safety between complete removal of PNA and incipient extraction of DNA was much smaller. (4) Prolonged hardening of nervous tissue in 10% formalin rendered PNA resistant to extraction by perchloric acid. (5) Perchloric acid extraction of fixed sections of nervous tissue removed insignificant quantities of nerve cell protein. (6) Cell structure was unaltered by this extraction procedure. (7) This extraction procedure did not alter the E_{2537}Å of two tissues without PNA, skeletal muscle and ribonuclease-treated nerve cells. It was suggested that this procedure could be employed to provide tissue "blanks" in ultraviolet microabsorption spectroscopy for PNA in tissue sections.

1. Ogur, M., and Rosen, G., *Arch. Biochem.*, 1950, v25, 262.

2. Erickson, R. O., Sax, K. B., and Ogur, M., *Science*, 1949, v110, 472.

3. Seshachar, B. R., and Flick, E. W., *Science*, 1949, v110, 959.

4. Cassel, W. A., *J. Bact.*, 1950, v59, 185.

5. Sulkin, N. M., and Kuntz, A., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 413.

6. Koenig, H., *J. Nat. Cancer Inst.*, 1950, v10, 1346.

7. Koenig, H., Groat, R. A., and Windle, W. F., *Stain Tech.*, 1945, v20, 13.

8. Windle, W. F., Rhines, R., and Rankin, J., *Stain Tech.*, 1943, v18, 77.

9. Stowell, R. E., *Stain Tech.*, 1946, v21, 137.

10. Pollister, A. W., and Moses, M. J., *J. Gen. Physiol.*, 1949, v32, 567.

11. Bensley, R. R. and Bensley, S. H., *Handbook of Histological and Cytological Technique*, University of Chicago Press, 1938.

12. Pollister, A. W. and Ris, H., *Cold Spring Harbor Sympos. Quant. Biol.*, 1947, v12, 147.

13. Lavin, G. I., *Rev. Sci. Inst.*, 1943, v14, 375.

14. Caspersson, T., *J. Roy. Micr. Soc.*, 1940, v60, 8.

15. Pollister, A. W., and Leuchtenberger, C., *Nature*, 1949, v163, 360.

16. Hamberger, C. A. and Hyden, H., *Acta Otolaryngolg.*, 1949, Supp. LXXV, 53.

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Determination of Polyglucose in Blood and Urine.* (19308)

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Polyglucose is a water-soluble polymer of glucose prepared by chemical polymerization. Preparations with molecular weights ranging from 3000 to 160000 have been obtained. Animal experiments have indicated that solutions are non-toxic, and may have value for use as a blood substitute in treatment of shock.†

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† Material for the present studies was obtained by courtesy of the E. I. du Pont de Nemours and Co. through the Medical Research Committee of the National Research Council. This material was prepared by Dr. P. T. Mora of the Rayon Dept., Pioneering Research Laboratory, Dupont Experimental Station, Wilmington, Del. Sample P-24-14-PIa and sample P-24-52-2 were the samples used in the majority of the experiments described in this paper.

Composition of polyglucose used as basis for analytical calculations. Polyglucose, like starch and glycogen, is difficult to dry to an anhydrous condition. Air-dried preparations contain 10% or more of moisture. After drying 8 days at 80° at atmospheric pressure, a preparation reached constant weight. Its carbon content then was 42.37%, corresponding to $(C_6H_{10}O_5)_2 \cdot H_2O$ (theory, 42.1% carbon), rather than to $C_6H_{10}O_5$ (44.44% carbon). It is uncertain whether the half molecule of water per $C_6H_{10}O_5$ unit in the dried preparation was moisture that could not be driven off under the conditions used, or whether it was a part of the molecular structure. In starch and glycogen Dumazert(3) has found the same composition (C = 42.2 to 42.4%) when these carbohydrates were dried at 80° under less than 1 mm pressure. His

