Rate and duration of net glycogen synthesis following glucose administration to fasted human leukocytes

Liselotte Plesner

Institute of Biophysics, University of Aarhus, DK-8000 Aarhus C, Denmark

Received 27 April 1984; revised version received 4 May 1984

When glucose was added to fasted human leukocytes in a final concentration of 0.5–5 mM there was a phase of glycogen synthesis followed by a phase of glycogen breakdown. The duration of the phase of net glycogen synthesis increased with increasing concentrations of glucose applied, but the net rate of glycogen synthesis was inversely related to this figure and decreased from approx. 7 nmol/10^7 cells per min at 0.5 mM glucose to an average of 4 nmol/10^7 cells per min at 5 mM glucose.

Leukocyte *Glucose load* *Glucose uptake rate* *Glycogen synthesis rate* *Glucose permeability* *Glucose phosphorylation*

1. INTRODUCTION

An extensive study of the synthesis and turnover of glycogen in human leukocytes has been published [1], showing that when glucose was added to fasted leukocytes, the glycogen content of the cells, measured 30 or 60 min later, increased with increasing glucose concentrations applied. At higher glucose concentrations, however, a maximum was reached, and thereafter a further increase in glucose concentration caused a decrease in glycogen content. Also, below a certain glucose concentration the glycogen content measured after 30 min always exceeded the value after 60 min. It therefore seemed of interest to follow glucose uptake and incorporation in glycogen as a function of time, when glucose in various concentrations was added to fasted leukocytes. This was done here, and subsequent to the glucose load a phase of glycogen synthesis folllowed by a phase of glycogen breakdown was always found. The duration of the phase of net glycogen synthesis increased with increasing concentrations of glucose obtained, but the net rate of glycogen synthesis was inversely related to this figure. From these findings emerge two main questions: (i) What determines the duration of the phase of glycogen synthesis, or what mechanism brings it to an end and starts glycogen breakdown instead? (ii) What determines the inverse relationship between the glucose concentration obtained and the glycogen synthesis rate?

This report deals mainly with the second question and the experiments were undertaken to elucidate at which step the observed down regulation is active, focusing on 3 possibilities: (i) the step catalyzed by glycogen synthase (EC 2.4.1.11), (ii) glucose transport through the cell membrane and (iii) the glucose phosphorylation step.

2. MATERIALS AND METHODS

Human polymorphonuclear leukocytes were isolated from freshly drawn blood from healthy blood donors as in [2].

The buffer used throughout contained 31 mM Mops, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$ and 1% gelatine (pH 7.4) at 37°C.

Cells were incubated in a volume of 2.5 ml in polyethylene flasks (scintillation vials, volume 20 ml) at 37°C and at $1–4 \times 10^7$ leukocytes/ml as indicated in the figure legends. Aeration and

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00 © 1984 Federation of European Biochemical Societies

