

# The Determination of Blood Sugar

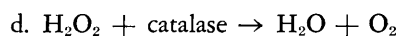
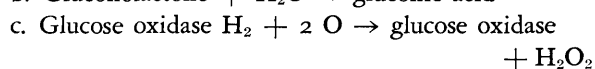
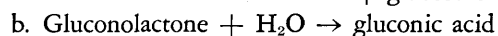
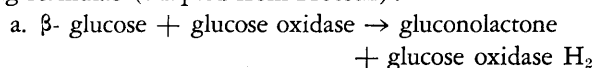
## A Rapid Screening Method Utilizing Glucose Oxidase Paper

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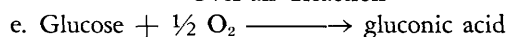
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Following the isolation of the enzyme glucose oxidase by Müller<sup>1</sup> in 1928 and Coulthard et al.<sup>2</sup> in 1942 from *Penicillium notatum* and *Aspergillus niger*, a specific method for the enzymatic determination of glucose became available. Early workers with this enzyme soon evolved methods for the determination of glucose in biologic fluids,<sup>3-6</sup> including blood and urine,<sup>7</sup> which are specific and accurate.

Glucose oxidase is a flavoprotein with a molecular weight of 152,000; alloxazine-adenine dinucleotide is the prosthetic group. The reactions by which glucose is oxidized by glucose oxidase are shown in the following formulae (adapted from Froesch):<sup>7</sup>



Over-all Reaction



The oxidation of  $\beta$ -glucose to gluconolactone (reaction a) involves the reduction of the prosthetic group of glucose oxidase and the hydration of the gluconolactone to gluconic acid (reaction b).

Molecular oxygen acts as a hydrogen acceptor for the re-oxidation of the reduced flavoprotein; the H<sub>2</sub>O<sub>2</sub> inhibits the reaction, hence a catalase is necessary to catalyze its decomposition to H<sub>2</sub>O and O<sub>2</sub> (reaction d).

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It has been shown that glucose in solution exists in two forms in the relatively constant proportions of 36 alpha to 64 per cent beta, and that glucose oxidase is virtually specific for oxidation of  $\beta$ -glucose. Studies by Keilin and Hartree<sup>8</sup> have demonstrated in the glucose oxidase preparations the presence of an enzyme, (gluco) mutarotase, which has the ability to convert the glucose rapidly to the  $\beta$ -form.

The over-all reaction results in the incorporation of one atom of oxygen into each glucose molecule, and glucose thereby loses its reducing properties. (See footnote column one.)

In 1956 filter paper impregnated with glucose oxidase, catalyzing enzymes and a color indicator was used clinically for the measurement of glucose in the urine. These products, Tes-Tape and Clinistix, have found extensive use in the detection of glycosuria. Such papers have a wide margin of error and reliance on them in achieving control of diabetes may be unsafe, since very high urine glucose levels may not be detected.<sup>9</sup> Both products have been found capable of detecting amounts of glucose in the urine as low as 0.1 per cent, but their use as a quantitative procedure for urine containing more than 0.5 per cent glucose is considered unsatisfactory. Nevertheless, we have found these products fairly reliable in the detection of asymptomatic glycosuria and particularly useful in screening the urine in a large diabetes clinic population for reducing substances other than glucose. Recently, we have used similar test paper designed to measure blood glucose.\*

### METHODS

This test material, filter paper in tape form impregnated with glucose oxidase, catalyzing enzyme and a suitable color indicator, is moistened with one drop of finger or ear-lobe blood, gently blotted, and the red color developing at the end of three minutes compared visually with a reference color scale calibrated to correspond to blood sugars of 50, 125, 250, and 350 mg. per cent. One technician performed all of the determinations, and the values obtained have been compared with a simultaneously obtained "true" blood sugar, as determined on finger or venous blood using the Somogyi-Nelson technic (normal values 60-95 mg. per cent).<sup>10</sup> The blood glucose oxidase paper values were determined and recorded before the completion of the Somogyi-

\*Supplied through the courtesy of Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Indiana.

Nelson determination. One hundred and seventy-seven paired observations were obtained. The results utilizing the tape were recorded in absolute values in an attempt to encourage the technician to interpolate readings for color changes falling between the four standard color scale values.

