Critical Analysis of Blood Sugar Measurements in Diabetes Detection and Diagnosis

M. J. Whichelow, M.A., A. Wigglesworth, B. D. Cox, W. J. H. Butterfield, M.D., F.R.C.P., and M. E. Abrams, B.Sc., M.B., M.R.C.P., London, England

SUMMARY

An analysis of 702 blood sugar levels during glucose tolerance tests shows that the site of blood sampling influences the blood sugar result. Capillary blood taken from the warm ear lobe gives an accurate reflection of the arterial blood sugar level. Compared to the arterial level the venous blood sugar level is unpredictable and differs from the arterial to a varying extent, e.g., from +26 mg. per 100 ml. to —4 mg. per 100 ml. at two hours in the glucose tolerance test depending on many factors.

Studies of the macromethod in the AutoAnalyzer indicate that although the reproducibility is good, the volume of blood pumped by the proportioning pump is inaccurate (too small). Analysis of the micromethod shows that the reproducibility is also good, though not quite so good as the macromethod. However, the sample volume taken for analysis by the proportioning pump is accurate.

Reasons are given for recommending that capillary, not venous, blood be taken, and that the ferricyanide-reducing micromethod on the AutoAnalyzer be used for blood glucose estimation. DIABETES *16*:219-26, April, 1967.

This paper is concerned with the accuracy of blood sugar estimations and the variations between the arterial, capillary and venous blood sugar levels in various types of subjects.

DEFINITIONS

Subjects

Nondiabetics: Arterial blood sugar less than 140 mg. per 100 ml. two hours after 50 gm. glucose by mouth.

Borderline diabetics: Arterial blood sugar 140 to 200 mg. per 100 ml. two hours after 50 gm. glucose.

Diabetics: Arterial blood sugar greater than 200 mg. per 100 ml. two hours after 50 gm. glucose.

Measurement of obesity

Skinfold thickness measured (in triplicate) with skinfold calipers in the midtriceps region for women, and the subscapular region for men.

Lean subjects: Skinfold thickness < 2.3 cm.

Obese subjects: Skinfold thickness > 2.3 cm.

Blood source

Arterial: Blood withdrawn through indwelling catheter in brachial artery.

Venous (mixed): Blood withdrawn in usual way from an antecubital vein without exclusion of the hand, the needle pointing centrally.

Venous (deep): Blood withdrawn through catheter threaded retrogradely into an antecubital vein draining deep muscle compartment, with hand excluded from the circulation by inflating cuff at wrist.

Venous (superficial): Blood withdrawn through catheter threaded retrogradely with the tip just under the skin in a superficial antecubital vein, with the hand excluded.

Capillary: Sample taken directly from blood flowing freely through skin puncture in warm ear lobe.

Blood sugar estimations using the AutoAnalyzer

Modification of ferricyanide-reducing method of Hoffman[1] analyzing forty samples per hour, either:

Macromethod: Using one dialyzer for whole blood, plasma or aqueous glucose solutions in the range of 10 to 650 mg. per 100 ml. Blood and plasma mixed with solid potassium fluoride and heparin to prevent glycolysis and clotting, or

Micromethod: Using two dialyzers for diluted blood and plasma or aqueous glucose solutions in the range 0.5 to 30 mg. per 100 ml. Blood or plasma, 0.1 ml., was diluted with 3 ml. of 1 per cent potassium fluoride solution, which prevents hemolysis and glycolysis.

INTRODUCTION

It is self-evident that easy, accurate measurements of blood sugar levels are essential for the reliable routine clinical diagnosis and management of diabetes mellitus.

There are several factors in the collection of blood samples which may influence the level of sugar found

From the Department of Medicine, Guy's Hospital Medical School, London, S.E.1, England.

in the blood and thereby affect artificially both the diagnosis and indirectly the apparent prevalence of diabetes. Some workers use capillary blood and some use venous, and these usually give different values. In an attempt to overcome this difficulty the World Health Organization[2] and the British Diabetic Association[3] have both suggested diagnostic venous as well as capillary levels during the glucose tolerance test for the recognition of diabetes, as set forth in table 1. It must be acknowledged that these venous values are not based directly on experimental data. Furthermore, they assume that the capillary-venous differences will be the same for all individuals—which may not necessarily be so.