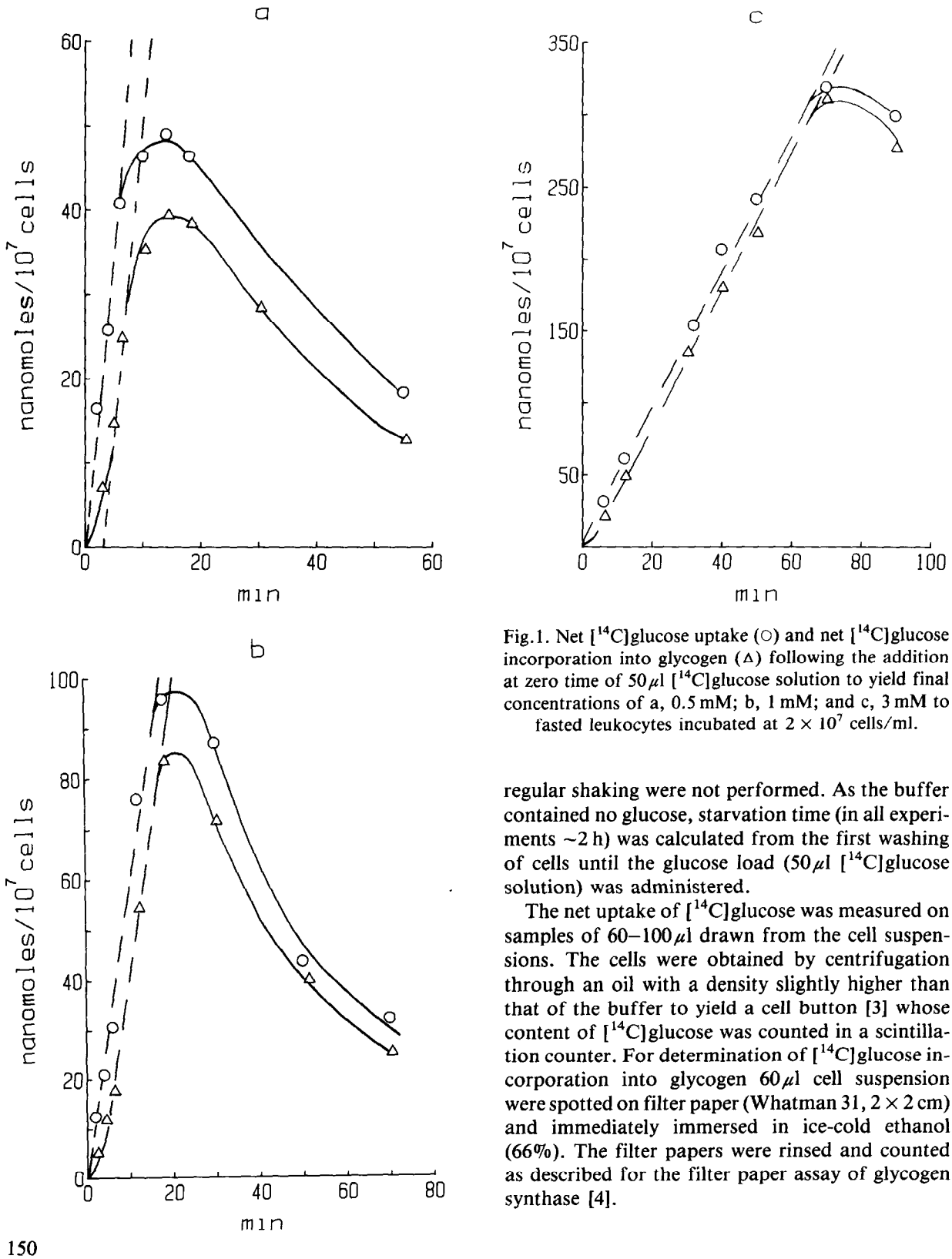

Fig.1. Net [^{14}C]glucose uptake (○) and net [^{14}C]glucose incorporation into glycogen (△) following the addition at zero time of 50 μl [^{14}C]glucose solution to yield final concentrations of a, 0.5 mM; b, 1 mM; and c, 3 mM to fasted leukocytes incubated at 2×10^7 cells/ml.

regular shaking were not performed. As the buffer contained no glucose, starvation time (in all experiments ~2 h) was calculated from the first washing of cells until the glucose load (50 μl [^{14}C]glucose solution) was administered.

The net uptake of [^{14}C]glucose was measured on samples of 60–100 μl drawn from the cell suspensions. The cells were obtained by centrifugation through an oil with a density slightly higher than that of the buffer to yield a cell button [3] whose content of [^{14}C]glucose was counted in a scintillation counter. For determination of [^{14}C]glucose incorporation into glycogen 60 μl cell suspension were spotted on filter paper (Whatman 31, 2 × 2 cm) and immediately immersed in ice-cold ethanol (66%). The filter papers were rinsed and counted as described for the filter paper assay of glycogen synthase [4].

Glycogen was determined by the filter-paper technique [5]. [U-^{14}C]glucose was from NEN and delivered in 90% ethanol, which was evaporated in a constant flow of N_2 before use. [U-^{14}C]Glucose was mixed with unlabelled glucose from Sigma to yield a specific activity of 800–1800 cpm/nmol. When the glucose concentration was varied (fig.1) the specific activity was kept constant. Other chemicals were from Boehringer, Sigma and Merck.

3. RESULTS

Fig.1 shows [^{14}C]glucose net uptake and [^{14}C]-glucose net incorporation into glycogen following administration of a [^{14}C] glucose load to human polymorphonuclear leukocytes (2×10^7/ml) starved for approx. 2 h in glucose-free buffer. The glucose concentrations obtained were 0.5, 1 and 3 mM in fig.1a, b and c, respectively.

The response is divided into a phase of net glucose uptake and net glycogen synthesis followed by a phase of decreasing content in the cells of [^{14}C]glucose and [^{14}C]glucose incorporated in glycogen. When cells were incubated at a higher cell concentration (4×10^7/ml) it was possible to measure the variation in glycogen content [5] subsequent to a glucose load and essentially the same biphasic pattern was obtained (not shown).

