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Creatinine Analysis in Single Collection Urine Specimens

REFERENCE: Needleman, S. B., Porvaznik, M., and Ander, D., **"Creatinine Analysis in Single Collection Urine Specimens,"** *Journal of Forensic Sciences*, JFSCA Vol. 37, No. 4, July 1992, pp. 1125–1133.

ABSTRACT: Controlled and uncontrolled fluid intake studies were conducted on series of volunteers over the 6 or 12 h of the study periods. Urine specimens were obtained from each subject randomly or at specified times relative to fluid ingestion. Creatinine analysis performed by a modification of the Abbott TDx procedure demonstrates that the values obtained from single collection specimens fall almost in the same range as the values from 24 h pooled collection specimens. The creatinine concentration can be used to indicate possible adulteration of urine specimens by dilution as a means of avoiding detection of use of drugs of abuse.

Between 4 and 7 h are required for a decrease in creatinine concentration to about 100 mg/dL from an initial mean of about 170 mg/dL. A minimum of 6 h is needed for any creatinine value to fall to 50 mg/dL or less. Thus, it appears that creatinine output is sensitive to the amount of fluid ingested, but the relationship is neither linear nor immediate.

The absence of a significant creatinine concentration in a specimen can be used as an indication of direct or indirect adulteration of the urine specimen by dilution or replacement with water. At NDSL-Great Lakes, a decline of the creatinine concentration to 30 mg/dL is used as a cutoff for differentiating between urine specimens that might have been tampered with to avoid detection of drug use and those specimens that are dilute for other reasons. Values at 10 mg/dL or less are suggestive of replacement by water. The information is provided to local commands for investigation prior to initiation of punitive action by the command.

KEYWORDS: pathology and biology, creatinine, urine, urine adulteration, single collection specimen, dilution, fluid-intake volume

A common practice to escape detection for drugs of abuse is to increase fluid intake prior to providing a specimen for drug urinalysis. The ingestion of large volumes of fluid is believed by some to sufficiently dilute the concentration of drug in the bladder to a point below that level determined by the testing laboratory to be called positive.

Technicians at the Navy Drug Screening Laboratory at Great Lakes, IL observe each specimen, as it is poured for testing, for unusual characteristics that might indicate adulteration or dilution. If a specimen is believed to be diluted based on lack of apparent odor and the clear and colorless appearance of the specimen, an analyst will check the specific gravity of the specimen, and perform a creatinine assay. The normal value for the specific gravity for a single collection urine specimen is 1.003 to 1.030 [*1*]. The

Received for publication 3 Sept. 1991; revised manuscript received 26 Oct. 1991; accepted for publication 18 Nov. 1991.

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creatinine concentration in a 24 h pooled urine will be approximately 0.80 to 1.70 g/mL for women and 1.00 to 1.90 g/mL for men [2]. Because the amount of creatinine formed is proportional to the muscle mass of an individual, the production rate will be dependent on both age and sex. Because creatinine is excreted at a relatively constant rate, as long as kidney function is not impaired, its measurement in urine for any individual should indicate whether or not the urinary concentration has been 'adjusted' by dilution either in vivo or in vitro.

Most methods for the measurement of creatinine in urine are based either on the Jaffe reaction between creatinine and picrate in alkaline solution [3] or on an enzymatic reaction [4]. Both methods have some drawbacks either from specificity or interference, or both.

The TDx REA Creatinine Assay (REA is Abbott Laboratories' name for the adsorption of fluorescent emission technique) was used in the present study [5]. The chromogen produced during the reaction between creatinine and alkaline picrate overlaps the excitation of the fluorescence signal [5]. The concentration of the specimen's creatinine is directly proportional to the amount of chromophore present [5].

It is often suggested that extensive dilution of urine may be achieved by ingesting large quantities of fluid prior to providing the specimen for analysis, or by using diuretics. In practice, severe physiologic considerations limit the actual utility of either approach—the quantity of fluid that can be ingested in a discrete period of time together with the time required for that fluid to clear the body limits the degree of dilution that can be achieved through exaggerated ingestion. Diuretics are limited by the increased output that can be promoted by such drugs.

