, 1956; Becker & Kreuzer, 1970; Klepping, Goudonnet, Didier & Escousse, 1971). The need for such normal data prompted this investigation of further aspects of catecholamine excretion including diurnal variation and its relation to sodium excretion. The effect of acute alteration of renal function on the urinary content of noradrenaline and adrenaline was also studied.

**SUBJECTS AND METHODS**

*Normal 24 h catecholamine excretion*

*Ambulant subjects.* Sixty 24 h urine samples (collected in 15 ml of 6 M-HCl to maintain the final pH between 2 and 3) were obtained from ten normal ambulant subjects working in the hospital or laboratory.

*Recumbent subjects.* Thirty 24 h urine samples were collected in similar manner from five recumbent, convalescent orthopaedic patients receiving no drugs and studied several weeks after any surgical procedure.

*Diurnal rhythm*

*Ambulant subjects.* Urine (collected in 6 M-HCl) was obtained over consecutive 8 h periods from eight subjects. One collection in each 24 h covered the period of sleep. The subjects were ambulant and active during the other two periods. Urine was also analysed for sodium and potassium content but sodium intake was not controlled.

*Recumbent subjects.* Similar collections were made in five male convalescent orthopaedic patients who were recumbent throughout.

*Diurnal rhythm in shift-workers.* Four healthy male shift-workers (technicians at a local steel works) collected 8 h urine samples throughout two 5 day periods when working day- and night-shifts respectively. Diurnal variation in catecholamine excretion was compared with that found in sodium and potassium loss.
Diurnal rhythm in other circumstances. Diurnal rhythm in catecholamine excretion was measured in one normal subject before and during vigorous exercise (a moorland walk) through an 18 h period including the night, and in a female patient with a phaeochromocytoma before and after operation. Diurnal variation was also looked for in patients who had sustained recent myocardial infarction.

Catecholamine excretion and alteration in renal function

Catecholamine excretion and renal vasodilatation. Urinary catecholamine excretion was measured before and during the diuresis produced by 20 M.R.C. units of porcine calcitonin given intravenously to five normal subjects. Porcine calcitonin has been shown to induce renal vasodilatation and diuresis in both animals and man (Salako, Smith & Smith, 1971; Edwards & Smith, 1972).

Catecholamine excretion and diuresis provoked by diuretic drugs. Measurements were made during the diuresis induced by oral diuretics. Four normal male subjects collected urine over five 2 h periods from 08.00 to 18.00 hours on two control days. On separate occasions they then took either frusemide (40 mg), chlorothiazide (500 mg) or chlorothiazide (500 mg) together with amiloride (20 mg). All drugs were taken orally at 10.00 hours.

Catecholamine excretion after acid load. Three subjects made 1 h urine collections for 7 h on a control day and after oral NH₄Cl (0-1 g/kg). Catecholamine excretion was assessed in relation to the changes induced in urinary pH.

Methods

Urinary catecholamine determination. Estimations were carried out fluorimetrically using a modification of the automated techniques of Merrills (1963) and McCullough (1968).

Using a batch technique, catecholamines from 2 ml aliquots of urine were adsorbed on to alumina at pH 8.5 and then eluted into 0.25 M-acetic acid. Extraction was carried out in 25 ml Sterilin containers (Sterilin Ltd, Richmond, Surrey). Eluates were stored at -20°C until needed.

Catecholamines were oxidized in the autoanalyser with ferricyanide at pH 5.8 and the resulting solution made strongly alkaline with 10% (w/v) NaOH, to produce the fluorescent trihydroxyindole derivatives. Ascorbic acid stabilizes the fluorescence of both adrenaline and noradrenaline derivatives, whereas thioglycollic acid stabilizes the fluorescence of the noradrenaline derivative only. Accordingly, eluates were cycled through the autoanalyser three times: first, with ascorbic acid as stabilizer; then with ascorbic acid replaced by water (to give 'blank' values) and, finally, with thioglycollic acid as stabilizer.

