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AN IMPROVED AUTOMATED METHOD FOR PLASMA GLUCOSE ANALYSIS WITH HIGH SPECIFICITY AND SENSITIVITY IN THE 5 TO 50 MICROGRAM RANGE

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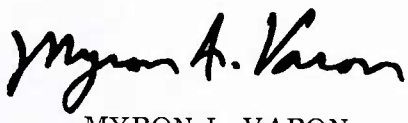

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ABSTRACT

An improved highly sensitive automated procedure for the analysis of plasma glucose in ultramicro sample volumes is presented. The procedure utilizes the glucose oxidase-peroxidase-dye reaction with an extract of gum guaiac as the chromogenic oxygen acceptor. Details for flow manifold construction and reagent composition are presented together with data concerning the precision of the method.

I. INTRODUCTION

Studies of blood glucose changes after radiation exposure of small laboratory animals produced a requirement for a specific and highly sensitive procedure for glucose analysis in ultramicro sample volumes. Numerous automated methods using the glucose oxidase-peroxidase-dye reaction for the determination of true glucose have been proposed.[1] These methods, however, either lack sufficient sensitivity for the determination of glucose in ultramicro sample volumes or require the use of some form of range expansion on the recording instrument.

A highly sensitive automated method employing an extract of gum guaiac as the chromogenic oxygen acceptor has recently been suggested by Hochella and Hill.[2] In our hands, however, the method yielded poor results which were primarily attributable to their design of the flow manifold. This report describes the modifications in manifold construction and reagent composition which are required to make the procedure usable for assaying plasma glucose in the 5 to 50 microgram range normally encountered in the ultramicro sample volumes obtained from small laboratory animals. The high sensitivity of the chromogen eliminates the need for recorder range expansion.

II. MATERIALS

The reagents used are those described by Hochella and Hill[2] with the following exceptions:

(a) enzyme: 5 ml of Fermcozyme 952 DM, a mixture of glucose oxidase and peroxidase (Fermco Laboratories, Chicago, Illinois), diluted to 100 ml with distilled water; no preservative is added since the reagent is stable at room temperature;

(b) buffer: 1.0 M sodium acetate, pH 5.2, to which 20 ml of Triton X-100 (Rohm and Haas Company, Philadelphia, Pennsylvania) is added per 1000 ml of buffer. Care should be taken to insure complete mixing of the buffer and Triton X-100.

III. PROCEDURE

The flow diagram for the procedure using the AutoAnalyzer (Technicon Corporation, Tarrytown, New York) is shown in Figure 1. We have routinely operated the system at a sampling rate of 40 per hour with a water wash between samples to obtain optimum resolution of sample peaks.

