If one seeks to express in a single word Dorothy Jordan Lloyd's predominant characteristic, that word is 'fearlessness'. It was in this spirit that she tackled the problems of an established industry—some of them so difficult to formulate as hardly to be thought susceptible to scientific attack. This characteristic also dominated her choice of relaxation. She was a notable mountaineer, and in later years a rider of steeplechasers. Her vigour in these pursuits, and in her equally adventurous intellectual excursions, will always be remembered by those who were privileged to enjoy them with her. She will be equally missed by her scientific colleagues, her friends in industry, and her partners in the pastimes she loved.

E. C. BATE-SMITH

Creatine and Creatinine Excretion in Women

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Although references to a possible relation between urinary creatine and/or creatinine and the female sexual cycle have appeared in the literature during the last thirty years, the existence of such a relation still remains far from certain. The data of Krause & Cramer (1910, 1911) suggested that the onset of menstruation is associated with a creatinuria, but Rose (1917), Stearns & Lewis (1921), Hodgson & Lewis (1928), Hyde (1942) and others have been unable to confirm this. Smith (1942) has observed the appearance of creatinuria during menstrual flow and has claimed, in addition, to observe cyclic variations in the excretion of creatinine. In view of the connexion between sex and creatine metabolism, as exemplified by the occurrence of creatinuria with avitaminosis-E, the pronounced creatinuria of late pregnancy and puerperium, the effects produced by administered androgens on creatine excretion in children, etc., a recognizable correlation of this kind in the non-pregnant female would obviously be of interest. The following observations on physiological creatine and creatinine excretion have been carried out with a view to supplying more data on this possible relationship.

METHODS

Choice of subjects. Four female subjects provided daily 24 hr. urine samples for unbroken periods of 60–70 days. For comparative purposes, two male subjects were similarly studied, one for 23 days, the other for two periods of 16 and 40 days respectively. Details of the daily dietary constituents, including number of meat meals, excess water and carbohydrate above normal ingested, were recorded, together with an account of any exercise above normal taken. Single 24 hr. urines were provided by ten other male subjects, and ten female subjects provided samples over menstrual periods only. All subjects were within the age group 19–26 years and were resident members of a student hostel, living under closely similar conditions and subject to the same dietary variations. The diet was a normal one without supplementation, and of moderate protein content (total urinary N values ranged from 8·47 to 12·02 g./day).

Estimation of creatine and creatinine. Urine samples were preserved with toluene and analyzed immediately or kept at the longest 2 days stored at 4°. Creatinine was estimated by a modification of the usual procedure, with a Spekker absorptiometer with Ilford 604 filters. Creatine was converted to creatinine by the boiling method of Folin (1914), since the increased pigmentation of urine occurring in dehydration methods which involve autoclaving with picric or mineral acids gives high colour blanks, equivalent to up to 150 mg. creatine/l. This blank value, although it is a reproducible quantity and can be accurately allowed for, is undesirable since it is comparable with the actual amounts of creatine found to be present in some normal urines. Folin's original method gives a colour blank equivalent to less than 40 mg. creatine/l., and when correction for such a blank value is made, urinary creatine may be satisfactorily estimated down to concentrations of 20–30 $\pm$5 mg./l.

RESULTS

Creatine excretion. In the urine of some 50 % of the female subjects studied, creatine was found to be occasionally present in amounts up to 150 mg./day, the daily excretion being generally in the region of 50 mg. No trace of creatine was found in the urine of any of the male subjects. The daily urinary creatine excretions of the four female subjects B–E are shown in Fig. 1. Only subject C (age 20, period of investigation 62 days) showed any extensive creatine excretion, the total weekly outputs being as follows: *0*, 123, 117, *161*, 66, 0, *77*, 276, 73 mg. The values in italics are those for weeks during which menstruation took place. Out of the eleven menstrual periods covered, two were associated with creatinuria, one with premenstrual creatinuria, one with a fairly continuous creatine excretion and the remaining seven with absence of creatinuria. The results for the second group of ten female subjects covering fourteen menstrual periods (seventy determinations) showed four associated with creati-

nuria and ten with its absence. In one of these subjects daily determinations made over a 45-day period showed a fluctuating creatinuria, ranging from 0 to 94 mg./day with an average level of *c.* 50 mg./day. No obvious or consistent relationship between creatine excretion, whenever it appeared in these two groups of subjects, and any phase of the menstrual cycle is apparent.