Urine samples were all analysed in duplicate; the batches included two control samples to which small amounts of adrenaline and noradrenaline (25% of the expected catecholamine content) had been added as a check that no urinary substances were interfering with the intensity of fluorescence produced. All estimations were made against a range of standards that had been subjected to the same extraction procedure. In this way all our results were fully corrected for recovery and therefore appear rather higher than those reported by other workers. Using this technique it proved possible to process sixteen extractions per hour and the automated analysis had a saturation capacity of sixty samples per hour.

Other methods. Urinary pH was measured using a Pye pH meter; sodium and potassium were measured using autoanalyser techniques (Technicon).
RESULTS

Reliability of methods

The coefficient of variation of repeated determinations of single urine samples was ±7.9% for noradrenaline and ±13.7% for adrenaline. The method for urinary sodium has a coefficient of variation of ±1.5%.

Normal 24 h catecholamine excretion

Ambulant subjects. The mean values for adrenaline were 18.2 μg ± 6.6 (SD) in 24 h (n = 60, range 6.4–35.4 μg) and for noradrenaline 51.9 μg ± 14.5 (SD) in 24 h (n = 60, range 20.4–84.0 μg).

Recumbent subjects. The values for adrenaline were 7.1 μg ± 3.3 (SD) in 24 h (n = 30, range 0–12.4 μg) and for noradrenaline 29.3 μg ± 14.2 (SD) in 24 h (n = 30, range 10.4–62.2 μg).

The difference between means for ambulant and recumbent subjects for both adrenaline and noradrenaline were statistically significant (P < 0.01 in both cases).

Diurnal rhythm

Ambulant subjects. A diurnal rhythm in free catecholamine excretion was apparent both for noradrenaline and adrenaline, the smallest amounts being excreted during the period 22.00–06.00 hours, the sleep period (Fig. 1). The variation in sodium excretion paralleled that for cate-

![Graph](image1)

**Fig. 1.** Mean 8 h urinary excretion of sodium, noradrenaline and adrenaline in five normal ambulant male subjects studied over a period of 7 days. N = sleep period (22.00–06.00 hours).
Urinary catecholamines. Mean values for this series and significance of the differences are shown in Table 1.

Recumbent subjects. Although all five patients were recumbent throughout the collections the same diurnal variations in catecholamine and sodium excretion were evident (Table 2). The absolute amounts of noradrenaline and adrenaline were, however, significantly less than in the active subjects.

| Table 1. Mean urinary excretion (± SD) of noradrenaline, adrenaline and sodium over 8 h periods in eight normal ambulant subjects (N = 60 for each period) |
|-----------------|-----------------|-----------------|-----------------|
|                 | 06.00–14.00     | 14.00–22.00     | 22.00–06.00     |
|                 | hours           | hours           | hours (Sleep)   |
|                  | Noradrenaline (µg) | 24.0±11.4       | 22.4±10.0       | 12.4±6.0**     |
|                  | Adrenaline (µg)  | 7.6±3.5         | 8.1±3.5         | 3.8±1.7**      |
|                  | Sodium (mmol)    | 50.6±24.4       | 64.2±27.2*      | 43.9±17.2      |

* Significantly higher than adjacent columns (P<0.001).
** Significantly lower than both preceding columns (P<0.001).

| Table 2. Mean urinary excretion (± SD) of noradrenaline, adrenaline and sodium over 8 h periods in five recumbent orthopaedic patients (N = 30 for each period) |
|-----------------|-----------------|-----------------|-----------------|
|                 | 06.00–14.00     | 14.00–22.00     | 22.00–06.00     |
|                 | hours           | hours           | hours (Sleep)   |
|                  | Noradrenaline (µg) | 12.2±5.1        | 11.0±6.1        | 7.3±4.9**      |
|                  | Adrenaline (µg)  | 2.6±1.5         | 3.4±1.9         | 1.6±1.2*       |
|                  | Sodium (mmol)    | 55.8±25.6       | 63.9±29.5       | 26.6±16.7*     |

* Significantly lower than both preceding columns (P<0.01).
** Significantly lower than both preceding columns (P<0.001).