TABLE 1

Recommended criteria for diagnosis of diabetes during glucose tolerance tests

Source	Classification	Time	Capillary	Venous
WHO*	Diabetic	2 hours	140	130
WHO	Normal	2 hours	120	110
WHO, BDA†	Subclinical diabetes	Fasting	130	125
BDA	Abnormal	2 hours	120	110
		peak	180	160

*WHO—World Health Organization
†BDA—British Diabetic Association

It is also well known that different methods of blood sugar analysis give different values.[4] For instance, the enzymatic methods using glucose oxidase—which is specific for glucose—give lower levels than older methods which depend on the reducing properties of glucose. These latter also estimate other reducing substances in blood and, although the quantities of these substances are small compared to the amount of glucose present, they can become important sources of difference between one investigation and another.

The problems which arose because different chemical methods give different values had never really been resolved before the demands for mass measurement generated by epidemiologic surveys led to an increasing use of automated methods for blood sugar analysis. The Technicon AutoAnalyzer has made the rapid analysis of large numbers of samples feasible and, indeed, without such methods many large-scale diabetes surveys would have been impossible. Unfortunately, not all workers can, or probably ever will, use the same methods, but since the AutoAnalyzer adaptation of the Hoffman method is so widely used currently and has been shown to be among the most reliable of the methods for sugar measurement,[5] we have undertaken a careful and critical analysis of the results from this method and examined various factors which influence the results.

METHODS AND RESULTS

1. Influence of sample source on the blood sugar level

a. *Comparison of arterial and capillary blood sugar levels*

In a study of glucose tolerance tests in one lean and one obese nondiabetic, a borderline diabetic and a diabetic, arterial and capillary blood samples were taken simultaneously on twenty-eight occasions. The blood sugars, ranging from 68 to 349 mg. per 100 ml., were estimated by the micromethod. The mean values for arterial blood were 169.5 mg. per 100 ml. and capillary blood 171.4 mg. per 100 ml.

There was no consistent difference between the arterial and capillary levels. On nine occasions the arterial level was higher, on thirteen occasions the capillary level was higher, and on six occasions they were the same. The capillary level varied by —8.80 to 4.2 per cent from the arterial.

b. *Comparison of deep and superficial vein blood sugar levels*

Simultaneous arterial (A) and deep (DV) and superficial vein (SV) blood sugar levels were measured in duplicate, using the macromethod, every half-hour during a glucose tolerance test on the four subjects mentioned in the previous section. The A-DV and A-SV differences are shown in table 2. After glucose, in just over half the analyses, the A-DV difference was greater than the A-SV difference, and this was particularly striking in the lean nondiabetic subject. The results indicate that since the blood sugar levels in the two *veins* can vary by as much as 24 mg. per 100 ml., the blood sugar level obtained will depend on the vein sampled.

TABLE 2

Comparison of arterio-deep vein* and arterio-superficial vein* sugar differences during glucose tolerance test

Time	Lean nondiabetic		Obese nondiabetic		Borderline diabetic		Diabetic	
	A-DV	A-SV	A-DV	A-SV	A-DV	A-SV	A-DV	A-SV
Fasting 1	4.0	5.5	1.0	1.0	3.0	2.0	2.0	6.0
Fasting 2	3.0	5.0	3.0	3.0	1.5	1.0	3.5	3.0
30′	23.0	8.0	12.0	15.0	13.0	11.5	23.5	17.5
60′	25.5	1.5	13.5	13.0	10.5	7.0	10.5	11.0
90′	8.5	6.0	—5.5	—8.0	6.0	0.5	—4.0	4.5
120′	—2.0	—1.0	—3.0	—1.5	—4.0	7.5	3.5	4.0
150′	—2.5	—1.5	—2.0	—3.0	—2.5	0	2.0	—1.5

*See "Definitions."

