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Is Glucose a Reliable Index of Carbohydrate Metabolism?

Report on the joint workshop conference "Glucose"
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Summary: The determination of glucose concentration is the most frequently used clinical laboratory test. It was the current vehemence in discussions about the judgement criteria for the diagnosis and monitoring of diabetes that motivated this discussion of pathobiochemical and analytical aspects in a circle of 38 experts. The composition of the working group made it possible to compare the needs of clinical diabetologists and diabetics with the analytical possibilties. Pathobiochemistry, sampling problems, diabetic self control, analytical methods and their standardisation, glucose monitoring, glucose sensors and glycosylated proteins were the topics of this glucose workshop.

Ist Glucose ein zuverlässiges Kriterium zur Beurteilung des Kohlenhydratstoffwechsels?

*Bericht über die gemeinsame Kleinkonferenz „Glucose"
der Deutschen Gesellschaft für Klinische Chemie und
der Deutschen Diabetes-Gesellschaft
am 15. und 16. Mai 1981
in Stuttgart*

Zusammenfassung: Die Bestimmung der Glucosekonzentration ist die häufigste Untersuchung im Klinik- und Praxislaboratorium. Die derzeit heftig diskutierten Beurteilungskriterien zur Diabetesdiagnostik und -überwachung gaben den Anlaß, im Kreise von 38 Experten die pathobiochemischen und analytischen Teilaspekte zu diskutieren. Die Zusammensetzung des Arbeitskreises ermöglichte eine Gegenüberstellung der Bedürfnisse des klinischen Diabetologen und des Diabetikers mit den analytischen Möglichkeiten. Pathobiochemie, Probleme der Probenahme, Diabetiker-Selbstkontrolle, analytische Methoden und deren Standardisierung, Glucosemonitoring, Glucosesensoren und glycosylierte Proteine waren Schwerpunkte dieser Glucose-Kleinkonferenz.

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Introduction

The determination of glucose concentration is the most frequently used clinical laboratory test. Nevertheless, at the beginning of this discussion of experts, some participants doubted that this topic could be treated in a conference of 1½ days. This discussion of the pathobiochemical and analytic aspects in a circle of experts was motivated by the current vehemence in discussions about the judgement criteria for the diagnosis and monitoring of diabetes. The composition of the working group facilitated a confrontation of the needs of clinical diabetologists and diabetics with the analytic possibilities. The intimate, convivial framework of the meeting made it possible to reconcile the otherwise often controversial standpoints of the clinic and the laboratory.

Pathobiochemistry

From the introductory pathobiochemical lectures, it became clear that the determination of glucose concentration in blood can only be a measure of the size of the extracellular glucose pool. This pool under euglycaemic conditions consists of 20 g glucose dissolved in 28 l extracellular glucose space in a 70 kg person (fig. 1). It is filled exclusively by nutrition and gluconeogenesis, while many organs contribute to the consumption of this pool (*Dietze*, Munich). Under fasting conditions, the brain, muscle and blood cells are the main organs involved in glucose uptake (6.8 g/h) while the liver refills the pool with a production of 7 g/h.

The determination of glucose concentration can neither disclose the cause of the measured changes in the pool in a normal subject nor in a diabetic, not even under loading conditions. The interpretation of a change in glucose concentration is only possible with the help of pathobiochemical knowledge, if we disregard special techniques (glucose clamp technique, determination of the glucose half-life time with radioactive isotopes). The relatively small reduction of the glucose pool during longer periods of fasting is only possible because of the simultaneous restriction of glucose consumption by muscle and brain and the increased reflux of substrates for hepatic gluconeogenesis. Hyperglycaemia can, on the other hand, be caused by a reduction of peripheral consumption or by an increase in hepatic production. Both mechanisms contribute to a disturbed glucose tolerance. Because of the insulin-dependent glucose transport, the role of muscle and fat tissue is especially important for carbohydrate tolerance. The increase in glucose uptake in a normal subject due to muscle activity is disturbed in an insulin-deficient diabetic. Small doses of insulin are capable of restoring the increased uptake of glucose by working muscles. In contrast to normal subjects, however, the liver of an insulin-dependent diabetic cannot react with a corresponding increase in glucose production, since his glycogenolysis and gluconeogenesis is inhibited by the therapeutically administered insulin. This often results in hypoglycaemia. The avoidance of this is a primary indication for monitoring blood glucose concentration in insulin-treated diabetics (*Kemmer*, Düsseldorf). On the other hand, the kidney plays a subordinate role in glucose homeostasis since gluconeogenesis of the proximal tubule in the kidney is compensated for by the insulin-independent glucose uptake of other sections of the nephron (*Guder*, Munich). Renal

