seem out of place to describe a method I have used for its preparation which is somewhat simpler than that given by Fischer.

A saturated solution of hydrobromic acid gas in acetic anhydride was found to react directly with several of the sugars and from the reaction mixture could be isolated the bromoacetyl derivative. By this reaction bromoacetylxylose,[1] bromoacetylcellulose and bromoacetyllactose, as well as bromoacetylglucose, were prepared in the crystalline condition with yields of respectively 26, 60 and 77% of the theoretical. Bromoacetylmaltose was also obtained by this method, though only in the amorphous condition. A detailed description of the preparation of bromoacetylglucose is given below; it is typical of the method of preparation of each of the above named compounds.

Twenty-five grams of finely powdered glucose were treated in a large Erlenmeyer flask at room temperature with 125 cc. of acetic anhydride saturated with hydrobromic acid gas. A very vigorous reaction followed, leaving a clear, straw-colored sirup. This was cooled, mixed with 300 cc. of chloroform and the solution washed twice with water, once with enough sodium bicarbonate solution to neutralize the dissolved acids, and then once more with water. After drying with calcium chloride, the chloroform solution was evaporated at 50° under diminished-pressure to a thick sirup which was washed into a beaker with a little dry ether. Fifteen to twenty volumes of petroleum ether were added, causing the bromoacetylglucose to precipitate as a thick sirup, which on cooling in an ice bath and being stirred vigorously, solidified in a few minutes to a crystalline mass. It was filtered off on a Büchner funnel and recrystallized by dissolving in 75 cc. of dry ether and evaporating the solution in a current of dry air until it crystallized. The yield of the once recrystallized substance was between 50% and 60% of the theory. Fischer obtained by his method 56% of the theoretical amount.

When bromoacetylglucose was sufficiently pure no trouble was experienced in preserving it in good condition for weeks at a time, but usually more than one recrystallization was necessary for this purpose.

WASHINGTON, D. C.

[FROM THE INSTITUTE OF THE MEDICAL CHEMISTRY OF KYOTO IMPERIAL UNIVERSITY, JAPAN.]

QUANTITATIVE DETERMINATION OF HIPPURIC ACID IN URINE, BLOOD, MUSCLES AND LIVER: A METHOD.

BY DR. HIIZU ITO.

Received July 28, 1916.

Since Bunge and Schmiedeberg[2] published a well-known method for quantitative determination of hippuric acid in urine in 1876, various

[1] THIS JOURNAL, 37, 2745 (1915).

[2] Bunge u. Schmiedeberg, *Arch. exp. Path. Pharm.*, **6**, 233 (1876).

investigators have suggested several methods. These methods may be conveniently classified in three ways: Hippuric acid is determined as such;[1] as nitrogen in glycocoll;[2] as benzoic acid.[3] The method here described follows the third scheme. It is generally acknowledged that, in extracting the experimental material, an extracting apparatus with boiling ether gives a better result than a separatory funnel. For this reason an extracting apparatus, designed as illustrated in Fig. 1 was used. The main tube *a* has two branch-tubes *b* and *c*, one of which, *b*, serves for the ascending petroleum-ether vapor, and the other, *c*, for the returning petroleum ether which, collecting at the lower small opening *d* of the branch tube *c* closes the latter, and prevents the ascent of vapor except through the other branch tube *b*. The flask *f* with petroleum ether is connected with the apparatus, and is immersed in an electric water bath, kept at 80–90°, which keeps the solution boiling. This apparatus is also adapted for extraction with ethyl ether.

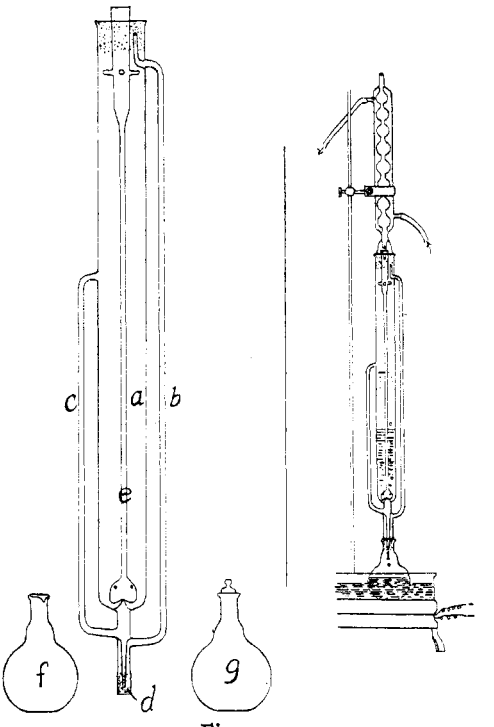

Fig. 1.

TABLE I.

