

THE ANALYSIS OF BLOOD GLUCOSE USING STABLE DRY SPECIMENS

ERIC G. COMSTOCK, M.D., AND BETSY S. COMSTOCK, M.D.

Biometric Laboratories, Houston, Texas

The determination of glucose concentration is 1 of the analyses most frequently performed on blood. Although many problems contribute to the errors encountered in this analysis, the lability of glucose in the blood specimen constitutes a major source of error.²⁻⁵ Ideally, all glucose determinations should be performed immediately after the specimen is drawn.

In an attempt to stabilize glucose in a specimen of blood when delay in analysis is anticipated, sodium fluoride and thymol or sodium fluoride alone may be included as preservative; however, problems such as omission of the preservative, inadequate mixing of the preservative and the blood, and aberrantly low values with no apparent explanation occur frequently and result in specimens that are unsatisfactory for analysis. The use of sodium fluoride makes the specimen unsatisfactory for analysis by enzymatic glucose procedures.³

Rapid drying of blood specimens on paper appeared likely to stop enzymatic degradation of glucose. The current report presents an investigation of the stability of glucose in blood specimens dried on paper at the time of collection.

REAGENTS AND EQUIPMENT

1. Deionized water is prepared by passage through a mixed-bed ion exchange resin.

2. *Glucose standard.* The glucose standard is prepared by weighing 100-mg. aliquots of anhydrous glucose dried over silica gel into small vials. Working standards are made up each day by dissolving the contents of 1 vial and diluting to 100 ml. for the standard of 100 mg. per 100 ml., and dissolving the contents of another vial and diluting to 50 ml. for the standard of 200 mg. per 100 ml.

3. *Copper reagent.* Twelve Grams Na_2CO_3

and 6 Gm. Rochelle salt (sodium potassium tartrate) are dissolved in approximately 100 ml. of water. Forty milliliters of 5 per cent copper sulfate (pentahydrate) are added and mixed. Eight Grams of sodium bicarbonate are added and stirred until dissolved. Ninety Grams of anhydrous Na_2SO_4 are dissolved in 250 ml. of hot water, which are cooled and added to the copper sulfate mixture. The whole solution is mixed and is made up to 500 ml. with water.

4. *Arsenomolybdate reagent.* Fifty Grams of ammonium molybdate are dissolved in 900 ml. of distilled water. Forty-two milliliters of concentrated H_2SO_4 are added. Six Grams of pulverized sodium arsenate ($\text{Na}_2\text{HASO}_4 \cdot 7\text{H}_2\text{O}$) are dissolved in 50 ml. of water, and added to the mixture. It is mixed well and allowed to stand overnight before use.

5. *Glucose oxidase reagent.* Worthington Biochemical Glucostat Reagent is prepared as directed in accompanying literature for double strength reagent (Freehold, New Jersey, 1965).

6. *Paper specimen carriers.* Whatman No. 4 filter paper for chromatography is imprinted, as illustrated in Figure 1, using an offset press. Some fountain solutions used in lithography contain reducing substances, and these must be avoided.

7. *Formalin fume fixation apparatus.* The chamber is a wide-mouth screw-capped 1-liter bottle. A perforated polyethylene basket holding as many as 10 specimens allows free circulation of formalin fumes and prevents direct contact of the paper specimen carriers with the formalin solution in the bottom of the bottle. The bottle should be kept closed tightly during the fixation interval (Fig. 2).

8. *Rotator for elution of glucose.* A disk cut from $\frac{3}{4}$ in. plywood is mounted in a vertical plane, attached at the center to the shaft of a ball-bearing arbor driven by a variable-speed motor. The speed of rotation is 15 to 20 r.p.m. Elastic bands of suitable size hold specimen vials in place (Fig. 3).

Received, July 12, 1965.

Dr. Betsy Comstock's present address is Department of Medicine, Baylor University College of Medicine, Houston, Texas.

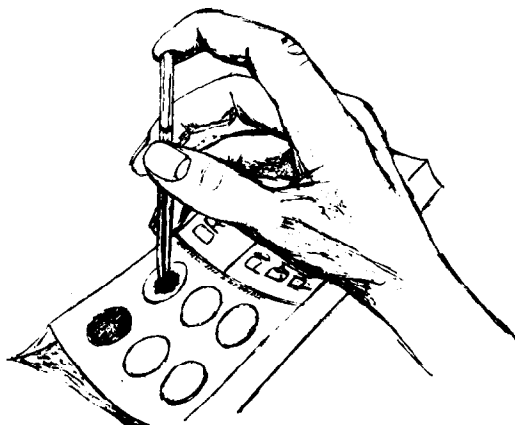


FIG. 1. Application of specimen to imprinted Whatman No. 4 filter paper.