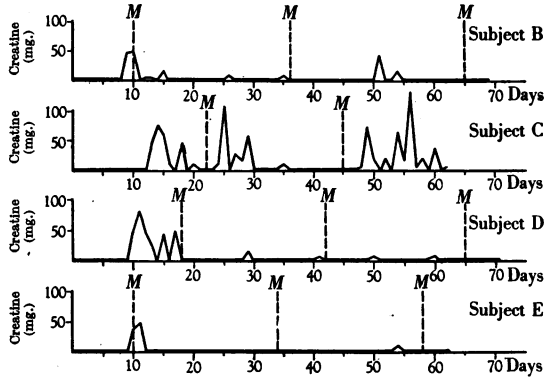

Fig. 1. Daily creatine excretion in subjects B–E. *M* signifies onset of menstruation.

Creatinine excretion. The daily creatinine excretion curves of the four female and two male subjects studied showed similarities both in the extent and frequency of the day to day fluctuations, and one typical graphical record only is therefore given (Fig. 2*a*), the data for the six subjects being summarized in Table 1.

Daily variations in creatinine output in men and women may be quite marked, and have been referred to by Cameron (1933), Espersen & Thomsen (1937), Dill & Horvath (1941), Wilkins, Fleischmann & Block (1941) and others, fluctuations of up to 25% of mean 24 hr. outputs being recorded. These observed variations undoubtedly represent the sum of a number of independent variations, each attributable to a separate source, and their consideration is necessary if a further cyclic variation of menstrual origin is to be established. Of the fluctuations which might exert a major effect on total excretion level the following have been considered: (*a*) a weekly periodicity resulting from a regular weekly environmental pattern, (*b*) irregular short term fluctuations caused by physiological compensatory mechanisms as a result of exercise, etc., and

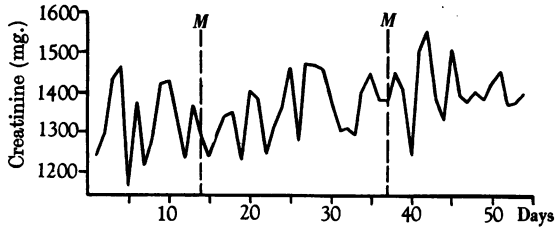

Fig. 2*a*. Daily creatinine excretion in subject C. *M* signifies onset of menstruation.

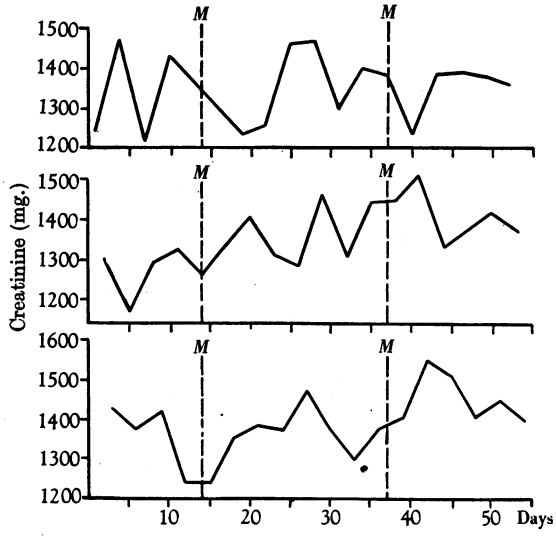

Fig. 2*b*. Creatinine excretion in subject C every third day. *M* signifies onset of menstruation.

(*c*) systematic trends due to growth, seasonal activities, etc. A periodic variation of type (*a*) might be expected in subjects on a hostel or hospital regimen with a fairly fixed weekly routine, and was looked for in the data of the four female subjects (the experimental periods of the two males not being

Table 1. *Creatinine excretion in male and female subjects*

Subject	Age	Period of study (days)	Average daily creatinine (mg.)	Maximum range of variation in excretion (mg.)	Maximum daily variation (mg.)
B, female	19	67	1306±102	421	391
C, female	20	62	1360± 90	382	298
D, female	26	70	1429± 84	330	233
E, female	19	62	1250± 80	394	253
A, male	19	40	1385±101	495	367
M, male	24	23	1866± 88	421	391

long enough to warrant detailed analysis of their data). The 24 hr. excretion values of each subject have been examined for variation between different days of the week and between weeks, by tabulation into columns of seven figures corresponding to the days of the week with omission of all values not covering a complete week. The results are given in Table 2.