Several members of the house staff at Jefferson Davis Hospital have been given the blood test paper to use in the routine diagnosis and care of patients. These house officers were shown the proper procedures in the use of the test paper, but they received no extensive instructions.

For statistical comparison twenty-nine blood samples were analyzed simultaneously by a modified Folin-Wu<sup>11</sup> and the Somogyi-Nelson<sup>10</sup> procedures.

### RESULTS AND DISCUSSION

The manufacturer designed the blood test paper to be used as a rapid method of determining the approximate level of blood glucose. For this purpose a color range from 50 to 350 mg. per cent was selected; therefore the blood paper shows no further change when the blood glucose drops below 50 mg. per cent and there is little increase in color intensity above 350 mg. per cent. It has been our experience that interns, residents and technicians, even those unfamiliar with the test paper, can quickly and easily determine whether a patient's blood glucose is low, normal or elevated. It does take some experience to make a finer differentiation, but with a little experience in reading the more subtle color changes this paper can be used as an accurate measure of the blood glucose level, within the limitations of the color scale.

In figure 1 are shown all 177 observations along with correlation as described by the regression equation. In figure 2 the first fifty-nine (one third) of the observations are shown, while figure 3 shows the last fifty-nine paired observations. It should be emphasized that the technician performing these determinations had no experience with this tape at the start of this series, yet satisfactory accuracy was achieved from the very first. As might be expected the accuracy improved with experience (figure 3).

Statistical analysis, appearing in table 1, further strengthens the impression that the blood tape is a satisfactory method of obtaining a quantitative blood sugar level. As it is known that the blood paper shows satisfactory color changes in the range of 50 to 350 mg. per cent, only those results in which the Somogyi-Nelson value fell within this range were used in these analyses. The results obtained by simultaneous analysis by the

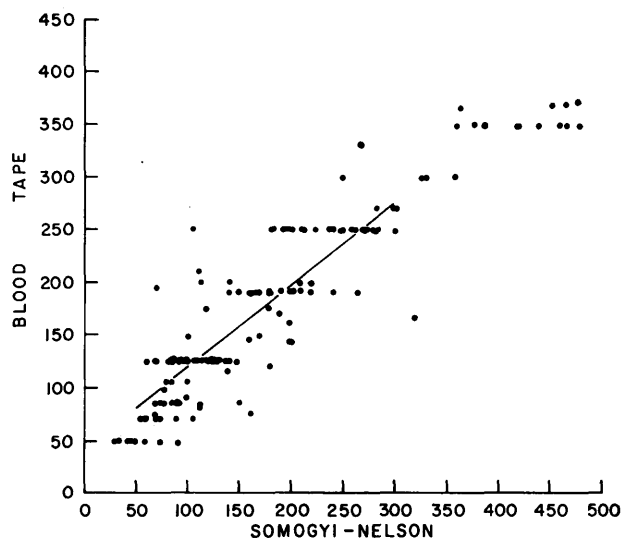


FIG. 1. The relation of the glucose oxidase paper blood sugar level to the simultaneously determined Somogyi-Nelson value in all 177 pairs of observations. The line represents the calculated regression equation.

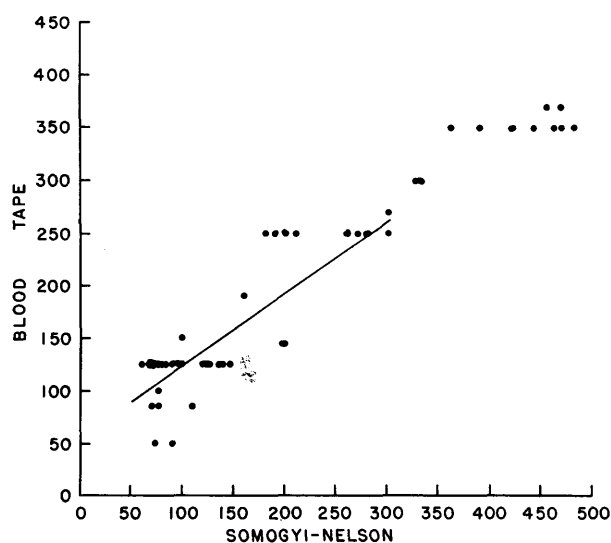


FIG. 2. A comparison of blood sugar levels determined by the glucose-oxidase paper and the Somogyi-Nelson technique (first fifty-nine observations).