The present study was undertaken to determine if the collection of single specimens for creatinine determination could be used to predict in vitro dilution of urine specimens by water and possible adulteration by dilution in vivo of the specimen as a means of confounding the laboratory findings for drugs of abuse.

Methods

Creatinine Analysis

Creatinine analysis was performed on the Abbott TDx (Abbott Laboratories, North Chicago, IL) using calibrators/controls prepared in the NDSL laboratory. The seven calibrators ranged from 25 to 300 mg/dL creatinine prepared in Abbott TDx Dilution Buffer (#9519) (bovine gamma globulin in phosphate buffer), together with a zero control consisting of Abbott TDx buffer. Calibrators and specimens were diluted 1:100 to a final volume of 0.5 mL. The excitation peak was at 485 nm and fluorescence was measured at 525 to 550 nm. Creatinine was obtained from Baker Chemical Co.

Normal Range

For the establishment of the normal urine creatinine range, 350 single collection random urine specimens were obtained from Navy recruit populations at different times of the day and on all days of the week.

Urinary Dilution

Fourteen volunteers were instructed to ingest fluids (water, beverages, soup, etc.) (no alcohol) over a 12 h period beginning upon awakening in the morning. The nature of the fluid ingested, the frequency of ingestion and the quantity taken was unrestricted except that the first ingestion had to occur within 15 min of rising in the morning. The quantity of fluid intake, as well as the volume of urinary output during the same period

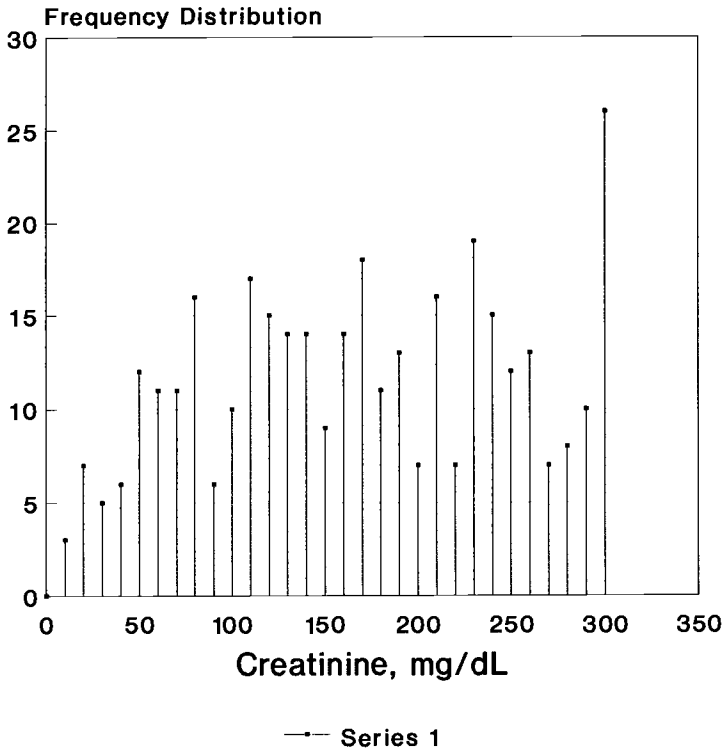
was measured and an aliquot of each voiding was taken for analysis of creatinine. Voidings were not tied to periods of intake.

In a second study, volunteers were assigned to one of two study groups, those in Group A ingesting 125 mL (low) of water hourly, and those in Group B ingesting 250 mL (high) of water on the same schedule. Fluid intake was restricted to water; no food was permitted over the 6 h study interval. A urinary voiding had to take place (regardless of volume produced) immediately following each water ingestion. The urinary output was measured and an aliquot taken for creatinine analysis.

