Studies on Urinary Excretion of Nitrogen and Electrolytes

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APPROXIMATELY 70 per cent of the body weight of man is water. Although the physiologic role of water has been the subject of a number of excellent reviews,[1-4] in many nutritional studies, it is hardly considered and its intake seldom measured. In studies of the adequacy of a given diet in human subjects, attention is often focused on the balance of protein and electrolytes without restriction of water intake, yet water is a limiting nutrient. In this report, we present the results of a series of feeding experiments in which human volunteer subjects were given a liquid dietary preparation of high nutritive value for the purpose of studying its adequacy as food in a fallout shelter. In the quantity offered, this preparation provided 80 gm. protein, 1,600 cal. and 1,400 ml. water per man per day.

Changes in the nutritive status of these subjects after fourteen days of feeding were estimated from a selected series of measurements taken before and immediately after the test period. These included the urinary excretion of nitrogen, changes in body weight and certain parameters related to fluid balance, namely, the specific gravity of urine and the urinary excretion of sodium and potassium. In addition, the hematocrit, hemoglobin and plasma protein levels were determined before and at the end of the feeding experiment.

The study was conducted on male volunteer subjects under strict supervision and under conditions designed to minimize errors in the collection of urine. Suitable groups of "young" and "old" subjects were observed. For comparison, we have also studied two other groups who were offered the same diet daily and two additional amounts of water.

EXPERIMENTAL

Eighteen male subjects from a state penal institution were selected and housed in separate rooms from which all plumbing was disconnected. Toilet facilities were made available only under surveillance of guards. The temperature of the rooms was kept at about 23° ± 2°c. and the relative humidity at 60 to 70 per cent. Selection of the commercial preparation used was based on convenience, acceptability by the subjects and high nutritive quality. The composition of the preparation is given in Table 1. One lot of the product was used for each experiment so that all subjects received a diet of identical chemical composition. In most studies the contents of cans selected at random were also analyzed in order to check the analytic data supplied by the manufacturer.

In each study, nine subjects were randomly divided into three groups of three each. Each man received four cans of Nutrament®‡‡ for a period of fourteen days. The amount of distilled water of-

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‡‡ Kindly supplied by the Edward Dalton Co., Division of Mead Johnson and Co.

TABLE I
Composition of Nutrament

Composition*	Amount
Vitamin A (U.S.P. units)	1,250
Vitamin D (U.S.P. units)	125
Vitamin C (mg.)	50
Thiamine (mg.)	0.5
Riboflavin (mg.)	0.6
Niacinamide (mg.)	5
Calcium (gm.)	0.5
Phosphorus (gm.)	0.4
Iron (mg.)	4
Iodine (μg.)	60
Vitamin E (I.U.)	2.5
Pyridoxine (mg.)	0.4
Vitamin B_{12} (μg.)	0.5
Calcium pantothenate (mg.)	2
Sodium (gm.)	0.2
Potassium (gm.)	0.9
Copper (mg.)	0.5
Manganese (mg.)	1
Fiber (gm.)	0.55

* In addition each 12.5 fluid ounce can of Nutrament liquid also supplied 20 gm. protein, 13.3 gm. fat, 50 gm. carbohydrate, 400 calories and 350 ml. water.

fered to the subjects in each group per day was as follows: group A, none; group B, 240 cc.; and group C, *ad libitum*. The daily total water intake of the last group was measured by taking the difference between 2 L. of distilled water provided for each of the three subjects every morning and the unconsumed portion. Individual urine collections were made, and the total volume was measured every twenty-four hours. Two sets of aliquots were coded and analyzed for sodium, potassium (flame photometry), chloride (Volhard titration method) and nitrogen (micro-Kjeldahl method). Specific gravity was determined by a hydrometer. Plasma protein was determined by the Kjeldahl method after precipitation with trichloracetic acid. Hematocrit reading was determined by centrifugation of heparinized blood, and hemoglobin was determined colorimetrically using cyanohemoglobin as a standard. All subjects were weighed each morning before breakfast.

RESULTS

Four separate studies were conducted with three groups of three young volunteer subjects (average age thirty-one, range twenty-seven to forty-six years), and two studies were conducted with three groups of three old volunteer subjects (average age fifty-six years, range fifty to sixty-six years).