Folin-Wu and the Somogyi-Nelson methods were submitted to the same statistical study. In table 1 it may be seen that the correlation coefficient is good in all instances and that the use of the blood glucose paper for quantitative determination is as good as, or possibly slightly better than, the Folin-Wu method (using the Somogyi-Nelson values as the basis of comparison). In addition the standard error of the difference of the mean indicated that the differences in the means be-

TABLE 1

Statistical evaluation of the blood test paper and Folin-Wu methods compared to the Somogyi-Nelson method

	Blood Glucose Oxidase Paper			Folin-Wu
	All observations	First One third	Last One third	
Number of observations	160			29
Mean $\pm$ standard deviation				
Somogyi-Nelson	155 $\pm$ 77 mg. per cent	150 $\pm$ 86 mg. per cent	163 $\pm$ 74 mg. per cent	144 $\pm$ 49 mg. per cent
Blood test paper	163 $\pm$ 66	165 $\pm$ 69	159 $\pm$ 59	
Folin-Wu				165 $\pm$ 49
Standard error of the difference of the mean	8.05	15.91	12.37	12.87
Correlation coefficient(r) $\pm$ S.E.*	0.89 $\pm$ 0.01	0.94 $\pm$ 0.01	0.94 $\pm$ 0.01	0.93 $\pm$ 0.01

\*Dunn, H. L. *Physiol. Rev.* 9:339, 1929.

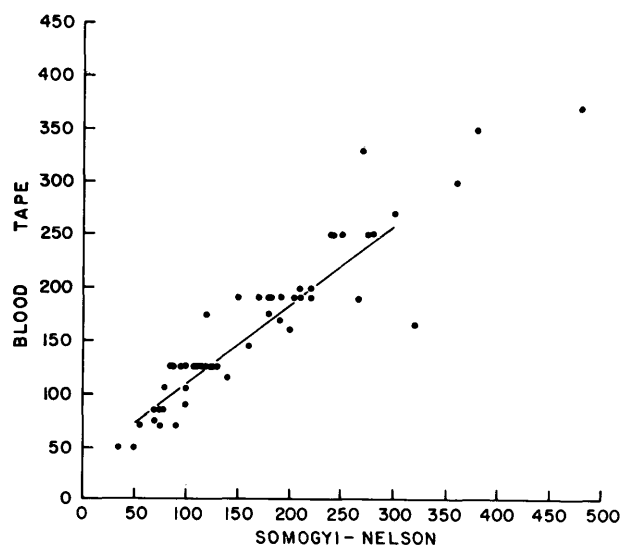


FIG. 3. A comparison of blood sugar levels determined by the glucose-oxidase paper and the Somogyi-Nelson technic (last fifty-nine observations).

tween the blood paper and the Somogyi-Nelson methods are probably due to chance alone. A comparison of the curves obtained by means of regression equation is shown in figure 4. This shows the tendency to read the low glucose values at somewhat higher values and the high glucose values at somewhat lower values when the blood test paper is used. In addition, the tendency for the Folin-Wu determination to average 20-25 mg. per cent greater than the Somogyi-Nelson values is also seen.

In addition to the statistical evaluation of the over-all precision there is also the problem of reliability of the tape in the transition of normal to elevated blood sugar

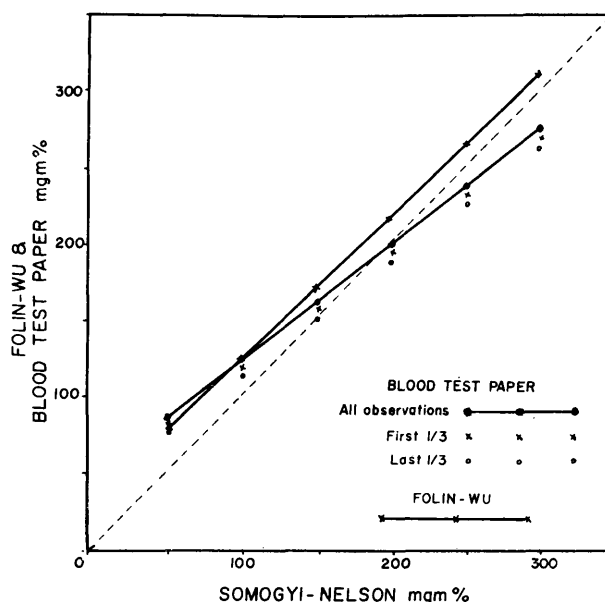


FIG. 4. The lines derived from the regression equations comparing the blood glucose oxidase paper and the Folin-Wu determinations to the simultaneously determined Somogyi-Nelson values. The dotted line represents theoretically perfect correlation.

