

Urinary Excretion of Vitamin B₁₂, Folic Acid, and Citrovorum Factor in Human Subjects on Various Diets.* (18941)

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Recent reports(1,2) have suggested that no appreciable vit. B₁₂ activity is present in the urine of normal subjects. In the present study small but measurable amounts of vit. B₁₂ have been found in the urines of 56 medical students who were maintained on various diets. Folic acid and citrovorum factor were also measured in the urines of these subjects to study the correlation, if any, in the excretion of these 3 vitamins.

Methods. Students in the medical school adopted various self-selected diets for a 3-day period and collected the 24-hour urine (under toluene) on the third day for metabolic studies in the biochemistry course. Aliquots of the urines were collected from the students, neutralized to pH 6.9, and stored in the frozen state until analyzed.

Vit. B₁₂ was measured microbiologically by a modification of the method of Thompson *et al.*(3). Stock cultures of the assay organism, *L. leichmannii* 4797 were transferred weekly in a skim milk medium containing 0.5% Difco Bacto-yeast extract, 0.5% whole liver powder,[†] and 10 mγ vit. B₁₂[‡] per 10 ml tube. The medium for growth of the inoculum was prepared by addition of 10 mγ vit.

B₁₂, 5 mg liver concentrate 1-20§ and 5 mg Difco Bacto-yeast extract per 10 ml tube of assay medium. The tubes of media for stock and inocula cultures were autoclaved at 120°C for 15 minutes and stored in the refrigerator until used. In preparing the assay medium(3) the ascorbic acid was added after the medium had been neutralized to pH 7.0. The tests were incubated at 37°C for 65 to 72 hours and titrated with 0.1 N alkali. Many of the urine samples were assayed at 2 or 3 different dilutions with good duplication of values. Marked inhibition was observed in a number of samples when the urine was undiluted; however when diluted 2 to 4 times, the inhibition was greatly reduced or completely eliminated. Active material, other than vit. B₁₂, was measured in some of the urines after destroying vit. B₁₂ by alkali treatment(4).

Folic acid activity (FA) was measured using *L. casei* 7469 with a medium modified from that suggested by Landy and Dicken(5) and synthetic pteroylglutamic acid (PGA)¶ as a standard. Citrovorum factor activity (CF) was assayed using *Leuconostoc citrovorum* 8081 with a medium similar to that of Sauberlich and Baumann(6) and synthetic CF¶ as a standard. All assays were done titrimetrically after 65- to 72-hour incubation. Fresh samples of synthetic CF were found to

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[†] Whole liver powder, dried at 70°, was kindly furnished by the Viobin Corp., through the courtesy of Mr. Ezra Levin.

[‡] Crystalline vit. B₁₂ was generously furnished by Merck and Co., Inc., Rahway, N. J.

§ Liver concentrate 1-20 was generously supplied by the Wilson Laboratories, through the courtesy of Dr. Claire E. Graham.

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¶ Synthetic PGA and CF were kindly furnished by the Lederle Laboratories Division, American Cyanamid Co., through the courtesy of Drs. E. L. R. Stokstad and H. P. Broquist.

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TABLE I. Average Excretion of Total FA, CF, and Vit. B₁₂ Activities in Urine of Human Subjects on Third Day of Dietary Regimen.

Diets	No. of subjects	Vit. B ₁₂ activity, mγ	FA activity, γ	CF activity, γ
Normal	14	31(16-55)	4.1(1.9- 6.6)	2.6(1.6-4.8)
Milk	7	37(11-70)	2.6(1.4- 4.7)	1.8(1.2-3)
Alkaline ash	6	28(6-54)	3.8(2.7- 6.4)	2.6(2.2-3.2)
Low protein	8	36(19-52)	3.3(2.3- 4.7)	2.8(2.1-4.1)
High "	7	33(13-52)	3.1(1.8- 5.2)	2.6(2.1-4)
" purine	8	43(15-79)	10.4(4 -25)	4.9(1.8-8.1)
Fasting	6	62(36-83)	3.9(2.7- 6.6)	2.8(2.2-3.8)

possess about 4% of the activity of PGA for *L. casei*. After 1 to 2 weeks the activity of the synthetic CF for *L. casei* was found to increase markedly when kept in dilute solution (30 γ/ml) in the refrigerator. The CF in the urines may therefore contribute variable amounts of activity to the FA values found with *L. casei*.