TABLE 3

Arterio-deep venous* differences during glucose tolerance test in diabetics and nondiabetics

Group	Number	0 Mean	0 S.D.	0.5 hour Mean	0.5 hour S.D.	1 hour Mean	1 hour S.D.	1.5 hours Mean	1.5 hours S.D.	2 hours Mean	2 hours S.D.	2.5 hours Mean	2.5 hours S.D.
Lean controls	16	2.4	± 2.1	19.4	± 10.4	14.0	± 11.5	12.9	± 9.4	7.0	± 7.2	6.9	± 5.0
Plump controls	16	1.6	± 1.5	13.4	± 8.5	10.8	± 8.4	7.3	± 9.2	4.8	± 7.2	3.0	± 7.2
Borderline diabetics	7	0	± 2.9	10.2	± 6.0	9.0	± 3.1	5.4	± 2.1	3.7	± 3.6	3.9	± 4.8
Diabetics	11	1.7	± 2.9	14.7	± 5.7	10.5	± 5.5	5.9	± 4.3	2.5	± 3.6	—1.6	± 4.7

*See "Definitions."

c. *Comparison of arterial and deep vein blood sugar levels*

Comparisons have been made of 300 arterial and deep venous blood sugar levels, estimated in duplicate using the macromethod during glucose tolerance tests on sixteen lean and sixteen obese nondiabetics, seven borderline diabetics and eleven diabetics. The results are shown in table 3.

In the fasting state the mean A-V fasting difference was greater in the lean controls than the other groups. The standard deviation in each group was not large but quite considerable in proportion to the mean A-V difference, and all the mean A-V values were smaller than the 5 mg. per 100 ml. difference suggested by the British Diabetic Association.

The mean A-V differences at one hour were widest in the lean nondiabetic group, next in rank came the obese nondiabetics, then the diabetics and the borderline diabetics. Again all the mean A-V values were less than the 20 mg. per 100 ml. suggested by the British Diabetic Association (see bottom line, table 1). However, the variation was considerable, particularly in the nondiabetics, as indicated by the large standard deviations. Some very small A-V differences occurred in the diabetic group, similar in magnitude to those in the fasting state. A like pattern was found at thirty and ninety minutes.

The variations in the A-V differences between the groups two hours after glucose were most germane to the present considerations since the values at this time are widely used as the basis for the diagnosis of diabetes. All the mean A-V differences were less than the 10 mg. per 100 ml. value suggested by both the British Diabetic Association and the World Health Organization, but the variation in values was wide. The mean A-V differences were greatest in the lean nondiabetics and smallest in the diabetics. At this time there were subjects in most groups with negative values. A similar pattern of results was found at two and one-half hours.

d. *Capillary-mixed venous blood sugar differences*

Comparisons were also made of capillary and mixed vein blood sugar levels taken two hours after 50 gm. of glucose in thirty control subjects attending the Bedford Borderline Diabetic Clinic. The venous blood was diluted and estimated using the micromethod in the same way as the capillary blood. The mean capillary-venous difference was 6.3 mg. per 100 ml. (S.D. ± 6.1), a result midway between that for the lean and obese nondiabetics described above.

2. INVESTIGATION OF BLOOD SUGAR MEASUREMENT

a. *Macromethod*

The standard macromethod has been modified slightly to give greater sensitivity and accuracy and to avoid troubles with fibrin clots. The manifold was made up on (1) the donor side of a 0.89-mm. diameter blood sample, a 0.42-mm. air and a 2.28-mm. diluent line, and (2) the recipient side of a 2.54-mm. reagent and a 1.42-mm. air line. With this manifold a larger volume of sample was taken, so that the accuracy and sensitivity were enhanced, although the range of values measured was smaller, i.e., about 200 mg. per 100 ml. To overcome the difficulties imposed by this restricted range of sugar levels, ferricyanide solutions of strengths varying from one fifth to four times as strong as the recommended standard (0.75 gm. per liter of solution) were used as necessary. These solutions cover ranges from 10 to 650 mg. per 100 ml. sugar.