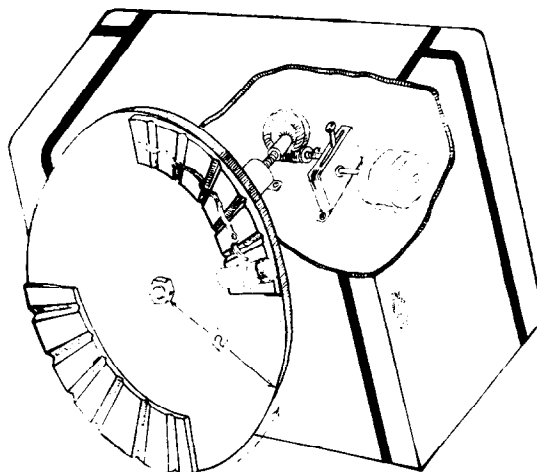


FIG. 3. Rotator for elution of glucose. This apparatus uses detachable trays with cradles cut for various sizes of tubes. The drive train consists of a motor, a variable-ratio speed reducer, and a worm drive.

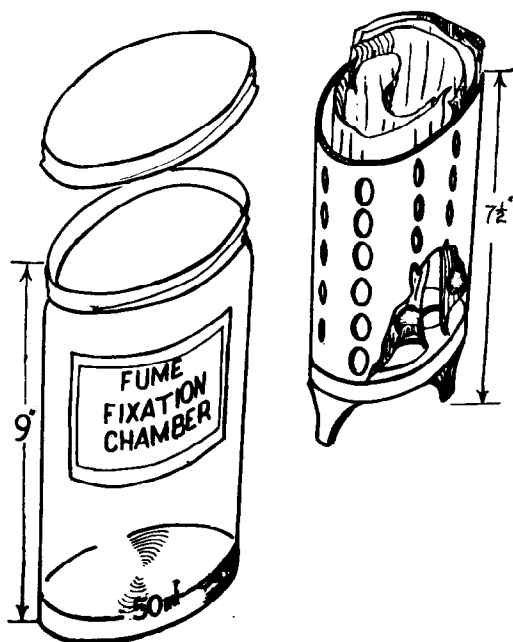


FIG. 2. Fume fixation chamber with cut away view of polyethylene specimen basket. Perforation in floor and wall of basket allow circulation of formalin fumes.

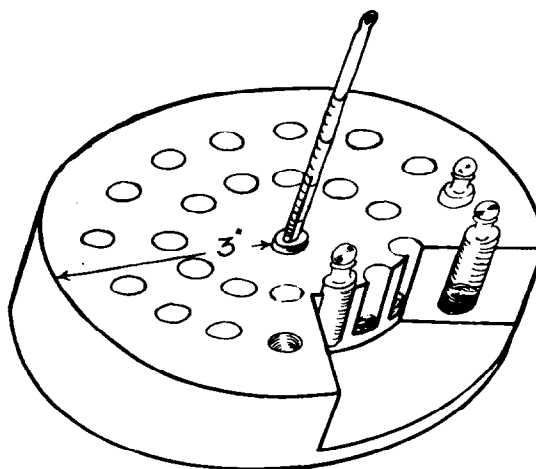


FIG. 4. Incubator for copper reduction, made from 6- by $1\frac{1}{4}$ -in. aluminum disk drilled to admit micro reaction vials 9 mm. in diameter.

9. *Micro-reaction vials.* Suitable containers for performance of the procedures may be prepared by selecting 1-ml. Neutraglas (Kimble) serum vials of matched absorbance at 520 m μ . Vials should be marked to indicate orientation within the light beam.

10. *Incubator for copper reduction.* A suitable incubator for the determination of reducing substances may be constructed by

using a solid aluminum block 6 in. in diameter and $1\frac{1}{4}$ in. in thickness. The upper surface is drilled with holes of a diameter to allow the vials to fit snugly to a depth of approximately $\frac{3}{4}$ in. The block may be maintained at 100 C. with any of several temperature-controlled hot plates available commercially (Fig. 4).

METHODS

Collection of specimen. Exactly 20 λ of finger blood are collected in a Sahli pipet and

transferred quantitatively to the circled area of the filter paper sample carrier (Fig. 1). Until the circled area is completely dry, it should not come in contact with any other surface.

Formalin fume fixation. Just prior to the analysis, the specimens are inserted vertically into the basket of the fume-fixation chamber in a water bath. The chamber is closed tightly. After 10 min., the specimens are removed and dried with warm air. No odor of formalin persists after about 10 min. Fifty milliliters of 37 per cent formaldehyde solution will provide adequate fixation for 8 to 10 groups of 10 specimens.