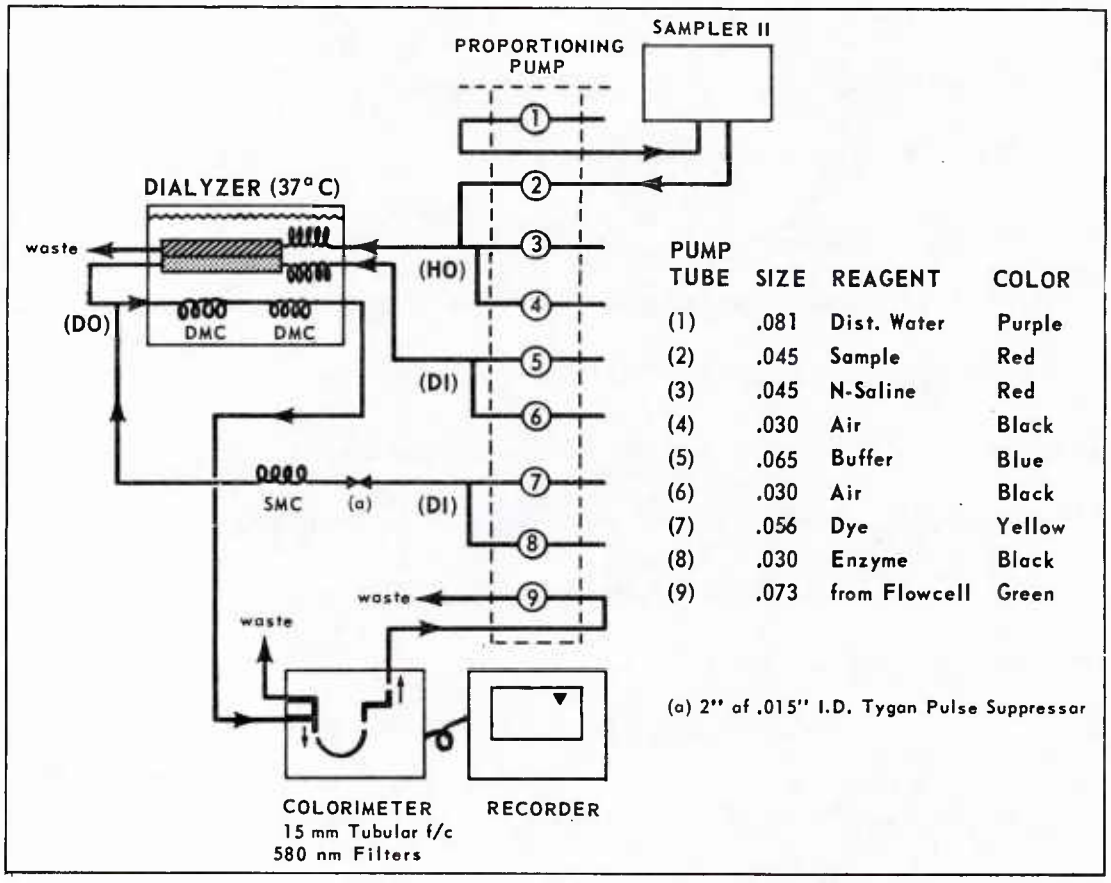

Figure 1. Diagram of the flow manifold for the automated determination of glucose. Glass fittings (Bulletin GF3, Technicon Corporation) and tube sizes are Technicon designations.

Before analysis, each sample must be diluted to a final volume of at least 1 ml to accommodate the volume of aspirated sample. We routinely make a 1 to 50 dilution with a 0.9 percent (w/v) aqueous solution of sodium chloride using a Fisher 240 diluter (Fisher Scientific Company, Pittsburgh, Pennsylvania).

IV. RESULTS

The sensitivity of the method is 0.210 O.D. units per 10 μg glucose. The optimum pH for the reaction mixture was found to be approximately 5.2. However, as shown in Figure 2, the sensitivity is not appreciably affected within the pH range 5.0 to 5.6.

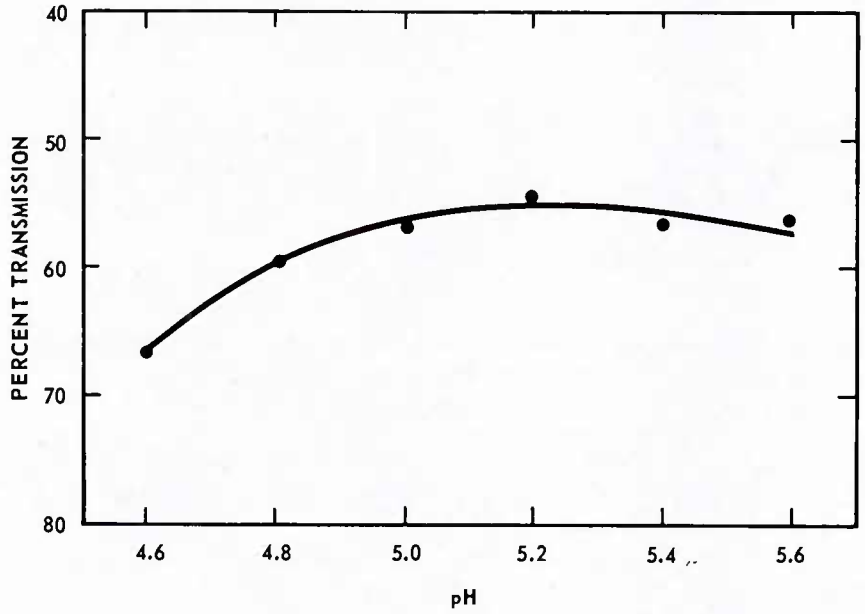

Figure 2. Color formed by a 5 mg/100 ml glucose solution at various pH values

Results of replicate analyses performed on two commercial control sera shown in Table I indicate that the dilution procedure does not affect the precision of the method. The standard deviation for the procedure is 2 mg/100 ml.

Table I. Replicate Glucose Analyses of Two Commercial Control Sera

Assay* #	Glucose (mg/100 ml)	
	Control A†	Control B‡
1	97.5	220.0
2	100.0	225.0
3	100.0	225.0
4	97.5	225.0
5	95.0	225.0
6	100.0	225.0
7	97.5	220.0
8	95.0	225.0
9	100.0	223.0
10	97.5	225.0
11	97.5	220.0
12	97.5	223.0
13	97.5	223.0
14	97.5	223.0
Mean	97.9	223.4
S. D.	1.7	2.0
Manufacturer's assay (method)	103 ± 5 (glucose oxidase, Hyland)	228 ± 10 (Nelson-Somogyi)

* Separate 1 to 50 aqueous dilutions (see text)

† Hyland Lot 0369D040A1, Hyland Division Travenol Laboratories, Inc., Los Angeles, California

‡ Moni-trol II Lot PTD-21 C. D., Dade Division, American Hospital Supply Corporation, Miami, Florida

Neither ascorbic acid, uric acid, nor reduced glutathione in physiological concentrations was found to interfere with the procedure.

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2. Hochella, N. J. and Hill, J. B. The collection and preservation of blood on filter paper for blood glucose determinations. In: Automation in Analytical Chemistry, Technicon Symposia, 1967, Vol. I, pp. 3-6. White Plains, New York, Mediad Incorporated, 1968.

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