Isolation and Identification of a New Ketone Body in Normal Human Urine.* (23031)

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Acetoacetic acid, its reduction product, β-hydroxybutyric acid, and its decarboxylation product, acetone, have long been considered the ketone bodies of blood and urine. In the course of a study involving quantitative determination of dihydroxyacetone in urine, we observed interference by a neutral ketone other than acetone on the colorimetric assay of the triose. This report describes the isolation and identification, and discusses the possible origin of a new ketone body, methyl ethyl ketone, in human urine.

Materials and methods. Ten liters of pooled urine from 5 apparently healthy adults collected under toluene in sterile containers were acidified with 800 g of metaphosphoric acid, and the small amounts of protein and other colored material were filtered with the aid of Celite. To the filtrate was added 42 g of recrystallized 2,4-dinitrophenylhydrazine hydrochloride prepared as a saturated solution in warm 2 *N* HCl. After 48 hours' standing at 25°, the dark orange precipitate was collected on a sintered glass funnel with a layer of Celite over the filtering surface. The contents of the funnel were washed twice with 250 ml of water and subjected to suction for removal of water. Precipitate and Celite were extracted with ethyl acetate repeatedly until no more colored material came off the Celite. The combined extracts measured about 2 l and were washed with 2 *N* HCl until the washings became nearly colorless, to remove unreacted 2,4-dinitrophenylhydrazine and other acid-soluble impurities. Then the ethyl acetate solution was washed repeatedly with 5% $NaHCO_3$ solution until washings became nearly colorless to remove hydrazones of keto acids. Finally the organic layer was washed with water and the solvent removed by stream of air overnight. The black syrupy residue was taken up in 100 ml of hot 95% alcohol and the solution was treated with 0.5 g of Norite charcoal, then filtered. The filtrate yielded a gummy precipitate on cooling. This process of purification was repeated 6 more times with minimum amounts of alcohol so that crystalline material was finally obtained. After 3 recrystallizations of the product from 95% alcohol and two recrystallizations from 50% alcohol, the pure material in the form of long plates had a melting point of 111-112° (corr.). Approximately 50 mg of 2,4-dinitrophenylhydrazone of the unknown neutral ketone was isolated from 10 l of urine.

Results. For identification of the unknown ketone the recorded melting points of 2,4-dinitrophenylhydrazones of known ketones were surveyed; methyl ethyl ketone had a melting point of 111°(1). The 2,4-dinitrophenylhydrazone of commercial methyl ethyl ketone (Eastman Kodak Co.) was prepared and the identity of the derivative of the unknown ketone with that of methyl ethyl ketone was established by the following observations: a) the mixed melting point showed no depression, b) chromatogram on Whatman No. 1 filter paper developed with methanol-saturated heptane(2) showed identical R_f of 0.64 (3), c) absorption spectra in 0.25 *N* NaOH (in 70% alcohol) were identical, showing the same maxima at 434 mμ and 535 mμ.

Discussion.† The most likely precursor of methyl ethyl ketone is α-methylacetoacetic acid which has been suggested as an intermediate of isoleucine catabolism(4,5). The steps in pathway from isoleucine to methyl ethyl ketone may be postulated as follows: oxidative deamination, decarboxylation, β-oxidation of long carbon chain(6), and finally decarboxylation. Since this ketone was found in the steam distillate of mulberry leaves(7),

* Aided by grant from Playtex Park Research Institute.

† We wish to thank Drs. I. A. Bernstein and M. J. Coon for their kind advice.

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.

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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)

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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.