Excretion of Water

It can be noted (Table II) that the mean amount of urine excreted by the nine volunteer subjects four days before the study was essentially the same. The urinary excretion (within the limits of error of twenty-four hour urine collection) remained unaltered for subjects in group C and diminished for those in groups A and B. The decrease was observed in several instances after the first day of the diet and appeared to level off to a stable value for the rest of the experimental period. In other studies, no urine collection was made before the study, and only the mean values of the urine volumes of the last thirteen days for each subject were calculated; the results are plotted in Figure 1. The young subjects (solid circles) in group C excreted an average of 1.89 L. of urine per day during the test period

TABLE II
Volume of Urine Excreted*

Period of Study (days)	Volume (L./day)		
	Study 1	Study 2	Study 3
	Group A		
−1 to −4	1.41 (1.88–1.21)	1.44 (1.84–0.92)	3.01 (3.44–1.88)
0 to 1	1.64	1.34	1.12
2 to 13	0.65 (1.64–0.41)	0.60 (1.34–0.19)	0.69 (1.12–0.37)
	Group B		
−1 to −4	2.06 (2.48–1.76)	1.58 (2.11–1.40)	2.14 (2.40–1.98)
0 to 1	1.22	1.52	1.18
2 to 13	0.66 (1.22–0.48)	0.56 (1.52–0.30)	0.17 (1.18–0.42)
	Group C		
−1 to −4	1.77 (2.20–1.46)	1.44 (2.10–1.02)	1.48 (1.88–0.96)
0 to 1	2.65	1.32	2.54
2 to 13	1.57 (2.25–1.10)	1.19 (2.5–0.67)	1.68 (2.54–0.96)

* Numbers outside of parentheses denote average and numbers inside parentheses range.

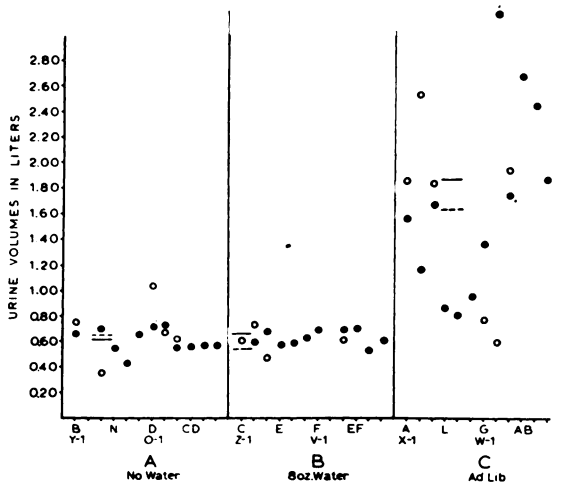

FIG. 1. Daily urine volume of young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

of two weeks, although the range was from 0.8 to 2.7 L. depending on the water intake (range 1.0 to 2.8 L.). The young subjects in group A excreted approximately the same amount of urine as those in group B, i.e., 0.63 and 0.65 L. per day, respectively. The variation among subjects was surprisingly small. In groups A and B the daily urine volume of old subjects (open circles) was essentially the same as that of the young subjects. In group C, the average urine volume of the old subjects was higher than that of the young subjects, but this difference is not statistically significant.

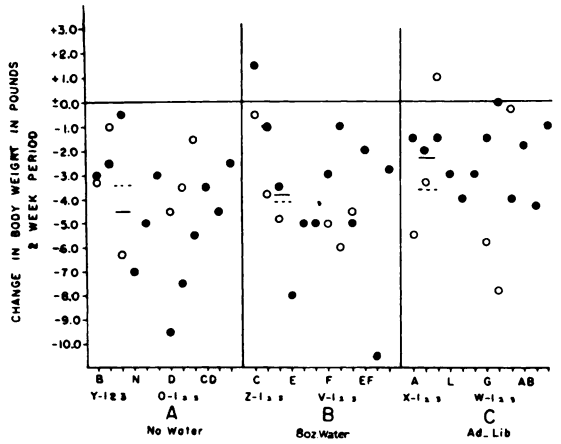

FIG. 3. Change in body weight of young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

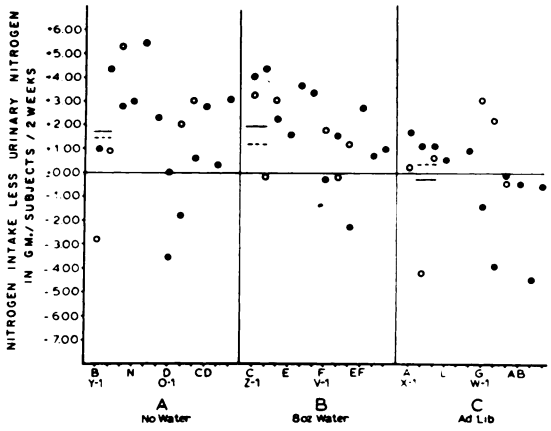

FIG. 2. Nitrogen intake less urinary excretion of nitrogen in young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

Nitrogen Studies

The average differences between nitrogen intake and urinary excretion is plotted for each subject in Figure 2. On the average, the urinary loss did not exceed the intake in any of the subjects. It appears that young and old subjects in groups A and B showed larger differences, suggesting more favorable nitrogen balance than those in group C (a trend which needs confirmation in future studies).

