one must not overlook the possible dietary origin of methyl ethyl ketone in normal human urine.

Summary. Methyl ethyl ketone has been isolated from normal human urine and identified. Its possible origin was briefly discussed.

1. Shriner, R. L., and Fuson, R. C., *The Systematic Identification of Organic Compounds,* John Wiley and Sons, 1940.

2. Meigh, D. F., *Nature,* 1952, v170, 579.

3. Robinson, W. G., Bachhawat, B. K., and Coon, M. J., *J. Biol. Chem.,* 1956, v218, 391.

4. Coon, M. J., and Abrahamsen, N. S. B., *ibid.,* 1952, v195, 805.

5. Coon, M. J., Abrahamsen, N. S. B., and Green, G. S., *ibid.,* 1952, v199, 75.

6. Carter, H. E., *Biol. Symposia,* 1941, v5, 47.

7. Sasaki, S., Watanabe, T., and Tasaka, Y., *J. Sericult. Sci. Japan,* 1951, v20, 448.

Received January 29, 1957. P.S.E.B.M., 1957, v94.

Vit. B_{12} Levels in Serum and Urine Following Parenteral Administration of Crystalline Vit. B_{12} or Liver Extract.* (23032)

L. L. SHEELY, O. NEAL MILLER AND W. G. UNGLAUB

Laboratory of Nutrition and Metabolism, Departments of Medicine and of Biochemistry, Tulane University School of Medicine, New Orleans, La.

Sokoloff, Sanneman, and Beard(1) on the basis of urinary excretion studies have suggested that there may be better retention of vit. B_{12} activity in tissues following parenteral administration of purified liver extract than following an equal amount of crystalline vit. B_{12}. It has been postulated(2) that vit. B_{12} must be bound to serum proteins to prevent its excretion and therefore facilitate its ultimate retention in tissues. This paper compares serum and urine vit. B_{12} levels of 2 groups of normal subjects, one group given crystalline vit. B_{12}, the other given an equivalent amount of vit. B_{12} in the form of purified liver extract.

Materials and methods. Subjects for this study were students and staff of Tulane University School of Medicine, and selected patients from Charity Hospital. One group of these subjects was given 50 μg of crystalline vit. B_{12}† in 1.0 ml of solution. The other group was given an amount of purified liver extract‡ containing 50 μg of vit. B_{12}, in 3.3 ml of solution. Both vit. B_{12} solution and liver extract were assayed prior to use by microbiological technic using *L. leichmannii* (ATCC-4797). In both groups, blood samples were drawn before injection, and at 1, 4, 8 and 24-hour intervals following injection. Urine was collected for 24 hours following injection. Diet was not controlled, as normal diets were not found to affect urinary excretion or serum levels of vit. B_{12}(3,4). Serum was separated from blood samples and stored at –20°C until assayed. From each sample of serum collected, an aliquot was treated with acid-washed charcoal, 75 mg/ml to adsorb free vit. B_{12}(5). These aliquots, along with untreated aliquots, were then assayed for vit. B_{12}§(6). Aliquots of 24-hour urine samples were stored at –20°C until assayed (3,6). Serum vit. B_{12} levels are expressed as mμg/ml and urinary excretion values are reported as μg/24 hours. To approximate the average amount of bound vit. B_{12} in serum over 24 hours and to relate this value to the average total vit. B_{12}, bound and total levels of the vitamin, of each subject, were plotted on coordinate graph paper for 0, 1, 4, 8, and 24 hours following the dose. The points were

* This investigation was supported by grants from Nutrition Foundation, P.H.S., and Williams Waterman Fund of Research Corp.

† "Cobione" was supplied by Merck Co., Rahway, N. J.

‡ Liver extract was supplied by Lederle Division of Am. Cyanamid Corp., Pearl River, N. Y.

§ "Vit. B_{12}" and "Vit. B_{12} activity" will be used synonymously.

TABLE I. Vit. B_{12} Values (mμg/ml) in Serum following Administration of 50 μg Crystalline Vit. B_{12} or Its Equivalent as Liver Extract.

Time of sampling, hr	Form of vitamin administered: Crystalline (8) Total	Crystalline (8) Bound	Crystalline (8) % bound	Liver extract (8) Total	Liver extract (8) Bound	Liver extract (8) % bound
Fasting	.27 ± .04*	.19 ± .04	70.4	.24 ± .03	.21 ± .03	87.5
1	1.74 ± .15	.75 ± .06	43.1	.96 ± .11	.70 ± .09	72.9
4	.94 ± .05	.69 ± .04	73.4	1.10 ± .08	.77 ± .03	70.0
24	.52 ± .04	.41 ± .04	78.8	.47 ± .04	.39 ± .03	82.9
Avg % bound, calculated†, 24 hr period			64.9 ± 3.4			81.5 ± 2.5

* Stand. error of mean. † Calculations described under methods.
No. in parenthesis is No. of subjects in group.

connected to form rough time-course curves, and the area under the curves determined. Average bound vitamin is expressed as % of total vit. B_{12}.

Results. Values for total vit. B_{12} in serum, drawn post absorptively, averaged 0.26 mμg (Table I) which agrees with values previously reported by this method(4,5,7). Bound vit. B_{12} values of all subjects in post absorptive state averaged 0.20 mμg, which is about 77% of total vit. in serum, and is in agreement with data obtained previously(5,8,9). Serum vit. B_{12} levels, both total and bound, determined 24 hours following injection of the vitamin are higher in both groups than pre-injection fasting levels (Table I).

