Original article
Scand J Work Environ Health 1980;6(3):188-196
doi:10.5271/sjweh.2617

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Key terms: catecholamine; circadian rhythm; circadian rhythmicity; excretion rate; mercury; potassium; shift-work system; shiftwork; spot sample; unconventional shift-work system; urinary excretion; urine

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/6937823
Circadian rhythmicity of the urinary excretion of mercury, potassium and catecholamines in unconventional shift-work systems

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VOKAC Z, GUNDERSEN N, MAGNUS P, JEBENS E, BAKKA T. Circadian rhythmicity of the urinary excretion of mercury, potassium and catecholamines in unconventional shift-work systems. Scand j work environ health 6 (1980) 188-196. The round the clock urinary excretion rates of mercury were assessed for two series of unconventional patterns of activity and sleep in subjects who were not exposed to occupational, medical, or other obvious sources of mercury. In the first series the urine was collected in 3-h periods from six subjects during the first and last 2 d of a four-week, continuous 6-h shift (car ferry, watches either 0800–1400 and 2000–0200 or 1400–2000 and 0200–0800). In the second series the urine was collected in 4-h periods from five subjects working an 8-h experimental rotation shift compressed into 5 d (work two mornings — 8-h interval — work two nights — 8-h interval — work two afternoons). The mean daily excretion rate of the 11 subjects (48 investigation days, 334 urine samples) was 14.5 pmol of mercury/min (range 5.5–24.4 pmol of mercury/min). The mercury excretion oscillated regularly during 24 h by ± 20–25% of the individual's daily mean excretion rates. The peak excretion rates were found at 0652 in the first and 0642 in the second series (cosinor treatment). Due to the circadian rhythm the mean 24-h excretion rates were best represented (correlation coefficient 0.92) by analyses of urine produced around noon (spot samples, collection periods 1100–1400 and 1000–1400, respectively). The circadian oscillations of mercury excretion were not influenced by the widely different and varying activity-sleep patterns of the two series. The rhythmicity of potassium excretion (peaks at around 1400) was more irregular. The stable oscillations of mercury excretion contrasted most with the excretion of adrenaline and noradrenaline, which, without losing the basic 24-h rhythmicity, closely followed the unconventional patterns of activity and sleep.

Key terms: circadian rhythms, excretion rate, spot samples, urine.

Several reports have suggested that the variability of mercury excretion found in urine collected at different times of the day might partly be due to an inherent circadian rhythmicity of mercury elimination (2, 10, 11, 12). In order to establish a physiological basis for these assumptions, we analyzed samples of urine collected around the clock at regular time intervals from subjects who were not exposed to industrial mercury hazards. A 24-h rhythm of mercury excretion was found in spite of unconventional and varying patterns of activity and sleep of subjects who were either on a continuous 6-h shift or on an experimental 2-2-2 rotation shift. So that the degree of stability of the rhythm could be judged from another point of view, a comparison was made with the circadian variations of urinary excretion of potassium and catecholamines under the same circumstances. Finally, since mercury excretion was found to vary regularly

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0355-3140/80/030188-9
throughout the day, we have proposed a
time for the collection of spot urinary
samples that would best represent the
daily excretion rate of mercury.

**Subjects and methods**

The conclusions of this paper have been
derived from two independent investiga­
tion series.

**Series 1**

Three engineers and three stokers (median age 37 a, range 19—54 a) on a coastal car
ferry served as subjects 1—6, each during
four weeks of a continuous 6-h shift on
board the ferry. The crew was divided
into team A (watches 0800—1400 &
2000—0200) and a complementary team B
(watches 1400—2000 & 0200—0800). Two
subjects were on shift A and two others
on shift B for the whole four weeks on
board. The fifth subject started with shift
A and switched to shift B after two weeks,
while the last one began with shift B and
changed to shift A after two weeks. The
subjects were examined for 48 h when
they came on board (days 1 and 2) and
again for 48 h before they left the ship
(days 25 and 26). No dietary restrictions
were imposed on the subjects, who freely
consumed caffeine-containing beverages
but, with the exception of one subject, no
alcohol. Urine was collected around the
clock at 3-h intervals. Due to the rotation
system of the crew an observation day in
the series began at 1400 and ended at
1400 the next day. An analysis of the body
temperature reactions to this unusual shift
system has been reported elsewhere (13).

