6284

Conjugation of Benzoic Acid with Glycine, a Test of Liver Function.

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In a recent study on the conjugation of benzoic acid in man, the author found that the excretion of hippuric acid proceeded at a constant rate irrespective of the amount of sodium benzoate administered.[1] Since it was further found that the output of hippuric acid was greatly increased by feeding glycine or foods rich in glycine such as gelatine, it became obvious that the rate of hippuric acid formation is dependent upon the speed with which the body can synthesize glycine. It was determined that a normal adult can produce enough glycine to conjugate from 0.90 to 1.30 gm. of benzoic acid per hour.

Since the main site of the synthesis of glycine is presumably the liver, it was considered probable that certain types of liver damage might show a diminution in the hourly excretion of hippuric acid. On this basis a test was carried out as follows: 5.9 gm. of sodium benzoate dissolved in 150 cc. of water is administered 1 hour after a breakfast consisting of coffee and toast. Complete hourly specimens of urine are collected for 4 hours. Hippuric acid is determined by the author's method.[2] A simpler method which is sufficiently accurate for clinical purposes can be employed. In this procedure the hourly specimen is measured, transferred to a small beaker, and acidified with concentrated HCl until acid to Congo red. (1 cc. of the acid is usually sufficient.) The solution is vigorously stirred until the hippuric acid has completely crystallized out, and then is allowed to stand at room temperature for 1 hour. The hippuric acid is filtered off on a Buchner funnel or a small filter plate, washed with cold water, allowed to air dry, and finally weighed. (Weighing to the second decimal place is sufficiently accurate.) To obtain the total hippuric acid, one adds to the weight of the precipitated crystals, the calculated amount remaining in solution. One hundred cc. of urine will hold in solution approximately 0.33 gm. of hippuric acid. If the volume of any specimen exceeds 150 cc., it should be slightly acidified with acetic acid, and concentrated on a water bath to about 50 cc. before precipitating the hippuric acid.

In applying the test to various liver diseases, the most striking

[1] Quick, A. J., *J. Biol. Chem.*, 1931, **92**, 65.

[2] Quick, A. J., *J. Biol. Chem.*, 1926, **67**, 477.

results were obtained in cases of toxic jaundice including catarrhal jaundice, and in obstructive jaundice of moderately long standing. In all these cases a definite diminution in the rate of hippuric acid excretion occurred, and significantly, the hourly output often remained low long after the jaundice had cleared up. In Table I a few typical results are recorded.

TABLE I.
Hourly Excretion of Hippuric Acid.

Time hr.	Normal I gm.	Normal II gm.	Normal III gm.	Obstructive Jaundice gm.	Obstructive Jaundice gm.	Catarrhal Jaundice gm.	Catarrhal Jaundice gm.
1	1.29	0.43	0.81	0.54	0.43*	0.23	0.35*
2	1.96	1.96	1.46	0.93	0.56	0.51	0.51
3	0.82	1.28	1.51	0.93	0.54	0.65	0.44
4	0.28	0.79	1.49	0.81	0.70	0.54	0.69

* 10 days after the first test.

6285

Appearance of Large Amounts of Non-Stainable Cultivable Granules in Bacterial Cultures on Saccharose Media.

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A Gram-positive spore bearing aerobic bacillus, isolated following accidental contamination of a saccharose broth tube, was found to grow on a saccharose agar plate as a thick soft spreading film. This film later became of semi-liquid consistency and in it the bacteria could be seen whirling about. Using a dark background preparation, the culture consisted for the most part of scarcely perceptible minute granules, often arranged in large clumps. Masses of these granules were seen in saccharose broth cultures as well as upon solid media. Using a very strong light or sunlight with the dark field microscope, these granules were also visible in strong Brownian movement. They were not stained by the usual methods. They were made visible by the following technique: a loopful of a broth culture or of an emulsion of the agar growth was mixed on a slide with a loopful of a crystal violet solution (a 1:5 or 1:10 dilution of the solution used for Gram stain). About one minute later a loopful of a 5% solution of silver nucleinate was added and the whole mixture was spread over the slide as a thin film. A sec-