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Microcalorimetric Assay for Glucose in Human Serum and Plasma

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Thermochemistry Section
Physical Chemistry Division
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National Bureau of Standards
Washington, D. C. 20234

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Interim Report

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This report is to be superseded by a future publication which will receive general distribution and should be cited as a reference. Please consult the NBS Office of Technical Information and Publications to obtain the proper citations.

Prepared for
National Institute of General Medical Sciences
National Institutes of Health
Bethesda, Maryland 20014

U. S. DEPARTMENT OF COMMERCE, Frederick B. Dent, Secretary
NATIONAL BUREAU OF STANDARDS, Richard W. Roberts, Director

Foreword

The use of clinical laboratory tests of physiological fluids for specific components has been rapidly increasing in the past few years. One aspect of this has been an increase in the number of tests for well-known particular substances for which satisfactory tests are available. Another equally important aspect is the increasing need of the physician and of the research biochemist for ready methods for determining other substances present in varying amounts in normal and pathological states. For this reason, the development of new techniques applicable to problems for which optimum solutions have not been found are of considerable interest in clinical and biological chemistry.

The universal occurrence of energy changes in chemical and physiological processes has caused the calorimeter to be considered as a potential tool for clinical chemical analysis and for observing biological processes for some years. The actual applicability, however, has been limited previously by lack or insufficiency of one or more necessary factors. Such factors include: instrument sensitivity and operational noise levels, instruments adapted to use of small samples, simplicity of instrumental operation and data reduction, and suitable mechanisms for sorting out specific processes from a melange of potential interferences. It was the judgment of the authors and of the National Institute of General Medical Sciences (NIGMS) that the understanding of these factors had developed to a point where a well coordinated attack on the application of microcalorimetry would now lead to successful results.

A program was established in the Thermochemistry Section (Physical Chemistry Division, Institute of Materials Research) at the National Bureau of Standards, and after some months was augmented with support from the NIGMS to demonstrate the feasibility of microcalorimetry in some specific areas useful in the Clinical Chemistry Laboratory. Dr. Robert S. Melville is the program officer for NIGMS. Collaborative support has been provided by the Clinical Chemistry Service (Dr. Donald Young, Chief), and by the Laboratory for Technical Development (Dr. Robert L. Bowman, Chief, and Dr. Robert L. Berger).

This is a report provided at the end of the first year of the NIGMS support of this work and summarizes a portion of the

work done. This report is one of four covering different aspects of the NBS Program. For the information of the reader, the titles and NBS Report numbers of the four reports are as follows:

NBS Report	73-178	Microcalorimetric Assay for Glucose in Human Serum and Plasma
	73-179	Design and Construction of the NBS Clinical Microcalorimeter
	73-180	Testing of the NBS Clinical Microcalorimeter
	73-181	Fine Structure in Thermal Growth Patterns of Bacteria by Microcalorimetry

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Microcalorimetric Assay for Glucose in Human Serum and Plasma

by

R. N. Goldberg, E. J. Prosen, B. R. Staples
R. N. Boyd, and G. T. Armstrong

Abstract

Microcalorimetry has been used to perform quantitative assays for glucose in human serum and plasma samples. The procedure was based upon the measurement of the heat effect brought about by the following enzyme catalyzed reaction:

$$\text{Glucose} + \text{ATP}^{-4} \xrightarrow[\text{Mg}^{+2}]{\text{Hexokinase}} \text{Glucose-6-Phosphate}^{-2} + \text{ADP}^{-3} + \text{H}^{+} \quad \text{(in buffer)}$$

For this reaction, calorimetric measurements have yielded a value of $\Delta U = -61.4\ \text{kJ}\cdot\text{mol}^{-1}$ in 0.2 M TRIS/TRIS·HCl buffer (pH = 7.60) at 30.8°C.

Using a procedure that seeks to minimize interfering heat effects and by application of a calibration curve determined using known glucose concentrations in water (not serum), fifty-one human serum and plasma samples have been assayed for glucose. The results cover a range of 35 to 408 mg glucose per 100 g of serum or plasma and are in satisfactory agreement with the results obtained using a conventional spectrophotometric clinical procedure. Since our results are based on the assumption of the essential specificity of enzymes, the agreement of the calorimetric results with the spectrophotometric results lends strong support to this fundamental hypothesis of biochemistry.

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1. INTRODUCTION

One may state as a general principle that chemical changes are, essentially without exception, accompanied by either the liberation to or the absorption of energy from the surroundings. This energy, in the form of a measurable heat effect, thus represents a signal, generated by the system itself, that may be exploited as a quantitative measure of the amount of reaction taking place. Exploitation of this principle for quantitative analysis is dependent on three factors: (1) the sensitivity of the calorimetric devices available for use, (2) the necessary specificity of reaction, and (3) a knowledge of the magnitude of the heat effect associated with a given quantity of substance and the various parameters that may affect it.

The first of these three requirements, high sensitivity, is presently accomplished by means of microcalorimetry [1, 2, 3]. The requirement of specificity is, in principle, obtainable by the use of an enzyme catalyzed reaction and is dependent on the availability of a pure enzyme if one is assaying for its substrate, or conversely, of a pure substrate if one is assaying for the enzyme. The final requirement, a knowledge of the thermochemistry, is essential if meaningful measurements are to be made.