Results

Normal Range

The distribution of creatinine values obtained for the 350 specimens is given in Fig. 1. Fifty-one of these (14.6%) fell within the range accepted as normal in general medical standard tables for 24 h urine collections, 130 to 160 mg/dL [6]. A total of 329 specimens (94%) had creatinine values greater than 50 mg/dL and 340 (97.1%) had values greater than 30 mg/dL. Twenty-six specimens had creatinine values of at least 300 mg/dL. Values greater than 300 mg/dL were reported only as 300 mg/dL. The mean creatinine concentration for the 350 specimens was 171.6 mg/dL with a standard deviation of 80.7 and a



(a). Based on 350 specimens

FIG. 1—*Creatinine normal range for study population.*

median of 173 mg/dL. Slightly more than 57% of the population fell within ± 1 S.D. and all but three individuals fell within ± 2 S.D. of the mean.

In Fig. 2 is shown the linearity of the NDSL controls when the actual creatinine values obtained are plotted against the theoretical values for these calibrators. The correlation coefficient r was 0.9999. Each assay point represents the average of ten independent assays.

Fluid Ingestion

In the uncontrolled fluid ingestion study, nine of the 14 volunteers had a fluid intake of 643 to 1858 mL over the 12 h study period. They had void volumes of 408 to 1830 mL during the same period. At the conclusion of the study, these volunteers were judged to be low level drinkers. The other five volunteers were classified as being high level drinkers based on their fluid intake of between 2003 and 6059 mL over the same period. These subjects had void volumes between 700 and 3750 mL. Among all subjects in the study, there was a 9-fold difference between the lowest and highest amount of fluid ingested. The individual fluid intake and urinary output is summarized in Table 1.

The fluid intake and urinary output for subjects in the second dilution study also is summarized in Table 1.

The creatinine values obtained for each specimen aliquot in the uncontrolled and controlled study groups are presented in Figs. 3 through 6. The specific gravity, pH and color of each specimen is given in Table 2.

In Fig. 3 are shown the creatinine values obtained for three subjects who ingested high levels of fluid over the 12 h of the study period. In each case, the creatinine dropped in about 2 h to a level of about 50 to 60 mg/dL and remained at that level for the remainder of the study. A drop in creatinine output to about the same level occurred for two subjects with moderate fluid intake after four hours and then remained low for the duration of the study. The results are seen in Fig. 4.

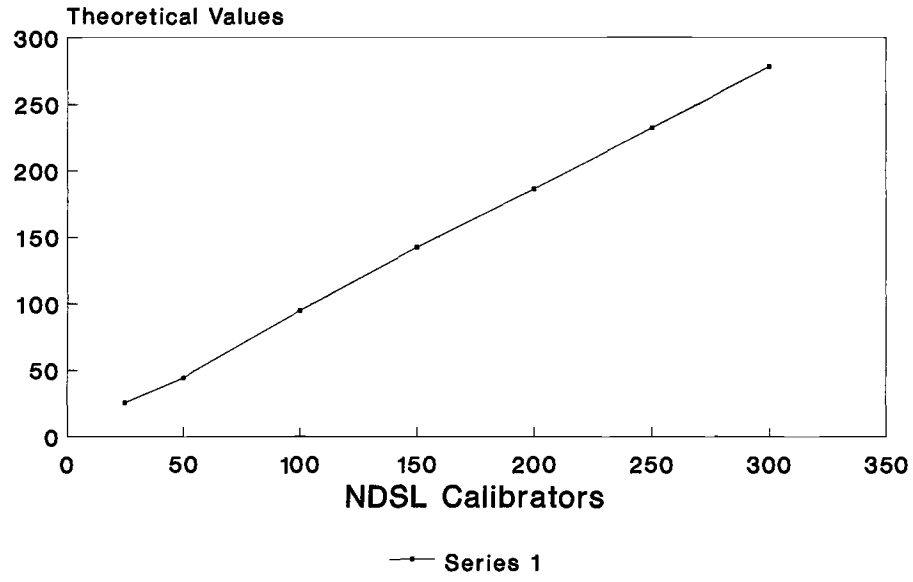


FIG. 2—*Linearity of NDSL controls.*

TABLE 1—*Fluid intake/output in urine dilution studies over 12 h study period.*

Subject	Uncontrolled Phase			Controlled Phase	
	Fluid Intake mL	Designation	Urinary Output mL	Fluid Intake mL	Urinary Output mL
A	6059	High	3750	750	100
B	3433	High	2216		
C	1745	Low	495	750	1155
D	2003	High	1392	1000	1420
E	1575	Low	1760	875	730
F	1560	Low	1450		
G	643	Low	1830		
H	1440	Low	1300		
I	960	Low	1200		
J	1858	Low	1080		
K	2061	High	2116	750	808
L	850	Low	1150		
M	2746	High	700	875	1825
N	1107	Low	408		