The duration of the phase of net glucose uptake and net glycogen synthesis increases with increasing glucose concentrations obtained (fig.1), but the rate of net glycose uptake and the rate of

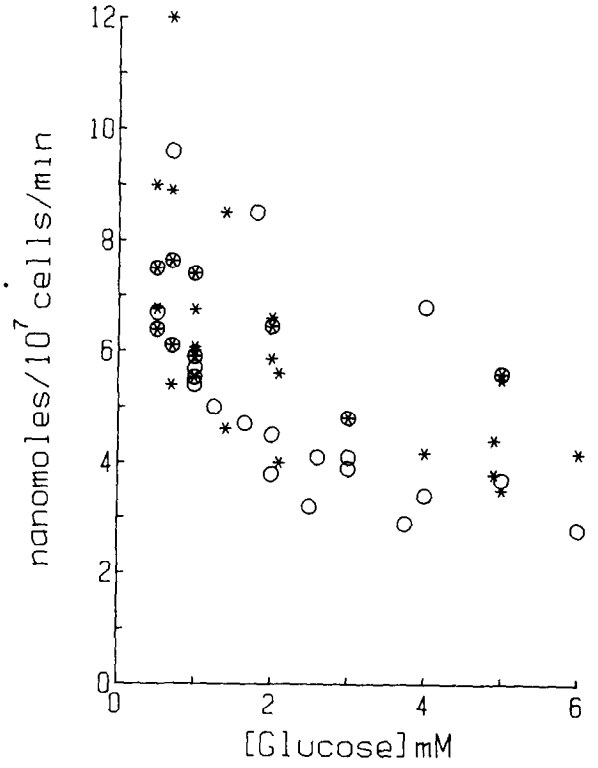

Fig.2. The net rate of [^{14}C]glucose uptake (★) and of [^{14}C]glucose incorporation into glycogen (○) in fasted human polymorphonuclear leukocytes following the addition of glucose in various concentrations. The rates were determined as the slope of the linear part of curves like those in fig.1. Cell concentrations were between 1 and 4×10^7 cells/ml.

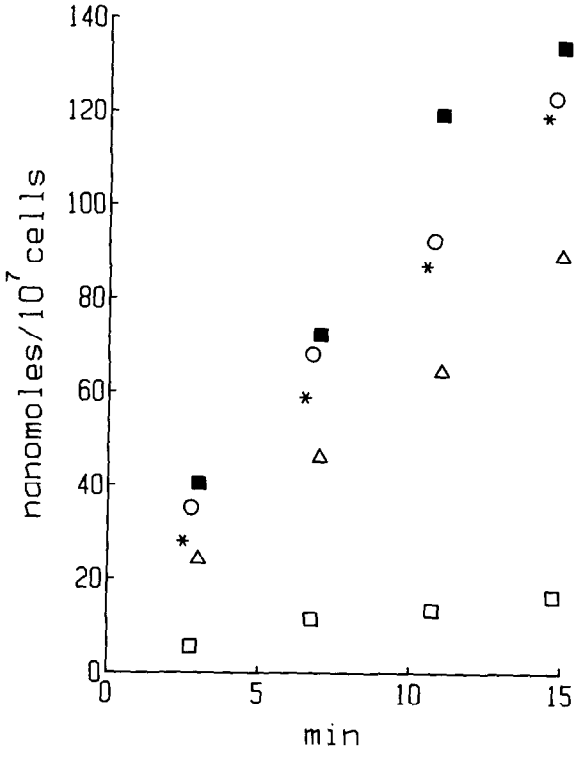

Fig.3. Net [^{14}C]glucose uptake in human polymorphonuclear leukocytes in the presence of various inhibitors of glucose transport. 10^7 cells/ml were incubated in glucose-free buffer for 2 h. At zero time [^{14}C]glucose was added to give a final concentration of 1 mM. One min before zero time 50 μl inhibitor solution was added to give a final concentration of 0.4 mM 2,4-dinitrophenol (△), 0.3 mM phloretin (□), 0.02 mM oligomycin in 5‰ ethanol (■) and 5‰ ethanol (○). To the control (★) was added 50 μl buffer.

