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.

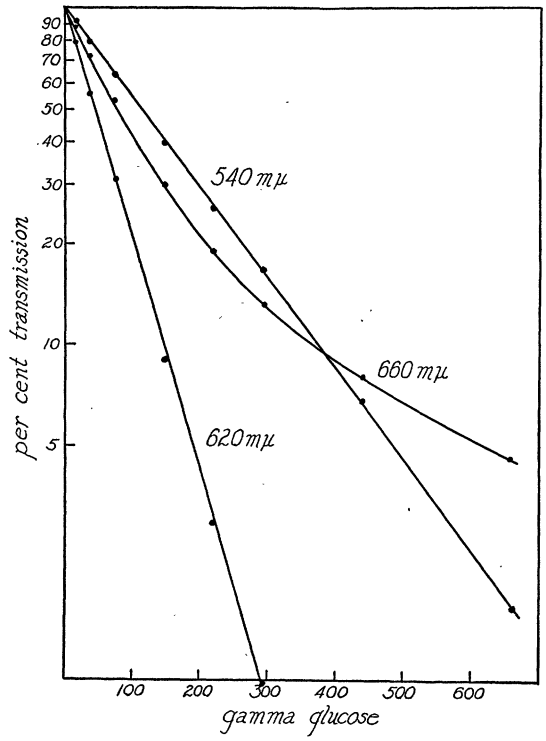

FIG. 1

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.

The illustrations in Fig. 1 show the aortic cannula (a), venous reservoir (b), and automatic overflow tubes (c), drawn to scale. The dimensions of the different parts are as follows: over-all length of aortic cannula, 7 cm; height to bend, 4 cm; spread from tip to vertical portion, 3.3 cm; internal diameter, 3 mm (constriction, 2 mm); over-all length of venous reservoir, 6.5 cm; internal diameter at top, 1.5 cm; length of side tube, 1 cm; diameter, 3 mm; length of overflow tube, 2.5 cm; diameter, 2 cm; length of inside straight tube, 3 cm; diameter, 3 mm; length of inside bent tube, 2 cm; diameter, 3 mm. The latter is connected by a rubber

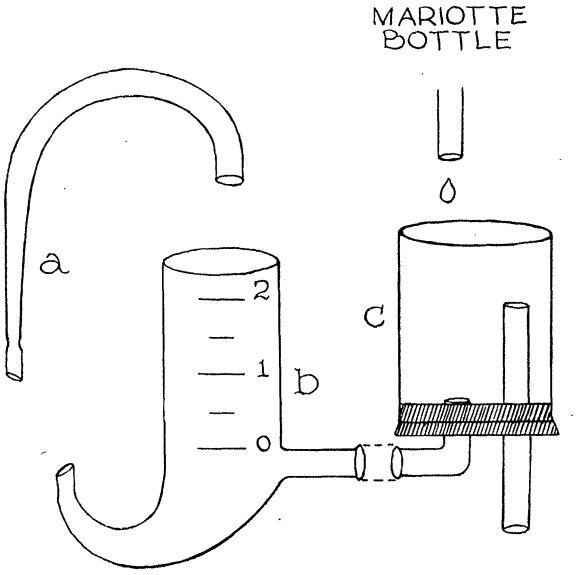

FIG. 1. Improved assembly of the Hartung-Clark double cannula for the isolated frog heart. The aortic cannula and venous reservoir and extensions for these are obtainable from Frank H. Osborn, 564 Howard Street, San Francisco 5, California.

tube (about 10 cm long) to the side tube on the venous reservoir. A rubber stopper is used for holding the tubes in the overflow tube, which is conveniently fixed to an iron stand with a clamp and adjusted to obtain the desired fluid level in the venous reservoir. A Mariotte bottle is placed conveniently on a top shelf for continuous dripping of Ringer's solution into the overflow tube. For observing drug action under changed pressures, convenient extensions for the aortic cannula are three pieces of the same glass tubing—2-cm proximal cannula end, 5-cm arc, and 9.5-cm length with arc, the maximum length permissible being 20 cm when cardiac embarrassment occurs. A piece of tubing about 4.5 cm in length and with 1.5-cm internal diameter is provided for joining (with a suitable rubber band cut from tubing) to the venous reservoir.

Checks were made on many hearts to justify the choice of the various parts used in this arrangement. A pressure of 2 cm of Ringer's fluid in the venous reservoir gave the most constant optimum performance, according to stroke output, minute output and rate, for the longest period (2 hrs) without auricular distention, confirming Clark (*3*). Departures from this pressure impaired the cardiac functional efficiency.

Frogs of 100- to 250-gm body weight are preferred for the improved double cannula. The frog is pithed first and the heart exposed. After the pericardium is opened, the right aortic arch and both superior vena cavas are ligated. Next, the frenum ligament is cut and a double silk thread passed under the inferior vena cava, after which the distal end of the venous reservoir is inserted and tied in just short of the sinus venosus, whose functioning should remain unimpaired. A few cc of Ringer's solution are introduced into the reservoir to empty the heart of blood and prevent clotting; this is also a check on correct insertion of the reservoir. Next, the aortic cannula is placed, all ligated vessels are cut away, the remaining thread under the inferior vena cava is tied, all pulmonary vessels are ligated, and the heart is removed and the cannulas mounted with suitable clamps on an iron stand. After any necessary adjustment of the cannulas, especially the aortic, which lifts the heart to allow free contraction of the sinus tissue, the circulation is started and the level of fluid in the venous reservoir adjusted with the aid of the overflow tube to secure optimum functioning of all cardiac chambers. The external surface of the heart should be kept moist by means of a slow, continuous drip of Ringer's solution. In this way the suspended heart may remain in good functioning condition for 12 hrs.

The chief advantages of the arrangement here described may be summarized as follows: (1) It may be used as a closed circulating system, or for continuous perfusion, without special aeration. (2) Perfusion (or venous) pressure is kept practically constant, regardless of changing conditions such as rate of flow, washing, measurement of stroke or minute output from the aortic cannula (with the aid of a trough of thin aluminum under the tip). (3) It allows ready change of pressures to desired levels. (4) With a tuberculin syringe (0.25-cc capacity for high concentrations) it permits easy drug application direct to venous reservoir (when closed circuit is used). (5) Fluid may be removed quickly from the venous reservoir with a medicine dropper. Ventricular volume is readily recorded by applying a small glass cardiometer (Camus type, *1*) with soft-rubber dam and small tambour recorder. Harvard heart levers are used for recording the activity of the ventricle or auricles, or both, on a kymograph.

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