To ensure that blood never came into contact with glass, the glass mixing coil on the sample side was replaced by a polyethylene coil of similar dimensions and the arrangement of the cactus, where the sample, diluent and air streams join, was altered to channel the sample through polyethylene tubing directly into the diluent stream before the air stream entered. These modifications have greatly reduced the obstruction of the tubes and contamination of the dialysis membrane with fibrin or blood clots.

It is well known that the glucose concentration of

plasma is higher than that of blood, and in this connection proper mixing of the blood from which the aliquot is drawn up by the pump is an important consideration. To demonstrate this, a single sample of well-mixed blood was divided into twenty-seven aliquots which were all placed in the sampler plate at the same time. Figure 1 shows that the last samples estimated twenty to forty minutes after the first gave considerably lower sugar levels. During this time the red cells had settled and, since the sample line dips down to the bottom of the cup, less plasma was drawn up. It is therefore important to mix the blood well immediately before estimation, either manually or with a mechanical stirring device.

To test the reproducibility of the method, ten samples each of five different aqueous glucose solutions of concentrations varying from 91.45 to 94.80 mg. per 100 ml. (group A) (table 4), and another ten samples each of five different glucose solutions, ranging from 213.5 to 221.1 mg. per 100 ml. (group B), were analyzed in random order to exclude bias by the machine operator. The results are shown in table 5. The S.E.M.'s

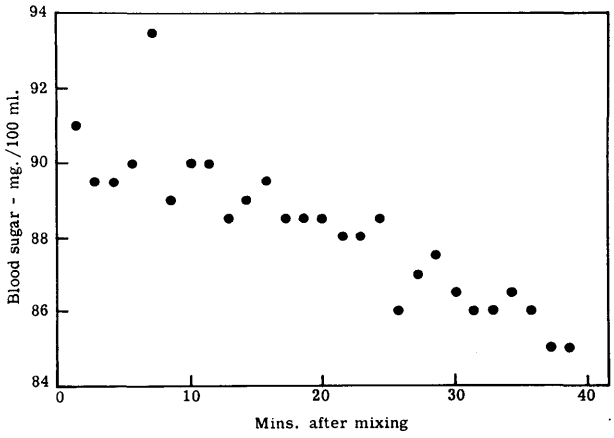

FIG. 1. Effect of degree of mixing of blood on estimated blood sugar level.

TABLE 5

Reproducibility of blood sugar levels compared to aqueous glucose solutions (macromethod)

		Range of glucose values	Number of duplicates analyzed	95 per cent duplicates apart by less than (per cent):
Aqueous solution	Series A	91.45 - 94.80	225	2.0
Aqueous solution	Series B	213.5 - 221.1	225	2.0
		50 - 99	72	2.1
		100 - 149	72	4.3
Blood	—	150 - 199	72	5.2
		200 - 249	72	2.8
		250 - 299	72	2.3
		>300	72	3.6

were small in all cases, ranging from 0.05 to 0.26 mg. per 100 ml., thus indicating that with ten replicates the method can easily distinguish between samples whose glucose concentration is less than 1.0 mg. per 100 ml. apart.

Since arterial and venous blood sugar estimations had been made in duplicate, and in view of the results above, it seemed relevant to determine how far the means of duplicate estimations differed from the observed mean values based on larger numbers of replicates. The means of all combinations of pairs in each group of ten samples of each aqueous solution were therefore calculated and compared to the mean of each group as a whole. For group A, 95 per cent of the means of pairs differed by less than ±0.76 mg. per 100 ml. from the mean of the group as a whole, and in group B, by less than ±1.51 mg. per 100 ml.