Weight Change

Changes in body weight expressed in pounds per subject, are plotted in Figure 3. It can be seen that the body weights of all subjects in groups A, B and C decreased slightly at the end of the experiment. This average loss represents no more than 4 pounds per two weeks or 140 gm. per day per person. By and large, we attach little significance to these losses because of the reduction of the daily caloric intake to 1,600 cal. from the period before the study. Our data do demonstrate that on the average, this dietary regimen caused only slight decrease in body weight during the test period.

Urinalyses

Specific Gravity. Specific gravity of the urine of each subject was measured daily.

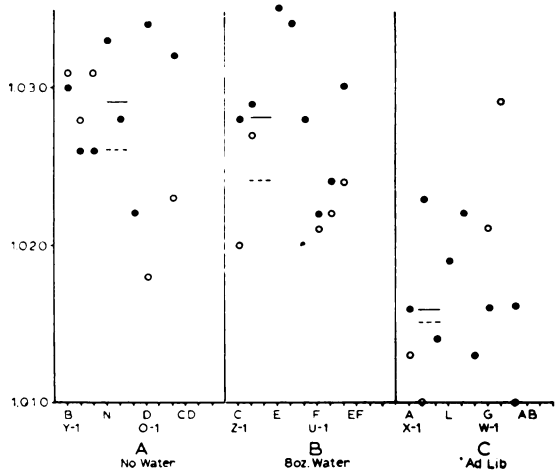

FIG. 4. Specific gravity of urine of young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

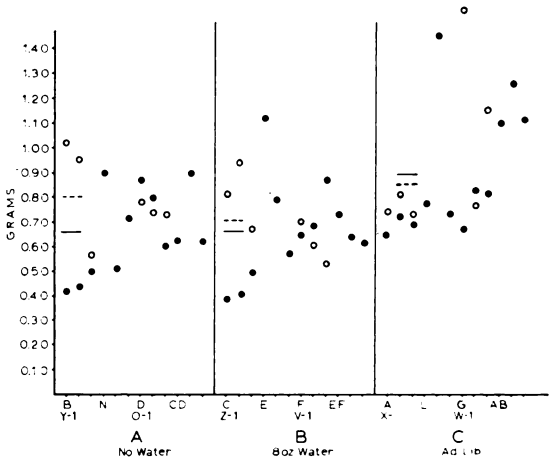

FIG. 5. Total urinary excretion of sodium by young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

The average results of fourteen day specimens in each subject are plotted in Figure 4. The specific gravity of the urine of subjects in group C was lower than that of the other two groups. All values were well within the limits of normal[5] although the urine concentration in group A was maximal.

Excretion of Sodium and Potassium. The average total amounts of sodium and potassium excreted by the young subjects over the entire test period are plotted in Figures 5 and 6, respectively. The data demonstrate that on the average the young subjects in groups A and B excreted less sodium than those in group C 0.66 ± 0.03, 0.66 ± 0.06 and 0.90 ± 0.05 gm., respectively. This difference is statistically significant using student's t test ($p<0.05$). No similar difference was observed in the old people. The excretion of potassium was the same among all subjects in the three groups.

Determination of Chloride. Daily excretion of chloride in the urine was estimated and was essentially identical in all groups (48.2 ± 2.4 for group A, 39.1 ± 2.3 for group B and 38.5 ± 1.7 for group C).

Hematocrit and Hemoglobin Determinations. Venous blood was drawn from the subjects before the study was begun and after one and two weeks for determination of hemoglobin and hematocrit. Hemoglobin and hematocrit values for all subjects (Table III) showed no systematic variations from the norm (45 ± 2 per cent for hematocrit and 15 ± 1 gm. per cent for hemoglobin). They indicate a lack of significant difference either in hemoglobin concentration or in hematocrit reading and thus present no evidence of dehydration. Similarly, there was no change in the plasma protein concentrations (data not present) determined at the beginning, the middle and the end of the experiment.

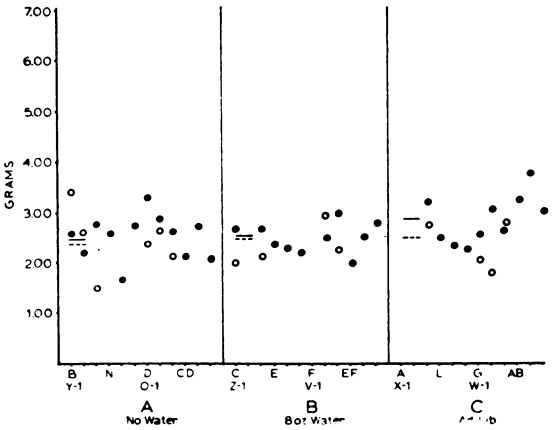

FIG. 6. Total urinary excretion of potassium by young subjects (solid circles) and old subjects (open circles) on a regimen of Nutrament. Solid line = average for the young subjects; broken line = average for the old subjects.

