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A Colorimetric Method for the Determination of Levulose in Blood and Urine.*

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One volume of the solution to be analyzed, a half volume of concentrated HCl, and a tenth volume of a 20% alcoholic solution of diphenyl amine, in a large test tube are heated in a boiling water bath for 15 minutes and then cooled. It has been found convenient to close the tube with a one-hole rubber stopper with the hole stuffed with glass wool. Shaking the solution with a third volume of liquid (melted) phenol causes the immediate absorption of the diphenyl amine together with the color. The addition of a half volume of 95% ethanol renders the mixture homogenous and suitable for colorimetric comparison, which may be made immediately, although this is not necessary. The color tends to darken slightly on standing. Standards are prepared similarly and simultaneously from solutions of levulose. It is felt preferable to make these latter at fairly frequent intervals from a 1% stock solution, using of course a preservative as toluene. Since 1 mg. of levulose per cc. of solution gives a fairly intense color, the standards have been made to range downwards from this concentration. Similarly if necessary the solutions to be analyzed have been diluted to within this range.

This method has been found satisfactory in the analysis of aqueous solutions, considerably diluted urines and tungstic acid blood filtrates. In more concentrated urines interference has been encountered, the color obtained being darker than it should be and frequently of a somewhat different shade. Tungstic acid filtrates contain substances that cause the development of a faint greenish blue coloration, that increases slightly the apparent color given by levulose. While theoretically dilutions and standards should probably be made from tungstic acid filtrates, as the influence is rather constant, it has been felt possible to use water, making a small frequently determined correction, without introducing significant error, except possibly when the levulose concentration is very low.

The diphenyl amine employed has been obtained from the Eastman Kodak Company. An old stock of this substance available in

* Aided by a grant from the David Trautman Schwarz Research Fund.

this laboratory was found absolutely unsuitable for this determination.

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Tolerance for Levulose in Several Types of Experimentally Produced Liver Injury.*

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In view of the unquestioned importance of the liver in carbohydrate metabolism, numerous attempts have been made to correlate hepatic efficiency and tolerance for various sugars. Among these fructose has attracted considerable attention. With experimental animals there seems but little question that impairment of the function of the liver is associated with a decreased ability to metabolize fructose, as evidenced by the greater hyperglycemia resulting. Having available a method for the determination of levulose in blood and urine, it has seemed of interest to study the effect of hepatic dysfunction on circulating levulose after administration.

In the normal rabbit, levulose disappeared from the blood in 90 minutes subsequent to its intravenous injection in doses of 2 gm. per kilo of body weight. The rate of removal was not strikingly affected by mild poisoning with various substances that are injurious to the liver, but more rigorous treatment with phosphorus, chloroform, and hydrazine sulfate did have an evident effect, levulose still remaining in the blood at the end of 90 minutes.

The oral ingestion of levulose in quantities as large as 7 gm. per kilo of body weight caused but slight increase in the total blood sugar and the appearance of very little levulose in the circulation. The liver poisons were without marked influence on the amount of levulose appearing in the blood stream, but the total blood sugar rose to very high levels when levulose was fed.

* Aided by a grant from the David Trautman Schwarz Research Fund.