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The accumulation of free and phosphorylated sugars in adipocytes based on a dynamic diffusion barrier

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The simple theory of a dynamic diffusion barrier is described and it is shown how this could account for the accumulation, in adipocytes, of those free sugars which are also phosphorylated. The standing concentration gradient established by this mechanism depends on the recycling of free sugar and sugar phosphate in submembrane structures which start in juxtaposition to conventional membrane hexose transporters. Although a continual expenditure of metabolic energy is involved, there can be a net gain from the potential-energy store of accumulated substrates. The hypothesis leads to a series of simple equations which can be used as the basis for computer simulations of experimental procedures.

Introduction

Adipocytes [1] and certain other animal cells [2–5] are able to accumulate 2-deoxy-D-glucose (and other sugars which are phosphorylated) against the concentration gradient. Sugars which are not phosphorylated (e.g., 3-*O*-methyl-D-glucose) equilibrate [6], but are not accumulated against the gradient. In mixtures of 2-deoxyglucose and 3-*O*-methylglucose, there is no mutual interference in their respective accumulation or equilibration (Thompson and Kleinzeller, unpublished results).

Since phosphorylation seems to be essential to accumulation, there have been various attempts to explain the mechanism. A model proposed by Van Steveninck [7,8] to account for a similar accumulation seen in yeast cells involved phosphorylation linked to the membrane transport of the sugar and a subsequent intracellular dephosphorylation of the hexose phosphate formed. This was to explain the excess of free sugar in the cytoplasm. For mammalian cells a tandem arrangement of influx, followed by phosphorylation, which in turn was followed by dephosphorylation and efflux was proposed [4]. Computer simulations of the tandem system to show the type of results to be expected were described by Wohlhueter and Plagemann [9].

The view proposed by Foley and Gliemann [1] was that, after phosphorylation,some of the sugar phosphate was dephosphorylated, with the release of free sugar in a cellular compartment where the newly form 2-deoxyglucose could not readily diffuse to the carrier system, and they therefore postulated a diffusion barrier. The free sugar, sequestered in this 'compartment', however, is rapidly exchanged with radioactively labelled sugar and is readily lost when the cells are placed in a medium with low sugar concentration.

A more recent theory proposed by Naftalin and Smith [10] envisages that a fixed-site transport system forms an integral part of a complex phosphorylating mechanism which reacts with sugar on the inward-facing sites of the sugar transporter and, by converting it to sugar phosphate, effectively inhibits efflux but not influx. So close an association of the phosphorylating enzymes with the hexose transporters would imply a plasma membrane localisation of the kinase enzymes. No hexokinase was found associated with the basal membranes of rat kidney proximal tubules [11], although this is one of the tissues capable of effecting against the gradient accumulation of sugars.

In this paper an alternative possibility is described based on the concept of a vectorially arranged series of phosphorylating enzymes which could create a dynamic diffusion barrier between the cytoplasm and the mem-

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brane foyers where the conventional sugar transporter clefts open.

A simple dynamic diffusion barrier

The following treatment of the dynamic diffusion barrier follows the lines used by A.V. Hill [12] and others to show how the concentration of oxygen would fall off towards the centre of a metabolising cell. The principle was that diffusion from any zone to an inner zone was less than the diffusion into that zone from an outer zone, by reason of the oxygen which was used in the zone. If cytoplasmic sugar at a high concentration is considered to be diffusing toward the membrane foyers along a series of zones where some of the sugar is removed as sugar phosphate, the same principles apply. This can be explained with the help of a simple diagram (Fig. 1).

Fig. 1 is a simple scheme showing a cylinder-shaped funnel leading from the bulk of the cytoplasm to a region of the membrane where the transporter cleft opens. For simplicity, only three zones of enzyme activity are shown. The concentrations of free sugar are S (in the bulk cytoplasm), S_1, S_2 and S_3 in the three enzyme zones and S_o in the foyer where one of the transporters operates.

In the steady state, the concentration in the foyer (S_o) will be the same as that in the outside medium, so that no net movement of sugar occurs across the membrane but, of course, isotopic fluxes of equal magnitude will flow in and out.

