8. Widner, W. R., Storer, J. B., Lushbaugh, C. C., *Cancer Research*, 1951, v11, 877.

9. Abercrombie, M., *Anat. Rec.*, 1946, v94, 238.

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Long Term Endogenous Creatinine Clearance in Man.* (23711)

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Endogenous creatinine clearances are similar to inulin clearances in the normal individual when run *simultaneously* for short periods of time (5-15 minutes)(1-3) and also when run *independently* (no inulin infusion) for long periods of time (1-24 hours)(4-5). Factors that might influence the endogenous creatinine clearance were investigated. Creatinine clearances were determined on 39 normal individuals and the values compared with reported short term inulin clearance values for individuals of the same age group—the determination of inulin clearances on the group of volunteer subjects was not possible.

Methods. All subjects were free from hypertension, muscle or kidney disease. Urine was collected without catheterization, a 1:1000 dilution made and 5 ml removed for analysis. Blood was obtained by venipuncture and placed in a tube containing potassium oxalate. Protein was removed from the plasma by the sodium tungstate-sulfuric acid method of Folin-Wu(6). Creatinine was determined by the method of Van Pilsum *et al.* (7).† Urea was determined by the Van Slyke(8) modification of the Folin-Wu(6) method.

Results. The effect of urinary volume variations on simultaneous creatinine and urea clearances was determined on 2 normal adult males. Clearances were of one or 2 hours duration and were run consecutively. Blood was withdrawn at the beginning and the end of each clearance period and the average of the 2 values was used in the calculation of the clearance. Urea clearance was calculated as standard clearance (C_s) when the rate of flow was less than 2 ml per minute; when greater than 2 ml per minute, it was calculated as maximum clearance (C_m). Creatinine clearance was calculated according to the clearance formula $\frac{UV}{P}$. Immediately preceding and during the clearance period, the subjects assumed normal activities, but avoided vigorous exercise or the ingestion of large amounts of meat. Diuresis was produced by ingestion of water. The results are shown in Table I. The range of variation of 139 to 169 ml per minute (subject 1) and of 123 to 139 ml per minute (subject 2) is of the same order of

TABLE I. Effects of Urinary Volume Variations on Simultaneous Creatinine and Urea Clearances in the Normal Adult Male.

	Urine vol (ml/min.)	Creatinine clearance (ml/min./1.73 m²)	Urea clearance (ml/min./1.73 m²) C_s	Urea clearance (ml/min./1.73 m²) C_m
1.	.88	169	63	56*
	1.08	139	72	69*
	3.00	161		115
	5.00	167		151
	3.00	161		94
	1.53	152	66	74*
2.	1.40	139	59	65*
	1.22	123	58	60*
	1.45	126	60	67*
	2.05	137		80
	9.95	131		114

Standard clearance (C_s) $= \frac{U\sqrt{V}}{P}$ maximum clearance (C_m) $= \frac{UV}{P}$(10).

* Clearances calculated according to the formula $\frac{UV}{P}$, regardless of rate of urine flow.

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† In this method creatinine is degraded to methylguanidine by O-nitrobenzaldehyde in the presence of alkali. The methylguanidine is measured with a modified Sakaguchi color reaction.

magnitude reported in consecutive inulin clearances(2-3). The urea clearances showed a greater range of variation, as has been previously reported(9).

The effects of protein ingestion, exercise, and length of clearance are shown in Table II. For the protein ingestion experiment the subjects fasted 12 hours and then a 3 hour clearance run. The subjects ingested large amounts of well cooked protein (approximately 300 g) and a 3 hour clearance run. Blood was withdrawn at the middle of each clearance period. The exercise experiment was determined similarly, except a period of ½ hour of vigorous exercise was substituted for protein ingestion. In the length of clearance experiment the one and 24 hour clearances were done consecutively, with blood samples withdrawn at the end of each clearance period, the 24 hour plasma creatinine value being the average of the two samples. Both exercise and a high protein meal seemed to increase the clearance a small amount. The 1 hour and 24 hour clearances were similar.

Creatinine clearances were determined on 39 individuals of both sexes varying in age from 3-99 years and the results compared with reported inulin clearances for the same age groups (Table III). All clearances were of at least 8 hours duration, the majority 24 hours. The plasma creatinine level was determined from a single sample at the middle of the clearance period or by the average of samples withdrawn at the beginning and at the end of the clearance period. In the age groups from 3-20 and 20-50 years there was good agreement between long term creatinine clearances and reported short term inulin clearances for these same age groups. In the age groups from 60-99 years there was a gradual consistent lowering of creatinine clearances, parallel with increase in age, which also agreed well with reported inulin clearances.

TABLE II. Effects of Protein Ingestion, Exercise, and Length of Clearance on Creatinine Clearance.

