

with NaOH. What color develops? Is it permanent? Compare test (2), page 8.

Salkowski's test. Add an excess of acetic acid to the solution obtained in the preceding test and heat in a boiling water bath. Is there a change of color? A ppt. of Prussian blue may separate.

Hippuric acid.—Dissolve about 2 grams of ammonium benzoate in half a glass full of water and take the solution before retiring at night. Collect the urine passed the following morning and bring the specimen to the laboratory for the isolation of hippuric acid, which has been formed in the body by the conjugation of glycocoll and benzoic acid. Write the equation.

To 50 cc. of the urine add 25 grams of solid ammonium sulfate and 1 cc. of conc. sulfuric acid. Stir until solution is complete and allow the mixture to stand for 15 minutes. Filter off the pigmented crystals of hippuric acid and dry them between folds of filter paper. Use the crystals in the following tests:

(a) Dissolve a small amount in 5 cc. of hot water and make a microscopic examination of the crystals which separate on cooling.

(b) Place a few crystals in a dry test tube and heat. They melt and on further heating the liquid assumes a red color and a sublimate of benzoic acid appears.

(c) Add 1 cc. of conc. nitric acid to a few crystals in a small evaporating dish and evaporate to dryness on a water bath. Transfer the residue to a dry test tube and heat. Note the odor of nitrobenzene (page 22).

(d) Boil the remaining crystals with about 5 cc. of conc. HCl for several minutes. Allow the crystals of benzoic acid to settle and pour off the supernatant liquid into a beaker. Add ammonium hydroxide until the solution is slightly alkaline and boil until the excess of ammonia is removed. Add a few drops of copper sulfate solution and note the color of the copper salt of glycocoll. Write the equation for the hydrolysis of hippuric acid.