Diurnal rhythm in shift-workers, The same diurnal variation was found in the urinary excretion of adrenaline and noradrenaline when day shifts were being worked as in our normal ambulant subjects. The peak sodium, noradrenaline and adrenaline outputs coincided with the periods of activity in the day and all three were minimal at night. On changing to night-shift work, urinary catecholamine excretion became maximal during the first period of activity following sleep (14.00–22.00 hours) and lowest in the day when the subject was sleeping (06.00–14.00 hours). The relation between catecholamine and urinary sodium excretion was lost for the first 3 days after changing to night shifts, was re-established in days 4 and 5 and was lost again on the first 2 days on resuming day-shift work (Fig. 2).

Diurnal rhythm in other circumstances. The urinary noradrenaline output of a normal subject
showing diurnal variation in catecholamine excretion in normal circumstances, approximately doubled with a smaller rise in adrenaline, during a long-distance moorland walk that started at 23.00 hours and finished at 20.00 hours the next day. Diurnal variation was not apparent during the period of exertion (Fig. 3).

A 31-year-old woman with a phaeochromocytoma of the left adrenal and persistent hypertension showed high overall excretion of noradrenaline and adrenaline and on the two days on which 8 h collections were made, some diurnal variation was found. Following the intro-

![Diagram showing mean 8 h urinary excretion of sodium, noradrenaline and adrenaline in four normal male shift-workers working a rota of five nights followed by 5 days on duty. N, night period 22.00–06.00 hours; S, period of sleep.]

duction of phenoxybenzamine and practolol in the pre-operative 10 days, the total free catecholamine excretion continued to be very high and no consistent diurnal pattern was evident. (Phenoxybenzamine and practolol did not interfere with the determination of either noradrenaline or adrenaline in the urine by our method although the possibility that their metabolites were detected by the assay cannot be excluded.) Post-operatively, total free catecholamine excretion fell to normal with a normal diurnal variation (Fig. 4).

Catecholamine excretion was measured in nine patients within the first 5 days of admission to hospital after myocardial infarction. Mean noradrenaline excretion was 102.7 pg ± 49.1 in 24 h and mean adrenaline excretion 16.8 pg ± 17.1 in 24 h. Total 24 h excretion of both adrenaline and noradrenaline significantly exceeded that in normal recumbent patients ($P < 0.01$ in both cases). Diurnal variation in free catecholamine excretion was less obvious than in controls and differences between the mean excretion for 8 h periods were not statistically significant (Table 3).
FIG. 3. The 8 h urinary excretion of noradrenaline and adrenaline, before, during and after a prolonged period of strenuous exercise in a single normal male subject.

FIG. 4. The 8 h excretion of noradrenaline and adrenaline in a 31-year-old woman with phaeochromocytoma. ■ Night period, 22.00–06.00 hours, that coincided with sleep.
Catecholamine excretion and alteration in renal function

Renal vasodilatation. Porcine calcitonin given intravenously to four volunteers produced a considerable diuresis and natriuresis, most marked in the hour following injection. No significant alteration occurred in the urinary excretion of noradrenaline or adrenaline in the 4 h after calcitonin administration. The urinary volume, sodium loss and free catecholamine excretion for the control and the first hour after calcitonin are shown in Table 4.

### Table 3. Mean urinary excretion (±SD) of noradrenaline and adrenaline over 8 h periods in nine recumbent patients with myocardial infarction. All samples were collected within 5 days of onset (N = 22 for each period). No significant difference exists between means.

<table>
<thead>
<tr>
<th></th>
<th>06.00–14.00 hours</th>
<th>14.00–22.00 hours</th>
<th>22.00–06.00 hours (Sleep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline (μg)</td>
<td>36.4±18.3</td>
<td>35.3±24.3</td>
<td>31.0±17.5</td>
</tr>
<tr>
<td>Adrenaline (μg)</td>
<td>7.0±8.2</td>
<td>5.4±6.5</td>
<td>4.4±4.1</td>
</tr>
</tbody>
</table>

### Table 4. Mean urinary excretion (±SD) of noradrenaline, adrenaline and sodium during the 1 h period after intravenous injection of 5 ml of dextrose-saline and of 5 ml of dextrose-saline containing 20 M.R.C. units of porcine calcitonin in four normal ambulant subjects.