**Series 2**

Six male members of the Institute of Work
Physiology changed their habitual pattern
of life (worktime 0800—1600) for a week
into an experimental 2-2-2 rotation shift
compressed into 5 d (work two mornings —
8-h interval — work two nights — 8-h
interval — work two afternoons). They
carried out their usual work at the In­
stitute from 0600 to 1400, from 1400 to
2200, or from 2200 to 0600. They traveled
by car to and from the Institute and slept
at home where they also spent their free
time. The habitual diet of the subjects was
not restricted with the exception that they
did not consume any alcoholic or caffein­
containing beverages. Urine was collected
around the clock at 4-h intervals for the
whole duration of the experimental shift
work. An observation day (days 1—5) in
this series began at 0600 and ended at 0600
the next day. The urinary mercury ex­
cretion of five of the subjects (subjects 7—
11, median age 29 a, range 27—33 a) was
assessed and the results are presented in
this paper. The results concerning the
circadian rhythms of other physiological
parameters will be presented elsewhere.

**Urine collection and analyses**

For both series urine was collected during
the respective periods (3 or 4 h) in 1-1
plastic bottles, and the time of the last
voiding in each period, usually at its end,
was noted. The subjects had to be
awakened and stood up for 2—3 min be­
fore some of the samplings. However, no
appreciable effect of these short rises
on the trends of the circadian variations
was seen in the results. The volume of
urine voided was measured for each period
in a graduated cylinder to ± 5 ml, and
two samples of 70—80 ml each were taken
for further analyses. One sample (for the
analysis of catecholamines) was acidified
to pH 3.0 with 6N hydrochloric acid, and
both samples were then stored at —15°C.

The mercury concentration of the urine
samples was determined by a direct cold
vapor method (4) with a Laboratory Data
Control mercury monitor, model 1235. The
concentrations were calculated from in­
ternal standards.

The stability and reproducibility of the
determinations were controlled in the
following manner: Urine containing a
known amount of added mercury was
divided into 10-ml plastic bottles and
stored at —15°C. In the course of the
analysis of each series of urine samples
one of the control samples was analyzed
in the same way as the investigated
samples. The coefficient of variation of
six parallel determinations of the control
sample (about 250 nmol of mercury/l) was
2.3 %. For the assessment of day to day
variation, the daily mean values of the
control samples were noted. Their coeffi­
cient of variation during half a year
amounted to 3.1 % (N = 19). The coeffi­
cients of variation at the concentration levels of 70 and 20 nmol of mercury/l were 4.7 \% (N = 8) and 7.4 \% (N = 12), respectively.

The potassium concentrations were assessed by flame photometry (Perkin-Elmer 403) after 1:500 dilution with an ionizing buffer containing 1,000 ppm of cesium.

The catecholamine analyses (adrenaline and noradrenaline concentrations) of the acidified urine samples were made with the trihydroxyindole method and the Technicon autoanalyzer II according to Andersson et al (1).

Calculations

Using the volumes of the collected urine samples and the durations of the collection periods, we converted the concentrations of the four substances analyzed in the 334 urine samples to their contents in each collected sample, as well as to the excretion rate (per min) in each collection period. Due to the sometimes irregular timing of voiding, the collection periods differed from the nominal 3 or 4 h by up to \( \pm 20 \) min. The calculations were therefore based on the noted durations of the collection periods.

In addition the excretion rates computed for each collection period were transformed into the relative values of the percentage of deviation from the individual's daily (24 h) mean (per min) excretion rate.

The dispersion of the data was characterized by standard errors (SE) of the means as well as by coefficients of variation. The significance of the results was tested by conventional parametric statistics (t-test for paired observations, two way analysis of variance).

The best fit sine curves and the acrophases (\( \phi \)) of the rhythms were estimated by the least-square fit cosine model with a fixed 24-h period (7), an iterative computing program and mini-computer Nord 20, 16 K, Norsk Data A/S being used.