This report gives the results of some experiments performed to test the feasibility of the application of the above principle to clinical chemistry. Specifically, the objective was to be able to perform quantitative analysis for glucose in human serum and plasma samples.

2. ENZYME SYSTEMS CONSIDERED

During the course of this work, two different enzymatic reactions were investigated, namely, those catalyzed by glucose oxidase and hexokinase. Initial work centered on the use of glucose oxidase, the reaction for which we write:

$$\beta\text{-D Glucose(buffer)}^{1} + O_2\text{(buffer)} \xrightarrow{\text{Glucose oxidase}} \delta\text{-Gluconolactone(buffer)} + H_2O_2\text{(buffer)} \quad (1)$$

The gluconolactone will eventually be converted to gluconic acid which in turn will react with the buffer present. If catalase is present, the hydrogen peroxide will decompose to water and oxygen:

$$H_2O_2\text{(buffer)} \rightarrow H_2O\text{(buffer)} + 1/2\ O_2\text{(buffer)} \quad (2)$$

Several factors may serve to complicate the chemistry. First, it should be noted that glucose oxidase is essentially specific for β-D Glucose [4] and thus one is dependent on the presence of the enzyme mutarotase or self rotation to accomplish the necessary $\alpha \rightarrow \beta$ conversion. Second, the conversion of δ-gluconolactone may require the presence of the enzyme lactonase [4] to speed up this otherwise slow conversion. Finally, one must contend with a constraint imposed on the system due to the limited solubility of oxygen in water, which requires one to insure that the glucose concentration is always kept below a given level ($\sim$18 mg $d\ell^{-1}$). Any measured heat effect will be attributable to a combination of the several processes described above, any one of which may serve as a source of experimental difficulty.

The second enzyme considered, hexokinase, catalyzes the following reaction:

$$\text{Glucose(buffer)} + \text{ATP}^{-4}\text{(buffer)} \xrightarrow[Mg^{+2}]{\text{Hexokinase}} \text{ADP}^{-3}\text{(buffer)} + \text{Glucose-6-Phosphate}^{-2}\text{(buffer)} + H^{\oplus} \quad (3)$$

The proton produced in the above reaction will in turn react with the buffer present:

$$H^{\oplus} + \text{buffer} \rightarrow \text{buffer}\cdot H^{\oplus} \quad (4)$$

---

[1] Throughout this report, the terminology (buffer) refers to an appropriately buffered state.

The measured heat effect will be the sum of the heat effects associated with reactions (3) and (4). That reaction (3) goes essentially to completion has been established by Burton and Krebs [5] who have determined the Gibbs's free energy change associated with (3) to be -21.3 kJ mol^{-1}. This corresponds to an equilibrium constant of ∿5500. Although the chemistry associated with the hexokinase reaction is simpler than that of the glucose oxidase reaction(s), hexokinase is known to be not totally specific for glucose in that mannose, fructose, and D-2-deoxyglucose will be phosphorylated to yield the corresponding 6-phosphates [4]. Nevertheless, this should not be a serious drawback for purposes of assay of human serum and plasma in that the quantities of these other carbohydrates are generally small in comparison with the amount of glucose present [4]. In principle, specificity could be enhanced by use of the enzyme glucose-6-phosphate dehydrogenase. This is done in the spectrophotometric procedure [6] out of the necessity to obtain a satisfactory absorption peak. However, for the calorimetric (heat measurement) procedure this is not needed, except perhaps to obtain added specificity.

3. EXPERIMENTAL

A. Materials[2]

The reagents used during the course of the experiments were:

Reagents	Source
α-D-Glucose, crystalline	National Bureau of Standards, Standard Reference Material No. 916
Adenosine-5'-triphosphate, disodium salt (ATP)	Cal. Biochem, Catalog No. 1191, Lot No. 200293 and 001775.
Hexokinase (yeast), lyophilized	Cal. Biochem. Catalog No. 376811, Lot Nos. 020031 and 220069 (Activity ∿75 IU/mg)

[2] Certain commercial equipment, instruments, or materials are identified in this report to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Reagents	Source
Hydrochloric Acid (concentrated)	Allied Chemical (B&A) Code No. 1090
Magnesium dichloride hexahydrate, crystalline	Allied Chemical (B&A), no Lot No. given
Potassium dihydrogen phosphate, crystalline	National Bureau of Standards, Standard Reference Material No. 186-I-C
Sodium hydrogen phosphate, crystalline	National Bureau of Standards, Standard Reference Material No. 186-II-B
Glucose oxidase, fungal	Sigma, Catalog No. G6125, Lot No. 45B-2050 (Activity ∿15 IU/mg). Sigma, Experimental Lot No. 42C-9500 (Activity ∿36 IU/mg).
Catalase, fungal (A. Niger)	Cal. Biochem, Catalog No. 21926, Lot No. 71259 (Activity ∿15,000 IU/mg) Sigma, Catalog No. C-40, Lot No. 121C-8010 (Activity ∿15,000 IU/mg).
Sodium chloride, crystalline	J. T. Baker Chemical Co., Catalog No. 3624, Lot No. 35082
Alkylphenoxy polyethoxy ethanol (Triton X-100)	Rohm & Haas Lot No. 5513
Distilled water	National Bureau of Standards

Contaminants of the hexokinase, as stated by the supplier, were:

Enzymatic Contaminant	Percent of Total Activity
glucose-6-phosphate dehydrogenase	0.004%
glutathione reductase	0.001%
phosphoglucose isomerase	0.007%
myokinase	0.001%
6-phosphogluconic dehydrogenase	0.001%

Other contaminants in this enzyme preparation included albumin, potassium phosphate, and magnesium aspartate.