Subject No.	Clearance (ml/min./1.73 m²)	
	Fasting	High protein
1	152	177
2	119	147
3	122	145
4	135	138
Avg	132	152
	Before exercise	After exercise
1	146	158
2	129	143
3	125	140
4	169	180
Avg	142	155
	1 hr	24 hr
5	139	141
6	163	150
7	137	150
8	147	167
9	175	160
10	150	152
11	118	123
Avg	147	149

TABLE III. Normal Endogenous Creatinine Clearances.

Age group	No. of subjects	Clearance (ml/min./1.73 m²)	Reported inulin clearance (ml/min./1.73 m²)
3-20	6	127 ± 16	*129 ± 17(2)
20-50 ♂	11	135 ± 14	131 ± 21(11)
20-50 ♀	7	127 ± 18	117 ± 15(11)
60-69	2	105 ± 7	96 ± 25(12)
70-79	3	104 ± 16	89 ± 20(12)
80-89	7	74 ± 22	65 ± 20(12)
90-99	3	38 ± 8	

All values are avg ± stand. dev.

* Calculated from data reported for age group 4-12.5.

Summary. The effects of urinary volume, diet, and exercise on long term endogenous creatinine clearance were studied on normal individuals using the ortho-nitrobenzaldehyde method for creatinine. Urinary volume variations had no effect on creatinine clearance; protein ingestion and exercise seemed to increase slightly the clearance values. One hour clearances were similar to 24 hour clearances. Long term creatinine clearances of 39 normal individuals of varying age groups were determined and were found to be similar to reported inulin clearance values of these same age groups.

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1. Hare, K., Goldstein, H., Barnett, H. L., McNamara, H., Hare, R. S., *Fed. Proc.*, 1949, v8, 67.

2. Mattar, G., Barnett, H. L., McNamara, H., Lauson, H. D., *J. Clin. Invest.*, 1952, v31, 938.

3. Miller, B. F., Winkler, A. W., *ibid.*, 1938, v17, 31.

4. Gazmuri, R., Canessa, M., *Rev. Med. Chile*, 1954, v82, 101.

5. Schwab, M., *Klin. Wochenschrift*, 1953, v31, 1030.

6. Folin, O., Wu, H., *J. Biol. Chem.*, 1919, v38, 81.

7. Van Pilsum, J. F., Martin, R. P., Kito, E., Hess, J., *ibid.*, 1956, v222, 225.

8. Peters, J. P., Van Slyke, D. D., *Quantitative Clinical Chemistry, Vol. II, Methods,* Williams and Wilkins Co., Baltimore, 1932, 557.

9. Moller, E., McIntosh, J. F., Van Slyke, D. D., *J. Clin. Invest.*, 1928, v6, 427.

10. Peters, J. P., Van Slyke, D. D., *Quantitative Clinical Chemistry, Vol. I. Interpretations,* Williams and Wilkins Co., Baltimore, 1946, 840.

11. Goldring, W., Chasis, H., *Hypertension and Hypertensive Disease,* Commonwealth Fund, N. Y., 1944, 56.

12. Davies, D. F., Shock, N. W., *J. Clin. Invest.*, 1950, v29, 496.

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Influence of Environmental and Skin Temperature on Threshold for Nicotine Axone Reflex Sweating in Humans.*† (23712)

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The major characteristics of axone reflex sweating and pilomotion, elicited by intradermally injected nicotinic drugs in the human, have been elucidated through the efforts of several workers(1,2). As yet no evidence exists either that these axone reflexes occur physiologically or, if they do, what role they might play. One possibility is that such a peripheral mechanism might serve to modify the response of the cutaneous effectors to their central nervous controls. Determination of effects of environmental and skin temperature changes on the characteristics of nicotine sweating appeared to offer an indirect test of this hypothesis. Measurement of threshold effects proved simpler and probably more accurate than measurement of changes in extent of suprathreshold responses, although some of the latter were essayed. The present study was restricted chiefly to the sweat response, although some corollary data on piloerection are also given. A very closely related investigation is that of Benjamin(3) who, studying the response of sweat glands to locally applied heat, showed an interaction between this stimulus on the one hand, and environmental temperature and locally injected acetylcholine on the other.

Methods. Healthy, white individuals, aged 20 to 34, were used as subjects; all but one experiment, noted below, were done on males. Injections of nicotine were made intradermally on the volar forearm with 26 gauge needles and 1 or ½ ml tuberculin syringes; in all cases, the volume injected was 0.05 ml. Solutions were made by diluting nicotine alkaloid to appropriate concentrations in sterile 0.9% NaCl; concentrations are expressed in terms of weight to volume. Sweating was detected by Randall's(4) iodine-starch paper method; the response was quantitated in terms of area and intensity on arbitrary scales. Piloerection was noted visually and, because of difficulty in quantization, noted only as

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