Results

Series 1

The left part of fig 1 presents the daily means of the mercury excretion rates (pmol/min) of the subjects working for the whole four weeks on schedule A (subjects 1 & 4) or on schedule B (subjects 3 & 6), as well as those subjects working on either schedule for two weeks (subjects 2 & 5). The intraindividual excretion rates were remarkably stable from day to day (day 1 to 2, and day 25 to 26), and comparatively small differences were observed between the first (days 1 & 2) and second (days 25 & 26) observation period. In contrast the levels of excretion varied from subject to subject (black columns, fig 1) by a factor of 4. Since the work conditions of both schedules were the same, the difference between the averaged excretion rates (\( \bar{x}_A \), \( \bar{x}_B \)) of shift A and shift B can be attributed to the random distribution of subjects with dissimilar excretion levels. This assumption is supported by the fact that the excretion levels of subjects 2 and 5 were the same on both schedules.

The average daily mean (\( \pm \) SE) excretion rate of the six subjects was 11.6 \( \pm \) 2.2 pmol/min. Consequently, the total urinary
excretion of mercury per 24 h was, on the average, 16.7 ± 3.2 nmol, with a range between 7.9 and 30.1 nmol/24 h (coefficient of variation 47%).

The average coefficient of variation of the mean excretion rates of mercury (pmol/min) in each of the 3-h collection periods was 47 ± 4% for shift A and 38 ± 1% for shift B. However, when the excretion rate was expressed as the percentage of deviation from the individual's daily mean, the average coefficient of variation in the 3-h collection periods decreased significantly to 20 ± 2% for shift A and to 21 ± 2% for shift B (p < 0.005 and p < 0.001, respectively). In this way the results became markedly more homogeneous in spite of the up to fourfold interindividual differences in the levels of daily mean mercury excretion. The circadian variations of the results are presented in the righthand side of fig 1.

As has been mentioned before, an observation day in this series lasted from 1400 one day to 1400 the next. In order to make the appreciation of the rhythmcity easier, the results of the two initial and two final collection periods are repeated in fig 1, to the left to 0800 and to the right to 2000 (broken lines). The horizontal bars show the corresponding positions of the work and sleep periods in the two shifts during the 24-h cycle. However, the watch changes were not as accurate during the night (nominally at 0200) as during the day. The irregular night schedule of the ferry boat sometimes enabled the subjects on shift A to go to bed earlier, around midnight, while shift B got up regularly at 0330.

The upper (A) and the middle (B) right panels of fig 1 show the averaged patterns of the 24-h variations of the excretion separately for shift A and shift B. A distinct circadian oscillation of the excretion by ± 20—25% of the mean daily values is discernible for both shifts, with peak excretions early in the morning and the lowest mercury elimination in the evening.

The circadian variation averaged over both shifts is shown in the lower right panel (A + B) of the figure, and it may be assumed that it approximates best the underlying rhythmic regulation of mercury elimination. The mean coefficient of variation of the collection periods was 21 ± 2%. The position of the acrophase of the computed sine curve that fitted the results best was found at 0652 (Ø 103°). The difference between mercury excretion in the 0500—0800 urine (peak) and the 1700—2000 urine (trough) was highly significant (p = 0.001, the coefficients of variation of the two collection periods being 27 & 22%, respectively). It is of interest that nearly the same degree of significance (p < 0.005) was found when the mercury excretion rates were expressed in picomoles per minute (coefficients of variation 54 & 49%, respectively) instead of as the percentage of deviation from the daily mean. This result indicates that the extent of the circadian variations was related to the 24-h mean levels of the mercury excretion of the subjects.

Finally, as seen in the righthand panels of fig 1, the mean 24-h mercury excretion (0% deviation) was best represented by urine collected around noon (collection period 1100—1400).

A corresponding evaluation of potassium urinary excretion under the same circumstances is given in fig 2. The intrapersonal 24-h mean excretion rates (µmol/min, left part of the figure) varied more from day to day than those of mercury. However, the interindividual, overall levels of excretion (black columns) varied much less. The average daily mean (± SE) excretion rate of potassium from the six subjects was 71.4 ± 2.1 µmol/min, ie, 102.8 ± 3.0 mmol/24 h (range 94.2—114 mmol/24 h). The coefficient of variation was only 7% as against 47% for the mercury excretion.