Hippuric acid 0.0328 g., equal to 0.0224 g. benzoic acid.				Hippuric acid 0.4264 g., equal to 0.2906 g. benzoic acid.			
Time for hydrolyses.	Conc. of NaOH.	Benzoic acid recovered (g.).	Benzoic acid accounted for.	Time for hydrolyses.	Conc. of NaOH.	Benzoic acid recovered (g.).	Benzoic acid accounted for.
8 hours	20%	0.0190	84.8%	8 hours	20%	0.2833	97.5%
8 hours	25%	0.0210	93.8%	8 hours	25%	0.2872	98.8%
12 hours	20%	0.0202	90.2%	12 hours	20%	0.2829	97.4%
12 hours	25%	0.0222	99.1%	12 hours	20%	0.2829	99.0%
16 hours	20%	0.0219	97.8%				

In order to determine the best conditions for hydrolysis of the hippuric

[1] *Loc. cit.*

[2] Henriques u. Soerensen, *Z. physiol. Chem.*, **63**, 27 (1909); **64**, 120 (1910); Blumenthal, *Z. klin. Med.*, **40**, 339 (1900).

[3] Jaarveld u. Stockvis, *Arch. exp. Path. Pharm.*, 10 (1879), 268; Folin, *J. Biol. Chem.*, 11, 257 (1912); Steenbeck, *Ibid.*, 11, 201 (1912).

acid varying concentrations of NaOH were tried. The results obtained after extracting the benzoic acid with petroleum ether are given in Table I.

It was thus found that the maximum result could be obtained by using 25% NaOH and 12 hours' boiling for the hydrolysis, and the following experiments were, therefore, carried out under these conditions. The results of determinations of hippuric acid in urine, blood, muscles and liver are shown in Tables II, III, IV and V.

To establish the purity of the recovered benzoic acid, its melting point was determined and found to be 116.0–120.0° ("uncorrected"). Furthermore, microscopical examination of the crystal form of the substance proved its identity with benzoic acid.

Procedures for the quantitive determination of hippuric acid in urine and in other mixtures will be described in detail in the following:

Experimental.

1. Urine.—A quantity of fresh rabbits urine was divided into two equal portions; to the one portion a fixed quantity of hippuric and benzoic acids was added and the other was used as a control. Each portion was made faintly alkaline with sodium carbonate, and evaporated nearly to dryness and the residue was thoroughly extracted with absolute alcohol. After evaporating off the alcohol the residue was dissolved in water. The solution was transferred to the apparatus described above and was extracted for 12 hours with pure dry ethyl ether (in this case the solution must be alkaline), which dissolved the resin substance, while the sodium hippurate and sodium benzoate remained undissolved. The solution was then transferred to a porcelain evaporating dish, a little animal charcoal was added and the ethyl ether was driven off completely. The solution, after having been poured into the apparatus, was made slightly acid with hydrochloric acid, and extracted with petroleum ether b. p. 30–60° for 48 hours. In this case the benzoic acid alone dissolved, while the hippuric acid remained undissolved. After cooling, the petroleum ether in the flask *f* was filtered into the weighed flask *g* and the petroleum ether was evaporated by a stream of dry air. The benzoic acid crystallized beautifully on the inner wall of the flask *g*.

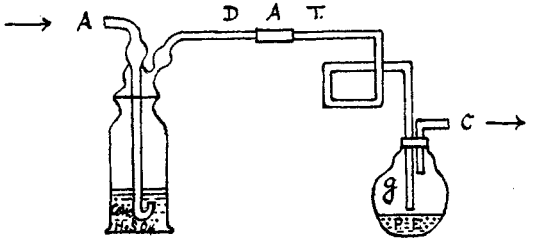

Fig. 2.—Evaporating apparatus. Petroleum ether in the flask (g); A = Air, D. A. T. = dry air tubing, P. E. = petroleum ether, and C = connection with a suction tube. Arrows show direction of air current.

The solution, which contained hippuric acid only, was neutralized

with sodium carbonate, and 15 cc. of 50% NaOH were added to it. The mixture was then carefully concentrated to 30 cc., and was boiled in a 200 cc. Kjeldahl flask with an upright condenser for 12 hours. It was then transferred completely to a porcelain evaporating dish concentrated to as small a volume as possible, after adding a little animal charcoal. After it was cooled, 30% hydrochloric acid was carefully added drop by drop with continuous stirring. The acidified solution was now transferred to the extracting apparatus, and extracted with petroleum ether for 72 hours. The petroleum ether was filtered off in the flask *g* mentioned above and evaporated as already described. The hippuric acid was weighed as crystalline benzoic acid. The results are given in Table II.

TABLE II.