Results and discussion. The range and average daily excretion of vit. B₁₂, FA, and CF compounds in the subjects on the various diets are shown in Table I. The values given for vit. B₁₂ may include other substances which are active for *L. leichmannii*. The average vit. B₁₂ excretion by the fasting subjects was much higher than that found in any of the other groups. Assay of the urine after alkaline destruction of the vit. B₁₂ showed that 4% (0 to 11%) of the total activity in the urine of the fasting subjects still remained. This was similar to the blank value 3% (0 to 8%) obtained for the urines of the subjects on the other diets with the exception of those on the high purine diets. For the high purine diets the average activity after alkaline treatment represented 32% of the total activity. This was presumably due to breakdown products of the nucleic acids found in the large amounts of liver and sweetbreads in these diets. After subtraction of these blanks, the average vit. B₁₂ excretion values fell in the normal range. The true vit. B₁₂ excretion value appeared to be about twice as high in the fasting groups as in any of the others. An increased excretion of other B vitamins has been observed during fasting in dog and man(7,8).

The controversial results in regard to the

presence of the antipernicious anemia factor (vit. B₁₂) in the urine of human subjects (9,10) would be expected in view of the small amounts of vit. B₁₂ excreted per day and the wide variation between different subjects. A concentrate of many liters of urine would be necessary to obtain sufficient vit. B₁₂ for a reticulocyte response(10).

The changes in excretion of FA and CF compounds with diet appear to be similar (Table I). The excretion of FA and CF compounds by the subjects on the alkaline ash, low protein, high protein and fasting regimens was comparable to that found for those on the normal diets. A decreased excretion of FA and CF was evidenced in the subjects on the milk diet and a greatly increased excretion was found in the group on the high purine diet. The high values in the latter group were probably due to the same nucleic acid derivatives which were found to interfere in the vit. B₁₂ assay. The excretion of FA and CF compounds was not increased during fasting, in contrast to that found for vit. B₁₂.

A part or all of the CF activity of the urine may be included in the FA values. In all individuals tested, the values for FA compounds were as high or higher than the corresponding values for CF compounds. This is summarized in the average figures (Table I) and suggests that other compounds in addition to CF may contribute to the total FA excretion.

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Summary. The average urinary excretion of vit. B₁₂ in 14 subjects on normal diets was found to be 31 my per day. Similar values were obtained in 28 subjects maintained for 3 days on the following diets: milk, alkaline ash, low protein, and high protein. A somewhat higher value was found in 8 subjects on high purine diets, and the increase over normal was shown to be due to interfering compounds. On the third day of fasting, 6 subjects were

found to excrete an average of 62 my vit. B₁₂ per day.

The average CF excretion by the subjects on the above diets accounts for only part of the total FA compounds excreted, and appears to be correlated with the total FA values. Differences in vitamin B₁₂ excretion resulting from dietary changes are not similar to those observed in CF and FA excretion.

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Effect of Cortisone upon Chemical Composition of Allantoic Fluid of the Chick Embryo.* (18942)

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The studies herein presented were undertaken in a search for biochemical correlates of the striking changes in the growth, development, and survival of chick embryos treated with cortisone or certain other steroids(1-5).

Methods. Distilled water or dilutions of cortisone† in distilled water were inoculated into the yolk sac of fertile White Leghorn eggs on the fourth day of incubation.

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† Vehicle in which cortisone acetate is supplied by Merck and Co., Inc:

1.5% benzyl alcohol

0.9% sodium chloride

0.4% polyoxyethylene sorbitan monooleate

0.5% sodium carboxymethylcellulose

(distilled water q.s. ad 100%)

This suspension was diluted with water to permit the injection of 0.25 to 4.0 mg of cortisone in a constant volume of 0.2 ml.

Allantoic fluids for chemical analysis were drawn from surviving embryos on the thirteenth day of incubation. This age was selected to avoid the excessive precipitate of urates in older embryos. The methods employed in the determination of sodium, potassium, chloride and sugar (reducing substance) levels are in regular use in this and in other laboratories(6).

Results. In Fig. 1 the chemical composition of the allantoic fluid from cortisone-treated chick embryos has been compared with the findings in controls inoculated with distilled water.

It is readily evident that cortisone treatment raised the mean value of the allantoic fluid sodium and lowered its potassium. This is a consistent finding irrespective of the amounts of hormone given. There is no dosage-effect relationship present despite the fact that the graphs suggest maximal increases in sodium in the 1.0 mg group with alterations in the same direction but of lesser magnitude with the 0.25, 0.5, 2.0, and 4.0 mg amounts. Statistically significant changes in the mean chloride and sugar (reducing substances) were

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