Extraction of glucose from the dried specimen. One of the stained specimen areas representing 20 λ of whole blood is torn from the carrier and inserted into a screw-capped 5-ml. vial containing 1.5 ml. of deionized water. The vial is closed tightly and placed on a continuous rotator (see above, Reagents and Equipment, 8) at a slow speed for 20 min. The agitation must not be vigorous or the paper may disintegrate, necessitating

centrifugation of the solution before samples are removed for analysis. If the eluate has a pink color, the formalin is exhausted and should be replaced.

Glucose oxidase procedure. Transfer 0.2 ml. of the eluate from the formalin-fixed specimen to a 1-ml. vial. Add 0.2 ml. of double strength Worthington Glucostat Reagent. Incubate at 37 C. for 30 min. Add 0.4 ml. of 50 per cent sulfuric acid. After mixing, allow the vial to stand for 5 min. at room temperature, then read the optical density at 520 m μ in the Beckman DU Spectrophotometer after adjusting the instrument so that the reagent blank reads zero optical density.

Analysis as reducing substances. Transfer 0.2 ml. of the eluate from the formalin-fixed specimen to a 1-ml. vial. Add 0.2 ml. of copper reagent and close the vial with a glass bead. Place the vial in an aluminum heat sink maintained at 100 C. for exactly 10 min., then cool it in water. Remove the bead and add 0.4 ml. of arsenomolybdate solution. Mix cautiously to avoid loss of specimen

TABLE 1
STABILITY OF BLOOD GLUCOSE

Type of Specimen, Conditions of Storage, and Method of Determination	Glucose Recovered in Storage Periods of													
	1/2 hr.	2 hr.	4 hr.	12 hr.	1 day	2 days	1 week	2 weeks	1 month	2 months	3 months	6 months	12 months	18 months
Whole blood with calcium disodium EDTA; room temperature; Somogyi-Nelson	76	70	65	40	40	20	<10							
Whole blood with NaF 5 mg./ml.; room temperature; Somogyi-Nelson	78	76	77	78	77	76	75	70	52	<10				
Dried specimen; 37 C.; fume fixation, reducing substances	74	76	77	77	76	75	74	75	74	75	74	—*		
Dried specimen; room temperature; fume fixation, reducing substances	76	76	76	77	76	75	76	77	75	76	76	77	74	—*
Dried specimen; room temperature in dessicator; fume fixation, reducing substances	76	77	76	76	77	77	76	74	75	76	77	78	75	76

* Specimen supply exhausted.

when foaming occurs. Allow the sample to stand at room temperature for 20 min. and read against a blank at 540 $m\mu$.

RESULTS

Examination of the data in Table 1 reveals that when no preservative was used blood glucose was reduced significantly after 2 hr. at room temperature and that blood retained essentially no glucose after 48 hr. A specimen with NaF added, 5 mg. per ml., retained its original glucose concentration after 7 days; glucose was reduced significantly after 14 days, and less than 10 per cent of the original glucose remained after 2 months.

The specimens dried on paper carriers and maintained in a humid incubator at 37 C.

for 3 months showed no decrease in glucose content. This supply of specimens was exhausted after 3 months. The specimens maintained at room temperature without desiccation were unchanged after 12 months, at which time the last aliquot was used. The specimens maintained at room temperature in a desiccator remain unchanged after 18 months. Three aliquots of this specimen remain for analysis. These will be analyzed after 3 years, 4 years, and 5 years.

Three methods for the determination of blood glucose have been used in this investigation. The determination of glucose in liquid specimens was performed with the Somogyi-Nelson procedure.⁴ The glucose recovered in solution after elution of formalin-fixed dry specimens was determined with an

TABLE 2

COMPARISON OF RESULTS OF GLUCOSE DETERMINATION BY 3 PROCEDURES: MULTIPLE ANALYSES IN THE SAME SPECIMEN*

Replicate No.	Dried Specimens Fixed with Formalin and Eluted		Liquid Specimen, NaF, Somogyi-Nelson
	Glucose oxidase	Reducing substances	
	<i>mg./100 ml. whole blood</i>		
1	98	101	100
2	102	102	102
3	102	100	98
4	100	100	103
5	102	102	101
6	102	103	103
7	102	102	100
8	102	102	102
9	101	104	101
10	102	102	100
Mean value	101.3	101.8	101.0
Sum of squared deviations†	16.1	13.6	22.0
Variance‡	1.79	1.5	2.4
Standard deviation	1.34	1.22	1.55

* Degrees of freedom: total, 29; between groups, 2; within groups, 27.

