sensitivity of the procedure employed for detecting the spots. With most good visual color reactions this lower limit is in the order of 1 μg (and frequently embarrassingly close to the upper limit), but with radioactive compounds of high specific activity quantities many orders of magnitude smaller may be easily detected, identified, and isolated in a small volume of solvent for further tests. Due to the limited top capacity of the filter paper (upper limit generally in the order of 10^{-4} gm of any single compound) and the consequent desirability of obtaining high specific activity in the radioactive metabolites, microorganisms which are resistant to radiation damage would seem to be the most suitable subjects for study by the procedure outlined. The present results indicate, however, that application of the technic to short-term experiments with small animals may be practical, since radioisotope doses of the order of 10^{-5} c/gm of tissue should be adequate for most purposes. Work is continuing to test the feasibility of obtaining data concerning the mechanisms of biological conversions by preparing a sequence of chromatograms at various periods after administration of an isotopic substance to show the order and rate at which various radioactive spots appear and fade. Preliminary data and calculations also indicate that it may be possible to use the chromatograms as a means of isolating radioactive intermediates for use in further metabolism experiments on a proportionately smaller scale.

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Quantitative Determination of Carbohydrates With Dreywood's Anthrone Reagent

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Roman Dreywood recently described a reagent for carbohydrates which is simpler to prepare and use than any of the standard reagents (*1*). Moreover, its specificity for carbohydrates is very high. Dreywood suggested that the reagent might be of value for quantitative determinations and has actually used it for the determination of cellulose and starch.[1]

The specificity that Dreywood claimed for the reagent has been fully confirmed in this laboratory: it has given a positive reaction with all pure mono-, di-, and polysaccharides tested, as well as with all samples of dextrins, dextrans, starches, and plant polysaccharides and gums. Positive reactions were also obtained with pneumococcus polysaccharides of types II and III (but not type I),[2] with all glucosides tested, and with the acetates of mono-, di-, and polysaccharides. No noncarbohydrates tested gave the characteristic blue color; a red color was produced by polyvinyl alcohol and by proteins. The common solvents gave no color, though solutions containing dioxane became fluorescent. The sugar alcohols likewise produced no color.

For a quantitative reagent, 2 gm of anthrone[3] is dissolved in 1 liter of 95% sulfuric acid (prepared by the cautious addition of 1 liter of concentrated sulfuric acid to 50 ml of water, and cooling). Four or 5 ml of the solution to be determined is measured into a test tube of 19- to 25-mm diameter, and 8 or 10 ml of the reagent added. The solutions are at once thoroughly mixed by swirling. After 10 min or more, the color is measured either in an electrophotometer against a blank containing only water and reagent or in a visual colorimeter against a glucose standard. The color varies with the amount of carbohydrate, in accordance with Beer's law, if color filters of 540 mμ (green) or 620 mμ (red) are used. The latter has been found preferable, since it gives higher sensitivity and decreases any errors caused by extraneous colors.

Inasmuch as the reaction is brought about by the heat developed when the reagent and water solutions are mixed, the shape and size of the reaction tube are important. Less color develops with a given amount of sugar in either small (less than 15-mm diameter) or very large tubes, and this error is greater at higher sugar concentrations. This is presumably due to the cooling of the solution before the reaction is finished. Use of small volumes of solution has the same effect. Thus, if a Klett-Summerson colorimeter is to be used, the reaction must be carried out, as described, in 19- to 25-mm tubes, and the solution poured into the Klett tubes just before the readings are made.

Fig. 1 shows absorption curves for glucose, measured with three different filters, in an Evelyn photoelectric colorimeter, with the use of 4 ml of the sugar solution and 8 ml of the reagent. Under these conditions the practical range is from about 8 to 200γ of glucose with the 620 mμ filter, and from 20 to 500γ with the 540 mμ filter. It is clear that the filter at 660 mμ cannot be used. In this laboratory only the 620-mμ filter is used.

[1] Personal communication.

[2] Obtained through the kindness of Dr. Michael Heidelberger.

[3] For convenience, there follows a condensed outline for the preparation of anthrone (*2*). A mixture of 104 gm of anthraquinone, 100 gm of granulated tin, and 750 ml of glacial acetic acid is heated to boiling under reflux. Over a period of 2 hrs, 250 ml of hydrochloric acid (sp. gr., 1.19) is added. The hot solution is then filtered through sintered glass, and 100 ml of water added. The mixture is cooled to 10°, and the precipitated anthrone filtered off with suction and washed with water. The crude product (about 80 gm) is dissolved in warm benzene (8–9 ml/gm), and 1/3 volume of petroleum ether is added. The anthrone that crystallizes is filtered off and air-dried. The yield is about 60 gm.