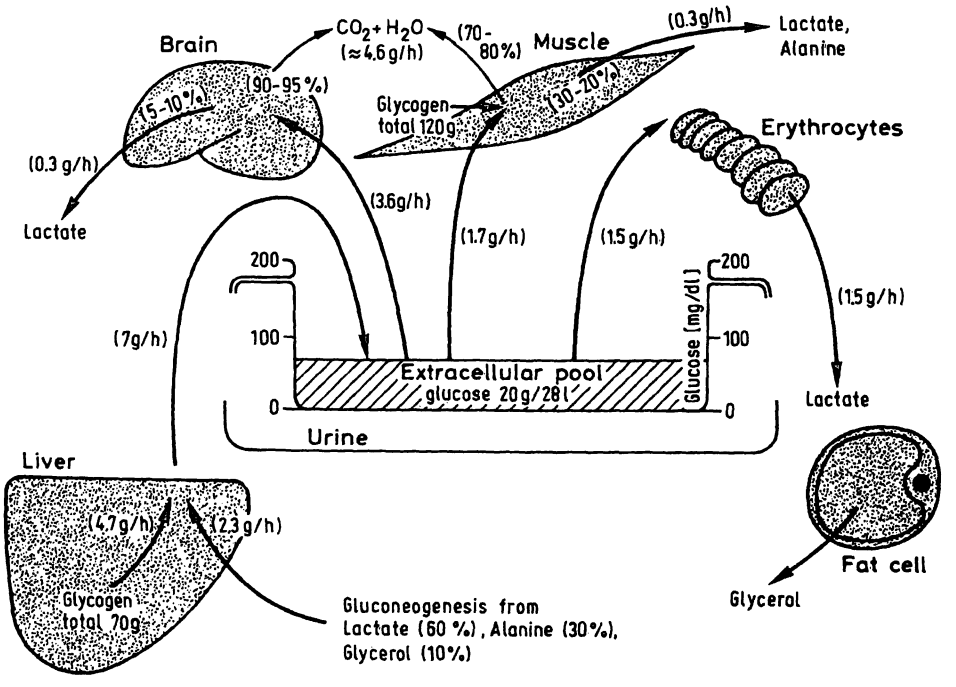

Fig. 1a. Glucose metabolism after fasting for 15 h overnight.

glucose production becomes significant only in cases of lactate acidosis and decompensation of hepatic gluconeogenesis. However, as an excretory organ for glucose, the kidney can be an essential regulator under hyperglycaemic conditions (*Henning*, Göttingen). Glucose determination in urine is therefore still important for monitoring hyperglycaemia. Its interpretation is, however, limited because of the lack of precision of routine methods to determine the renal threshold. The widely used method of polarimetry was judged to be obsolete for hospitals (*Borner*, Berlin) but sufficiently exact and economic for outpatient care (*Willms*, Bad Lauterberg; *Kurow*, Berlin). In an unselected group of patients, polarimetry showed false results in 9% of urine samples and was inferior to the estimation with enzymatic test strips (*Appel*, Karlsruhe). Above all, with increasing renal insufficiency, not only the interpretation of urine glucose but also of blood glucose becomes difficult. In spite of these limitations, the determination of glucose concentration in blood and urine remains the most important procedure for monitoring carbohydrate metabolism. This is underlined by the new findings which assign a pathogenetic role to hyperglycaemia for diabetic late complications (*O. Wieland*, Munich).