TABLE 2—*Specific gravity, pH, and color of urine specimens.*[a]

Subject	Uncontrolled Phase					Controlled Phase		
	Number Specimens	Specific Gravity		pH		Number Specimens	Specific Gravity	
		Initial	Final	Initial	Final		Initial	Final
A	12	1.020	1.002	6.5	6.0	5	1.024	1.006
B	10	1.022	1.002	5.0	5.0			
C	5	1.019	1.024	6.0	6.0	7	1.014	1.004
D	8	1.030	1.002	5.0	5.0	7	1.030	1.003
E	8	1.022	1.016	6.0	5.0	6	1.016	1.010
F	5	1.019	1.007	5.5	6.0			
G	6	1.022	1.021	7.0	6.0			
H	5	1.020	1.004	5.0	6.5			
I	5	1.030	1.004	5.0	7.0			
J	7	1.023	1.002	5.0	5.0			
K	7	1.018	1.006	6.0	5.0	8	1.030	1.009
L	4	1.008	1.022	6.0	6.0			
M	5	1.022	1.003	5.0	6.0	7	1.017	1.009
N	4	1.021	1.007	6.0	5.0			

[a]In each case the initial color was dark yellow and the final color was straw.

The creatinine output for four of the subjects who ingested lower quantities of fluid per hour is seen in Fig. 5. With one exception, the creatinine output did not begin to drop until near the end of the study period. The creatinine pattern, seen in Fig. 6 for the final four subjects in the low intake group, is erratic and does not show the drop seen in the other three categories (Figs. 3–5).

In Fig. 7 is shown the creatinine level for each void following ingestion of 125 mL of water per hour by subjects of Group A. A drop to about 50 mg/dL occurred after 2 h. The creatinine value for two of the three subjects in the 250 mL group (Group B) dropped to about 50 mg/dL after 3 h. In one case, the creatinine output dropped slowly over a period of 5 h. This is summarized in Fig. 8.