dried starch and glycogen showed zero water content by the chemical method of K. Fischer; consequently, it appeared that the H_2O of the residual $(C_6H_{10}O_5)_2 \cdot H_2O$ was part of the composition of these polysaccharides, which Dumazert accordingly expressed as $(C_{12}H_{22}O_{11})n$. The question is raised, whether it is preferable to base calculations of polyglucose on the anhydrous formula, $C_6H_{10}O_5$, or on $(C_6H_{10}O_5)_2 \cdot H_2O$. Because of uncertainty regarding the nature of the half molecule of water per $C_6H_{10}O_5$ unit in the apparently dried polyglucose, and because of the established use in analytical chemistry of the anhydrous formula in calculations of starch analyses, we have adhered to the conventional anhydrous formula $C_6H_{10}O_5$ in the present analyses.

Reactions used for determination of polyglucose.‡ Two reactions of polyglucose are applied. One is that with anthrone, which produces a green color, adaptable to photometry, with apparently all carbohydrates. The reagents and procedure are simple. The color intensity produced per unit of carbon has been found, by experiments with solutions standardized by carbon determination(10,11), to be the same whether the glucose is free or is combined in polyglucose. The other reaction is the increase in reducing sugar caused by acid hydrolysis. Polyglucose has some reducing power, but it is greatly increased by hydrolysis.

Reaction of anthrone with polyglucose and glucose. Dreywood(2) showed that when a solution of anthrone in concentrated sulfuric acid is added to one volume or less of aqueous carbohydrate solution a green color results, that the reaction is given by all types of carbohydrates, and by none other of the substances tested except glycerol and furfural. The reaction was placed on a quantitative basis for colorimetric analyses by Morris(7), and was used by Durham and coworkers(4) for the determination of blood sugar. Polyglucose reacts like the naturally occurring carbohydrates. The preparation of standard polyglucose solutions by weight is difficult because of the extremely hygroscopic nature of the substance. However, when the chromogenic power of polyglucose solutions was compared with that of glucose solutions containing the same weight of carbon per unit volume (10,11), it was found that the color intensities produced were exactly equal, and obeyed Beer's law. Hence, assuming $C_6H_{10}O_5$ as the composition of anhydrous polyglucose, 1.111 mg of glucose is equal in chromogenic power to 1 mg of polyglucose, and glucose solutions prepared on that basis can be used as standards for photometric determination of polyglucose. As found for other carbohydrates (1,2,4,7), polyglucose develops in 15 minutes a color with anthrone that remains constant for several hours at room temperature. However, the *conditions at the time of the color development* have an effect on the intensity of the color that has not been mentioned in previous publications. When 10 ml of anthrone solution in 95% sulfuric acid were added to 5 ml of aqueous polyglucose solution in a test tube cuvette of 17 mm bore, with shaking of the tube during the addition(4,7), duplicate photometric readings gave a standard deviation of 1.8% of the mean, which was several times the instrumental error. In order to obtain more rapid and uniform mixing, the anthrone solution was added to the carbohydrate in 50 ml Erlenmeyer flasks, with constant rotation, 75 seconds being taken for the addition. The result was an optical density only 60 to 70% as great as when the mixing occurred in the test tubes. The cause of the difference was not temperature, which was found to rise to 94-95° in both the tubes and the flasks. Various procedures of mixing were tried, including heating the cuvettes for 3 minutes in a water bath after the mixing. As constant an optical density per unit of polyglucose was obtained by the following procedure as by any more elaborate. To 5 ml of polyglucose solution in a test tube cuvette of 17 mm bore, 10 ml of anthrone solution were added from an automatic burette of that capacity with the cock fully opened so that addition was made during a constant

‡ The determination of polyglucose has also been studied at the Merck Institute for Therapeutic Research, Rahway, N. J., by R. H. Silber and H. J. Robinson. They have developed turbidimetric procedures for the determination. Their methods are as yet unpublished.

interval of 75 seconds. The anthrone solution was dropped directly onto the surface of the aqueous polyglucose solution, and the tube was shaken constantly during the addition and for a few seconds afterwards, until the disappearance of refraction differences in the solution indicated that mixture was complete. The tubes were then let stand at room temperature, and readings were made after 15 minutes. With amounts of polyglucose giving optical densities of 0.3 to 0.5 the standard deviation of replicates from the mean was ±1.8%. The dependence of the optical density on the conditions under which the two solutions are mixed makes it essential to run duplicate analyses, with the color developed in different cuvettes.