Preparing the Patient and Selecting the Time for Taking the Sample

The influence of food on glucose is generally known. However, besides the definition of fasting glucose (12–15 hours after the last meal), the dietary pretreatment of the patient is not standardized. 125–150 g of carbohydrate per day, as recommended by WHO for the oral glucose tolerance test, is not considered to be sufficient (*Otto*, Bremen). While the glucose sample is usually taken between 6.00 and 9.00 a.m., opinions differ as to the optimal selection of the times for postprandial blood glucose and 24 h pattern. In general, it is recommended to take blood 1 h after a meal. A general recommendation cannot be given for the 24 h curve. It is crucial that blood sampling is adapted to the individual demands of the clinicians and the meal times of the patient rather than the set times layed out by the laboratory (*Willms*, Bad Lauterberg). Although a dependence of the behaviour of glucose on the time of day is observed by clinicians, a rigid, endogenous diurnal rhythm of glucose cannot be demonstrated (*Wisser*, Stuttgart). The time differences in the behaviour of blood glucose during the oral glucose tolerance test or insulin therapy are apparently dependent upon exogenous, nutrition-dependent regulatory mechanisms (e.g. fatty acids, catecholamines).

Sampling

The capillary blood sample is seen as the optimal specimen for judging metabolic status. Although there is no significant difference between venous and capillary glucose concentration in fasting persons, the difference can reach a maximum of 30–60 mg/dl (1.7–3.4 mmol/l) depending upon the amount of orally administered glucose (*Haslbeck*, Munich; *Förster*, Frankfurt). Under nonfasting conditions, the capillary-venous differences are highest after breakfast and can then reach a maximum of 20 mg/dl. They also depend on the time interval after glucose administration, the degree of overweight and the severity of diabetes (*Haslbeck*, Munich). The

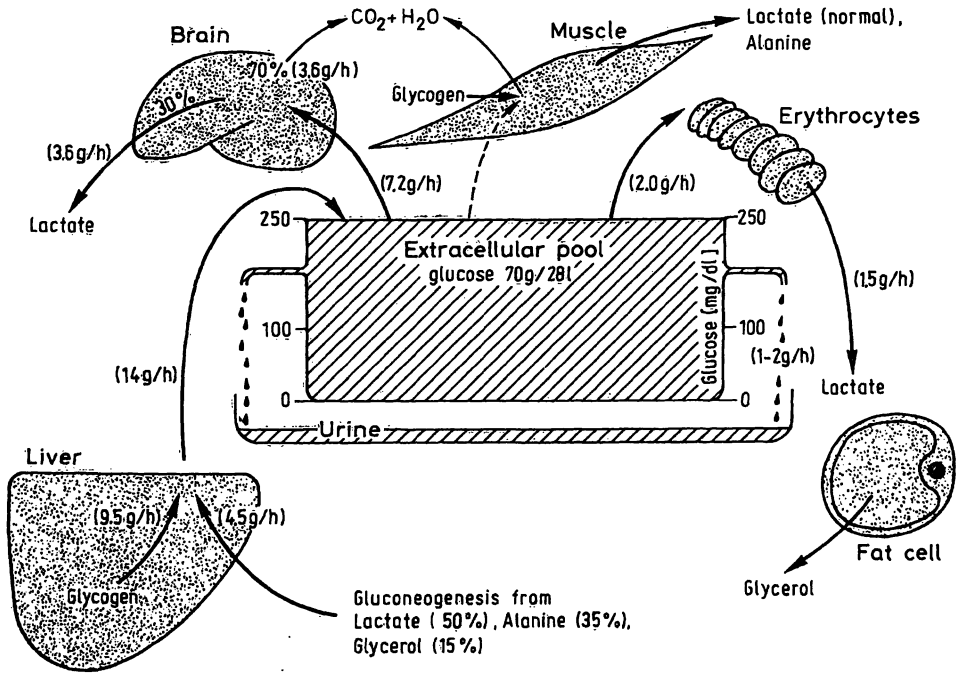

Fig. 1b. Glucose metabolism in acute insulin deficiency.