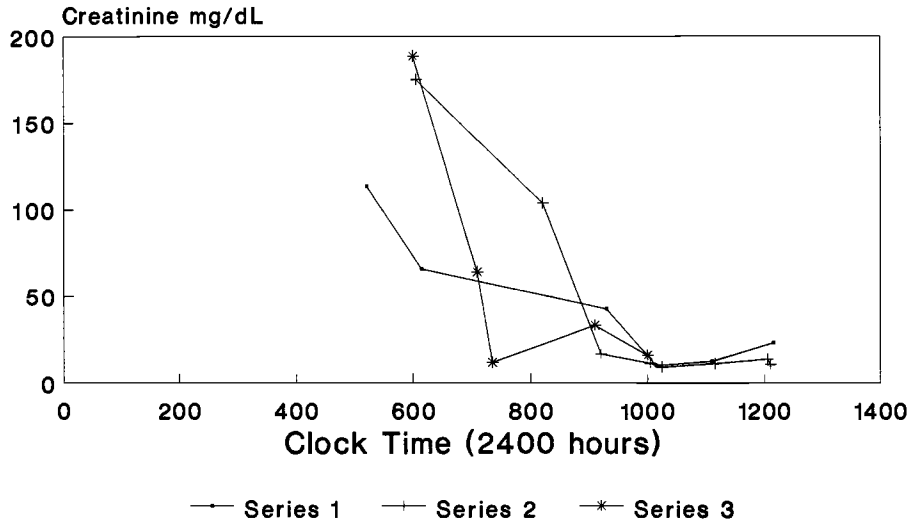


FIG. 3—*Rapid dilution. Group 1—High level ingestion.*

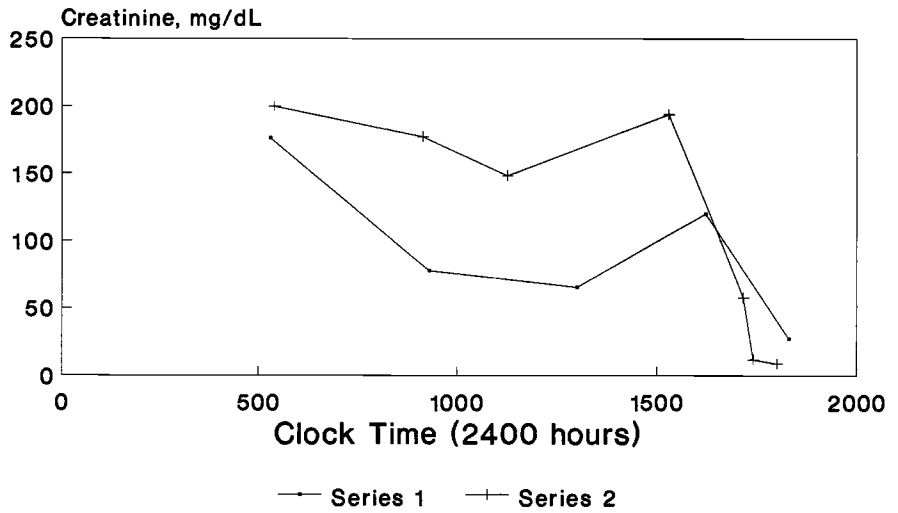


FIG. 4—*Moderate dilution. Group 1—High level ingestion.*

Discussion

The Abbott controls/calibrators for creatinine contain albumin. In our hands, these reagents have resulted in very low but definite creatinine values for buffer blanks when assayed against similar reagents prepared in our laboratory, in phosphate buffer, without albumin. Accordingly, laboratory-made reagents were used throughout this study, although Abbott TDx reagents were used in other parts of the study as secondary controls.

The analytical procedure used in this study is a variant of the typical alkaline picrate assay for creatinine. A general method for fluorescence polarization immunoassays of simple analytes on the TDx with no hardware changes to the instrument has appeared