Reducing power of polyglucose before and after Hydrolysis. For measurement of reducing substance, the sugar method of Miller and Van Slyke(6) was used, in which the sugar acts on an excess of ferricyanide and the ferrocyanide formed is measured by titration with ceric sulfate. Polyglucose of average molecular weight about 90000 analyzed by this method, without preceding hydrolysis, showed per unit of carbon 3.4% of the reducing power shown by glucose. A preparation of average molecular weight about 8000 showed 9.7%. Per mg of polyglucose calculated as $C_6H_{10}O_5$, their reducing values are 3.7 and 10.6% of the reducing power of 1 mg of glucose. For determination of the rate of hydrolysis the polyglucose solution was mixed with an equal volume of 2 N hydrochloric acid and heated in a test tube immersed in a boiling water bath with the tube loosely covered to prevent loss of water by evaporation. Reducing power reached a plateau in 2 hours. Per mg of carbon the reducing power was then 92 ± 0.5% that of glucose, for both of the preparations mentioned in the preceding paragraph. With the higher molecular weight polyglucose preparation the increase in reducing power caused by hydrolysis was 92 − 3.4 = 88.6% of the theoretical increase that would have occurred if the reduction had been zero before hydrolysis and if all the combined glucose, estimated from the carbon content, had been set free by the hydrolysis. With the lower molecular weight preparation the increase was 92 − 9.7 = 82.3% of theoretical. Failure to obtain theoretical yields of glucose by acid hydrolysis has been noted with starch and glycogen. In a recent careful study Dumazert(3) has found that the maximal reducing power obtained from both of these carbohydrates by hydrolysis was 95% of that calculated from the carbon content on the assumption of complete conversion to glucose. The increase in reducing power caused by hydrolysis serves to measure polyglucose in the presence of pre-formed glucose, and is the procedure used at present for blood analysis.

Photometric determination of polyglucose in glucose-free urine with anthrone. In the absence of glycosuria the anthrone method is the one of choice for urine. The non-carbohydrate reducing substances of urine, which in their reaction with the reagents used for reducing sugars may be equivalent to as much as 2 grams of sugar per liter of urine(8,9), do not give an appreciable color with anthrone.§

Reagents. Anthrone solution. 1 g of anthrone is dissolved in 95% sulfuric acid, prepared by mixing 475 ml of sulfuric acid of specific gravity 1.84 with 25 ml of water. The solution is stored in a refrigerator. It has remained satisfactory for several weeks. The anthrone used was obtained from the Montclair Research Corp., Montclair, N. J. The blank obtained with different batches of sulfuric acid varied considerably. A bottle of Eimer and Amend's "C.P. Tested Purity Reagent" was satisfactory. *Stock glucose solution.* 111.1 mg of glucose are dissolved in water and made up to 100 ml. In chromogenic power with anthrone this is equivalent to a solution of 1 mg of anhydrous polyglucose, calculated as $C_6H_{10}O_5$, per ml. *Determination of standard optical density or transmission curve.* Portions of 1, 2, 3, and 4 ml of the stock glucose solution are diluted

§ Methods for determining polyglucose in glucose-containing urine have not been tested. Presumably the anthrone procedure could be used for total carbohydrates, with subtraction of 0.9 of the glucose to calculate polyglucose. However, because of non-glucose reducing substances of urine, the correction would have to be made by determining the glucose by fermentation(8,9).