Benzoic acid added (80 cc. urine).					Hippuric acid added (80 cc. urine).				
Gram benzoic acid added as sodium benzoate.	Gram benzoic acid recovered.	Benzoic acid in control urine.	Difference.	Percentage of benzoic acid accounted for.	Gram benzoic acid calc. from hippuric acid added.	Gram benzoic acid recovered.	Gram benzoic acid in control urine.	Difference.	Percentage of benzoic acid accounted for.
0.2768	0.2770	0.0039	0.2731	98.7	0.2677	0.2691	0.0090	0.2601	97.2
0.2054	0.2075	0.0045	0.2030	98.8	0.1747	0.1742	0.0077	0.1665	95.3
0.1961	0.2024	0.0067	0.1957	99.8	0.1938	0.2018	0.0136	0.1882	97.1
0.1124	0.1138	0.0030	0.1108	98.6	0.0812	0.0848	0.0064	0.0784	96.6
0.0631	0.0652	0.0042	0.0610	96.7	0.0460	0.0512	0.0081	0.0431	93.7

2. Blood.—The defibrinated blood of a cow or a rabbit was divided into two equal portions; to one a fixed quantity of hippuric acid was added and the other was used as a control. Each portion was diluted five times with water, neutralized with weak hydrochloric acid, and heated on a water bath. In this treatment, protein coagulated completely with the fats, and deposited at the bottom of the beaker with a clear layer on its top. After 24 hours' standing, it was filtered through by suction, and repeatedly washed with hot water. The residue together with the filter paper was ground in a glass mortar, adding water, and again filtered with suction. This treatment was repeated several times. The filtrate was then made faintly alkaline, evaporated nearly to dryness, and the residue was thoroughly extracted with absolute alcohol. In the case of blood, it was not necessary to remove the benzoic acid, for none was added to it and normal blood does not contain benzoic acid. The subsequent treatment was the same as in urine. The results are tabulated as follows:

TABLE III.

Cc. of blood.	Gram hippuric acid added.	Gram benzoic acid calc. from hippuric acid.	Hippuric acid as benzoic acid recovered.	Hippuric acid, as benzoic acid in control blood.	Difference.	Percentage of benzoic acid accounted for.
40 (blood of cow).....	0.3943	0.2687	0.2508	0.0042	0.2466	91.8
50 (blood of cow).....	0.3245	0.2211	0.2064	0.0032	0.2032	91.9
40 (blood of rabbit)...	0.2840	0.1935	0.1735	0.0024	0.1734	89.6

3. Muscle.—Muscles, which were finely cut, were divided into two equal portions. Each portion was ground in a glass mortar with addition of some water and was then heated. A fixed quantity of hippuric acid was added to the one portion and stirred well, the other was the control. Each portion was filtered through muslin, having been washed with hot water repeatedly, and was pressed dry. The residue was again ground in glass mortar and filtered through muslin as before and this treatment was repeated several times. The filtrate was made neutral or weakly acid, and evaporated. The protein in the filtrate coagulated with the fats upon heating on a water bath and the whole was filtered by suction, and was washed with water several times. The filtrate was evaporated nearly to dryness and the residue was thoroughly extracted with absolute alcohol. After this treatment, the procedure was the same as in the case of blood. The results are given in Table IV.

TABLE IV.

Grams of muscles.	Gram hippuric acid added.	Benzoic acid calc. from hippuric acid.	Hippuric acid, as benzoic acid recovered.	Hippuric acid, as benzoic acid in the control.	Difference.	Percentage of benzoic acid accounted for.
50	0.3572	0.2434	0.2243	0.0008	0.2235	91.8
70	0.3348	0.2282	0.2077	0.0016	0.2061	90.3
100	0.2197	0.1497	0.1326	0.0011	0.1315	87.8

4. Liver.—The same treatment as in the case of muscles was applied. The results are given in Table V.

TABLE V.

Grams of liver.	Gram hippuric acid added.	Benzoic acid, calc. from hippuric acid.	Hippuric acid, as benzoic acid recovered.	Hippuric acid, as benzoic acid in the control.	Difference.	Percentage of benzoic acid accounted for.
50	0.4132	0.2816	0.2628	0.0016	0.2612	92.8
50	0.3750	0.2556	0.2331	0.0010	0.2321	90.8
50	0.2581	0.1759	0.1566	0.0012	0.1554	88.3

For the determination of hippuric acid the investigators mentioned in the foot-note of p. 2189, took great pains to obtain the purest possible hippuric acid and at the same time to secure the largest quantitative yield. The effort here described has been to simplify the method by means of a suitable extraction apparatus and to obtain the most satisfactory results in both of these respects.

Summary.

(1) The method proposed can be well applied to the quantitative determination of hippuric acid in urine and similar materials.

(2) The method has the advantage of simplicity of treatment and of giving fairly accurate results.

NEW HAVEN, CONN.