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Gasometric Determination of Glucose.

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The method depends on the reduction of ferricyanide by the sugar, and the determination of the excess ferricyanide, by measuring the nitrogen gas evolved by the reaction with hydrazine:
$4\ K_3Fe(CN)_6 + N_2H_4 + 4\ NaOH = 4\ K_3NaFe(CN)_6 + 4\ H_2O + N_2$
The reagent solution contains 6 grams of potassium ferricyanide and 150 grams Na_2CO_3 per liter. One volume of this is mixed in a tube of 10 mm. diameter with 2 volumes of sugar solution, or of Folin-Wu blood filtrate, containing 0.04 to 0.4 mg. of glucose per cc. This is heated 4 minutes by immersion in boiling water. The solution is cooled to room temperature, and is resaturated with air by stoppering and shaking the tube 1 minute. Two cc. of a solution, prepared by mixing 1 volume of saturated hydrazine sulfate solution with 1 volume of 40 per cent sodium hydroxide, is placed in the chamber of the Van Slyke-Neill[1] manometric blood gas apparatus. Three cc. of the sugar-cyanide solution are then measured into the chamber from a rubber-tipped pipette (described by Van Slyke and Neill). The reaction of ferricyanide and hydrazine is practically instantaneous. The chamber is evacuated, the mixture is shaken 1 minute, the gas volume is reduced to 0.5 cc., and the pressure in the manometer is read as p_1. A control without glucose is run, and the pressure read as p_0. The decrease in pressure, p_0-p_1, due to reduction of ferricyanide by sugar, is the measure of the glucose. One control serves for an entire series of sugar determinations, which can be run off at the rate of about 1 each 3 minutes. One mg. of sugar causes a drop in the pressure reading of 238 mm. at 15°, 242 at 20°, 246 at 25°, 250 at 30°. The factor per mg. of sugar is constant, within 1 per cent, for amounts of sugar varying within the limits given above for use with the method.

[1] Van-Slyke, D. D., and Neill, James N., *J. Biol. Chem.*, 1924, lxi, 523-73.