All plasma and serum samples were obtained from the clinical laboratory (Dr. Donald Young, Chief) at the National Institutes of Health. These samples were kept frozen at approximately -10°C until ready for use. Samples were thawed at room temperature (∿23°C) a few hours prior to use and inverted

many times by hand to mix the sample.

B. Apparatus and Procedures

All calorimetric measurements summarized in this report were performed using the NBS Clinical Microcalorimeter [3]. The calibration constant of this instrument, as determined from electrical calibrations, is 16.70 J v^{-1} s^{-1} at 30.8°C. The thermopile signal was amplified by a factor of 10^5 using a precision nanovolt amplifier and recorded both in analog and in digital form. Calculation of the peak areas was accomplished by numerical integration [3b, 8].

The calorimeter cells were of the type "F" design and made of Kel-F. Each compartment of a cell holds slightly more than 150 μℓ of solution. Since these cells are not yet disposable, it was necessary to denature any enzyme remaining in a cell after an experiment by means of a concentrated urea solution. Cells were then rinsed a minimum of five times with the buffer being used and sucked nearly dry by means of a vacuum line. Beginning with run no. 415 (between serum nos. 18 and 19), this procedure was modified in that a cell, following treatment with concentrated urea, was then rinsed at least five times with distilled water and air dried completely. The advantage of this procedure is that it avoids any heats of dilution which may be caused by traces of water or buffer present on either side of the cell compartment that cause slight mismatch of the solutions in the two cell compartments. However, by having the cell completely dry one then encounters a difficulty in mixing due to the thinness of the calorimeter cell and the associated capillary effect which will not permit the liquid to flow after the hydrophilic surface treatment wears off. This problem was minimized by tilting a cell to one side following loading of that side of the cell (the

compartment for the enzyme solution). Occasionally, however, a mixing problem did occur. Thus, an inert wetting agent, Triton X-100, was added to the buffer being used in the preparation of solutions, the volumetric ratio of buffer to wetting agent being 10^6 to 1. To date, no mixing problems have been encountered using this improved procedure.

Gravimetric procedures were used in the preparation of all solutions. Weighings were done either on a microbalance sensitive to ±5 μgm or a semi-microbalance sensitive to ±0.1 mg. All weights given in this report refer to apparent mass in air.

Measurements of pH were performed on a Corning Model 12 pH meter. The meter was standardized both before and after any measurements using standard buffers.

Interfering heat effects due to a mismatch of the reacting solutions contained in the respective compartments of a microcalorimeter reaction cell were minimized by use of two different procedures followed in the preparation of solutions. The first (method I) was to add a known amount of the limiting substrate to a given buffered solution containing an excess amount of all other reagents required to carry out the reaction. This solution was designated as the substrate solution. The enzyme solution was prepared by addition of the required enzyme(s), preferably in lyophilized form and free of all contaminants (buffer salts in particular), to the same buffered solution used in the preparation of the substrate solutions.

The second procedure (method II) that was followed was to prepare first the substrate solution by addition of a known amount of substrate to a buffered solution containing excess quantities of all other required reagents. The enzyme solution was then prepared by addition of the necessary enzyme(s) again in lyophilized form, to a portion of the substrate solution. Thus, the substrate contained in the enzyme solution has been pre-reacted. The reason for the use of this procedure is that in performing assays for glucose in serum and plasma samples, it is not possible to resort to the first procedure (method I) described above without having mismatched enzyme and substrate solutions (in particular, there would be no proteins on one side) that result in interfering heat effects.

The detailed procedure used for the preparation of solutions for assays of glucose in serum and plasma was as follows:

1. Buffer Solution. To a vial, containing 220 mg of the disodium salt of ATP and 30 mg of $MgCl_2 \cdot 6H_2O$, add 1.0 ml of 2.0M TRIS/TRIS·HCl buffer, pH = 7.60. Mix to dissolve all solids and store this solution at approximately 4°C.

2. Substrate Solution. To 1.0 ml of a serum (or plasma) sample to be assayed for glucose, add 0.1 ml of the solution prepared in (1) above. Mix thoroughly. The amount of dilution of the serum (or plasma) sample must be known quantitatively.

3. Enzyme Solution. To at least 100 µg of lyophilized hexokinase, add 400 µl of the substrate solution. Mix thoroughly.

The above solutions should suffice for at least three calorimetric measurements for glucose in serum.

To illustrate the nature of this analysis, a sample experiment showing raw data and calculation procedures has been included in the Appendix to this report.