Net diffusion of sugar will occur at the cytoplasmic end of the funnel and will be proportional to the concentration gradient. If we assume a diffusion permeability factor, P_d, which takes into account the free diffusion mobility, the restricted area of all the funnels relative to the total membrane and other factors, such as solvent drag due to the counter-diffusion of phosphorylated sugar along the funnels, we can write the following equations:

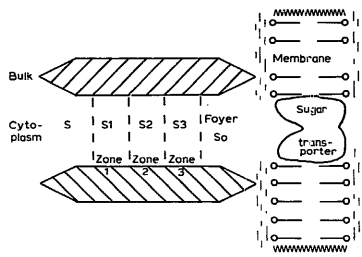

Fig. 1. Schematic diagram showing a cylinder-shaped 'funnel' with three equally spaced zones of enzyme (kinase) activity between the cytoplasm and the 'foyer' where the sugar transporter operates. For further details see text.

$$P_d(S - S_1) = P_d(S_1 - S_2) + V(1) \quad (1)$$

where $V(1)$ represents the loss of free sugar by the phosphorylating enzyme(s) in zone Z1.

Similarly, for the other zones we have:

$$P_d(S_1 - S_2) = P_d(S_2 - S_3) + V(2) \quad (2)$$

$$P_d(S_2 - S_3) = P_d(S_3 - S_o) + V(3) \quad (3)$$

$$P_d(S_3 - S_o) = 0 \text{ (steady-state)} \quad (4)$$

Thus

$$P_d(S_2 - S_3) = V(3) \quad (5)$$

$$P_d(S_1 - S_2) = V(3) + V(2) \quad (6)$$

$$P_d(S - S_1) = V(3) + V(2) + V(1) \quad (7)$$

Adding the two sides of Eqns. 4–7 we obtain the relationship:

$$P_d(S - S_o) = 3V(3) + 2V(2) + V(1) \quad (8)$$

If the values of $V(1)$, $V(2)$ and $V(3)$ are each equal to V, then it will be seen that there will be an amplifying effect, since $P_o(S - S_o)$ will be equal to twice the sum of the enzyme activities considered separately. The amplification depends on the depth of the funnel in terms of enzyme zones. If there are N enzyme zones the general equation becomes:

$$P_d(S - S_o) = ((N + 1)/2) \cdot V \quad (9)$$

Eqn. 9 gives the magnitude of a dynamic diffusion gradient which could be maintained between the cytoplasm and the membrane foyers. It depends on the V of the phosphorylating enzymes in the funnel complexes, the diffusional permeability of free sugar in the funnels and the amplification factor. The funnel complexes could be submembrane structures of very small volume relative to the bulk cytoplasm. If the funnel complexes were about 1 μm long and 10 nm outside diameter, the total volume of 125000 funnels would be less than 0.1% of the watery cytoplasm of each cell.

The net diffusion of free sugar along the funnels will be determined by the initial gradient at the mouth of the funnels i.e., $P_d(S - S_1)$ of Eqn. 1. It can be shown that if $S_o = 0$, then $S_1 = S/2$ in the example used; thus the net diffusion into the funnels would only be half $P_d \cdot S$ and generally is equal to $(S \cdot P_d/\text{AF})$, where AF is the amplification factor ($\text{AF} = (N + 1)/2$). Assuming there is an optimal distribution of enzymes, then, in the steady state, the total quantity of free sugar diffusing

along the funnels must be balanced by the phosphorylation of the funnel enzymes. This can be represented as

$$P_d/AF \cdot (S - S_o) = V_p \quad (10)$$

where $V_p = V_{max} \cdot$ saturation.

Phosphorylation of free sugar in the funnels will lead to the build-up of sugar phosphate but, since it is impermeable, its only means of egress is to diffuse back along the funnels to the cytoplasm. Indeed, if P_p is the diffusional permeability of the sugar phosphate, an equation equivalent to Eqn. 10 is readily derived i.e.:

$$P_p(X_f - X_c) = V_p \cdot AF \quad (11)$$

where X_f and X_c represent the concentrations of sugar phosphate in the foyers and cytoplasm, respectively.

In the steady state, the diffusion of sugar phosphate into the cytoplasm must be the same as the diffusion of free sugar from the cytoplasm along the funnels towards the foyers. Thus

$$P_d(S - S_o) = P_p(X_f - X_c) = V_p \cdot AF \quad (12)$$

As the steady state is approached, it may be assumed that influxing sugar is phosphorylated by enzymes nearest the transporters and that enzymes further along the funnels are phosphorylating (and recirculating as sugar phosphate) the cytoplasmic free sugar diffusing along the gradient in the funnels.