Average maximum concentrations of total vit. B_{12} and bound vit. B_{12} were 1.8 and 0.8 mμg respectively in the group given crystalline vitamin. In contrast, average maximum concentration of total vit. B_{12} was lower in the group receiving liver extract, 1.2 compared to 1.8 mμg. However, average maximum concentration of bound vitamin was the same in both groups.

The time required for maximum vit. B_{12} level in serum to be reached was different in the 2 groups. In the group given crystalline vitamin, 7 of 8 subjects reached maximum values in one hour, however, 6 of 8 subjects given liver extract required 4 hours. These observations are reflected in data shown in Table I. One hour after injection of vit. B_{12}, average level in serum was greater in the group receiving crystalline vitamin than in the group receiving liver extract, although no difference was observed in serum levels taken at 4 hours.

Average proportion (%) of bound vitamin to total vitamin was calculated over the 24 hour period as described above. Data shown in Table I demonstrate that a greater % of vit. B_{12} was bound following administration of liver extract than was observed following injection of crystalline vitamin, 81.5 ± 2.5% and 64.9 ± 3.4% ($p<0.01$). These data suggest that a greater proportion of vitamin is free, following administration of crystalline vit. B_{12}, and therefore it would be anticipated that a greater amount of vit. B_{12} would be excreted in urine following administration of crystalline vit. B_{12}, rather than liver extract. Urinary excretion of the vitamin in the group receiving crystalline vit. B_{12} (16 subjects) averaged 9.0 ± 0.75 μg as compared to 6.0 ± 1.1 μg for 8 subjects that received liver extract. The groups are significantly different at the 2% level.

Although crystalline vit. B_{12} is completely adsorbed on charcoal and completely dialyzable against 100 volumes of saline; vit. B_{12} in liver extract, although completely adsorbed on charcoal, was only 67% dialyzable under these conditions. It seemed probable that the differences in urinary excretion resulted from unavailability of the vitamin to the blood and hence to the kidney, when administered as liver extract.

Discussion. Ability of human serum to bind vit. B_{12}, *in vitro* and *in vivo,* has been studied by several investigators(5,7,8,9). Mollin and Ross(2) studied this phenomenon in normal subjects, and showed that human serum has a limited ability to combine with vit. B_{12}, with maximum serum bound vit. B_{12} levels of 0.8 to 1.4. It is possible that bind-

ing of vit. B_{12} with serum may be necessary to prevent its urinary excretion, and that the binding mechanism is capable of handling a limited amount of absorbed vitamin. Urinary excretion of vit. B_{12} may be proportional to the level of free vitamin in serum(2), although this point has not been confirmed in this laboratory.

Upon examination, the data demonstrate that total concentration of vit. B_{12} in serum is not the only factor influencing maximum concentration of bound vit. B_{12}. This is suggested by the observation that maximum concentration of serum bound vitamin is not different in the 2 groups one hour after administration of the vitamin while maximum concentration of total vitamin is different. However, concentration of total vit. B_{12} does influence to some degree the concentration of bound vitamin, inasmuch as the highest bound vitamin levels occurred at 1 hour in the group receiving crystalline vitamin and at 4 hours in the other group.

The higher proportion (%) of bound vitamin in serum of the group receiving liver extract was probably due to slower absorption from site of injection, inasmuch as total concentration of vit. B_{12} in serum does not seem to be directly related to the binding maximum. This slower absorption, perhaps influenced by the non-dialyzable fraction of liver extract, resulted in less vitamin present in free or uncombined form. However, absorption was rapid enough to saturate the binding mechanism. This slower absorption continued over a longer period of time, and apparently resulted in less excretion of the vitamin by the kidney.

Summary. Liver extract and crystalline vit. B_{12} were administered in equivalent amounts to 2 groups of normal subjects, and total serum vit. B_{12} levels, bound vit. B_{12} levels, and urinary excretion of vit. B_{12} compared in the 2 groups. Concentration of free vit. B_{12} was higher in the group receiving crystalline vitamin. Over the 24-hour period, percentage of bound vitamin serum was greater in the group receiving liver extract. Urinary excretion of vit. B_{12} was significantly lower in the group receiving liver extract.

The authors wish to express appreciation for interest and assistance of Dr. R. G. Yaeger, Jean Snider and Barbara Geer.

1. Sokoloff, M. F., Sanneman, E. N., Jr., and Beard, M. F., *Blood,* 1952, v7, 243.

2. Mollin, D. L., and Ross, G. I. M., *J. Clin. Path.,* 1953, v6, 65.

3. Register, U. D., and Sarett, H. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 837.

4. Unglaub, W. G., Rosenthal, H. L., and Goldsmith, G. A., *J. Lab. Clin. Med.,* 1954, v43, 1.

5. Miller, O. N., *Archives of Biochem. and Biophysics,*

6. Thompson, H. T., Dietrich, L. L., and Elvehjem, C. A., *J. Biol. Chem.,* 1950, v180, 184.

7. Rosenthal, H. L., and Sarett, H. P., *ibid.,* 1954, v199, 433.

8. Mollin, D. L., and Ross, G. I. M., *J. Clin. Path.,* 1952, v5, 129.

9. Pitney, W. R., Beard, M. F., and Van Loon, E. J., *J. Biol. Chem.,* 1954, v207, 143.

Received January 7, 1957. P.S.E.B.M., 1957, v94.