As is the case with most measurements, and in particular those for which a high state of reliability has not yet been achieved, it was necessary to drop the results of some calorimetric experiments. Results were dropped for several legitimate reasons that included leaks, the most frequent cause, premixing of the solutions in the two reaction cell compartments, as evidenced by excessively low values for the measured heat, lack of sufficient enzymatic activity, inability of the solutions in a reaction vessel to mix, and miscellaneous operator errors such as failure to obtain a complete record of the data.

4. RESULTS AND DISCUSSION

A. Glucose in Water/Glucose Oxidase runs

A summary of results for the glucose oxidase system is shown in Table I. As is evident from these results, some experimental factor was not under control. Since the hexokinase system seemed more promising, we went on to study that system rather than trying to improve the glucose oxidase system.

B. Glucose in water/Hexokinase runs.

Numerical results for the glucose/hexokinase system in buffer (not in serum) are given in Table II. A plot of these results is shown in figure 1. A molar energy of reaction ($\tilde{\Delta U}$) equal to -61.4 kJ mol^{-1} was obtained from a least squares fit to this data. This measured energy is the sum of the energies associated with processes (3) and (4) described in Section 2. The energy of buffer protonation, process (4), has been reported [9] to be -47.5 kJ mol^{-1} and represents 78 percent of the measured exothermic heat effect. Thus, the molar energy of reaction for process (3), the phosphorylation of glucose-6-phosphate by ATP, is -13.9 kJ mol^{-1}. A check on this value could be obtained by measurement of the energy of reaction in a second buffer system with a corresponding correction for the heat of protonation of that buffer. This has not been done yet.

C. Assays Performed on Human Serum and Plasma Samples

The results of microcalorimetric assays for glucose in human serum and plasma samples are given in Table III. In most cases, calorimetric measurements were performed in triplicate. The results of these individual measurements, expressed in terms of mg glucose per 100 g of serum or plasma, are given in column three. The average of these determinations are presented in column four, the expressed uncertainty being the average deviation. In column five are given the results of glucose determinations performed on these same samples at the clinical laboratory of the National Institutes of Health on an autoanalyzer using the enzyme glucose oxidase. Numbers in this column that are not in parentheses were obtained chronologically prior to the microcalorimetric measurements, while those in parentheses were obtained after the calorimetric measurements had been performed. This was done to test for

any possible changes in glucose concentration with time or our handling of the serum samples. Since the autoanalyzer results are expressed in units of mg glucose per dℓ of serum or plasma, a rigorous comparison of these results would require a knowledge of the density of the individual samples. This is expected to change the values by only a few percent. Nevertheless, it should be noted that the mean of the fifty-one calorimetric analyses (110.0 mg glucose per 100 g serum or plasma) is in good agreement with the mean of the autoanalyzer results (110.4 mg glucose per dℓ serum or plasma).

A plot of the microcalorimetrically determined glucose concentration (units of mg glucose per 100 g serum or plasma) as a function of the glucose concentration (mg glucose per dℓ serum or plasma) determined by NIH using the conventional glucose oxidase method with an autoanalyzer is shown in figure 2. The plot covers a range from 35 to 408 mg glucose per 100 g serum (plasma). The mean of the absolute deviations between the two sets of numbers is $6._7$ mg glucose per dℓ of serum (plasma), assuming a density of unity for the samples.

Several factors still remain to be investigated before the spectrophotometric method as applied to the quantitative determination of glucose is fully satisfactory. These factors include:

(1) Investigation into possible causes of the several large discrepancies between the calorimetric and spectrophotometric results.

(2) A study of several possible interferences, such as the effect of pH, addition of mannose, fructose, etc.

(3) An understanding of the cause of and a better control of the endothermic heat effect observed when serum or plasma is mixed with itself, and the endothermic remixing energy (see appendix).

It is worthwhile to point out that the closeness of the agreement of the results obtained between the calorimetric and spectrophotometric methods may have been fortuitous, in the sense that both methods, and in particular the newer microcalorimetric method, may have associated with it several possible sources of systematic error that have not yet been recognized. Nevertheless, the results

do serve to indicate the essential validity of the calorimetric approach, as performed in a research laboratory, to the assay of glucose in human serum and plasma samples. It should also be noted that since the basis of the measurement technique rests on the assumption of the essential specificity of enzymes, the results do lend strong support to the correctness of this basic hypothesis of biochemistry. It should also be pointed out that the calibration curves used were performed on glucose in water (not in serum) and still gave reasonably correct values for glucose in serum.

5. ACKNOWLEDGEMENTS

We wish to thank Drs. Sam Margolis and Robert Schaffer of the Organic Chemistry Section (NBS) and Dr. Jo Everse of the Department of Chemistry at the University of San Diego for their helpful discussions pertaining to the biochemistry pertinent to this investigation. We also thank Dr. Donald Young for supplying serum and plasma samples, as well as the results of glucose analyses for these samples performed in the clinical laboratory of the National Institutes of Health, and Dr. Robert L. Berger of the Laboratory for Technical Development, Heart and Lung Institute, National Institutes of Health, for his assistance and encouragement in this project.