calculation of capillary blood glucose values from venous results can therefore not be recommended. Similarly, blood values should not be calculated from serum or plasma results. The plasma value is generally higher than that for blood (3–10 mg/dl for fasting blood (*Schmidt*, Mannheim)), but in individual cases calculation is not possible (*Thomas*, Wiesbaden). Last, but not least, since the arteriovenous or the capillary-venous difference depends on the method of analysis (*Weiss*, Munich), one should uniformly use capillary blood for blood sugar determination. For this purpose, small volume capillaries (10–50 µl) are preferable to larger ones. Thin, long capillaries allow more exact blood sampling than short, thick ones (*Weiss*, Munich). With fluoride or maleinimide, the glucose concentration in capillary blood can be sufficiently stabilized so that mailing of capillary blood samples is possible without deproteinization (*Schmidt*, Mannheim). The filter papers introduced for phenylketonuria screening may, after impregnation with boric acid, also be used for mailing capillary whole blood samples (*Kruse-Jarres*, Stuttgart). An exact quantification of capillary blood can be achieved in the laboratory if more than 50 µl of stabilized blood is drawn. Because of the danger of glycolysis, samples without additive as well as venous blood samples should not be used for analysis at all. Even if these factors are considered, the sampling error is twice as high as the analytical errors (*Stamm*, Munich).

Demands Made by the Clinical Diabetologists and the Diabetic on the Laboratory

The demands of the clinician presented by *Otto* (Bremen) correspond largely with those of the diabetologist in practice (*Kurow*, Berlin): a noninvasive method would be optimal; considering the current possibilities, the sampling procedure should be as painless as possible and allow quick, quantitative determination without prior calibration of the blood sample. Simple apparatus which allows a determination with a maximal variation of ± 15% would be sufficient for self control of diabetics. Independence from measuring devices would also be desirable for that purpose.

Diabetic Self Control

Monitoring of carbohydrate metabolism by the diabetic patient himself is, with the corresponding training and intelligence, better than sporadic control of blood glucose by the doctor. Blood sugar self control should be restricted to special indications such as hypoglycaemia without warning symptoms, changes in renal threshold in patients with renal insufficiency, or during pregnancy. Otherwise it can provide very little information in addition to urine glucose self control (*Willms*, Bad Lauterberg). There are considerable differences in the evaluation of the methods for self control. Selected and trained patients can measure blood glucose very accurately with test strip reflectometers (*Busch*, Mannheim), but if the reflectometers are not properly controlled, considerable differences can arise. Some diabetics educated in natural sciences may use quantitative photometry for glucose determination in their own laboratory, but this is certainly an exception (*Thomas*, Wiesbaden).

Analytical Methods and Their Standardization

In contrast to the United States, in Germany the chemical methods for blood glucose determination have been largely replaced by enzymatic procedures. However, there is still a wide range of methodological variants, which result not only from the choice of material under investigation (plasma, blood, haemolysate, deproteinized supernatant), but also from the secondary reagents used in the glucose oxidase method, the temperature and kind of measurement (kinetic, fixed time, end point), and the form of the enzyme (wet, matrix-bound, dry) (*Guder*, Munich). The interlaboratory surveys of the Bayerische Kassenärztliche Vereinigung, which represent a large field study with 5000 participants, gave a blood glucose deviation of up to ± 20 mg/dl at an assigned value of 100 mg/dl, not considering the methodology. This variation is caused chiefly by matrix effects, thus making a comparison of the methods questionable (*Stamm*, Munich). Smaller differences between the hexokinase method and the glucose dehydrogenase method have been eliminated in the meantime by changing the reagents (*Lang*, Darmstadt). Nevertheless, there are still considerable differences between the different glucose oxidase methods, especially if the samples contain uric acid or other interfering substances (*Stamm*, Munich).