The metabolic cost of creating the dynamic diffusion barrier is not insignificant and may be considered to be equivalent to the hydrolysis of one ATP molecule for every sugar phosphate formed. However, it may be assumed that one molecule of sugar phosphate (if the sugar is glucose) undergoing aerobic oxidation yields 39 molecules of ATP, so that, if a deduction from the sugar phosphate pool equal to $1/39\ V_p$ is provided for, a balance will be maintained. When 2-deoxyglucose is the test sugar an alternative source of ATP must be presumed.

From an inspection of Eqns. 10–12 it will be clear that in an experimental situation V_p will start at a low value and consequently the efficiency of the dynamic diffusion barrier will also be low. In order to use these equations to represent experimental conditions (e.g., in computer simulations) some discussion of determining the average saturation of the enzymes and the diffusional permeability is appropriate.

The average saturation of the phosphorylating enzymes will clearly depend on the range of concentrations in the gradient between the foyers and the cytoplasm. Assuming a linear distribution between the top and bottom concentrations the average effective concentration can be calculated. With a computer program designed to do this, it was found that the best approximation was to assume a median value of $0.5 \cdot (S + S_o)$ and to determine the saturation on the basis of the usual Michaelis formula. Thus:

$$\text{saturation} = 0.5(S + S_o)/(0.5(S + S_o) + K_{mp}) \quad (13)$$

where K_{mp} is the Michaelis constant for the phosphorylating enzymes.

The diffusional permeability used in Eqns. 1–10 refers to the steady state concerned, but it should not be taken as an invariable constant. If the funnels have a relatively restricted cross-section for diffusion, the inward diffusion of sugar phosphate could affect the rate of outward diffusion of free sugar.

Since the rate of influx of sugar will determine the phosphorylating activity of the enzymes nearest to the foyers and hence the initial build-up of the sugar phosphate concentration, it seemed likely that sugar influx would also be a factor.

In general, P_d is likely to start high when the efficiency of the dynamic diffusion barrier is low and to settle down to a steady value as the concentration reaches the optimal conditions.

The amplification factor must also be taken into account, but since P_d and AF always occur in the form P_d/AF, they may be combined as a single parameter. From an analysis of experimental results for non-insulin-stimulated cells, and assuming a V_{max} of 0.1 mmol $\cdot l^{-1} \cdot s^{-1}$ and a K_{mp} of 1.3 mM, the following empirical formula was deduced:

$$P_d/AF = 0.016 + 0.033/(1 + 1360 \cdot \text{influx}) \quad (14)$$

where influx is also in mmol $\cdot l^{-1} \cdot s^{-1}$. The dimensions of P_d/AF are s^{-1}.

This empirical formula was based on a particular V_{max} and K_{mp} value. Consequently, P_d/AF must be recalculated using Eqns. 10 and 13 if the V_{max} or K_{mp} for the kinase reactions are altered.

In writing a computer program to simulate experimental results certain simplifying assumptions have been made. These will be described here.

(i) In the initial stages of an incubation it is assumed that the production of sugar phosphate is equal to the sum of the net entry of sugar at the transporters plus the diffusion of free sugar from the cytoplasm into the funnel complexes.

$$\text{Flux (sugar phosphate)} = \text{net entry} + (P_d/AF) \cdot S \quad (15)$$

However, this flux cannot exceed the capacity of the enzymes for phosphorylation i.e., $(V_{max} \cdot \text{saturation}) = V_p$. Also, provision is made to deduct $1/39 \cdot V_p$ to cover the metabolic cost of the dynamic diffusion gradient when it is desired to simulate a glucose experiment.

(ii) The production of free sugar in the bulk cytoplasm is presumed to be due to the dephosphorylation of sugar phosphate as proposed by Foley and Gliemann

[1]. However, once formed, the free sugar will start to diffuse down the funnel complexes towards the foyers and the overall flux will be the production minus the diffusional loss via the funnels.

$$\text{Flux (free sugar)} = V(\text{phosphatase}) - (P_d/\text{AF}) \cdot S \qquad (16)$$

If Eqn. 15 predicts a flux for sugar phosphate production greater than V_p then the production is set equal to V_p and the excess is added to the flux for free sugar in Eqn. 16. The extra free sugar coming directly into the cytoplasm in this way will be limited since, when the phosphorylating capacity has been reached, the foyer concentration will progressively rise and cut off the net entry rate.