6. APPENDIX - MICROCALORIMETRIC ASSAY FOR GLUCOSE (Sample No. 6211)

Raw Data

To 1.02278 g of serum (sample no. 6211) was added 0.10827 g of buffered solution containing ATP and magnesium chloride. This solution was mixed thoroughly and is the substrate solution described previously in this report (see Section 3.B). The enzyme solution was prepared by addition of 0.51599 g of substrate solution to 277 μg of hexokinase.

The microcalorimeter cell was then loaded with 0.11030 g of enzyme solution and 0.15405 g of substrate solution into the respective cell compartments. An area of +2.7821 millivolt-seconds (mv·s) was obtained for the first mixing, while the remix gave an area of -0.399 mv·s.

Treatment of Data

The heat effects to be associated with the first and second mixings are, respectively,

$$Q_1 = (16.7\ \text{mJ}\cdot\text{mv}^{-1}\cdot\text{s}^{-1})(+2.7821\ \text{mv}\cdot\text{s}) = +46.461\ \text{mJ (exothermic)}$$

$$Q_2 = (16.7\ \text{mJ}\cdot\text{mv}^{-1}\cdot\text{s}^{-1})(-0.0399\ \text{mv}\cdot\text{s}) = -0.666\ \text{mJ (endothermic)}$$

It should be noted that this endothermic heat effect, +0.666 mJ, in this specific instance, was not observed in glucose in water determination. The origin of this effect is not yet known and constitutes a source of ambiguity in the data treatment on the order of a few percent in the case of the glucose determinations. If it is assumed that this effect is a thermal interference, characteristic of some property of serum or plasma, one can reason that the heat effect legitimately to be associated with the glucose/hexokinase reactions is equal to $(Q_1 - Q_2)$ or +46.461 - (-0.666) = +47.127 mJ in this instance. It should be emphasized that at present, the cause of this endothermic effect is not yet known and that some other treatment of the data, e.g. taking $(Q_1 + Q_2)$

or just Q_1 alone, might be the more accurate procedure. Nevertheless, for purposes of this report, the procedure of exclusively taking $(Q_1 - Q_2)$ was used to obtain the results of all glucose analyses reported.

Using our measured heat effect of +47.127 mJ and a knowledge of the equation of the calibration curve of the glucose/hexokinase reaction (see fig. 1), we calculate that 0.730 μ moles of glucose have been reacted in 0.15405 g of substrate solution. Applying a correction for the dilution of the serum by the buffered solution containing ATP and magnesium chloride, one can calculate the concentration of glucose in the serum sample:

$$\text{glucose concentration } (\mu \text{ moles per g serum}) = \frac{0.730}{0.15405} \times \frac{(1.02278 + 0.10827)}{1.02278} =$$

5.240.

This corresponds to a $94._4$ mg of glucose per 100 g of serum. The spectrophotometric determination performed at the National Institutes of Health yielded a value of 93 mg of glucose per dℓ of serum.

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Table I

Summary of Glucose/Glucose Oxidase Results

Run No.	μ moles of glucose reacted	Q(mJ)	$-\Delta H(kJ\ mol^{-1})$
82	0.0890	16.65	187
83	0.0891	9.03	101
84	0.0917	10.65	116
85	Zero	0.45	--
116	0.0826	8.41	102
117	0.0854	17.72	207
119	0.0861	16.90	196
120	0.0845	15.55	184

Average = (156 ± avg. dev. 43) kJ mol^{-1}

Procedure used for preparation of Solution: Method I

Buffer: 1/15 M Sørensen's phosphate [7] containing 0.146 gm of sodium chloride per liter, pH = 6.0.

Glucose Concentrations: 5.86×10^{-7} mol $(g\ soln)^{-1}$ in runs no. 82 to 85, or 10.6 mg $d\ell^{-1}$.

5.47×10^{-7} mol $(g\ soln)^{-1}$ in runs no. 116 to 120, or 9.9 mg $d\ell^{-1}$.

Enzymes: Sigma Glucose Oxidase (Lot No. 45B2030) and Catalase (Lot No. 121C-810) were used in runs no. 82 to 85.

Sigma Glucose Oxidase (Lot No. 42C-9500) and Cal Biochem Catalase (Lot No. 71259) were used in runs no. 116 to 120.

In all cases the enzyme activity should have been sufficient to bring about complete reaction within a few seconds.

Table II

Results for the Glucose/Hexokinase System in Buffer

Run No.	μ moles of glucose reacted	Q_{meas} (mJ)	Q_{calc} (mJ)	*Difference Q(meas)-Q(calc), mJ	% Difference
176	0.00	3.25	2.31	+0.94	-40.7
177	0.00	1.88	2.31	-0.43	+18.6
178	0.00	2.18	2.31	-0.13	+ 5.6
179	0.3849	25.18	25.94	-0.76	+ 3.0
180	0.3797	25.89	25.62	+0.27	- 1.1
181	0.3860	25.33	26.01	-0.68	+ 2.6
173	0.7503	48.39	48.37	+0.02	- 0.0
174	0.7582	50.40	48.86	+1.54	- 3.0
175	0.7529	48.03	48.53	-0.50	+ 1.1
182	1.1193	70.30	71.03	-0.73	+ 1.0
184	1.0954	71.66	69.56	+2.10	- 3.0
185	1.1146	69.08	70.74	-1.66	+ 2.4

Procedure used for preparation of solutions: Method II

Buffer: 0.2 M TRIS/TRIS·HCl, pH = 7.60

Glucose Concentrations: 0.00 μ moles glucose (g soln)$^{-1}$ in runs no. 176 to 178, or 0.0 mg dℓ$^{-1}$.