To assess any additional scatter of results using blood rather than glucose solutions, we have compared the difference between duplicate estimations on 432 whole blood samples of sugar content ranging from below 100 mg. per 100 ml. to more than 300 mg. per 100 ml., with duplicate estimations for groups A and B above. Table 5 brings all these results together and shows that

TABLE 4

Reproducibility of macromethod (series A and B) and micromethod (series C and D)

		1	2	3	4	5	Mean
Series A	Mean mg. per 100 ml.	94.80	94.25	93.05	92.05	91.45	
	S.E.M.	0.08	0.24	0.19	0.05	0.26	0.16
Series B	Mean mg. per 100 ml.	219.4	221.1	216.9	215.0	213.5	
	S.E.M.	0.21	0.18	0.15	0.22	0.19	0.19
Series C	Mean mg. per 100 ml.	81.70	82.00	76.80	76.80	75.00	
	S.E.M.	1.30	1.30	1.40	0.60	0.80	1.08
Series D	Mean mg. per 100 ml.	289.7	290.0	284.8	283.3	278.0	
	S.E.M.	1.71	1.59	1.18	1.35	1.02	1.35

whereas 95 per cent of the duplicate estimations on glucose solutions were apart by less than 2 per cent, in the case of blood the range was somewhat greater. Although we do not know the true mean blood sugar of the samples it may be deduced that 95 per cent of our blood sugar results based on the mean of duplicate estimations are likely to be within 1.5 mg. per 100 ml. of the true mean.

b. *Micromethod*

The standard micromethod (two dialyzers) has been employed for the estimation of sugar in diluted blood. No modification of the glass parts of the system has been necessary but the manifold has been adapted to enhance sensitivity and the same range of strengths of ferricyanide solutions used as for the macromethod. On the donor side of the manifold are 1.42-mm. blood sample and diluent lines and a 1.01-mm. air line and on the recipient side a 1.85-mm. reagent and a 1.42-mm. air line.

To test the reliability and reproducibility of the method we have taken groups of six samples each of five glucose solutions of concentrations ranging from 75 to 82 mg. per 100 ml. (group C) and another group ranging from 278 to 289.7 mg. per 100 ml. (group D), diluting them 1:31 before analysis in exactly the same way as the capillary blood samples. The results of the mean values and S.E.M.'s are shown in table 4. It should be noted that the S.E.M.'s for the macromethod were based on ten replicates as against six for the micromethod. When fifteen replicate 0.1-ml. aliquots from a single blood sample with a mean blood sugar level of 86.2 mg. per 100 ml. were analyzed by the micromethod, the S.E.M. was found to be 0.152 mg. per 100 ml.

As expected, the micromethod was not so reproducible as the macromethod. To investigate the sources of error, ten 0.1-ml. replicates were pipetted from a single blood sample and diluted in the usual way. The samples were then analyzed in duplicate and the results subjected to analysis of variance. The major part of the variance was due to pipetting and dilution. Against this can be set the fact that blood diluted 1:31 with 1 per cent potassium fluoride can be stored for at least a week at 4° C. without affecting the results. There was a mean rise of 1.4 mg. per 100 ml. in the blood sugar estimate of twenty-four samples kept for seven days at 4° C. after the first analysis.

c. *Comparison of blood and plasma*

Comparisons were made between plasma and blood sugar levels, using both macro and micromethods (table 6). The mean differences between blood and plasma estimated with the macromethod were 28 mg. per 100 ml. for the diabetics and 15.5 mg. per 100 ml. for the nondiabetics, but the percentage differences were similar: 13.5 and 15.5 per cent, respectively. For the micromethod the mean absolute and percentage differences were 23.5 mg. per 100 ml. (9.4 per cent) for the diabetics and 9.0 mg. per 100 ml. (9.6 per cent) for the nondiabetics. Thus, as found by other workers,[6] there is a proportional difference between the glucose concentrations of blood and plasma.

d. *Comparison of macro and micromethods*

Blood: For a comparison of the results given by the macro and micromethods venous blood was taken and replicate (usually five) 0.1-ml. samples withdrawn, diluted and analyzed by the micromethod. Replicate (usually five) whole blood samples were analyzed using the macromethod.