(iii) The concentration of free sugar in the foyers is assumed to obey the relationship in Eqn. 10, which can be rewritten in the following form:

$$S_o = S - (V_{max} \cdot \text{Saturation}) \cdot \text{AF}/P_d \qquad (17)$$

S_o is not allowed to go negative and if Eqn. 17 makes it so, the value of S_o is made equal to one-tenth the outside concentration.

In this form it is clear that in the absence of any phosphorylating activity (i.e., if $V_{max} = 0$) the sugar concentration in the foyers would be the same as in the cytoplasm. Thus, intracellular diffusion (and that in the funnels) is presumed to be fast relative to the rates of membrane transport. Eqns. 10 and 17 also emphasize that the concentration gradient set up is a dynamic one depending on the kinase activity.

(iv) Besides Eqns. 13–17 it is necessary to provide parameters for the sugar transporters. For this purpose the standard Regen and Tarpley [13] kinetic parameters have been used. It was found that the modification proposed by Baker and Widdas [14] made only insignificant differences and may be neglected in this context.

(v) A single computer program based on the five simple equations referred to above can give a satisfactory explanation of the accumulating effect for 2-deoxyglucose in experiments carried out over the concentration range from 0.01 to 20 mM. The concentrations of 2-deoxyglucose phosphate are also reasonably well predicted by a suitable choice of the V_{max} and K_m parameters used to determine the rate for dephosphorylation in Eqn. 16 (see Table III).

(vi) Basically, the stimulating effect of insulin may only involve an acceleration of influx at the transporters. However, it was found that for a reasonable computer simulation of the time-course of the effects of results, such as those of Foley and Gliemann [1], it was also necessary to increase the V_{max} for phosphorylation by a factor approaching that for the acceleration of influx. This also involved a readjustment of the V_{max} for dephosphorylation and a recalculation of P_d/AF to give a value which fitted Eqn. 17 to the steady state for the particular experiment.

Methods

The methods used to prepare the suspension of adipocytes were as described by Foley and Gliemann [1]. Three or four estimations were made for each experimental point and the standard deviation (or standard error) determined. Where there was considerable variation between experiments only the means of the three or four results for each are given.

Results

Experiments on the time-course of accumulation

Most of the experiments in the present study were carried out with 0.1 mM 2-deoxyglucose in the outside incubating medium. In three of these, uptake was measured sequentially at intervals up to 60 or 90 min. There was considerable variation in the 60-min values but the percentages of the 60-min values reached at intermediate times were reasonably consistent (Table I).

Fig. 2 is a print-out from a computer program based on the five equations referred to above and which used a reiterative procedure. In this figure the mean percentages of Table I calculated for the 60 min steady value are also shown and it will be seen that, with the parameters chosen and given in the legend, the computer program simulates a time-course similar to that of the experimental results.

It is noteworthy that the activity constant F_g of the Regen and Tarpley [13] kinetics was only about one-hundredth of that which would be required for the hexose transport in red cells at 37°C.

TABLE I

Time-course of accumulation of 2-deoxyglucose at 37°C

Percentage uptake of accumulated 2-deoxyglucose at different times of sampling. Each estimate was the mean of three or four determinations. The outside medium contained 0.1 mM 2-deoxyglucose.

Time (min)	Expt. 1 (%)	Expt. 2 (%)	Expt. 3 (%)	Mean (%)
10	36.7	37		36.85
15			55	55
20	52.5	63		57.8
30	71.2	76.5	76	74.6
40	88.5	83		85.8
45			88	88
50	88.5	98		93.3
60	100	100	100	100
60 Min Value (mM)	1.39	1.83	0.95	1.39

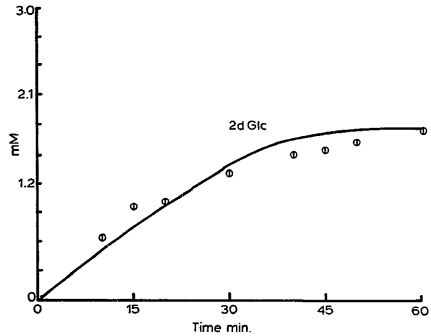

Fig. 2. Computer print-out of program based on Eqn. 14 and with V_{max} (kinase) = 0.1 mmol·l^{-1}·s^{-1}, K_{mp} = 1.3 mM, V_{max} (phosphatase) = 0.105 mmol·l^{-1}·s^{-1}, K_m = 3.6 mM. The Regen and Tarpley parameters were F_g = 0.02 s^{-1}, K_{go} = 5 mM, K_{gi} = 5 mM, B_g = 5 mM. The outside concentration was 0.1 mM. 2dGlc, 2-deoxyglucose.