2.583 μ moles glucose (g soln)$^{-1}$ in runs no. 179 to 181,or 46.5 mg dℓ$^{-1}$.

5.013 μ moles glucose (g soln)$^{-1}$ in runs no. 173 to 175, or 90.3 mg dℓ$^{-1}$.

7.492 μ moles glucose (g soln)$^{-1}$ in runs no. 182 to 185, or 135.0 mg dℓ$^{-1}$.

Enzyme: Hexokinase (Cal Biochem Lot No. 020031).

In all cases, the enzyme activity should have been adequate to bring complete reaction within 10 seconds.

*Values given under the heading Q_{calc} were obtained from a least squares fit of the experimental data.

Table III

Summary of Microcalorimetric Assays for Glucose in Human Plasma and Serum

No.	Plasma No.	NBS Microcalorimeter (Glucose Hexokinase) mg glucose/100 g plasma	Average	NIH Autoanalyzer (Glucose Oxidase) mg glucose/dℓ plasma	Difference NBS-NIH
1	103	101 - 105 - 103	103±2	103	0
2	105	167 - 175 - 168	170±3	162	+8
3	115	231 - 239 - 233	234±3	224	+10
4	111	81 - 76 - 76	78±3	87	-9
5	113	57 - 59 - 64	60±3	67	-7
6	108	189 - 199	194±5	207	-13

No.	Serum No.	NBS Microcalorimeter (Glucose Hexokinase) mg glucose/100 g serum	Average	NIH Autoanalyzer (Glucose Oxidase) mg glucose/dℓ serum	Difference NBS-NIH
1	30208	91 - 90 - 91	91±0.3	90	+1
2	30219	76 - 71 - 74	74±2	73	+1
3	31206	63 - 61 - 60	61±0.8	72	-11
4	1274	188 - 189 - 192	190±1	170	+20
5	2003	207 - 199 - 196	201±4	194	+7
6	2008	66 - 68 - 69	68±1	70	-2
7	2012	62 - 62 - 66	64±2	67	-3
8	2017	93 - 89	91±2	88	+3
9	2009	73 - 77 - 88	79±6	79	0
10	2002	95 - 100 - 97	98±2	93	+5
11	1316	342 - 351	346±5	330	+16
12	1276	65 - 65 - 69	66±2	67	-1
13	2027	69 - 65	67±2	66	+1
14	2010	91 - 83	87±4	90	-3
15	2061	86 - 83	85±1	85	0
16	5237	63 - 56 - 60	60±2	67	-7
17	5263	81 - 79 - 79	79±1	75	+4
18	5216	94 - 95	94±0.4	87	+7
19	9279	102 - 104 - 104	103±1	96(89)	+10
20	9216	411 - 406 - 408	408±2	378(365)	+26

No.	Serum No.	NBS Microcalorimeter (Glucose Hexokinase) mg glucose/100 g serum	Average	NIH Autoanalyzer (Glucose Oxidase) mg glucose/dℓ serum	Difference NBS-NIH
21	5233	92 - 90 - 92	91±1	87	+4
22	5234	79 - 78	78±0.5	82	-4
23	5235	62 - 65 - 66	64±2	66	-2
24	5253	289 - 296 - 296	294±3	318	-24
25	6211	94	94	93	+1
26	6247	95 - 98	97±2	91	+6
27	6248	64 - 67	66±1	77	-11
28	6237	64 - 59 - 64	63±2	63	0
29	6253	70 - 68 - 74	71±2	75	-4
30	9219	116 - 113 - 118	116±2	122(108)	+1
31	9243	72 - 67	69±3	79(70)	-6
32	9310	76 - 75 - 70	74±2	83(70)	-3
33	10392	146-145-148	146±1	152(143)	-1
34	9251	34 - 36 - 36	35±1	42(38)	-5
35	10364	126 - 125 - 130	127±2	116(81)	+28
36	10401	146 - 141 - 147	145±2	150(137)	+1
37	6272	58 - 62 - 60	60±1	64(65)	-4
38	6276	90 - 92 - 88	90±2	92(93)	-2
39	23288	105 - 106 - 108	106±1	115(110)	-7
40	24327	89 - 86 - 87	87±1	93(90)	-5
41	6286	73 - 74	74±0.5	83(80)	-7
42	23292	110 - 114 - 114	113±2	125(124)	-12
43	23265	70 - 68 - 69	69±0.6	80(81)	-11
44	23266	68 - 67 - 67	68±0.6	80(81)	-12
45	23275	67 - 66 - 65	66±0.4	75(75)	-9
	Average for 51 samples =		110.0 (Mean Avg. Dev. = 1.9)	110.4	-0.4

See discussion in text for explanation of various aspects of this table.