† Sums of squared deviations: total, 54.97; between groups, 3.27; within groups, 51.7.

‡ Variance: between groups, $(3.27/2) = 1.64$; within groups, $(51.7/27) = 1.91$. Variance ratio: $F = (1.91/1.64) = 1.16$. F (0.05 error level), critical value = 19.43.

TABLE 3

COMPARISON OF RESULTS OF GLUCOSE DETERMINATION BY 3 PROCEDURES: ANALYSES OF 10 CONSECUTIVE SPECIMENS*

No. of specimen	Dried Specimens Fixed with Formalin and Eluted		Liquid Specimen, NaF, Somogyi-Nelson
	Glucose oxidase	Reducing substances	
	<i>mg./100 ml.</i>		
1	88	86	88
2	76	73	75
3	82	78	81
4	85	84	87
5	77	78	78
6	76	79	78
7	75	74	76
8	76	74	73
9	83	79	81
10	80	80	78
Mean value	79.8	78.5	79.5
Sum of squared deviations†	183.6	160.5	214.5
Variance‡	20.4	17.8	23.8
Standard deviation	4.5	4.22	4.87

* Degrees of freedom: total, 29; between groups, 2; within groups, 27.

† Sums of squared deviations: total, 567.86; between groups, 9.26; within groups, 558.6.

‡ Variance: between groups, $(9.26/2) = 4.6$; within groups, $(558.6/27) = 19.26$. Variance ratio: $F = (19.26/4.6) = 4.19$. F (0.05 error level), critical value = 19.43.

enzymatic procedure,¹ and it also was determined by its reducing capacity. In order to compare these procedures with respect to variability inherent within the technics, replicate analyses using each method were performed. The samples all were drawn from a pool of 5 normal specimens drawn with EDTA. Prior to withdrawing specimens, the glucose content of the pool was adjusted to approximately 100 mg. per 100 ml., as determined by the Somogyi-Nelson procedure. The 20- λ aliquots required for analysis were measured with the same pipet, so that sampling errors would be comparable. Ten analyses were performed simultaneously by the same technologist.

The data presented in Table 2 with analysis of variance show the ratio of variances (F) as 1.16. Since the critical value for F at an error level of 0.05 is 19.43, the means do not differ significantly.

For statistical analysis, the data from 10 consecutive specimens are presented in Table 3.

DISCUSSION

This procedure should be useful in any area of medical practice where there is need for reliable evaluation of blood glucose on a specimen obtained by finger puncture. The stability of the specimen is so striking that an analysis will be reliable whether it is performed within 5 min. or after 1½ years. The specimen carrier has none of the potential defects of glass test tubes or plastic bottles. Consisting merely of a few drops of blood dried on paper, it cannot break or spill. It needs no refrigeration. It can be mailed inside a first class letter without special handling and without absorbent packing material. If it is delayed in transit, no harm is done to the reliability of the results. Since identification is written on the specimen, it cannot be interchanged with other speci-

mens and identification cannot be lost. Multiple samples can be collected on a single specimen carrier; thus a glucose tolerance test can be performed on specimens obtained by finger puncture and applied in sequence along the paper, with no opportunity for the specimen sequence to be altered.

SUMMARY

A method is presented for the collection and preservation of blood specimens by drying aliquots of blood on a paper carrier.

Glucose, in specimens dried on paper and stored in a desiccator, has been demonstrated to be stable for 18 months.

A method for fixation of protein on the paper carrier which allows glucose to be eluted with water is demonstrated. Comparison with usual methods for preparation of protein-free solutions shows the new method to yield results equivalent to those obtained by procedures which measure true glucose.

Other soluble nonprotein molecules, ordinarily more stable than glucose, can also be expected to be stable in dry specimens.

These methods are promising because they avoid analytic errors due to glucose lability and because they present an opportunity to the physician to order procedures "in retrospect" on samples collected and stored.

REFERENCES

1. Beach, E. F., and Turner, J. J.: An enzymatic method for glucose determination in body fluids. *Clin. Chem.*, 4: 462-475, 1958.
2. Meites, S., and Bohman, N.: *In vitro* stabilization of blood glucose with water. *Clin. Chem.*, 9: 594-599, 1963.
3. Natelson, S.: *Microtechniques of Clinical Chemistry for the Routine Laboratory*. Springfield, Ill.: Charles C Thomas, Publisher, 1957, p. 208.
4. Nelson, N.: Photometric adaptation of the Somogyi method for determination of glucose. *J. Biol. Chem.*, 153: 375-380, 1944.
5. Ruiter, J., Weinberg, F., and Morrison, A.: The stability of glucose in serum. *Clin. Chem.*, 9: 356-359, 1963.