Variability in the results

The variability of the results is illustrated by Table II, which collects the results of the 60-min accumulations in eight experiments with 0.1 mM incubations of 2-deoxyglucose.

Fig. 3 is a print-out of the same computer program as shown in Fig. 2, but including the accumulation of 2-deoxyglucose phosphate as well as that of 2-deoxyglucose. The mean and standard error of the eight results were calculated to be 1.57 ± 0.17 mM for 2-deoxyglucose and 2.72 ± 0.52 mM for 2-deoxyglucose phosphate. The steady-state values of the computer print-out were 1.76 mM and 2.37 mM for 2-deoxyglucose and 2-deoxyglucose phosphate, respectively.

TABLE II

Variability of results with 0.1 mM 2-deoxyglucose incubations

Values were from 60-min incubations; each value was the mean of three or four determinations.

Expt. No.	2-Deoxyglucose (mM)	2-Deoxyglucose phosphate (mM)
1	2.07	4.10
2	2.30	5.20
3	1.39	1.31
4	1.83	3.96
5	0.95	1.90
6	1.71	1.61
7	1.14	2.05
8	1.16	1.66
Mean	1.57	2.72
S.E.	0.18	0.52
(*n*)	(8)	(8)

Experiments at different incubating concentrations

A few experiments have been done at concentrations ranging from 0.01 to 20 mM but with emphasis on the final values obtained with either 60 or 120 min incubation. Again, there was large variability of the final values obtained, particularly at higher concentrations, but some results seemed to show a regular pattern. Table III lists the results for non-insulin-stimulated cells together with the steady-state values from computer print-outs similar to those shown in Figs. 2 and 3. The same computer program was used and the parameters were identical except for that of the outside concentration.

The effect of insulin

Foley and Gliemann [1] showed that adipocytes which were maximally stimulated by insulin could rapidly accumulate 2-deoxyglucose and 2-deoxyglucose phosphate. With 10 mM 2-deoxyglucose in the outside medium there was a rapid accumulation during the first minute and a tendency to flatten off after about 5 min.

Fig. 4 is a computer simulation of the Foley and Gliemann result based on the same equations used for Figs. 2 and 3 but fitted to the time-course for their results by increasing the Regen and Tarpley [13] parameter F_g 10-fold, the V_{max} for phosphorylation 6-fold and the P_d/AF value by a factor of 5.5. It was also necessary to increase the V_{max} for dephosphorylation from 0.105 to 0.9, but the extra part (0.8) was only 'activated' as the sugar phosphate concentration rose. A modulator term with a half-saturation of 20 mM was used for this purpose but the K_m for the 'activated' phosphatase was

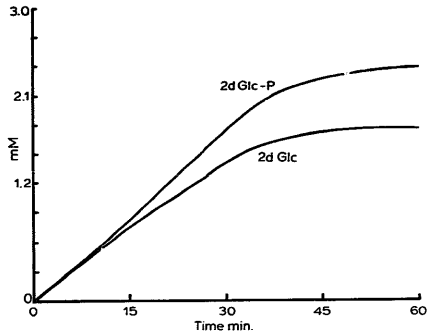

Fig. 3. Computer print-out showing the accumulation of 2-deoxyglucose and 2-deoxyglucose phosphate when the outside concentration was 0.1 mM. The parameters were the same as in Fig. 2. 2dGlc, 2-deoxyglucose; 2dGlc-P, 2-deoxyglucose phosphate.

unchanged at 3.6 mM. The combination of the higher V_{max} and the modulator term effectively allowed a steeper rise in the sugar phosphate curve and ensured that it approached a more stable end-point. At the end of the 20-minute run the V_{max} for all the 'activated' phosphatase was about the same as that for the kinase (0.6 mmol $\cdot l^{-1} \cdot s^{-1}$). The modulator term had a negligible effect on the 2-deoxyglucose curve.