<table>
<thead>
<tr>
<th></th>
<th>After intravenous injection of dextrose-saline</th>
<th>After intravenous injection of dextrose–saline containing calcitonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline (μg)</td>
<td>2.0±1.0</td>
<td>3.0±1.9</td>
</tr>
<tr>
<td>Adrenaline (μg)</td>
<td>1.0±0.3</td>
<td>1.1±0.9</td>
</tr>
<tr>
<td>Sodium (mmol)</td>
<td>7.6±0.5</td>
<td>27.4±5.4*</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>64.4±27.8</td>
<td>293.8±189.4</td>
</tr>
</tbody>
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* Significantly different from control \((P<0.01)\).

Response to oral diuretics. Urinary sodium excretion was increased by all the diuretic regimes. Potassium excretion was increased by frusemide and chlorothiazide and to a lesser extent by the combination of chlorothiazide and amiloride. However, none of the diuretics produced significant changes in the urinary excretion of free catecholamines during the period of diuresis (Fig. 5).

Urinary acidification. The acidification of urine produced by an oral NH₄Cl load in three subjects was not associated with any significant alteration in urinary excretion of catecholamines (Fig. 6).
DISCUSSION

The results presented here confirm and extend those of other workers: our values for the urinary excretion of free noradrenaline and adrenaline in normal control subjects are similar to those reported by Becker & Kreuzer (1970) and Mabry & Warth (1969) and the decrease produced by recumbency has also been recorded (Euler, Luft & Sundin, 1955; Kärki, 1956).

Diurnal rhythm has been examined in the past by Kärki (1956) and Euler et al. (1955) who found a decrease in excretion of catecholamines during sleep and an increase during the day.

![Graph](image)

**Fig. 5.** Mean 2 h excretion (+SD) of sodium, potassium, noradrenaline and adrenaline by four normal ambulant male subjects. Frusemide (40 mg), chlorothiazide (500 mg) or chlorothiazide (500 mg) with amiloride (20 mg) were administered at 10.00 hours.

This cycle resembles that described for sodium, potassium and corticosteroid output (Stanbury & Thomson, 1951; Liddle, 1966) and plasma renin activity (Brown, Davies, Lever & Robertson, 1966). Diurnal variation in arterial blood pressure follows the same pattern (Bevan, Honour & Stott, 1969).

Earlier work on the relationship between sodium and water retention during the treatment of hypertension with anti-adrenergic drugs (Smith, 1965) suggested that the causal factor might be the inhibition of adrenergic activity and a connection between sodium metabolism and sympathoadrenal activity has been suggested by many authors. It was, therefore, of interest to
compare the diurnal rhythm in catecholamine excretion with that for urinary sodium and potassium. Under normal circumstances the urinary cation rhythm is similar to that in catecholamine excretion. This applies to subjects working by night and sleeping by day but only after the initial period of re-adjustment.

Our results obtained in shift-workers indicate that the two cycles become separated in the first day or two after commencing night-shift work when sodium and potassium cycles retain their original pattern, only reverting to a bigger urinary excretion during the work period after

Fig. 6. Mean urinary excretion (+ SD) of noradrenaline and adrenaline over a period of 7 h under control conditions and following oral administration of NH₄Cl (0·1 g/kg body weight) in three normal subjects. The pH of each urine sample for each subject is shown to indicate the pH range achieved.

2 or 3 days. Noradrenaline and adrenaline excretions, however, change immediately in direct relation to the alteration in periods of physical activity. A similar lag in the sodium–potassium cycle is seen on resumption of day-shift working. This, again, contrasts with the catecholamine cycle which immediately comes into phase with the periods of activity and rest.