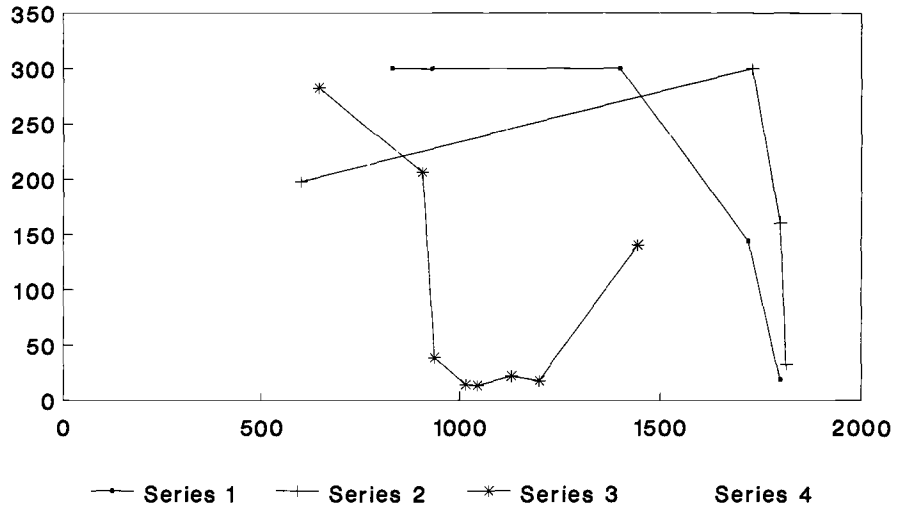


FIG. 5—*Minor dilution. Group 2—Low level ingestion.*

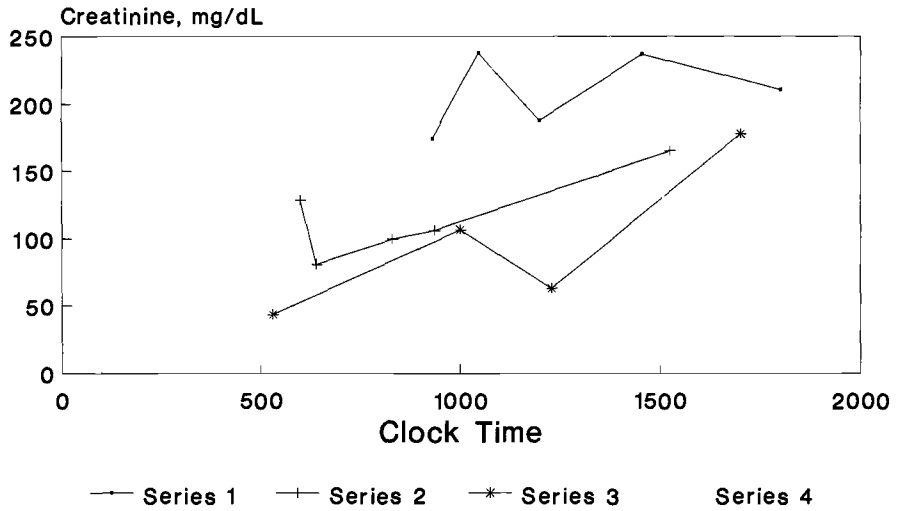


FIG. 6—*No dilution. Group 2—Low level ingestion.*

in the literature [7]. The use of fluorescein and quantitation by means of fluorescence provides the novelty of the current assay.

The 'normal range' for creatinine obtained from analysis of the 350 single collection specimens showed strong coincidence with the values generally reported for 24 h pooled collection specimens. In our experience, first-void specimens have a somewhat higher creatinine level than do specimens obtained later in the day under normal intake/output conditions. The amount of creatinine excreted varies between individuals, but for each individual, the daily output is almost constant, with a variation reported not greater than 10 to 15% under ordinary assay circumstances [7]. In general, the variation, however, is not so great as to negate use of any single collection specimen to assess possible tampering with the specimen to avoid detection of drug use.