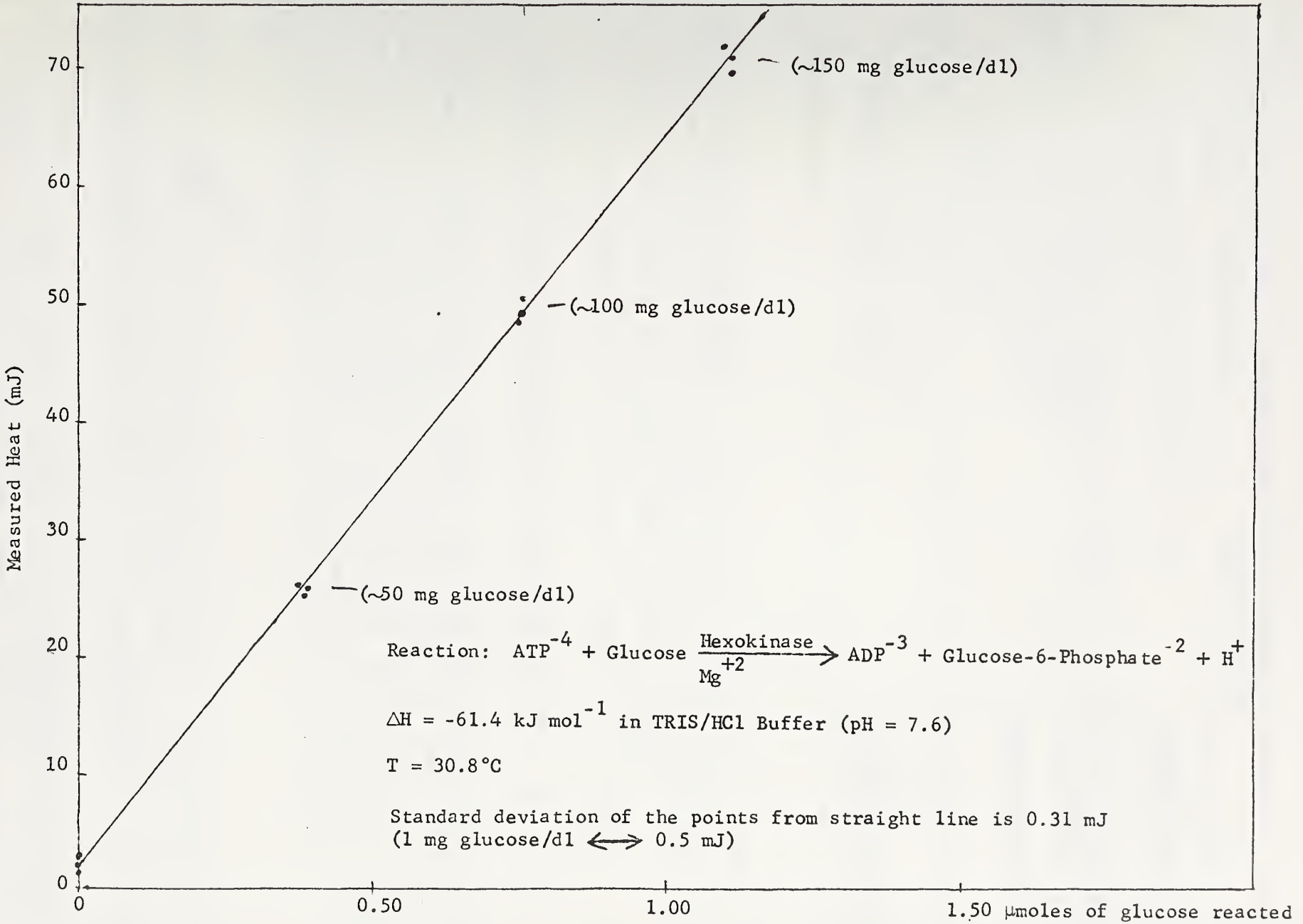
70
60
50
40
30
20
10
0
Measured Heat (mJ)
(~150 mg glucose/dl)
(~100 mg glucose/dl)
(~50 mg glucose/dl)
Reaction: ATP^{-4} + Glucose $\xrightarrow[Mg^{+2}]{\text{Hexokinase}}$ ADP^{-3} + Glucose-6-Phosphate^{-2} + H^{+}
ΔH = -61.4 kJ mol^{-1} in TRIS/HCl Buffer (pH = 7.6)
T = 30.8°C
Standard deviation of the points from straight line is 0.31 mJ
(1 mg glucose/dl ⟷ 0.5 mJ)
0
0.50
1.00
1.50 μmoles of glucose reacted