The average coefficients of variation for the potassium excretion rates (µmol/min) in the 3-h urine collection periods were 30 ± 2% for shift A and 40 ± 3% for shift B. In contrast to those of mercury, they decreased only insignificantly to 29 ± 3% and 33 ± 2%, respectively, when the excretion rates were expressed as the percentage of the deviation from the individual's daily means. The upper (A) and middle (B) right panels of the figure show that the extent of the circadian variation of potassium excretion was about twice as high as that of mercury. While the 24-h rhythmicity was unmistakable,
the curves of the circadian variations of potassium excretion were not as regular as those of mercury excretion, and the degree of dispersion of the values (SE) was greater.

The calculated acrophase of the average 24-h oscillation for both shifts (A + B, lower right panel, mean variation coefficient of the collection periods 33 ± 2 %) was found at 1430 (0° 217.5°). The difference between the peak and trough excretion rates was highly significant (p < 0.001).

That the apparent curve of a circadian rhythm can be deeply affected by unconventional hours of activity and sleep is demonstrated by the urinary excretion of adrenaline (fig 3), which was similar to mercury in that the intraindividual day-to-day variability of the daily mean excretion rates (ng/min, left part of the figure) was much lower than the interindividual differences in the overall levels of excretion (black columns), which varied by a factor of 3. The average daily mean (± SE) excretion rate of adrenaline from the six subjects was 9.5 ± 1.5 ng/min with a coefficient of variation of 40%. The average excretion per 24 h was 13.7 ± 2.2 μg with a range of 8.4–23.4 μg.

Again, as in the case of mercury elimination, the average coefficients of variation of the adrenaline excretion rates (ng/min) in the 3-h collection periods decreased significantly from 55 ± 2 % to 27 ± 3 % for shift A (p < 0.001) and from 51 ± 5 % to 37 ± 5 % for shift B (p < 0.005) when the excretion rates were calculated as the percentage of deviation from the individual’s daily mean values.

The righthand side of the figure shows that in both shifts (panels A and B) the amplitude of the circadian oscillation of the excretion was very high, amounting to about 75 % of the mean daily excretion rate. However, marked changes in the

Fig 2. Urinary excretion of potassium. Left: Daily means of the potassium excretion rates of the subjects working either shift A or B. Right: Averaged circadian variations of potassium excretion expressed as the percentage of deviation from the individuals’ daily mean excretion rates.

Fig 3. Urinary excretion of adrenaline. Left: Daily means of the adrenaline excretion rates of the subjects working either shift A or B. Right: Averaged circadian variations of adrenaline excretion expressed as the percentage of deviation from the individuals’ daily mean excretion rates.
levels of excretion, synchronous with the alternating periods of activity and sleep, were superimposed on the expected curve of the 24-h rhythm. In both shifts the excretion was distinctly enhanced when ever the subjects worked, and it was depressed whenever they slept. The two peaks of the average curve for both shifts (panel A + B) indicate the extrinsic effect on the expression of the 24-h rhythmicity of adrenaline elimination. An impression was thus created of a phase difference of 6 h for the two shifts. However, it is probable that for both shifts the acrophase of the basic underlying rhythm was about the same as the common computed phase 1508 (Ω 227°).

Fig 4 shows that the mode of circadian urinary excretion of noradrenaline was similar to the elimination of adrenaline. However, both the day-to-day variations (left part of the figure) and the interindividual differences (black columns) were smaller than those found for adrenaline. The average daily mean (± SE) excretion rate of noradrenaline from the six subjects was 34.3 ± 2.9 ng/min, and, accordingly, the average excretion per 24 h amounted to 49.4 ± 4.1 µg (range 38.3—61.9 µg, coefficient of variation 20%).

The comparatively low average coefficient of variation of the excretion rates (ng/min) for the 3-h collection periods of shift A changed only insignificantly from 15 ± 2% to 19 ± 3% when the excretions were expressed as the percentage of the daily means, while the higher, corresponding coefficient of shift B decreased highly significantly (p < 0.001), from 36 ± 2% to 24 ± 2%.

The amplitude of the circadian variations (right part of fig 4, panels A and B) was smaller than that of adrenaline, and the curve was, for both shifts, more distorted by enhanced excretion during work and, vice versa, by restrained excretion during sleep. The acrophase of the presumed, underlying basic rhythm probably fell again within the early afternoon hours (panel A + B, Ω, computed phase 1438, 219.5°).