net glucose incorporation into glycogen both decrease with increasing glucose concentrations. The rate calculated from the slope of the broken lines is 7.5 nmol/10^7 cells per min at 0.5 mM glucose (fig.1a), 5.9 nmol/10^7 cells per min at 1 mM glucose (fig.1b), and 4.8 nmol/10^7 cells per min at 3 mM glucose (fig.1c). Before the net incorporation of glucose into glycogen became linear with time the incorporation curves were slightly upward corresponding to a lag time before glucose incorporation into glycogen began. However, there does not seem to be any difference between the net glucose uptake rate and the rate of [^{14}C] incorporation into glycogen as determined from the linear parts of curves such as those shown in fig.1. The inverse relationship between the rates thus measured and the glucose concentrations obtained is shown in fig.2. Between 6 and 10 mM glucose there was no further decrease in rates (not shown).

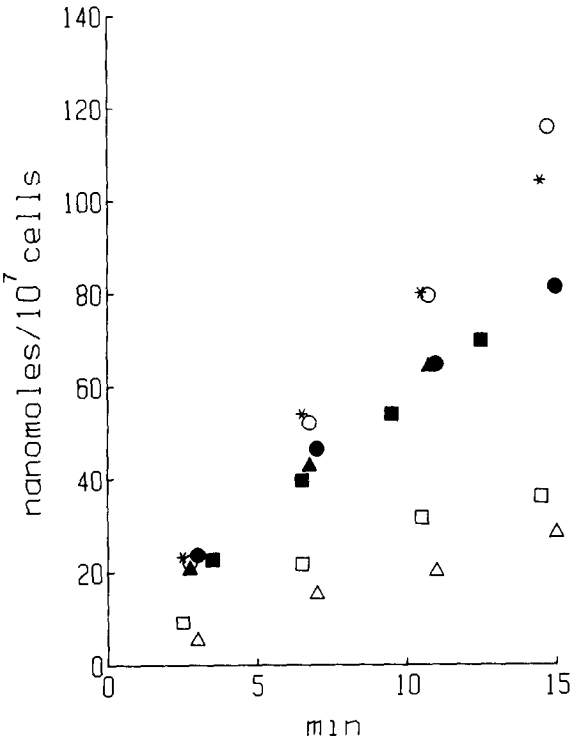

Fig.4. Net [^{14}C]glucose uptake in human polymorphonuclear leukocytes in the presence of various glucose analogues. 10^7 cells/ml were incubated in glucose-free buffer for 2 h. At zero time [^{14}C]glucose was added to give a final concentration of 1 mM. 1 min before zero time 50 μl glucose analogue solution was added to give a final concentration of 1 mM 3-*O*-methylglucose (○), mannose (△), 2-deoxy-glucose (□), galactose (●), glucosamine (▲) and fructose (■). To the control (★) was added 50 μl buffer.

Fig.3 shows the effect on net glucose uptake of certain inhibitors of glucose transport. Oligomycin (19 $\mu g \cdot ml^{-1}$) stimulated before it completely stopped further increase in the [^{14}C]glucose content of the cells. The stop was effective from ~20 min (not shown). In the presence of phloretin (300 μM) there was still an uptake which became linear in time indicating in two experiments an uptake rate of 0.6–0.7 nmol/10^7 per min. 2,4-Dinitrophenol (400 μM) in 4 experiments gave rise to linear net uptake curves but the rate of uptake was decreased by about 20–30%. With the inhibitors mentioned above, ethanol was added and the maximal concentration obtained in the cell suspension was 5‰. A control containing 5‰ of ethanol rules out inhibition caused by this concentration of the agent.

Insulin, at a final concentration of 37 and 70 m units/ml as well as ouabain (0.75 mM) administered alone or together with insulin [6] (37 or 70 m units/ml) were without effect on the net glucose uptake rate when added to cell suspensions 1 min before glucose (1 mM) (not shown).

When hexoses at a final concentration of 1 mM were administered to incubations 1 min before glucose (1 mM) (fig.4), the net glucose uptake rate was decreased by mannose and 2-deoxyglucose, while the decreasing effect of glucosamine, fructose and galactose was less pronounced. However, 3-*O*-methylglucose did not seem to influence the glucose uptake.

In each of the 5 incubations shown in fig.3 and in each of the 7 in fig.4, ^{14}C incorporation into glycogen was measured along with glucose uptake, and in every case (after the initial 5 min as discussed above) essentially the same line was obtained.

