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A Practical Method for Preparation and Isolation of *l*-beta-Hydroxybutyric Acid.*

HARRY BLUNDEN. (Introduced by H. J. Deuel, Jr.)

From the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles.

The increasing number of studies on the metabolism of ketone bodies has made a method for the preparation of pure *l*-beta-hydroxybutyric acid desirable. The easiest way to accomplish this is to synthesize it in the animal body. Although it may be obtained from the urine of fasting human subjects, it may also be readily produced in large amounts from the urine of fasted rats fed sodium butyrate according to the earlier procedures from this laboratory (0.237 g of butyric acid daily per 100 g rat in solution by stomach tube in divided doses morning and evening). When 85 female rats weighing 125-150 g were used, 58 g of *l*-beta-hydroxybutyric acid were excreted in a 4-day period of which 34 g were recovered as the pure Ca-Zn double salt. Although a similar procedure might be employed with a smaller number of dogs, our experience has been that rats are particularly well adapted because they can not regurgitate. The following method was employed in separating the acid in pure form.

To the pooled urines is added sufficient solid copper sulfate to obtain a concentration of 20%. To the resulting solution is added 0.5 g calcium hydroxide (in 10% aqueous suspension) for each gram of copper sulfate present. This amount of the base usually suffices to bring the pH slightly alkaline to litmus. If the pH is not alkaline to litmus after standing ½ hour more calcium hydroxide should be added. The mixture is then filtered by suction, working the precipitate with a spatula to a dry, hard cake, to express as much of the liquid as possible.

The filtrate is evaporated under reduced pressure to a syrup. At this point the precipitated salts may be filtered off. The resulting syrup is then acidified to litmus with 50% sulfuric acid. Caution must be exercised here to prevent the temperature of the syrup rising above 10°C, otherwise conversion to crotonic acid may occur. Then one full equivalent of 50% sulfuric acid is added to the syrup for each equivalent of *l*-beta-hydroxybutyric acid present as determined by assay according to the Van Slyke procedure.

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Plaster of Paris is stirred into the acidified syrup in sufficient quantity to make a thick paste. As soon as the paste begins to set, one stirs it vigorously until a fairly coarse meal results. More plaster of Paris may be incorporated until the mixture is fairly dry.

The meal is then extracted with redistilled ether in a Soxhlet apparatus for 8 hours, after which the ether is evaporated off and the residue taken up with a minimum quantity of water.

The cloudy, slightly colored liquid is then shaken cold with a small amount of Norite and filtered; the resulting clear solution is divided into 2 equal volumes and neutralized by warming it with calcium carbonate and zinc carbonate respectively as outlined by Shaffer.[1] It is desirable to allow the 2 equal portions of the solution of beta-hydroxybutyric acid to stand for 12 hours in contact with an excess of the respective carbonates.

The excess carbonates are filtered off and the 2 solutions poured together. If more than 10% of the double salt of *l*-beta-hydroxybutyric acid is present, the calcium zinc double salt crystallizes out immediately and the addition of an equal volume of hot ethyl alcohol causes almost all of the remaining salt to crystallize out in 24 hours. The calcium zinc double salt may be purified by recrystallizing from water to which ethyl alcohol is added and finally from water.

The pure free *l*-beta-hydroxybutyric acid can now be recovered by acidifying a solution of the salt, setting with plaster of Paris and extracting with ether. After evaporation of the solvent the syrupy acid remains free from any contaminating substance.

After 3 recrystallizations from alcohol and one from water a preparation having the following constants was obtained:

	Found	Theoretical
$[\alpha]^{D}_{18°}$	—16.21°	—16.26°[1]
Ash (CaO + ZnO)	26.50	26.55%
Van Slyke assay	79.1%	75.9%
	oxidation to Hg acetone precipitate	oxidation to Hg acetone precipitate

This method of preparation and solution has been employed several times successfully by the author and independently by another investigator in this laboratory (B. Bobbitt) who obtained a higher percentage recovery than is reported here.

[1] Shaffer, P. A., and Marriott, W. M., *J. Biol. Chem.*, 1913-14, **16**, 265.