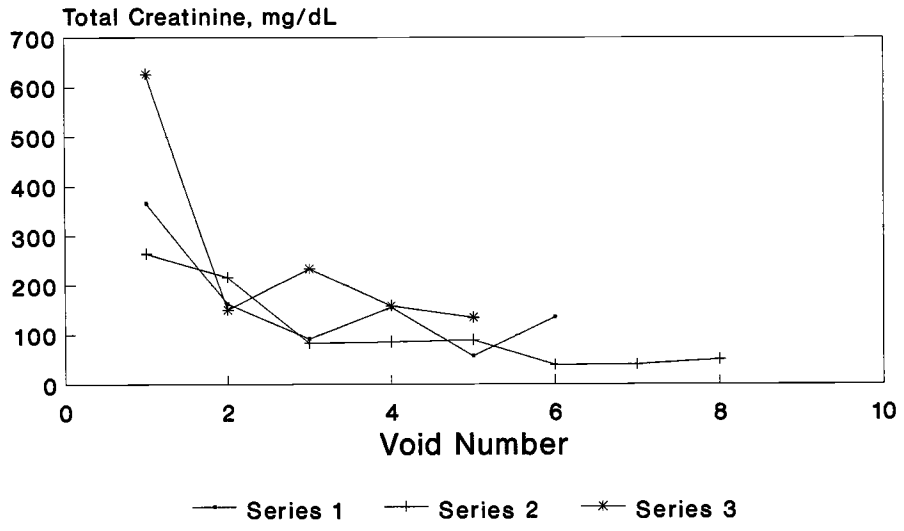


FIG. 7—*Creatinine excretion per void. Group A—125 mL ingestion.*

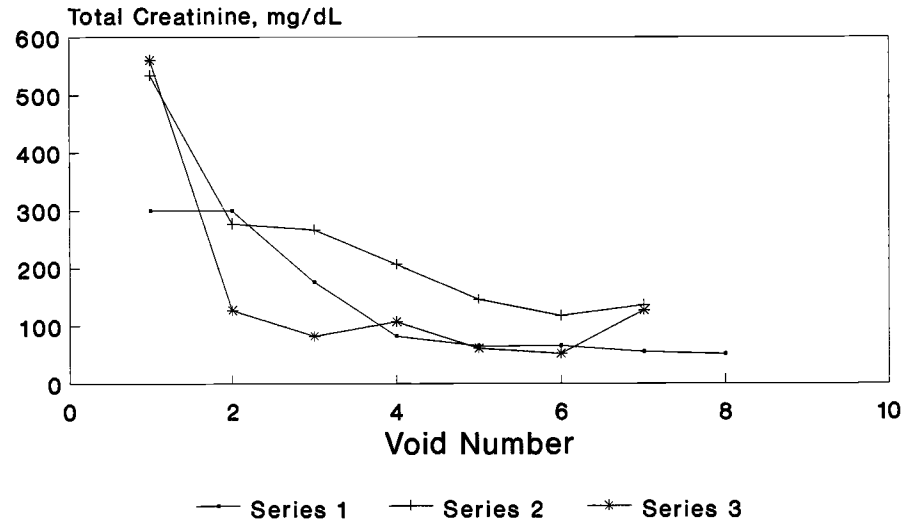


FIG. 8—*Creatinine Excretion per void. Group B—250 mL ingestion.*

The uncontrolled fluid intake study conditions paralleled the usual intake/output conditions for the subjects. The fact that among those subjects drinking large volumes of fluid there was almost a two to one disparity between fluid intake and void output suggests that the body does not process large quantities of fluid within a short time after intake. Several hours were required to evidence a fall in creatinine level to about 50 mg/dL for most subjects. None showed a very rapid drop in their creatinine output within a short time (for example, 1 h) of ingesting fluid. For the low intake group, the void volume more nearly matched the intake volume. However, even among these subjects, the drop in creatinine level did not occur in less than two hours and in some cases took as much as five hours. It would appear from these data that the body does not process excess fluid as efficiently as it does more nominal levels of fluid.

In the parallel, controlled part of the study, four to seven hours were required to reach creatinine values of about 100 mg/dL and a minimum of six hours for any of the creatinine values to reach 50 mg/dL or lower. Thus, it appears that the creatinine output is sensitive to the amount of fluid ingested but the relationship is neither linear nor immediate. The absence of a significant creatinine level in a specimen then can be used as a clear indication of tampering with the specimen either by direct replacement with water or, at least, of some attempt to adulterate the specimen by internal or external dilution.

At the Navy Drug Screening Laboratory-Great Lakes, a creatinine value of 30 mg/dL has been used as the cutoff for differentiating between urine specimens that have been tampered with in order to avoid detection of drug use and those specimens which are dilute (but 'natural') for other reasons. The high degree of correspondence between the normal distribution curve for creatinine reported in the medical literature for 24 h collection specimens and the curve obtained here for single collection specimens strongly suggests that single collection specimen values are useful in predicting urine adulteration. Although the lower limit for 24 h collection specimens is about 130 mg/dL, allowances for possible natural dilution of urine—and of its creatinine content—have suggested that a range of 90 to 100 mg/dL might be a reasonable cutoff value signaling tampering. Nevertheless, in order to safeguard individuals who might have dilute urine for other reasons, we have elected to lower still further the cutoff value used in this laboratory to 30 mg/dL to indicate adulteration. When this value is taken together with lack of specimen color, lack of typical urine (or any) odor and a specific gravity 1.0000, we have further defined our system to reporting 'suggestive of adulteration with water' for creatinine assay values between 10 and 30 mg/dL and 'appears to be water' at values of 10 mg/dL and below. Reports of analytical results to client commands ask that the command contact the laboratory for an explanation of the findings prior to initiating disciplinary action against the specimen donor for alleged use of drugs of abuse.

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