It appears, therefore, that the diurnal rhythm in catecholamine excretion, although normally
Urinary catecholamines

parallel to that of sodium and potassium, may not be controlled by the same mechanism. It is not possible to identify fully the controlling factors but activity and wakefulness both play important roles. A similar pattern has recently been suggested for the nucleotide, adenosine 3':5'-cyclic monophosphate and its urinary excretion may also be determined in part by physical activity (Eccleston, Loose, Pullar & Sugden, 1970).

Confirmatory evidence of this hypothesis is provided by the effect of sustained physical activity throughout one night and the next day in one individual showing normal diurnal rhythm at other times. The endogenous cycle is easily overridden by physical activity.

In clinical practice urinary catecholamine excretion is commonly high in the early days after myocardial infarction. Our results show that diurnal rhythm in catecholamine excretion is lost in this condition. This may imply the existence of persistent sympathoadrenal stimulation despite minimal physical activity.

Tolson, Mason, Sachar, Hamburg, Handlon & Fishman (1965) reported some increase in catecholamine excretion in normal volunteers on the first day of admission to hospital and attributed this to psychological stress. However, in these subjects night-time excretion remained significantly different from that found during the day.

In a single patient with phaeochromocytoma, diurnal rhythm was inconsistent in the pre-operative medication period but was restored after successful removal of the tumour. In this case the total catecholamine excretion was high enough to be diagnostic. No evidence has yet been obtained from patients with adrenal medullary tumours and normal or high-normal urinary catecholamine output but it would be of interest to examine their diurnal rhythm in the hope that lack of normal variation might be of help in the diagnosis (cf. loss of the diurnal rhythm of changes in plasma cortisol concentrations in Cushing’s syndrome).

If physical activity and wakefulness are the main determinants of the diurnal rhythm in catecholamine excretion and this rhythm is lost during exercise or after myocardial infarction, it is possible that the renal handling of noradrenaline and adrenaline may be altered by changes in blood flow or urinary pH that might accompany either exercise or the low cardiac output state of myocardial infarction—although these effects are opposite in direction—i.e. reduction in renal blood flow and acidosis in myocardial infarct and increase in renal blood flow and acidosis in exercise.

Catecholamines are excreted in part by the activity of the ‘base-pump’ in the proximal renal tubule (Rennick & Quebbemann, 1970) and experiments were, therefore, performed to examine urinary catecholamine excretion during renal vasodilatation and during the acidosis induced by oral administration of NH₄Cl. Porcine calcitonin was used as a vasodilator since alterations in p-aminohippurate clearance induced by this substance were under investigation at this time (Edwards & Smith, 1972). Despite substantial increases in renal plasma flow and electrolyte excretion, urinary excretion of catecholamines was not increased. Induced acidosis also failed to affect urinary catecholamine content. It seems unlikely, therefore, that alteration in the renal handling of catecholamines can be responsible for the increases seen after exercise or myocardial infarction.

Finally, to confirm the lack of dependence of urinary sodium excretion on catecholamine excretion, previously deduced from the experiment in shift-workers, urinary catecholamine excretion was measured during the diuresis induced by frusemide, chlorothiazide and chlorothiazide with amiloride. Despite large alterations in sodium excretion, with or without potassium loss, no significant alteration occurred in catecholamine excretion. This finding
conflicts with those of Heidland & Hennemann (1969) who used intravenous diuretics to produce large and rapid effects in their subjects.

We conclude that urinary catecholamine excretion is related to physical activity and wakefulness and that the normal diurnal rhythm may be easily overridden by strenuous exercise or sustained sympathoadrenal activity. The relationship between the diurnal catecholamine rhythm and that of urinary sodium excretion is apparent rather than real and the cycles can be separated by altering the sleep/wake periods. Although catecholamines are transported by an active pump mechanism in the kidney, it is unlikely that the alterations in urinary excretion we have observed are caused by changes in the renal handling of noradrenaline and adrenaline.

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Urinary catecholamines