The most frequently used deproteinizing agent in the hexokinase and glucose dehydrogenase method is perchloric acid, while uranyl acetate and the *Somogyi-Nelson* deproteinization with zinc sulfate/barium hydroxide is suited for the glucose oxidase method. Stabilized haemolysate and whole blood are, however, being increasingly used as samples. With haemolysate, the disturbance caused by erythrocyte enzymes due to fructose must be considered; the use of whole blood in instruments with an oxygen electrode requires the standardization of the oxygenation of the sample (*Renner*, Munich). A coefficient of variation of 2–3% should be obtained in an optimal procedure in order to reach the precision of 5% desired by clinicians and diabetics (*Haeckel*, Hannover). The day to day variation of 5% is not adequate for optimal diabetes diagnosis.

In addition, there are several interfering factors that the clinical chemists should try to eliminate: Thus uranyl acetate can only be used as a deproteinizing agent when the sample contains enough protein. Otherwise, the surplus uranyl ions must be precipitated from the supernatant with a phosphate buffer (*Weiss*, Munich). Bilirubin interferes with double wavelength methods which

measure at 340 and 380 nm (*Haeckel*, Hannover). A large number of drugs and metabolites (e.g. acetylsalicylic acid, DOPA, uric acid) can interfere with unspecific methods (also glucose oxidase methods) in vitro and in vivo; these therefore interfere in urine analysis (*Borner*, Berlin). Even glucose determination with dry reagents which fulfills the demands of a precise method, are susceptible to disturbances due to matrix effects (*Wisser*, Stuttgart). In general, the latter methods show a good correlation with the hexokinase/glucose6-phosphate dehydrogenase method (*Thomas*, Wiesbaden).

In view of the large number of methodological variants, standardization seems advisable. There is, however, no internationally accepted reference method to date. Isotope dilution, gas chromatography, mass spectrometry (*Björkhem* et al. and National Bureau of Standards, U.S.A.) and hexokinase/glucose-6-phosphate dehydrogenase (United States' Department of Health Education and Welfare) are relatively well tried methods. They show an analytical coefficient of variation of 2% at the most (*Dybkaer*, Copenhagen). In practice, the means of the assigned values for the hexokinase and glucose dehydrogenase method of the German interlaboratory surveys meet this standard (*Stamm*, Munich). The description of the manual methods for glucose determination as a DIN-norm has been proposed for hexokinase and glucose oxidase (*Stähler*, Tutzing). Efforts are being made to give the assigned values and the range of scattering for the internal and external quality control for the reference method only. Then the variety of modifications can be adjusted accordingly. With this procedure, standardization and simplification of the quality control procedure are achieved at the same time.

Glucose Monitoring

The continuous recording of blood glucose is not so much an alternative to the usual random control (24 h pattern) but rather a valuable supplement to the solution of particular problems in diabetology. This kind of analysis can solve therapeutic problems together with a coupled insulin infusion system (e.g. paradoxical insulin effectiveness, questionable routes of administration) (*Kritz*, Vienna). One can usually do without the continuous glucose recording in the open-loop systems. Such analytical devices may be of use for scientific problems concerning the imitation of physiological patterns of insulin secretion and the question of infusion therapy with more than one hormone, as well as the socalled "motion induced glycaemic excursions" (*Renner*, Munich). Even though some centres appear successful in achieving an improvement in the normalization of blood glucose by using a real feedback system (*Kerner*, Ulm; *Jipp*, Stuttgart), we have still not reached the stage where those not having this equipment available must reproach themselves for offering inferior diabetes treatment (*Otto*, Bremen). As yet, there exist no defined indications for the diabetologist as to the therapeutic application of such insulin-infusion systems which are self-operating in response to the actual blood glucose concentration.