4. DISCUSSION

Polymorphonuclear leukocytes in exudates are exposed to low and varying glucose concentrations. Here we studied the effect of glucose at concentrations ranging from blood-glucose level to 1/10 of this value on net glucose uptake and glycogen synthesis of these cells.

When fasted polymorphonuclear leukocytes incubated at $1-4 \times 10^7$/ml are fed glucose in this concentration range there is a phase of net glycogen synthesis followed by a phase of net glycogen breakdown, and the duration of the phase of glycogen synthesis increases with the glucose concentration of the load administered. The occurrence of the turning point, where glycogen synthesis is replaced by glycogen degradation, could only be correlated at very high glucose concentrations to the occurrence of saturating amounts of glycogen in cells ($66\,\mu g/10^7$ cells [7], $74\,\mu g/10^7$ cells [1], $100\,\mu g/10^7$ cells [8], $115\,\mu g/10^7$ cells [9]) corresponding to 370–640 nmol glucose as glycogen in 10^7 leukocytes. From the net glucose uptake rate measured the remaining amount of glucose in the suspension at the time of the 'turning point' may be calculated. In fig.1a–c the turning point occurs when approx. 20% of the glucose present in the medium has been taken up by cells. However, this figure varies, in some cases, reaching almost 40% at low glucose and decreasing below 5% at sufficiently high glucose concentrations. High cell concentrations decreased the duration of the phase of net glucose uptake and net glycogen synthesis.

During the phase of glycogen synthesis the net glucose uptake rate and rate of [^{14}C]glucose incorporation in glycogen are (within the accuracy of the methods used) indistinguishable, and both decrease with increasing concentrations of glucose administered in the glucose load (fig.2).

To explain the observed down regulation the glycogen synthesis step is considered first, as glycogen synthase is thought to be rate-limiting in mammalian glycogenesis [10–13].

In [7], where the enzyme was shown to be activated upon addition of glucose to starved leukocytes, the activation was found to be inversely related to the final concentration of glucose in the range 0.5–10 mM, i.e., in the range of the observed down regulation of net glucose uptake rate and [^{14}C]glucose incorporation rate observed here. In [7] 'activation of glycogen synthase' was measured as increasing glucose 6-phosphate-independent activity (I activity) compared to glucose 6-phosphate-dependent activity (D activity). This figure, the I%, does not correlate to the ^{14}C incorporation rate when glucose analogous are present (fig.4). Glucosamine, 2-deoxyglucose and mannose were able to elicit a D to I conversion when added to starved leukocytes [7] and their 6-phosphorylated derivatives did stimulate the conversion in gel-filtered homogenates [14]. Also, when galactose (1 mM) was added to cells at the same time as a glucose load of 1 mM, the I% was increased ~30% above the increase resulting from addition of glucose only (unpublished). Although [^{14}C]glucose incorporation into glycogen as well as glucose net uptake was thus seen to be down regulated in some situations where the I% increased, it is still possible that the down regulation remains with the synthase, as this enzyme is known to have more active forms [9,15] than the I-form.

Down regulation of the glucose transport system seems a priori a less likely hypothesis. However, in fibroblasts a down regulation of glucose transport was elicited by glucose, mannose, glucosamine, oligomycin and 2,4-dinitrophenol [16], the same agents, which in fig.3,4 are seen to decrease net glucose uptake and [^{14}C]glucose incorporation in glycogen. Also, at low glucose concentrations the glucose net uptake rate yields a permeability comparable to those calculated from transport studies: At 0.5 mM glucose a net uptake rate of 7 nmol/10^7 cells per min may be estimated (fig.2), and calculating the cell surface from a diameter of 11 μm, a permeability is obtained dividing the uptake rate (mol/10^7 cells per s) by the surface area ($m^2/10^7$ cells) and the concentration gradient (mol/m^3 = mM)

$$P = \frac{J}{\Delta C} = \frac{7 \times 10^{-9}\,\text{mol} \cdot 10^{-7} \cdot \text{min}^{-1} \times 1/60\,\text{s} \cdot \text{min}^{-1}}{3.8 \times 10^{-3}\,\text{m}^2 \cdot 10^{-7} \times 0.5\,\text{mol} \cdot \text{m}^{-3}}$$

$$= 6 \times 10^{-8}\,\text{m} \cdot \text{s}^{-1} = 6 \times 10^{-6}\,\text{cm} \cdot \text{s}^{-1}$$

Even if in this calculation the cell surface is underestimated the permeability obtained does not rule out that transport may be rate limiting thus rendering down regulation on this system a possibility.