each to 100 ml. Of these solutions triplicate portions of 5 ml, containing glucose chromogenically equivalent to 0.05, 0.10, 0.15, and 0.20 mg of polyglucose, respectively, are pipetted into cuvettes. To each solution in its cuvette 10 ml of the anthrone solution are added slowly with shaking. A blank solution is prepared by adding 10 ml of anthrone solution to 5 ml of water in another cuvette. After the mixtures have stood 15 minutes or longer, readings in a photometer are taken of transmission or optical density with light of 630 millimicrons wave length, the blank being used to set the zero point. For the standard curve, optical densities (optical density = 2 – log of percentage transmission) or the logarithms of the transmissions are plotted against the equivalents of mg of polyglucose in the cuvettes, 1.111 mg of glucose being equivalent to 1 mg of polyglucose. If the scale of the photometer reads directly in optical densities, it is convenient, instead of plotting the linear curve, to use the readings to determine the K value of the equation: (1) Mg polyglucose in cuvette = KD where D is the optical density.

In the present work, the photometer used was a Coleman Junior, with a density as well as a transmission scale. The cuvettes were Pyrex test tubes, 19 by 150 mm, tested for uniformity of transmission. With this equipment the K value of Equation 1 was 0.239, with a standard deviation of ± 0.004, or 1.8%.

Analysis of urine. Dilution. Urine is so diluted that 5 ml of the diluted solution contain about 0.1 mg of polyglucose. If x = mg of polyglucose expected per ml of urine, one volume of urine is diluted to the nearest convenient approximation of 50 x volumes. *Color development and reading.* Duplicate 5 ml portions of the diluted urine are treated with anthrone and the photometer readings are taken as described for the standard curve. *Calculation.* The mg of polyglucose in the cuvette are estimated by interpolation on the standard transmission curve, or from optical density by Equation 1 and the concentration of polyglucose in the urine is calculated as: (2) Mg polyglucose per ml urine = mg in cuvette x 0.2 n where n is the number of times the urine was diluted before the 5 ml sample was taken.

Determination of polyglucose in blood or plasma. Bloom and Willcox(1) have used anthrone for blood dextran, with preliminary quantitative separation of the dextran from glucose by the procedure commonly used for glycogen in tissues, the material being digested with strong KOH solution, and the polysaccharide in the digest precipitated by alcohol in approximately 60% concentration. This procedure was not found applicable to these particular samples of polyglucose, because precipitation was incomplete. For accurate results the polyglucose has been determined by measurement of the reducing sugar before and after acid hydrolysis. The reducing sugar has been determined by the ferricyanide reduction method of Miller and Van Slyke(6). The ferrocyanide formed by reduction is titrated with standard ceric sulfate solution, of which 1 ml is equivalent to 0.1 mg of glucose. The method has a precision of 1%. The range of glucose covered is wide, going up to the equivalent of 8 mg of glucose per ml of blood, which is of advantage for the great range of polyglucose concentrations that is encountered after infusions. The glucose equivalent of the ferricyanide reagent is not affected by the NaCl present in the hydrolyzed samples. Presumably any other reduction method with similar advantages would serve. For rapid approximate analyses the anthrone method can be applied to the Folin-Wu tungstic acid filtrate, the total carbohydrate thus determined being corrected by subtracting 1.0 mg per ml of blood for the usual amount of glucose plus polysaccharide present in normal blood. The correction can be made more exact by determining the blood glucose, but this procedure, involving two different types of analysis and control of their reagents, offers no advantage in convenience or speed over the hydrolysis method, and is likely to be less accurate. Polyglucose penetrates the cells to a negligible extent. The plasma polyglucose can either be determined directly, or can be estimated by multiplying the blood concentration by 100/(100 – hematocrit). For precipitation of blood proteins to obtain a filtrate for polyglucose determination, we have used the Folin-Wu tungstic acid method.

Protein precipitates formed with zinc or cadmium hydroxides carry down considerable amounts of polyglucose.