Table 2. *Variation in creatinine excretion in four female subjects*

(Based on daily outputs in mg.)

Source of variation	Mean squares (degrees of freedom in brackets) B	C	D	E
Days	72633 (6)	21001 (6)	11084 (6)	11783 (6)
Weeks	105912 (6)	10335 (6)	17411 (6)	14432 (6)
Residual	342338 (36)	4613 (36)	4439 (43)	4448 (36)

The three subjects C–E exhibit mean squares for different days of the week significantly greater than the corresponding residual mean squares, which are measures of random variation. This suggests that some factor, dietary or environmental, is operating to produce a variation in excretion associated with the days of the week. In the case of subject B, however, the very large residual variation obscures any day of the week effect on the basis of an analysis of variance. The conclusion that the level of creatinine excretion is related to the days of the week is borne out by the curves in Fig. 3, which are plots of the mean excretion values for the different days of the week calculated as percentages of the average daily excretion over the whole experimental period, thus expressing the different sets of results in comparable terms. The marked rise and fall in all four curves points to a significant periodicity and further suggests that the same factor producing this effect is operable in subject B despite the greater random variations, since the curve for B clearly resembles those of the other three subjects.

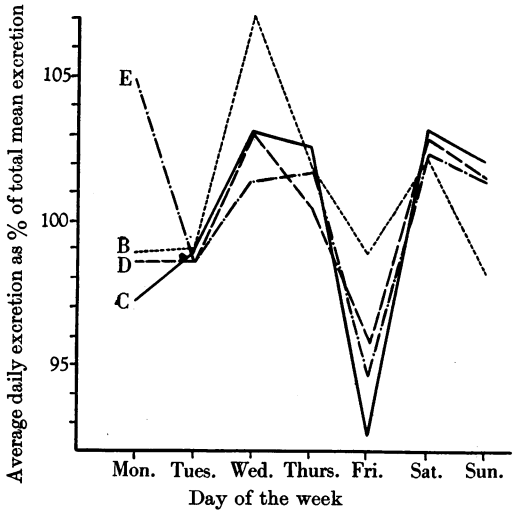

Fig. 3. Average daily excretion of creatinine in four female subjects.

Irregular short-term fluctuations with their probable compensatory characteristics are considered to be the major cause of deviations from the mean excretion level, but an interpretation of data minimizing or eliminating their effects might reveal the existence of more fundamental phenomena. Such a method of interpretation has been employed, although deductions from it are not necessarily significant owing to the difficulty of obtaining continuous data for a large number of subjects over many menstrual cycles.

The average creatinine outputs over the periods 1st–7th days, 2nd–8th days, 3rd–9th days, etc., have been computed, giving the mean daily excretion for weeks centred on days 4, 5, 6, etc., and these values have been plotted against the numbers of the middle days of the corresponding 7-day period. Each value is an average of one each of the days of the week so that the effects of both a weekly periodicity and sudden but compensatory variations occurring within the particular 7-day period will be largely eliminated. Further, each original daily excretion figure appears seven times in the moving averages so that any uncompensated variations will be spread out over periods of 3 days on either side of the day of their actual occurrence. This will effectively smooth out the large short-term fluctuations, but should not markedly affect a long-term periodicity such as a menstrual one. In addition, a plot of these 7-day moving averages should bring out steady trends in excretion level.

This treatment has been applied to the data for subjects B–E and male subject A and shows the females to give rise to two distinct types of excretion pattern. The curves for subjects C and D closely resemble one another, as do those of subjects B and E (Fig. 4). C and D exhibit a periodic rise and fall in daily output together with a pronounced tendency for the daily output to increase steadily with time. This general trend is represented by the sloping lines on the diagrams for these subjects. B and E show a less distinct periodicity, as far as can be judged

from the available data, and any trend in output is not so apparent. The general level of 7-day moving average excretion is given by the approximately horizontal broken lines on the diagrams. If we relate the curves to the occurrence of the menstrual phenomena, of the eight periods now falling within the period of investigation, five are associated with a fall in excretion level while three are associated with a rise. In six out of seven instances a maintained rise is apparent at the 14th day of the menstrual cycle.