TABLE 6

Comparison of blood and plasma sugar levels by macro and micromethods

Subject	Macromethod				Micromethod			
	Blood mg. per 100 ml.	Plasma mg. per 100 ml.	Difference mg. per 100 ml.	Per cent	Blood mg. per 100 ml.	Plasma mg. per 100 ml.	Difference mg. per 100 ml.	Per cent
13	69.5	84.0	14.5	17.3	75.5	88.0	12.5	14.2
15	70.5	93.5	23.0	24.7	74.0	92.5	17.5	18.9
16	74.0	85.0	11.0	12.9	76.0	81.0	5.0	6.2
14	74.0	90.5	16.5	18.3	85.0	91.0	6.0	6.6
12	81.0	95.0	14.0	14.7	87.0	91.0	4.0	4.4
11	85.5	95.5	10.0	10.5	88.0	95.0	7.0	7.4
1	118.0	142.0	24.0	16.9	158.0	173.0	15.0	8.7
6	157.5	182.5	25.0	13.7	172.0	188.0	16.0	8.5
9	189.5	211.0	21.5	10.3	201.0	212.0	11.0	5.2
3	199.0	232.5	33.5	14.4	217.0	243.0	26.0	10.7
7	210.5	246.5	36.5	14.8	217.5	248.0	30.5	12.3
4	254.0	289.5	35.5	12.3	271.5	305.0	33.5	11.0
2	297.0	338.5	41.5	12.3	314.5	348.0	33.5	9.6

TABLE 7

Comparison of macro and micromethods of blood and plasma sugar levels

Subject	Blood				Plasma			
	Macro mg. per 100 ml.	Micro mg. per 100 ml.	Difference mg. per 100 ml.	Per cent	Macro mg. per 100 ml.	Micro mg. per 100 ml.	Difference mg. per 100 ml.	Per cent
13	69.5	75.5	6.0	8.7	84.0	88.0	4.0	5.0
15	70.5	74.0	3.5	5.0	93.5	92.5	—1.0	—1.0
16	74.0	76.0	2.0	2.7	85.0	81.0	—4.0	—5.0
14	74.0	85.0	11.0	14.9	90.5	91.0	0.5	0.5
12	81.0	87.0	6.0	7.4	95.0	91.0	—4.0	—4.0
11	85.5	88.0	2.5	2.9	95.5	95.0	—0.5	—0.5
1	118.0	158.0	40.0	34.0	142.0	173.0	31.0	22.0
8	145.5	162.5	17.0	11.7	—	—	—	—
6	157.5	172.0	14.5	9.2	182.5	188.0	5.5	3.0
9	189.5	201.0	11.5	6.1	211.0	212.0	1.0	0.5
3	199.0	217.0	18.0	9.1	232.5	243.0	10.5	4.3
7	210.5	217.5	7.5	3.6	246.5	248.0	1.5	0.5
10	245.0	255.0	10.0	4.1	—	—	—	—
4	254.0	271.5	17.5	6.9	289.5	305.0	15.5	5.0
2	297.0	314.5	17.5	5.9	338.5	348.0	9.5	2.8
5	390.0	391.0	1.0	0.25	—	—	—	—

The results of studies on ten diabetic and six nondiabetic subjects are shown in table 7. The micromethod invariably gave higher results and the difference between the micro and macromethods was greatest with the elevated blood sugar levels of the diabetics. The mean percentage difference between the two methods was found to be similar for the diabetics and nondiabetics (6.3 per cent and 6.9 per cent, respectively).

Plasma: Forty milliliters of venous blood were drawn, and from half of this the plasma was separated by centrifugation. Comparison was made between the results of the sugar levels measured both by macro and micromethods as with blood, above. The results are also shown in table 7. There was much less difference between the two methods for plasma, but the micromethod usually gave slightly higher results—of the order of 1 to 2 per cent.

e. *Volume of sample removed by proportioning pump*

The greater viscosity of blood than the glucose standards might mean that the plastic manifold tubing of the proportioning pump did not deliver exactly the same volume of blood as aqueous standard. To investigate this, five AutoAnalyzer cups (each holding 2 ml.) containing blood, and five cups containing plasma all from the same blood sample and five cups containing glucose standard of approximately the same glucose concentration as that of the blood were weighed before and after the aliquot for sugar estimation had been removed by the proportioning pump. The difference gave the weight of fluid removed, and by allowing for the specific gravity of the fluids (blood 1.060, plasma 1.027, glucose standard 1.000)[7] the volume of liquid removed from the cups could be calculated.