Reagents. Redistilled water. Ordinary distilled water may contain contaminants that reduce significant amounts of the dilute standard cerate solution on standing. Hence, redistilled water is used to prepare the standard cerate solutions. Distilled water is treated with 5 ml of concentrated sulfuric acid and 5 g of potassium dichromate per liter, and is redistilled. *Ten per cent sodium tungstate.* Ten grams of $Na_2WO_4 \cdot 2H_2O$ per 100 ml. Deviation of the solution from neutrality is tested by adding a drop of phenolphthalein to 10 ml of the solution and titrating with either 0.1 N H_2SO_4 or NaOH to neutrality. If the solution is alkaline and requires more than 0.4 ml of 0.1 N acid, enough 1 N H_2SO_4 is added to the stock solution to render it neutral to phenolphthalein. If the solution is acid, it is similarly neutralized with 1 N NaOH. *1/12 N sulfuric acid. Tungstic acid solution*(9). One volume of the tungstate is mixed with 8 volumes of 1/12 N sulfuric acid. The solution slowly loses strength by precipitation of the tungstic acid, and should be prepared fresh every two weeks. *2 N hydrochloric acid. 1 N sodium hydroxide.* This is adjusted by titration against the 2 N HCl so that 2 volumes of the alkali are equivalent to 1 volume of the acid. *Alkaline ferricyanide solution*(6). 5 g of recrystallized, reagent grade, $K_3Fe(CN)_6$ and 10.6 g of anhydrous Na_2CO_3 are dissolved in water and made up to 1 liter. The solution must be free of ferrocyanide. The presence of the latter will be revealed by a high blank titration. Ferrocyanide can be removed by the recrystallization process of Folin(4a). *18 normal sulfuric acid (approximate)*(6). Add 465 ml of concentrated sulfuric acid (sp. g. 1.84) to 535 ml of water. Reagent grade sulfuric acid must be used, and must be first tested for reducing material as follows: To 20 ml of the acid add 60 ml of water and 0.05 ml of approximately 0.1 N $KMnO_4$ solution. The pink color must last for at least 5 minutes. *Indicator solution*(5). Dissolve 6.95 g (0.025 moles) of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in water and make up to 1 liter. Into the solution stir 14.85 g (0.075 mole) of ortho-phenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$) until all is dissolved. The indicator, either in solid form or prepared in solution as above, can be obtained from the G. Frederick Smith Co., Columbus, Ohio. In the sugar titration the color change at the end point is sharp, from golden brown to light yellow. On standing, the golden brown slowly returns. The end point is, however, stable for at least a minute. For use in the titrations, the above stock solution is diluted 5-fold with water. *Stock 0.1377 solution of ceric sulfate*(6). Approximately 110 g of anhydrous ceric sulfate (obtainable from the G. Frederick Smith Co.) is placed in an 800 ml beaker, and 35 ml of concentrated H_2SO_4 and 35 ml of water are added. The mixture is stirred and heated, and small amounts of water are added until practically all of the ceric sulfate is dissolved. The solution is filtered, cooled, and the clear filtrate diluted to 1 liter. The solution is standardized by titration against an 0.1 N solution of ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$), which is prepared by adding 39.214 g of the salt (analytical reagent grade, for use as analytical standard) to 200 to 300 ml of water, adding 25 ml of the 18 N H_2SO_4, and dissolving with stirring. The solution is then diluted to 1 liter. For the standardization of the ceric sulfate solution, 15 ml of the latter are pipetted into a 150 ml beaker, diluted with 50 ml of water and 3 ml of 18 N H_2SO_4, and titrated with the ferrous ammonium sulfate solution until the yellow color almost disappears. Then 1 or 2 drops of the diluted indicator solution are added and the titration is continued until the color changes from light yellow to golden brown. The normality of the Ce^{IV} solution is calculated as (ml ferrous salt solution)/(ml Ce^{IV} solution) $\times$ 0.1. The ceric sulfate should be more than 0.15 N. From it an exactly 0.1377 N solution is made by dilution with distilled water. *E.g.*, if the normality is 0.1560, then (0.1377/0.1560) $\times$ 1000 = 883 ml are diluted to 1 liter to make the 0.1377 N solution. The stock solution stored in a dark bottle will keep for a year. It should be kept at room temperature, not in the ice box, as some of the ceric salt may crystallize out in the cold.

