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## A Modification of the Diphenylamine Method for Determination of Inulin.

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Colorimetric methods for the determination of inulin in blood serum or plasma and urine based on the reaction of inulin with diphenylamine have been described in recent years. The most widely used procedure is that of Alving, Rubin and Miller,[1] in which the inulin-containing solutions are heated in closed tubes with diphenylamine and hydrochloric acid dissolved in ethyl alcohol. Tightly capped tubes are necessary because of the volatile solvent, and because the solution must be heated for 60 minutes at 100°C to obtain the maximum color. In this laboratory as well as in others, this

[1] Alving, A. S., Rubin, J., and Miller, B. F., *J. Biol. Chem.*, 1939, **127**, 609.

method has been troublesome and the results not consistently accurate.

For this reason a modification has been devised in which a less volatile solvent is used, thus permitting the use of open tubes. With this reagent a shorter period of heating is necessary for maximum color development and accurate results have been obtained in the determination of inulin in blood serum or heparinized plasma in concentrations as low as 4 mg per 100 cc. The determination can be carried out on 0.2 cc.

*Method. Reagent.* Three grams of diphenylamine, reagent grade, are dissolved in 100 cc of glacial acetic acid and to this solution are added 60 cc of concentrated hydrochloric acid.

This reagent is stable if kept in an amber bottle in a refrigerator at 6°C, and the color production with inulin is found to remain constant for at least one month. A few crystals of diphenylamine may precipitate out of solution but this does not affect the reagent. If kept at room temperature the color in the blank determination becomes progressively darker, but if correction be made for the blank as is automatically done in a photoelectric colorimeter of the Evelyn type the calibration curve is not changed. The color production with different batches of reagent shows only slight variation.

*Determination of inulin in blood plasma.* It is necessary to remove glucose from the blood plasma since this sugar reacts with the reagent to produce a blue color although to a much less extent than inulin. The glucose is removed by fermentation with washed, starch-free yeast as in the method of Alving *et al.*[1] The plasma proteins are then precipitated by the zinc sulfate method of Somogyi.[2] The protein-free filtrate is diluted so that the final solution contains between 2 and 12 $\mu$g of inulin per cc. For most determinations the micro precipitation technique of Somogyi in which the filtrate represents a 1 :40 dilution of the plasma will be found suitable.

Two cc of the filtrate are measured into a pyrex test tube, 150 by 16 mm. Four cc of the diphenylamine reagent are accurately pipetted into the tube. (The reagent is drawn into the pipette with a rubber bulb.) The tube is agitated to mix the solutions thoroughly and is then placed in a rack in a boiling water bath. The bath should be adjusted so that the rate of boiling is uniform and the level of water is kept constant at about the level of the solution within the tube. A blank, containing 2 cc of distilled water and 4 cc of reagent is heated simultaneously. After 30 minutes the tubes are removed from the boiling water and cooled to room temperature. The color is then quantitatively measured in a photoelectric colorimeter using a light

2 Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

filter with maximum transmission at 620 millimicrons. We have used the Evelyn photoelectric colorimeter with the aperture set at 6 cc and the 620 filter. For the most accurate results it is advisable to run a blank determination on a sample of serum or plasma obtained before the administration of inulin. This blank value is small, if freshly prepared yeast suspensions are used, and does not vary widely with different samples of serum or plasma so that an average blank value may be determined for several samples and this value used thereafter. The average blank value for a group of human sera was found to be equivalent to 1 mg inulin/100 cc serum.

The method is calibrated by treating known solutions of inulin with the diphenylamine reagent. The color formation follows the Lambert-Beer law as indicated by the linear relationship between the concentration of inulin and the logarithm of the light transmission.

*Determination of inulin in urine.* Normal urine contains solutes which react with diphenylamine to give a blue color. The blank values obtained vary with the degree to which the urine is concentrated by the kidney. Samples of normal human urine excreted at the rate of approximately 1 cc per minute were found to give a color equivalent to 10 mg of inulin per 100 cc of urine. In the determination of renal clearances of inulin the concentrations of inulin in the urine are so great that the urine corresponding to an excretion rate of 1 cc per minute may be diluted to 1:1000 or more and at these dilutions the blank values are so small that they may be disregarded. If glucose is present it must be removed by fermentation. Moreover, if the urine contains significant amounts of protein, deproteinization must be carried out as in the plasma method. Ordinarily, however, it is only necessary to dilute the urine so that

TABLE I.
Recovery of Added Inulin.

| | Inulin added, mg/100 cc | Inulin recovered, mg/100 cc | % |
|---|---|---|---|
| Serum | 3.2 | 3.25 | 101.5 |
| | 4.0 | 3.68 | 92.0 |
| | 4.0 | 3.95 | 98.6 |
| | 8.0 | 8.05 | 100.6 |
| | 20.0 | 19.4 | 97.0 |
| | 20.0 | 20.2 | 102.0 |
| | 33.3 | 33.7 | 101.2 |
| | 33.3 | 33.1 | 99.4 |
| | 33.3 | 33.0 | 99.1 |
| Urine | 100 | 100 | 100 |
| | 200 | 196 | 98 |
| | 500 | 500 | 100 |
| | 500 | 510 | 102 |

the concentration of inulin is between 2 and 12 μg per cc. Two cc of the diluted urine are measured into a test tube, 4 cc of the reagent added and the tube heated in a boiling water bath as previously described for plasma.

In Table I are shown the results of recovery experiments. Measured amounts of inulin were added to blood serum and the removal of glucose and protein carried out as described. Blank determinations were made on each sample of serum. In the case of the urine, the inulin was added in the concentrations shown, and the determinations made directly following suitable dilution. The urine specimen was a highly concentrated one and the blank value was equivalent to 51 mg inulin per 100 cc of undiluted urine.

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## The Nervous Factor in Burns.

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(Introduced by M. B. Visscher.)

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While evidence has been accumulating concerning the importance of a nervous factor in the etiology of traumatic shock,[1-3] similar studies of the problem of burns have not been carried out.

A comparison was made of the effects of thermal trauma to one hind leg in control cats and in spinal animals. Transection of the spinal cord at $L_1$ or $T_{11}$ effectively eliminated the influence of afferent nerve impulses from the burned leg but left the major vasoconstrictor outflow from the spinal cord intact.

The experiments were performed on 83 adult cats, anesthetized throughout the procedure with pentobarbital sodium. One hind leg was burned with a Bunsen flame for 10 or 15 minutes. The spinal cord was transected about 30 minutes preceding application of thermal trauma. Observations were made of blood pressure (carotid cannula), hemoglobin and specific gravity of whole venous blood,

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1 O'Shaughnessy, L., and Slome, D., *Brit. J. Surg.*, 1935, **22**, 589.

2 Lorber, V., Kabat, H., and Welte, E. J., *Surg. Gyn. and Obst.*, 1940, **71**, 469.

3 Freedman, A. M., and Kabat, H., *Am. J. Physiol.*, 1940, **130**, 620.