Discussion

If the accumulation of 2-deoxyglucose against the gradient follows the principles outlined here, this implies that there is a biological adaptation involving a vectorial arrangement of phosphorylating enzymes in submembrane structures which have a special relationship to the foyers where the sugar transporter clefts

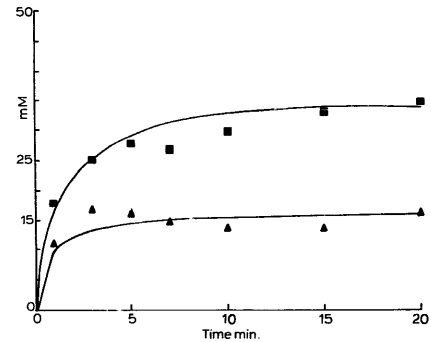

Fig. 4. Computer print-out simulating the effect of insulin stimulated adipocytes with 10 mM 2-deoxyglucose outside. The experimental results published by Foley and Gliemann [1] are shown as. ▲, free 2-deoxyglucose and ■, 2-deoxyglucose phosphate. The lines are print-outs from the modified computer program; F_s was increased 10-fold ($F_s = 0.20\ s^{-1}$), V_{max} (kinase) was increased 6-fold (V_{max} (kinase) = 0.6 mmol $\cdot l^{-1} \cdot s^{-1}$), the value of P_d/AF was 0.09 s^{-1}. The phosphatase V_{max} was 0.9 mmol $\cdot l^{-1} \cdot s^{-1}$ but part was modulated as described in the text. Other parameters were as in Fig. 2.

TABLE III

Effect of varying the outside incubating concentration

Results for 5–20 mM were for 120-min incubations. The others were 60 min. The computer simulations were all based on Eqn. 14. V_{max} (kinase) = 0.1 mM·s^{-1}, K_{mp} = 1.3 mM, V_{max} (phosphatase) = 0.105 mM·s^{-1}, K_m = 3.6 mM and the following Regen and Tarpley [13] parameters: F_g = 0.02 s^{-1}, K_{go} = 5 mM, K_{gi} = 5 mM, B_g = 5 mM. Only the outside concentration was varied. Anomalous results (in parentheses) can be fitted only if Eqn. 14 is altered in each case to give lower values for (P_d/AF). The V_{max} (phosphatase) must also be changed.

Outside concn. (mM)	Experimental results		Computer simulations	
	2-deoxy-glucose	2-deoxy-glucose phosphate	2-deoxy-glucose	2-deoxy-glucose phosphate
0.01	0.14	0.18	0.13	0.18
0.05	1.02	1.17	1.0	1.35
0.1	1.57	2.72	1.76	2.37
1.0	4.76	12.31	5.0	7.18
	4.65	11.61		
	(11.96)	(10.02)		
5.0	–	–	10.2	15.6
	(21.49)	(16.23)		
10.0	15.70	22.05	15.3	22.0
	14.24	18.66		
	(34.67)	(11.65)		
20.0	22.93	17.54	23.9	28.2
	22.74	22.92		

open on the inner surface of the cell membrane. No such structures have so far been described in adipocytes but they could be too small to be observed under the light microscope. Although electron-microscope studies have revealed numerous submembrane structures, such as rosettes, small vacuoles and other complex invaginations, all characterised as being of cell membrane origin [15], no specific functions could be ascribed to them. As the number of sugar transporters in the membrane may be few on a surface-area basis, this wealth of other submembrane structures may make the resolution of the 'funnels' particularly difficult.

There are also problem involving energy considerations. These arise because underlying the concentrating mechanism is a continual phosphorylation in the funnel complexes and dephosphorylation at different sites on the membrane, or somewhere in the cytoplasmic reticulum. Phosphorylation will presumably involve the breakdown of ATP as part of the kinase activity. The recycling could thus represent a serious drain of cellular energy, which has not been fully considered in earlier models involving phosphorylation and dephosphorylation, although Van Steveninck [7] has drawn attention to the decrease in ATP, orthophosphate and polyphosphate which accompanied the accumulation of free sugar in yeast cells. Experiments with 2-deoxyglucose, while providing useful analogies with which to study and follow the accumulating mechanism, present their own energy problem. Thus there is usually assumed to be a block in aerobic metabolism at the 2-deoxyglucose phosphate stage [9] and the supply of ATP to maintain the concentration gradient of 2-deoxyglucose may have to rely on other sources of acrobic metabolism possibly involving amino acids, glycerol or lipids. Foley and Gliemann [1] have shown that no accumulation of free 2-deoxyglucose occurred in cells which had been preincubated with 1 mM dinitrophenol to deplete them of ATP.