TABLE I. Volume of 1:10 Blood or Plasma Filtrate to Take for Analysis.

mg polyglucose/ml of blood or plasma	ml of filtrate to use for titration samples (V of equations 4 & 5)	ml of filtrate to dilute to 25 ml for anthrone method (W of equation 6)
<1	2	5
1 to 3	1	2
3 to 8	.5	1
>8	.2	.3

0.002754 N standard ceric sulfate for blood sugar titrations(6). Exactly 2.000 ml of the stock solution are pipetted into a 100 ml volumetric flask. Five ml of the 18 N H_2SO_4 solution are added, and the solution is made up to 100 ml with the *redistilled water*. If the dilution is made with ordinary distilled water, the strength of the ceric sulfate may decrease measurably within 2 or 3 hours. If prepared with water redistilled as directed, the strength does not change significantly for 6 hours at room temperature in diffuse daylight, and will not change in a week in a refrigerator.

Analysis of blood or plasma. Precipitation of proteins. One volume of blood or plasma is run with stirring into 9 volumes of tungstic acid solution. Filtration is through either paper or cotton, either of which should be prepared by previous washing and drying. *Measurement of filtrate samples.* Aliquots of tungstate filtrate are measured to contain preferably not over 0.5 mg of polyglucose. The titration method can handle samples containing up to 0.8 mg of total glucose, but it is convenient to keep the total of polyglucose plus blood glucose in the sample below 0.5 mg, so that the volume of cerate solution required will be under 5 ml. From the expected concentration of polyglucose in the blood or plasma, the volume of desirable aliquot of the filtrate to take as sample may be estimated from the second column Table I.

Preparation of samples with and without hydrolysis. For analyses in duplicate, measure into each of four 40 ml heavy-walled test tubes the volume of filtrate estimated by Table I, and add from a burette enough water to bring the volume up to 3 ml. Two of the tubes are used for duplicate determination of reducing sugar without hydrolysis, the other 2 for determination after hydrolysis. To each of the 2 tubes for hydrolysis add 3.00 ml of 2 N hydrochloric acid. Immerse the tubes, loosely covered, in boiling water for 2 hours. Then cool, add 3 ml of water, and neutralize by adding 6.00 ml of the 1 N sodium hydroxide. To the other 2 tubes, for analysis without hydrolysis, add 12 ml portions of redistilled water. *Reduction.* To each of all 4 tubes add 2 ml of the alkaline ferricyanide solution. Mix by gently whirling the tubes. Place all the tubes in a rack or wire basket which can be immersed in a boiling water bath deeply enough to immerse the tubes at least as far as the level of the surfaces of the solutions. The tubes must be so arranged that they do not touch each other. If a wire basket is used, it should have a raised floor. The tubes are immersed in actively boiling water for 15 minutes, then cooled at once by immersion in running water for 3 minutes. *Titration of the ferrocyanide formed.* The titration should be performed within an hour after the reduction. Just before each solution is titrated, approximately 1 ml of the 18 N H_2SO_4 is added, followed by one drop of the diluted indicator solution. The solution is then titrated with the 0.002754 N ceric sulfate. The titration must be performed in a white light against a white background. A rapid stream of the ceric sulfate may be run in until the color begins to change. *Blanks.* Two blanks are determined, one for the analysis without hydrolysis and one for the analysis with hydrolysis. For each the blood filtrate is replaced by water, and for the blank for the hydrolyzed filtrate the amounts of HCl and NaOH used for the latter are employed. The usual blank found for the unhydrolyzed solution has been 0.10 ml of the cerate solution, for the hydrolyzed solution 0.14 ml of the cerate. The blanks, once determined, serve for analyses until the reagents or filter papers, used for filtering the blood filtrates, are changed.