Series 2

Table 1 presents the distribution of the daily mean excretion rates of mercury (pmol/min) from subjects 7—11 in the 5 d of the investigation. A twoway analysis of variance of the data confirmed that there was no significant difference between the average results obtained from day to day (\(\bar{x}_D, F_{4,16} = 0.790, p > 0.05\)). On the other hand, the average levels of the
excretion of the subjects ($\overline{X}_N$) varied by a factor of 2 (range 11.9—24.4 pmol/min), and the differences were highly significant ($F_{1,16} = 42.72, p < 0.001$). The application of fiducial limits showed that the mean levels of excretion of subjects 9 and 11 were significantly higher ($p < 0.01$) than those of the other three subjects.

The average daily mean ($\pm$ SE) excretion rate of the five subjects calculated from $\overline{X}_N$ was 18.0 $\pm$ 2.5 pmol/min, ie, 25.9 $\pm$ 3.6 nmol/24 h (range 17.1—35.1 nmol/24 h, coefficient of variation 31%). Though the mercury excretion in series 2 was, on the average, about 55% higher than that in series 1, the difference was not significant ($p > 0.05$).

The average coefficient of variation of the excretion rates (pmol/min) in the 4-h collection periods ($N = 30$) was $37 \pm 2\%$, and it decreased highly significantly ($p < 0.001$) to $19 \pm 1\%$ when the excretion was expressed as the percentage of deviation from the individuals' daily mean values. Fig 5 shows that the circadian variations of the mercury excretion were of the same order of magnitude ($\pm 20—25\%$) as they were for series 1. Obviously the rhythmicity of the excretion was not affected by the continuous daily changes of the work/free and sleep periods in the 24-h cycles. The acrophases computed separately for each day varied only slightly around 0700. The average computed acrophase of a composite curve for the 5 d was 1452 ($\phi 223\circ$). In addition, fig 5 shows that in series 2, as well as in series 1, the mercury excreted in the urine collected around noon (collection period 1000—1400) represented the best estimate of the mean 24-h excretion of the subjects ($0\%$ deviation).

It has been mentioned before that a detailed description of other parameters investigated in series 2 will be presented elsewhere. However, it may be stated that the circadian variations of potassium were affected little by the experimental shiftwork system of this series and that the computed average acrophase for the 5 d was $1452$ ($\phi 223\circ$). As in series 1, the catecholamine excretion was enhanced whenever the subjects were awake and partly suppressed whenever they slept.

In spite of the widely ranging sleep/wake cycles and different dietary habits, especially with regard to the consumption of caffeine-containing beverages, the mode of mercury excretion was essentially the same in both series, and the results can be combined. Thus, the average ($\pm$ SE) mercury excretion rate of the 11 subjects included in this study was $14.5 \pm 1.9$ pmol/min with a range of $5.5—24.4$ pmol/min and a coefficient of variation of 43%. The average mercury excretion per 24 h amounted to $20.9 \pm 2.7$ nmol (range 7.9—35.1 nmol). The excretion varied regularly during 24 h by, on the average, 20—25% of the mean daily excretion, a peak occurring around 0700. Finally, fig 6 shows the high correlation ($r = 0.92$) between the mercury excretion rates around noon (urine collection periods 1100—1400 and 1000—1400, respectively) and the daily mean rates of the subjects.
Discussion

A regular oscillation of urinary mercury excretion during 24 h was established in this study of persons nonexposed to occupational, medical, or other obvious sources of mercury. In agreement with Jacobs et al (8), it may be said that this excretion reflected the inevitable day-to-day intake of mercury from food, water, air, and other components of an essentially nonhazardous environment, as well as the body burden of mercury. Even in such an environment the amount of mercury excreted per day varied from person to person by a factor of 4. In contrast the intraindividual variations were small from day to day, as well as over periods as long as four weeks (fig 1 & table 1).

The circadian variation of the excretion (fig 1 & 5) is regarded as evidence of an inherent, internal regulatory rhythm of the urinary elimination of mercury. Though the amplitude of the circadian oscillations was, on the average, only moderately high (± 20—25 % of the mean daily excretion rates), the difference between the amount of mercury eliminated during the early morning (θ ~ 0700) and early evening hours was highly significant. The rhythm appeared to be fairly strong and firmly fixed, as is evident from the fact that the circadian variations were not appreciably affected by the unconventional, as well as changing, periods of activity and sleep that differed widely from the habitual pattern of life.