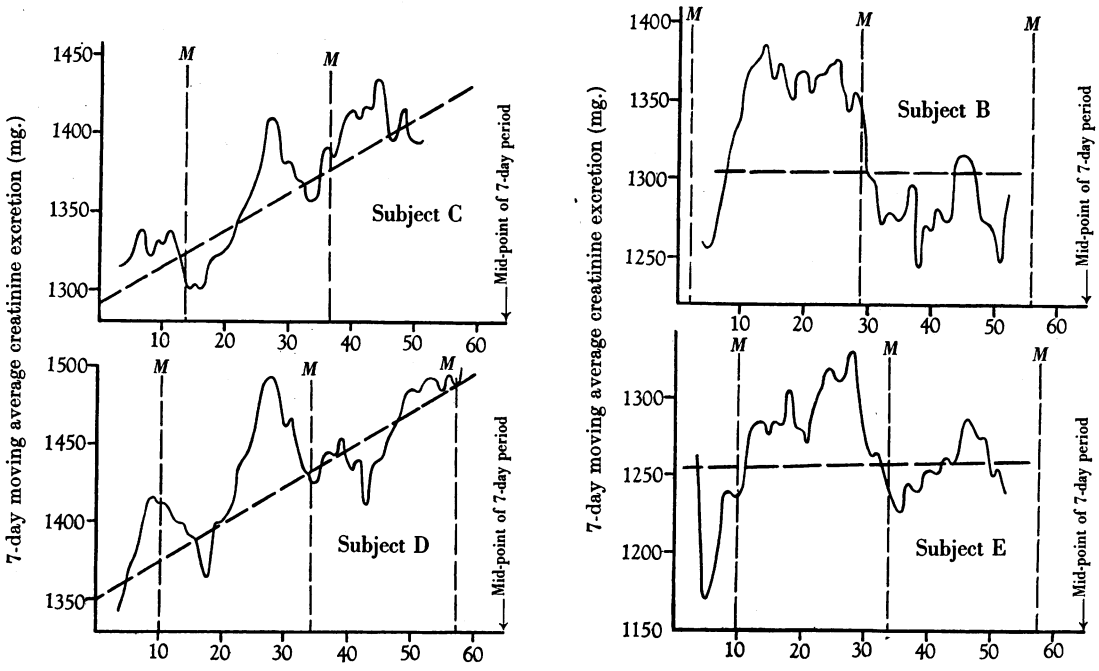

Fig. 4. Weekly moving average excretion of creatinine in four female subjects.

Comparable data for male subjects are not available, but the 40-day period of subject A shows a similar rise and fall in weekly average excretion over a period of about 28 days, and suggests that a long term periodicity may quite possibly be also a characteristic of males. On the basis of the data from these four subjects alone, there seem grounds for the existence of a menstrual effect on creatinine excretion. However, it is so strongly masked by other environmental effects that a substantial amount of further data would be required to establish it with certainty.

DISCUSSION

It is generally recognized that an intermittent physiological creatinuria is characteristic of some women and the data presented agree with this view. No obvious relationship between creatine excretion and menstruation has been observed in the fourteen subjects investigated. This is contrary to the observations of Smith (1942), who followed the output of urinary creatine and creatinine in four women on every second or third day for a number of cycles and found creatine appearing in the urine at the onset of menstruation, or rising to peak values in subjects normally exhibiting a creatinuria. It must be pointed out, however, that since all the other workers who have observed cases of significant creatine excretion in women have stressed its marked day to day irregularity, creatine excretion curves based solely on such periodic estimations cannot be justifiably interpreted as indicating any more than the qualitative existence of a creatinuria. There does not appear to be sufficient evidence from the data to hand, either in the literature or as a result of the present work, to support the existence of a clear-cut quantitative relationship.