Macromethod: The weights and volumes removed from each cup were found to be: whole blood 0.525 gm. or 0.495 ml., whole plasma 0.518 gm. or 0.504 ml. and standard 0.515 gm. or 0.515 ml., each value being the mean of five measurements. Thus, 4 per cent less blood is taken by the proportioning pump than standard, and 2 per cent less plasma.

Micromethod: With the micromethod the differences in the volumes of diluted blood, diluted plasma and standard pumped were undetectable. We observed an approximate 2 per cent difference between plasma sugar levels measured by the macro and micromethods, and thus we can account for all of this difference by the smaller volume of plasma pumped in the macromethod.

Of the 6 per cent difference between the macro and micromethods observed for blood we can account for two thirds (a 4 per cent difference) by the smaller volume of blood pumped by the macro manifold. We are inclined to attribute the other 2 per cent difference to the red cells blocking the pores in the dialyzer membrane, but further investigations of this point have not been made.

f. *Recovery of glucose added to blood*

A study of the recovery of glucose added to blood demanded accurate volume measurements, and it was found that blood could not accurately be delivered from a 10-ml. volumetric pipette. Weighing experiments indicated that whereas 0.1 ml. *water* remained in the pipette after draining according to the makers' instructions, 0.3 ml. *blood* remained. Thus, the volume of

blood measured is too small by 2 per cent, and allowances have been made for this when the recovery of weighed amounts of glucose added to measured quantities of blood were estimated. The recovery of glucose as determined by the micromethod was 100, 108 and 101 per cent on three separate occasions (each the mean of five or six determinations), whereas that determined by the macromethod was 97, 100.5 and 97 per cent, respectively.

DISCUSSION

The standardization of blood sugar measurements is becoming of increasing importance. They apply to the diagnosis and management of a large proportion of some populations. For example, as many as 12 per cent of the adult English population may be considered to be diabetic by certain very strict criteria.[8] There are also indications that the prevalence of diabetes mellitus is of the same magnitude in most highly mechanized countries, but lower in more primitive societies.[2] However, the point cannot be substantiated easily: perusal of the reports of different population surveys quickly reveals the difficulties involved in comparing one survey with another. If, as has been recommended by the WHO Expert Committee on Diabetes Mellitus,[2] epidemiologic surveys are to be carried out with a view to identifying, before it is too late, cultural, social, dietary, climatic and other factors which may contribute to diabetes mellitus, it is clearly important to standardize measurement procedures between countries with respect to the levels of blood sugar taken as diagnostic of diabetes, the procedures for obtaining blood for sugar analysis and the methods of analysis.

Diagnostic levels of blood sugar

Regarding the diagnostic levels of blood sugar, in the present paper we have departed from the WHO definitions because we are not convinced they are correct. The exact relationship between hyperglycemia and the complications of diabetes is still uncertain. In view of the very high proportion of adults in Western societies with blood sugar levels at two hours in the glucose tolerance test over 120 mg. per 100 ml. (13 per cent) and over 140 mg. per 100 ml. (9 per cent), we hold the view that persons with blood sugars over 200 mg. per 100 ml. (3 per cent) at two hours, whether or not they recognize any symptoms, are highly likely to be in need of treatment to control their hyperglycemia and perhaps to protect their vascular systems against the long-term consequences of unrecognized hyperglycemia.[9] These patients are therefore defined here as diabetics.

For the very large number of persons, here called borderline diabetics, with two-hour blood sugars between 140 and 200 mg. per 100 ml. (some 6 per cent of the adult population), the question of the need for treatment is open. There is no difficulty in reaching a decision when a patient in this range has symptoms of metabolic decompensation or complications, but further work is required to settle the indications for therapy in individuals without either. This problem will only be answered by studying and examining such a borderline group, as is being done currently by Keen and his colleagues at the Bedford Borderline Clinic.[10,11]

Blood source

The venous blood sugar level is quite variable compared to the arterial and is, therefore, a less reliable reflection of the events in the whole body since it is also affected by events in the local tissues the vein has drained. A comparison of the blood sugar levels in veins draining deep and superficial tissues (table 2) showed that the venous level depended on the vein sampled, which means that glucose metabolism or blood flow is not the same in all tissues.