Glucose Sensors

A significant breakthrough to the solution of glucose measurements either invasively or noninvasively cannot be reported (*Schaldach*, Erlangen). All invasive systems so far have only limited application since they have considerable disadvantages (e.g. energy supply, sensitivity to interfering factors etc.). The best known procedure is a fuel cell which oxidizes glucose itself. The disadvantage is that a number of other components in the blood falsify the energetic interaction between the electrodes and the medium to be analyzed, especially during metabolic decompensation. With respect to the noninvasive methods, IR spectrometry with attenuated total reflection (ATR) plates optimized by a CO_2 laser is the only currently feasible procedure which is however, far from being successfully put into practice (*Kruse-Jarres*, Stuttgart). An alternative which seems ingenious in theory and would make therapy possible at the same time, would be the use of glycosylated insulin which has sufficient biological activity. This could be implanted as a biologically inactive complex with lectin and then set free from lectin again by glucose (*Wieland*, Munich). Determinations of insulin, proinsulin, and C peptide are not necessary for routine diabetology and do not therefore represent alternatives or regular complements to glucose determinations. Besides research, their domain is the differentiation of conditions for which glucose is an unreliable parameter (e.g. hypoglycaemia). In addition there are considerable methodological difficulties, which, for example, lead to poorly comparable normal ranges due to the heterogeneity of standard materials and the antisera used (*Gerbitz*, Munich).

Glycosylated Proteins

Our information on enzymatic and nonenzymatic glycosylation of proteins in relation to blood glucose levels is still incomplete. However, beyond the known data, there are hopeful beginnings in both areas, which are of possible diagnostic value.

HbA_1 is widely seen as the main representative of nonenzymatically glycosylated proteins. There are currently at least 6 different methods of determination available. Some techniques include only N-terminal glycosylated haemoglobin; others apparently also include other amino acids (lysine) of glycosylated haemoglobin. On the basis of his comparative study, *Reinauer* (Düsseldorf) suggests using the HPLC method as a reference method.

For routine analysis however, the thiobarbituric acid method and the macrocolumn procedure are recommended. An external quality control for improving the comparability of results is proposed and urgently indicated. According to *Dolhofer* (Munich), the reliable determination of the stable ketoamine form of HbA_1 will only succeed with sufficiently long columns and slow elution. Under the conditions of commercial microcolumns, not only the stable forms are collected, but also the labile forms, even after dialysis. Whether the simultaneous determination of the labile forms (*Schiff* base) and the stable form (ketoamine) offers valuable additional information in diabetologic practice will have to be proven by further experience. According to *Henrichs* (Quakenbrück), a significant increase of the unstable form of HbA_1 is observed if the mean blood glucose remains above 160 mg/dl for 12 h. In principle, simultaneous determination of several glycosylated proteins with differing association and turnover kinetics should open the possibility of gaining closer insight into the glycaemia history of the patient.

The determination of lysine bound glucose (furosine) in albumin of human serum seems to be of great interest. The method used for glycosylated albumin is more specific than others for determining glycosylated proteins because only the ketoamine form is measured (*Schleicher*, Munich).

The lipoproteins (HDL, VLDL and LDL) in plasma also contain 2–3% carbohydrates. This is due to enzymatic glycosylation involving mainly neutral sugars (glucose, galactose and mannose), amino sugars (N-acetyl-glucosamine, N-acetyl-galactosamine) and N-acetylneuraminic acid. The determination of these carbohydrate components in lipoproteins may become diagnostically interesting as the carbohydrate content of VLDL is found to be in good correlation to blood glucose and to HbA_1 in insulin dependent diabetics (*Kattermann*, Mannheim). When analyzed chromatographically, galactose and mannose as well as the hexosamines GlcNAc and GalNAc are found to be increased in diabetes.

During therapy, the hexosamine concentration drops from a maximum of about 300 nmol/mg apolipoprotein to normal values within a few days. This means that the changes, like those in glycosylated albumin, are more rapidly reversible than those in HbA_1. In addition, it seems interesting that an increased nonenzymatic glycosylation of lipoproteins is also found in diabetics (*Wieland*, Munich).

The latter findings extend our knowledge far beyond the information of blood glucose concentrations. The increased glycosylation of VLDL apolipoprotein could, for example, be responsible for the delayed triglyceride clearance and thus for the hypertriglyceridaemia in diabetes. Changes in glycosylation of LDL or HDL could lead to an alteration in binding to the specific lipoprotein receptors at the cell surface. By such a mechanism these findings would deserve much interest with respect to the pathogenesis of the diabetic vascular complications.

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