TABLE III
Hematocrit Reading and Hemoglobin Concentrations of Subjects Receiving Nutrament and Different Amounts of Water

Study No.	Hemoglobin		Hematocrit	
	Before Study	After 2 Weeks	Before Study	After 2 Weeks
Group A				
1	45 ± 2.0	46 ± 2.4	15.2 ± 0.4	15.2 ± 0.3
2	46 ± 1.6	48 ± 1.2	14.8 ± 0.2	14.9 ± 0.2
3	46 ± 2.1	45 ± 2.2	16.1 ± 0.5	15.1 ± 0.4
4	45 ± 1.8	48 ± 2.4	14.3 ± 0.3	14.6 ± 0.4
5	43 ± 2.2	44 ± 1.6	15.1 ± 0.4	15.6 ± 0.1
6	47 ± 1.8	44 ± 1.9	14.6 ± 0.6	14.9 ± 0.2
Group B				
1	46 ± 2.6	47 ± 1.8	15.2 ± 0.1	15.6 ± 0.4
2	49 ± 1.2	44 ± 1.9	15.6 ± 0.3	15.1 ± 0.2
3	42 ± 1.3	47 ± 3.1	14.2 ± 0.2	14.8 ± 0.1
4	46 ± 3.2	46 ± 1.0	16.2 ± 0.2	15.1 ± 0.4
5	44 ± 1.8	46 ± 2.6	14.1 ± 0.3	14.6 ± 0.2
6	43 ± 1.7	45 ± 1.9	15.3 ± 0.2	15.6 ± 0.3
Group C				
1	45 ± 2.0	46 ± 2.5	14.2 ± 0.2	15.2 ± 0.1
2	42 ± 1.8	47 ± 3.0	14.6 ± 0.3	15.6 ± 0.5
3	46 ± 2.1	47 ± 1.6	16.2 ± 0.4	14.8 ± 0.3
4	48 ± 1.9	45 ± 1.1	14.4 ± 0.4	14.2 ± 0.2
5	42 ± 2.3	44 ± 2.0	15.4 ± 0.4	15.0 ± 0.2
6	46 ± 2.1	43 ± 1.6	13.8 ± 0.6	15.1 ± 0.4

NOTE: Studies 1 through 4 were conducted on young subjects and studies 5 and 6 on old subjects.

COMMENTS

As already stated, the parameters used to determine the nutritional status of our volunteer subjects before and after experiments were maintenance of body weight, urinary excretion of nitrogen and indexes of fluid balance shifts. In the measurement of body weight, daily variation of a given subject must be considered as it is probably great enough to make any change of 3 to 4 pounds during the entire period of study physiologically unmeaningful. Although the average weight loss in the three groups was about 4 pounds, a few of the subjects lost as much as 7 to 10 pounds. It is possible that these larger losses were due to insufficient caloric intake.

Inasmuch as this preparation produces less than 10 gm. of feces per day per person, we believe that the fecal nitrogen loss was insignificant as compared to the total intake. Likewise, nitrogen loss through other routes, such as the skin, was insignificant. Therefore, it does not seem unreasonable to assume that the difference between the dietary intake and urinary excretion of nitrogen is a satisfactory representation of the nitrogen balance of the subjects during this period. Examination of the detailed data indicate that the urinary excretion values were high in four subjects in different groups. We are inclined to regard this as an artifact of collection difficulties, because it is well known that even when meticulous supervision is exercised, human subjects are prone to forget the importance of consistent urine collections. Others concerned with this problem have frequently invoked the constancy of creatinine excretion as a measure of completeness of urine collection. However, our experience during the past ten years with urine collections from volunteer subjects from a state penal institution failed to support this concept and made more credible the fact that creatinine excretion is just as variable as any other component in the urine.

The purpose of other studies in this same general area has been to establish minimum water requirements. In the experiments described herein, our objective was to establish whether or not the water content of the diet offered was sufficient to keep our subjects in good condition without adverse evidences of dehydration. There were no complaints of thirst or signs of skin dryness and, clinically, there was no sign of dehydration. Consequently, the appropriate control group for this study is a group of similarly fed subjects allowed access to additional sources of water *ad libitum*. When the usual criteria of state of hydration were applied to our experimental subjects and to such control subjects, no differences were seen in hematocrit values, specific gravity of urine or total excretion of electrolytes in the urine. Under these circumstances, the conclusion that the water content of the preparation studied was sufficient as the sole source of water for these subjects over the period of time studied seems amply justified.

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