Previous studies have shown that forearm glucose uptake is variable in nondiabetic persons during a glucose tolerance test.[12] Lean subjects have a much greater glucose uptake than obese subjects, so that in lean subjects the arteriovenous difference will be greater and the venous level lower than for the same systemic level in obese subjects.

Another unpredictable factor affecting venous glucose concentration is the blood flow, which is affected by ambient temperature, pain and emotion. For any one level of glucose uptake, the faster the blood flow the smaller the arteriovenous difference will be.

Finally, in the later stages of glucose tolerance tests, the A-V difference may be negative in those cases where cell glucose uptake from the extracellular fluid is slower than the demand for glucose elsewhere (e.g., glycosuria in diabetes). Thus, by two hours in the glucose tolerance test, the time recommended for assessment of glucose tolerance, the range of A-V differences we observed was +26.0 to —4.0 mg. per 100 ml.

By contrast the negligible differences found between the glucose concentrations of arterial and capillary blood indicate that capillary blood sugar levels (at any rate, those taken from the warm ear lobe) are a valid measurement of systemic blood sugar levels. Since the arterial blood sugar level is more representative of extracellular fluid glucose concentration, it is recommended that glucose tolerance tests be based on arterial or

capillary blood sugar levels.

Blood sugar measurements

The AutoAnalyzer offers many advantages over the manual methods, particularly where large numbers of samples have to be analyzed, such as in surveys for diabetes. It is quicker than the manual methods since a blood sugar estimation can be performed in eight minutes; it can handle many samples a day (up to 300) and, using the ferricyanide-reducing method, the reagent and running costs are low.

There are undoubtedly disadvantages in estimating blood sugar levels by methods which are based on the reducing properties of glucose, but the concentration of reducing substances other than glucose in the plasma is small. The red cells contain glutathione, but care can be taken not to lyse the red cells to avoid the release of glutathione. Experiments have shown that when the red cells have been lysed, for instance by freezing and thawing, the apparent glucose concentration is appreciably increased.

Some workers have argued in favor of estimating plasma sugar levels[13] involving the separation of the plasma by centrifugation. But in order to obtain plasma samples, a relatively large volume of blood is needed, which has to be taken by venepuncture, and is therefore subject to all the objections mentioned above for venous blood.

The present results indicate that the reproducibility of both the macro and micromethods on the AutoAnalyzer is as good as any, and probably better than most, manual methods for estimating blood sugar levels. The reproducibility of the macromethod is better than that of the micromethod. Against this is the disturbing fact that the macromethod underestimates blood sugar levels, because the manifold pumps a smaller blood volume than aqueous standard volume.*

In screening for diabetes or assessment of the diabetic state, where the absolute blood sugar level is important, the micromethod is preferable. There are other advantages, too, in using diluted blood and the micromethod, since the sample can be stored for up to a week at 4° C. without affecting the result, a serious consideration wherever blood samples have to be transported from the area of a survey to the laboratory. Finally, to take 0.1 ml. of capillary blood from an ear lobe puncture is simple and almost painless and saves the expense of using needles and syringes.

*For studies of glucose metabolism in tissues dependent upon precise measurements of the arteriovenous difference, the *absolute* blood sugar levels are not so important and the macromethod is the method of choice.

RECOMMENDATIONS

In view of all the foregoing data, it seems important to urge the standardization of the methods for blood sugar analysis for diabetes surveys and screening and the assessment of the diabetic state. We recommend that venous blood sugar estimation be abandoned and capillary blood samples taken. We also recommend using the ferricyanide-reducing micromethod on the AutoAnalyzer, since this has already been used for many surveys and epidemiologic studies of glucose tolerance. Wherever this method is not used, workers should be encouraged to compare their method at a number of blood sugar levels against the ferricyanide-reducing method.

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