levels (100 to 150 mg. per cent). In the first seventy-five pairs of observations there were twenty-three instances in which the difference between the values obtained by the two methods could be of diagnostic significance. Fifteen of these observations were in the 95 to 125 mg. per cent range. Errors here would be of importance mainly in the evaluation of the fasting blood sugar levels. It is obvious that the untrained observer experiences some difficulty in learning to interpolate the color changes in this range. In all instances the paper

was read as showing a level of 125 mg. per cent whereas the Somogyi-Nelson value lay in the 95-100 mg. per cent range. Six determinations had variations of diagnostic significance in the 140 to 200 mg. per cent range, where the variation is of significance in the evaluation of the postprandial blood sugar levels. The remaining two determinations showed such wide variation between the two methods of analysis that it was assumed that errors in analysis were involved.

In the last seventy-five observations there were only four instances in which the difference in the two methods would be of clinical significance, again reflecting the increasing accuracy as experience is gained in the use of the tape. Three of these determinations fell in the 80 to 125 mg. per cent range and one in the 115 to 150 mg. per cent range.

The remarkable degree of correlation is due in part to the consistency of technic employed by a single technician; however, it has been observed that similar accuracy is approached by the ward and staff physicians with very little practice, which bespeaks the reproducibility of the blood test paper method.

This tape has been found to be very useful in the day-to-day management of the hospitalized diabetic, conveniently rapid, and of inconsequent cost compared to the usual methods for the determination of blood sugar. In certain situations, notably diabetic acidosis, post-operative diabetic care, and the obstetric care of the diabetic mother at term, the method has become indispensable. In addition, it has been invaluable in a large charity hospital emergency room as a rapid screening method for differentiating hypoglycemia from hyperglycemia. This is particularly true in comatose patients where head injuries, ethanol intoxication and poisonings must also be quickly recognized and time is a major factor. Recently it has been found that the blood test paper is useful in the rapid diagnosis of episodes of hypoglycemia not originating from insulin therapy, when laboratory facilities have not been available. The regulation of insulin dosage has also been facilitated by this test paper. The only objection is the color scale, which should be expanded to include more color standards if the tape is to be used for quantitative blood sugar determination. The present scale, or one containing even fewer color standards, is adequate where the paper is used only as a screening procedure.

#### SUMMARY

A new rapid method for the determination of the blood glucose is described that utilizes paper impregnated with the enzyme glucose oxidase. Any person trained in technical observation can utilize this test paper

to detect gross alterations in the blood glucose level. With practice and observation the blood glucose oxidase paper can be used as an accurate quantitative method within certain blood sugar ranges. This method is particularly useful in emergencies, in the diagnosis of coma due to extremes in the blood sugar level and as an aid in regulating insulin dosage.

#### SUMMARIO IN INTERLINGUA

*Un Rapide Methodo Pro Le Determination De Suco Sanguinee, Utilisante Papiro A Oxydase De Glucosa*

Es describe un nove e rapide methodo pro le determination de glucosa del sanguine, utilisante papiro impregnate del enzima oxydase de glucosa. Omne individuo experite in observation technic pote utilizar iste papiro testatori pro deteger grossier alterationes in le nivello del glucosa del sanguine. Post practica e experientia, on pote usar le papiro a oxydase de glucosa sanguinee pro le accurate determination quantitative de suco sanguinee intra un certe spectro de valores. Le methodo es specialmente utile in casos de urgentia, in le diagnose de coma causate per extremos in le nivello de suco sanguinee, e in le regulation del dosage de insulina.

#### ACKNOWLEDGMENT

This study was supported by a grant from Eli Lilly and Company, Indianapolis, Indiana.

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