It has been claimed [17] that glucose is taken up by a simple diffusion process in human granulocytes, and a passive transport of 1 nmol/10^7 per min was obtained for 3-*O*-methylglucose [17]. The glucose consumption figure given would however necessitate a net glucose uptake rate of approx. 10-times this value. Some mechanism other than simple diffusion seems to be necessary to explain this observation which corresponds to our finding

above (fig.3) that the net glucose uptake rate is decreased by a factor 10 in the presence of phloretin.

An effect of insulin on glucose consumption measured after 1 h incubation with 16.7 mM glucose was also reported [17], but no influence of insulin on the glycogen content of cells was found. This is in accordance with the results reported here and in [7,18].

A third possibility emerges from fig.4, which shows that the only hexose not giving rise to a down regulation of net glucose uptake and [^{14}C]glucose incorporation into glycogen is 3-*O*-methylglucose, the only sugar that is not 6-phosphorylated by the cells. At low glucose concentrations glycogen synthase may be rate-limiting (cf. fig.2) where glucose net uptake rate and [^{14}C]glucose incorporation rate extrapolate to the maximal velocity of the enzyme (D + I activities) determined in these cells to be between 8 and 12 nmol/10^7 cells per min [5,12]. At higher glucose concentrations the synthase or some intermediate reaction may no longer be able to prevent accumulation of glucose 6-phosphate. The down regulation could then be explained by glucose 6-phosphate and 6-phosphorylated glucose analogue inhibition of the hexokinase activity of the cells. More detailed glucose uptake data should be compared to the data on glycogen synthesis to answer these questions, and to elucidate whether in human polymorphonuclear leukocytes glucose is stored as glycogen before it becomes available for other purposes in the cell, as indicated here.

ACKNOWLEDGEMENTS

The author wishes to express here gratitude to Professor J. Gliemann for helpful advice and to Professor F. Kissmeyer, Head of the Blood Bank Service, Aarhus Kommunehospital, for supply of blood samples. The expert technical assistance of Mrs Vinni Ravn is gratefully acknowledged.

REFERENCES

[1] Scott, R.B. (1968) J. Clin. Invest. 47, 344–352.
[2] Esmann, V. (1964) Metabolism 13, 354.
[3] Andreasen, P., Schaumburg, B., Østerlind, K., Vinten, J., Gammeltoft, S. and Gliemann, J. (1974) J. Anal. Biochem. 59, 110–116.
[4] Thomas, J.A., Schlender, K.K. and Larner, J. (1968) J. Anal. Biochem. 25, 486–499.
[5] Sølling, H. and Esmann, V. (1975) Anal. Biochem. 68, 664–668.
[6] Sobrino, F., Ruiz, G. and Goberna, R. (1982) Biochem. J. 208, 261–268.
[7] Wang, P., Plesner, L. and Esmann, V. (1972) Eur. J. Biochem. 27, 287–300.
[8] Plesner, L., Salsas Leroy, E., Wang, P., Rosell Perez, M. and Esmann, V. (1972) Biochim. Biophys. Acta 268, 344–353.
[9] Saugmann, P. and Esmann, V. (1977) Biochem. Biophys. Res. Commun. 74, 1511–1519.
[10] Larner, J. and Villar-Palasi, C. (1971) Curr. Top. Cell. Regul. 3, 195–236.
[11] Soderling, T.R., Hickenbottom, J.P., Reimann, E.M., Hunkeler, F.L., Walsh, D.A. and Krebs, E.G. (1970) J. Biol. Chem. 245, 6317–6328.
[12] Roach, P.J. (1981) Curr. Top. Cell. Regul. 20, 45–105.
[13] Cohen, P. (1978) Curr. Top. Cell. Regul. 14, 117–196.
[14] Wang, P., Bantle, G. and Sørensen, N.B. (1977) Biochim. Biophys. Acta 496, 436–447.
[15] Saugmann, P. (1977) Biochem. Biophys. Res. Commun. 74, 1520–1527.
[16] Ullrey, D.B., Franchi, A., Pouyssegu, J. and Kalckar, H.M. (1982) Proc. Natl. Acad. Sci. USA 79, 3777–3779.
[17] Leroux, J.P., Marchand, J.C., Hong Taun Ha, R. and Cartier, P. (1975) Eur. J. Biochem. 58, 367–373.
[18] Esmann, V. (1963) Diabetes 12, 545.