The extent of the dispersion of the mercury excretion rates in the consecutive 3- to 4-h collection periods of the urine was assessed by coefficients of variation. The average coefficient of about 40 % for the 6—8 collection periods of a 24-h cycle decreased significantly to about 20 % when the excretion rates (pmol/min) were expressed as the percentage of the deviation from the daily mean excretion rates of the subject. This result can be interpreted as evidence that the extent of a subject's circadian variation of mercury excretion was related to his individual mean excretion level. In other words, the amplitudes of the excretion rates assessed in picomoles per minute were generally higher for subjects with high mercury excretion than for subjects with low daily excretion.

In this connection it is of interest to compare the pattern of mercury elimination with the patterns of excretion of the other substances that were investigated in this study. As to the urinary elimination of potassium, the interindividual differences in the daily mean excretion (fig 2) were smaller than those of mercury excretion. On the other hand, the intraindividual day-to-day variations were greater, and the average coefficient of variation for the collection periods decreased only insignificantly when the excretion rates were expressed as the percentage of deviation from the daily mean excretion. Although the average amplitude of the rhythm of potassium excretion was higher than that of mercury, the comparatively high dispersion of the relative values of the excretion indicates a greater heterogeneity of the excretory rhythmicity. This finding is in agreement with those of other studies, which demonstrated that the excretion may be directly influenced by the amount of ingested potassium, that the peak excretion usually occurring at or shortly after midday may appear much later (as for one of our subjects in series 1), and that there is a great variability in the extent to which the basic rhythmicity of the excretion may be overruled by unconventional habits of life (3).

The catecholamine excretion (fig 3 & 4) measured in the present study presented a good example of such an overriding of a basically rhythmic elimination. Our results were similar to those from another investigation (9) in showing that the daily mean excretion of adrenaline and noradrenaline varied markedly from person to person but remained fairly stable under the same circumstances. The average dispersion of the results obtained throughout the 24-h cycles diminished significantly to one-half when the excretion rates were expressed as the percentage of the mean daily elimination. As in the case of mercury, this result indicates that the direction and the relative extent of the changes of the excretion rates during 24 h were fairly homogeneous for subjects living under the same conditions.

A regular and distinct circadian rhythm of adrenaline excretion with a crest (θ) shortly after midday was reported in a cross-sectional study of subjects who were
in bed for 24 h and slept only at night (5), as well as in a longitudinal study of subjects who did not sleep at all and were active for 75 h (6). Our investigation showed that the expected curve of the circadian rhythm of adrenaline excretion was heavily distorted by the unconventional patterns of activity and sleep. A markedly higher than expected excretion was always superimposed on the recognizable background of the basic circadian variation of adrenaline elimination whenever the subjects were awake and active, while a lower than expected excretion was recorded whenever they slept. A similar overriding of the circadian variation was also found for the excretion of noradrenaline.

The contrasting stability of the circadian variation of mercury excretion under the same circumstances provides an opportunity to simplify the assessment of the daily excretion of mercury in nonoccupationally exposed subjects. Since the widely different excretion rates assessed in urine samples taken around midday correlated with the mean 24-h excretion (fig 6), daily excretion can be fairly well estimated from spot samples of urine collected around noon. However, it is necessary to point out that the assessment of mercury concentration alone is not sufficient for such a purpose, the concentration of any substance in the voided urine being related to the urine flow. Thus, both the volume of the urine produced during such a spot sampling and the duration of the collection period (about 3—4 h) must be recorded for the calculation of the excretion rates.

Since most published papers dealing with mercury elimination are based solely on the concentration of mercury in urine, it is uncertain how far the mercury excretion in exposed industrial workers may be affected by the described circadian rhythm. The small amount of data on excretion rates available in the literature (11, 12) suggests that such a possibility exists.

Acknowledgments

We wish to thank Ms A Bolling, Ms M Bull, Ms I Liaaen and Mr H Olsen for their skilled technical assistance, Ms S Kurseth for typing the manuscript, and Mr M Vokac for programming the calculations of urine analyses on the TI-59 100B calculator.

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Received for publication: 25 February 1980