The reagent darkens in the course of time, and there is a shift in the position of the absorption curves. The curves obtained with the 620-mμ filter remain linear, however, even after a month (by which time the solutions are so dark that measurement is exceedingly difficult). In practice, the reagent may be used for 4 or 5 days with either the 540- or 620-mμ filter. The aging of the reagent precludes the use of a standard calibration curve, and hence a known sugar standard must be included with each series of unknowns. Alternatively, fresh reagent may be prepared daily.

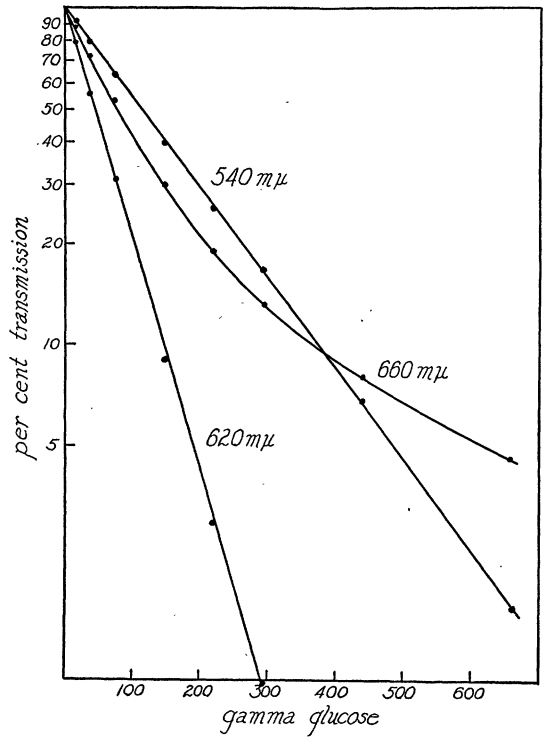

FIG. 1

The calculation of results will depend on the type of colorimeter used. In the case of the Klett type of instrument, the sugar concentration is directly proportional to the dial reading. With the Evelyn type, where readings are in per cent transmission, results can be plotted on semilogarithmic paper as in Fig. 1, or calculations can be done with a slide rule.[4]

Duplicate determinations rarely vary by more than 2% from the mean. Greater errors than this can usually be ascribed to dirty tubes, lint from filter paper, etc.

The reagent is remarkable in that the same depth of color is given by a compound of a sugar as if the compound were first hydrolyzed and the sugar then determined. Thus, for 100γ amounts of each, glucose gives a value of 100γ; maltose, of 105γ; and glycogen, of 111γ. Duplicate samples of glycogen, determined before and after hydrolysis with 1N sulfuric acid at 100° C for 3 hrs, give identical colors. Equal amounts of glucose and fructose give identical colors, and 100γ of sucrose is equivalent to 105γ of glucose. Similarly, 100γ of α-methyl glucoside gives a value of 93γ (theoretical 93), and 100γ of glycogen triacetate, a value of 61γ (theoretical 62.5). (This compound was dissolved in 0.2 ml of acetone, and 3.8 ml of water was added just before the reagent.) Galactose gives much less color than glucose: 100γ gives a reading corresponding to 54γ of glucose. Lactose hydrate (100γ) gives a value of 77γ as glucose; this would be predicted from the 50γ of glucose and 50γ of galactose in the sugar.

The reagent has been used with success in this laboratory for the determination of glucose in blood and lactose in milk, glycogen in blood and liver, carbohydrates in urine, etc. In cases where the amount of carbohydrate is not too small, compared with the extraneous material, the determinations may be exceedingly simple. Thus, lactose in milk can be determined after simple dilution of the whole milk; the result obtained is the same as that found after deproteinization of the milk. Glycogen determinations are greatly simplified, since acid hydrolysis can be omitted. Detailed data on some of the determinations just mentioned will be published later.

[4] The logarithm of I_0/I can be read directly on the *log* scale of the slide rule opposite the transmission on the *CI* scale. This logarithm is directly proportional to the sugar concentration.

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Improved Assembly of the Hartung-Clark Double Cannula for the Isolated Frog Heart

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In 1911, Hartung (*4*) first described a double cannula for perfusion of the isolated frog heart which he used in an extensive study of aconitine. A slight modification of the same arrangement was used in 1912 by A. J. Clark (*2*) in his studies of digitalis and of metabolism and electrical responses of the heart. The arrangement used by Clark and its adaptation to various purposes has been described and illustrated (*3*).

After some usage of a double cannula arrangement[2] in demonstration exercises for students and in research on cardiac drugs in this department, it has seemed that this method, further improved, has merits that other more complicated (*1, 6*) or uncontrolled (*5*) methods do not.

[1] Fellow, American Bureau for Medical Aid to China, Inc. The author wishes to thank P. J. Hanzlik for suggestions and continued guidance.

[2] A similar cannula, but without the improvements described in this paper, was first demonstrated in this department by F. P. Luduena, now with the Sterling-Winthrop Research Institute, Rensselaer, New York.