Calculation. The titrations are made with cerate solution of which 1 ml is equivalent to 0.1 mg of glucose. H = ml of cerate, minus the corresponding blank, to titrate the hydrolyzed sample. U = ml of cerate, minus the corresponding blank, to titrate the unhydro-

lyzed sample. f = % of theoretical increase in reducing power caused by hydrolysis of the polyglucose used. (See "Reducing power of polyglucose before and after hydrolysis.") V = volume in ml of 1:10 filtrate used as sample. The general equation is: (3) Polyglucose in sample $= 0.9\Delta \times \frac{100}{f}$ where Δ = increase in reducing sugar, calculated as glucose. When the Miller-Van Slyke titration is used as above described, $\Delta = 0.1(H-U)$, and the ml of blood represented in the sample = 0.1 V. Hence: (4) Mg polyglucose per ml blood or plasma $= \frac{H-U}{V} \times \frac{90}{f}$ For the higher molecular polyglucose tested, similar to that which will probably be used for infusions, f was found to be 88.6. For this value of f the calculation becomes: (5) Mg polyglucose per ml blood or plasma $= 1.016 \frac{H-U}{V}$.

To correct for the slight amount of naturally occurring polysaccharide present in the blood, a determination of the increase in reducing power caused by hydrolysis can be made on a sample of blood drawn before the polyglucose infusion, and the amount found can be subtracted from the polyglucose calculated by Equation 4 or 5. Since the correction is not usually over 0.10 or 0.12 mg per ml of blood (1), it can be neglected, except for accurate estimations of small concentrations of polyglucose.

Approximate determination of polyglucose in blood or plasma with anthrone. Reagents. Anthrone solution, described for urine analyses. *Tungstic acid solution,* described above.

Analysis of blood or plasma. Precipitation of proteins with tungstic acid as described for the reduction procedure. *Dilution of the tungstic acid filtrate.* The volume of filtrate, W, indicated in the last column of Table I is diluted to 25 ml with water. *Determination of total polyglucose plus glucose in filtrate.* Five ml portions of the diluted filtrate are treated with 10 ml portions of anthrone solution, and the optical density read as described for urine. *Calculation.* The "mg of polyglucose in the cuvette" are calculated as described for urine analysis. (6) Mg polyglucose per ml blood $= (\frac{50}{W} \times$ "mg polyglucose in cuvette") $- 0.9$ G, where W is the volume of tungstate filtrate diluted to 25 ml before the 5 ml portion was taken for analysis, and G represents preformed blood carbohydrate in mg per ml. Ordinarily G can be taken as 1 mg per ml of blood or plasma.

Precaution in use of anthrone method. Anthrone develops color with minute amounts of all carbohydrates. There is enough cellulose in dust and lints to affect analyses. All glassware must be kept clean and free from dust, and the reagents must be protected from dust.

1. Bloom, W. L., and Willcox, M. L., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 3.

2. Dreywood, R., *Anal. Chem.,* 1946, v18, 499.

3. Dumazert, C., *Bull. Soc. Chem. Biol.,* 1950, v32, 988.

4. Durham, W. F., Bloom, W. L., Lewis, G. T., *U. S. Public Health Rep.,* 1950, v65, 670.

4a. Folin, O., *J. Biol. Chem.,* 1928, v77, 421.

5. MacFadyen, D. A., and Van Slyke, D. D., *J. Biol. Chem.,* 1943, v149, 527.

6. Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.,* 1936, v114, 583.

7. Morris, D. L., *Science,* 1948, v107, 254.

8. Van Slyke, D. D., and Hawkins, J. A., *J. Biol. Chem.,* 1929, v83, 51.

9. ———, *J. Biol. Chem.,* 1928, v79, 739.

10. Van Slyke, D. D., and Folch, J., *J. Biol. Chem.,* 1940, v136, 509.

11. Van Slyke, D. D., Plazin, J., and Weisiger, J., *J. Biol. Chem.,* 1951, v191, 299.

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