Figure 1

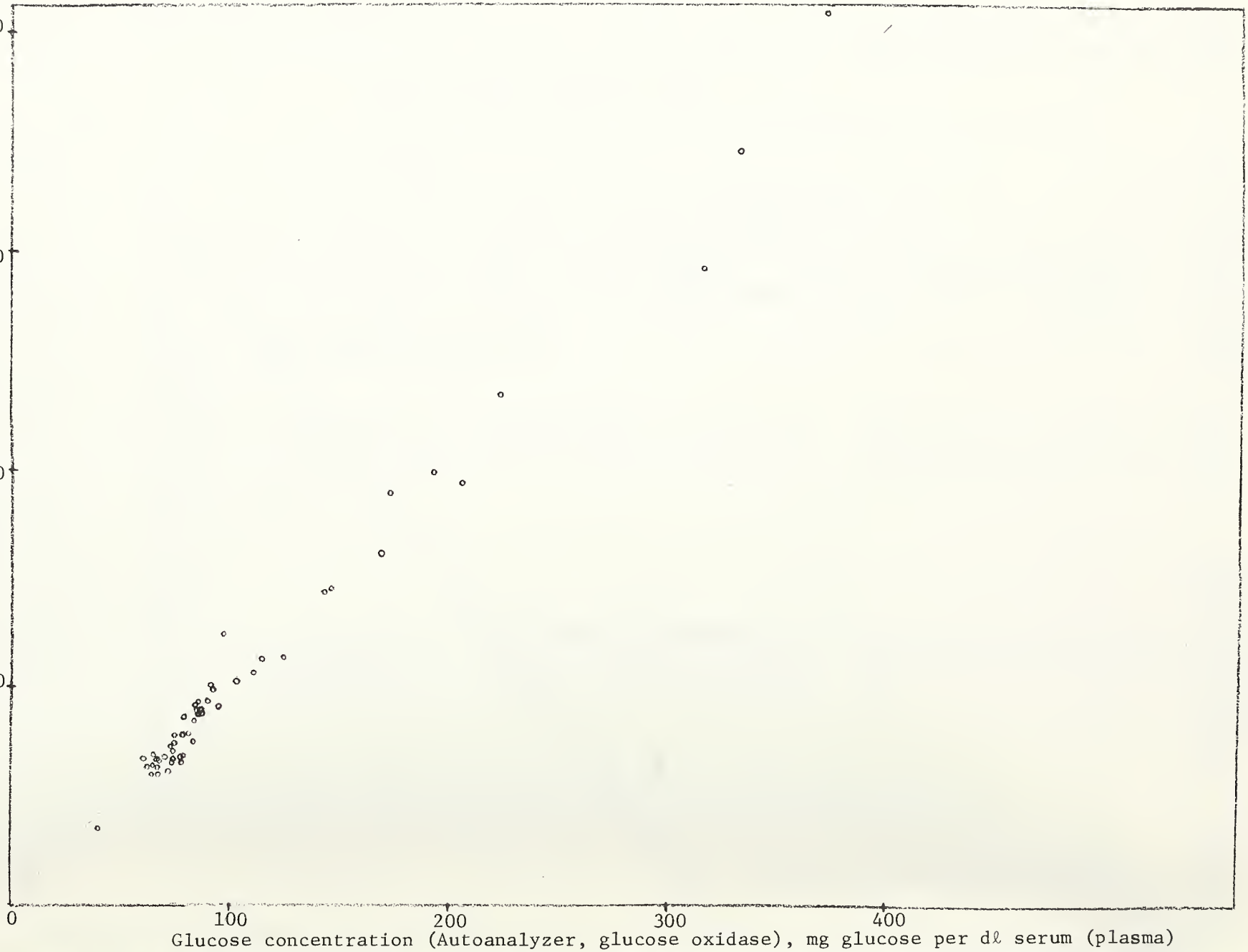
Glucose concentration (Microcalorimetric, hexokinase), mg glucose per 100 g serum (plasma)
400
300
200
100
0
100
200
300
400
Glucose concentration (Autoanalyzer, glucose oxidase), mg glucose per dℓ serum (plasma)

Figure 2

FORM NBS-114A (1-71)

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16. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here.)

Microcalorimetry has been used to perform quantitative assays for glucose in human serum and plasma samples. The procedure was based upon the measurement of the heat effect brought about by the following enzyme catalyzed reaction:

$$\text{Glucose} + \text{ATP}^{-4} \xrightarrow[\text{Mg}^{+2}]{\text{Hexokinase}} \text{Glucose-6-Phosphate}^{-2} + \text{ADP}^{-3} + \text{H}^{+} \text{ (in buffer)}$$

For this reaction, calorimetric measurements have yielded a value of $\Delta U = -61.4$ kJ·mol^{-1} in 0.2 M TRIS/TRIS·HCl buffer (pH = 7.60) at 30.8 °C.

Using a procedure that seeks to minimize interfering heat effects and by application of a calibration curve determined using known glucose concentrations in water (not serum), fifty-one human serum and plasma samples have been assayed for glucose. The results cover a range of 35 to 408 mg glucose per 100 g of serum or plasma and are in satisfactory agreement with the results obtained using a conventional spectrophotometric clinical procedure. Since our results are based on the assumption of the essential specificity of enzymes, the agreement of the calorimetric results with the spectrophotometric results lends strong support to this fundamental hypothesis of biochemistry.

17. KEY WORDS (Alphabetical order, separated by semicolons) Analysis; biochemical microcalorimetry; clinical microcalorimetry; clinical chemistry; enzyme specific reactions; glucose in serum; hexokinase; microcalorimetry; serum.

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