As has been indicated, the daily excretion of creatinine in humans may vary by up to 300 mg. from day to day. Fluctuations of this order render meaningless the identification of cyclic changes in excretion from daily output curves alone. Day to day fluctuations in the six subjects in this study were frequently comparable with the maximum limits of variation over the whole period and masked any obvious cyclic changes. Nevertheless, analysis of the data for the females has revealed a significant correlation between level of creatinine excretion and

day of the week, presumably due to the influence of a weekly routine common to all subjects. It is probable that this weekly periodicity is a factor to be generally considered in all experiments on creatinine excretion in humans, and some such treatment of observations as that described must be used to eliminate this periodicity and also to reduce the effect of short-term fluctuations if particular deviations from the mean excretion level are being sought.

From excretion curves based on estimations made at intervals of 2–3 days, Smith (1942) has reported a regular premenstrual rise in excretion followed by a fall during the menstrual flow. Such curves smooth out the day to day fluctuations without taking into account their origin or their possible composition as a summation of several separately operating fluctuations, and thus give a false impression of the nature of creatinine excretion. To illustrate this, the data for the six subjects in this study have been interpreted in a similar manner, the representative results for subject C being given. In Fig. 2*b* are shown the three corresponding curves of values for every third day only, constructed from the complete excretion curve shown in Fig. 2*a*, and which resemble closely the curves obtained by Smith. It is obvious that not only are these 'periodic' curves unsatisfactory approximations of the complete excretion curve but that they bear no consistent relation to one another, and deductions drawn from them would have little significance. A connexion between menstruation and creatinine elimination has not been conclusively proved, and the data available indicate that if it is existent, it is by no means a clear-cut relationship as was proposed. The further long-term investigation of many subjects is clearly necessary, including examination of the excretion characteristics of males, before this relationship can be established with certainty. It is thought that treatment of results along the lines presented in this paper may be of value in clarifying this problem.

SUMMARY

1. Studies of the day to day creatine and creatinine excretion of a number of men and women, aged 19–26 years, revealed the absence of creatinuria in the males and its presence, in amounts up to 150 mg./day, in a proportion of the females.

2. No evidence has been obtained to relate creatinuria, whenever it appeared, to any phase of the menstrual cycle.

3. A weekly periodicity in creatinine excretion was observed, but there was no conclusive evidence to link the level of urinary creatinine with menstruation.

4. The nature of the daily fluctuations in creatinine output is discussed.

The author is indebted to Dr R. B. Fisher for his invaluable help in the analysis of the data obtained and to the University of London for a grant from the Dixon fund.

REFERENCES

Cameron, J. D. (1933). *Quart. J. exp. Physiol.* **23**, 351.
Dill, D. B. & Horvath, S. M. (1941). *Amer. J. Physiol.* **133**, 520.
Espersen, T. & Thomsen, A. (1937). *Acta med. Scand.* **92**, 39.
Folin, O. (1914). *J. biol. Chem.* **17**, 469.
Hodgson, P. & Lewis, H. B. (1928). *Amer. J. Physiol.* **87**, 288.
Hyde, E. C. (1942). *J. biol. Chem.* **143**, 301.
Krause, R. A. & Cramer, W. J. (1910). *J. Physiol.* **40**, 61*P*.
Krause, R. A. & Cramer, W. J. (1911). *J. Physiol.* **42**, 35*P*.
Rose, W. C. (1917). *J. biol. Chem.* **32**, 1.
Smith, O. W. (1942). *J. clin. Endocrinol.* **2**, 1.
Stearns, G. & Lewis, H. B. (1921). *Amer. J. Physiol.* **56**, 60.
Wilkins, L., Fleischmann, W. & Block, W. (1941). *J. clin. Endocrinol.* **1**, 3.

Oxidation of Glucose, Glycerol and Acetate by *Staphylococcus aureus**

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Because of the importance of *Staphylococcus aureus* as a test organism in assay of antibiotics, detailed information about its metabolism is desirable. While comparing the oxidation of glycerol and glucose in microrespiration experiments, we observed that resting suspensions rarely consumed enough oxygen for complete oxidation of these two substrates. Krebs (1937) also reported incomplete oxidation of glucose, glycerol, lactate and pyruvate by 18–24 hr. old cells of *Staph. aureus*. Usually about 2·5 mol. of oxygen/mol. of glucose is rapidly consumed followed by a slow uptake. The observed uptake of oxygen and respiratory quotients in our experiments could be accounted for if acetic acid is assumed to be an

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