For these reasons, separate computer programs have been used for simulations of 2-deoxyglucose and glucose experiments. There is not much difference for normal runs in which provision is only made for glucose phosphate to be available as the energy source for maintaining the concentration gradient. The chief effect was that the concentration of sugar in the 'foyers' was automatically maintained at a value just less than the outside concentration, so that a net influx of sugar – to balance the metabolism – ensued. This requirement was smaller at low concentrations, since the saturation of the kinase enzymes in the funnels was lower but, at higher concentrations, amounted to the equivalent of about 7 mmol of sugar per litre of cell water per hour. Nevertheless, the extra store of substrate available as free sugar and sugar phosphate provided a considerable net gain, which could be an important physiological bonus for cells which use sugar phosphate for other purposes, such as lipid storage.

Indeed, one justification for having a computer program which can simulate cellular activity is to illustrate this possible physiological function (Fig. 5).

Adipocytes are subject to insulin stimulation. Thus, after a meal when the blood sugar may have risen towards 10 mM, they will rapidly accumulate glucose and glucose phosphate. Considering only that there could be an accumulating mechanism for glucose which was analogous to that for 2-deoxyglucose, then the free glucose might typically rise to about 16 mM and glucose phosphate to about 20–25 mM. After the meal had been absorbed, the blood sugar would fall back to 5 mM and the transporters would revert to the low rate of the resting condition. When this had happened there would be an exit of some of the free sugar, which had been accumulated in excess, but this would cease when the dynamic diffusion barrier was that appropriate to 5 mM, even though there may be very little further net entry. Fig. 5 shows that most of the free sugar and sugar phosphate would be available to the cell for over an hour with a minimal loss.

Thus in terms of substrate available for other cellular activities the potential store would be nearly 1.5-times as large as it would be if it was sugar phosphate only. As the free sugar and sugar phosphate are rapidly recycling in the funnel complexes, cellular activities

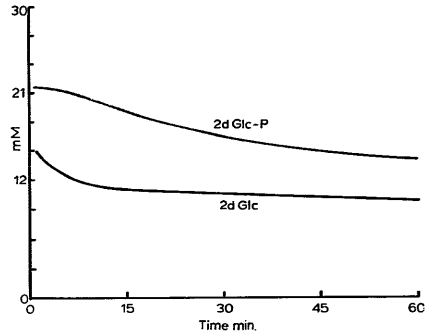

Fig. 5. Computer simulation of the physiological effect of the 'exit' which would occur if cells, equilibrated at 16 mM sugar and 25 mM sugar phosphate were placed in a medium of 5 mM and the transport activity reverted to resting levels. For this print-out the same parameters as in Fig. 2 were used, except for the Regen and Tarpley [13] parameters, which were adjusted for glucose, i.e., $F_g = 0.011\ s^{-1}$, $K_{go} = 9$ mM, $K_{gi} = 9$ mM, $B_g = 9$ mM. 2dGlc, 2-deoxyglucose; 2dGlc-P, 2-deoxyglucose phosphate.

which required sugar phosphate as a starting point would not be handicapped by the fact that part of the store was in the form of free sugar. However, since glucose 6-phosphate readily enters other metabolic pathways, the hypothetical situation represented in Fig. 5 would not be seen in practice, as the 'other' pathways would consume the store too rapidly.

The effects of draining off additional quantities of sugar phosphate for 'other' cellular activities can also be illustrated by computer simulations. Table IV gives a summary of the results of one series of runs in which the 'other' activities were given V_{max} values over a 10-fold range. Table IVA shows the decline in the steady-state levels of the cytoplasmic sugar phosphate and free sugar which would occur contemporaneously with such activity. The depression of the 'foyer' concentration of glucose needed to obtain a net entry which balanced the rate of overall metabolism was also simulated and recorded. It will be seen that with significant rates of 'other' activities there would either be little, or no, apparent accumulation of free glucose in the cytoplasm relative to the outside concentration. However, if

TABLE IV

Computer simulations of the effects on cellular activities of the removal of sugar phosphate

The data A were from computer simulations similar to Fig. 5 and represent the steady-state results which would be given by cells having a dynamic diffusion barrier concentrating mechanism. The data B are from computer simulations for cells with a simple equilibrating system for sugars and with kinase and phosphatase enzymes in the cytoplasm. The outside concentration was 5 mM in all cases. The parameters were as in Fig. 5; the K_m for 'other' activities was arbitrarily set at 3.6 mM.

V_{max} 'other' activity ($mM \cdot s^{-1}$)	Concn. in foyer (mM)	Concn. in cytoplasm (mM)	Average saturation-kinase (%)	Steady-state sugar-*P* (mM)	'Other' activity (mM/h)
A:					
0.10	0.35	3.94	62	1.51	106
0.05	1.10	5.17	71	2.87	80
0.02	2.38	6.88	78	5.55	44
0.01	3.24	7.91	81	7.88	25
0.00	4.70	9.57	85	13.63	0
B:					
0.10	1.18	1.18	48	1.05	81
0.05	1.72	1.72	57	2.00	64
0.02	2.71	2.71	68	3.99	38
0.01	3.43	3.43	73	5.73	22
0.00	4.81	4.81	79	10.20	0

the low concentrations in the foyers are taken into account, the accumulation in the cytoplasm is still seen to be considerable. The sugar phosphate was low as this was the immediate precursor for the 'other' activities. Table IVB shows similar data from a computer simulation for cells which had only a simple equilibrating system for sugar.

The most noticeable difference of the cells with the dynamic diffusion barrier was their ability to maintain a higher saturation of the phosphorylating enzymes and hence a higher steady-state level of sugar phosphate for use for 'other' metabolic activities. The cells without the dynamic system were not able to maintain the same level of sugar phosphate in the steady state and the higher rates of 'other' activities were substantially reduced.

Thus the advantages of the accumulating mechanism for free sugars are two-fold; firstly, it supplies an additional potential store of substrate for cells with other synthetic functions and secondly, it ensures that the immediate precursor, glucose 6-phosphate (or perhaps fructose 6-phosphate), is available at a more optimal concentration.

The computer simulations used to represent the results of experiments carried out with maximal insulin stimulation as reported by Foley and Gliemann [1] are also of interest. To use the same computer program for this purpose it was necessary to increase (i) the rate of sugar transport (F_g of the Regen and Tarpley [13] kinetics) by a factor of 10 (ii) the V_{max} of the kinase enzymes by a factor of 6–10 and (iii) the value of P_d/AF by a factor of 3–7. These values were relative to those used in computer simulations for the non-insulin-stimulated experiments of this work and the one in the above report [1]. The phosphatase activity also required individual adjustment for each experiment, and a modulator term, which gave an additional activity at higher sugar phosphate levels, improved the fit.

The nearly parallel increase in (i) sugar transport (ii) kinase activity and (iii) the P_d/AF values was strongly suggestive of a 'recruitment' of additional sugar transporters and their associated 'funnel' complexes being involved. However, a physical recruitment into the membrane, as proposed by Wardzala et al. [16] may not be necessary. In the resting state, the sugar transporters may be biased to have their empty clefts predominantly facing inward [17] and due to a falling cytoplasmic glucose level the concentration in some of the foyers may be brought to zero by the dynamic diffusion barrier mechanism. The transporters with inward-facing clefts but lacking substrate would then become quiescent and this could result in a loss of water from the 'foyer' end of the 'funnels', followed by a partial shut-down of the complexes. Such shut-down funnels would come close to a physical diffusion barrier and their energy expenditure would be greatly reduced.

It was also proposed by Widdas [17] that one action of insulin may be to reverse the bias on the sugar transporters and to favour the empty clefts facing the outside. If this occurred, the transporters with clefts exposed to the extracellular glucose at 5–10 mM would become active and transport glucose into the 'foyers' of the 'closed' funnels. Sugar would bring in water osmotically and when the enzyme zones became active, additional water would be drawn in by the ions associated with the sugar phosphate, and so the funnels would open up or be 'recruited'. Thus an action of insulin on the sugar transporters could have a 'knock-on' effect on the accumulating mechanism as a whole.

At present, it is assumed that the recycling of sugar and sugar phosphate is associated with the expenditure of metabolic energy from the breakdown of ATP. However, in addition to the partial 'shut-down' of funnel complexes in the resting state there may be some economy from the amplification factor in the dynamic diffusion barrier hypothesis which may be significantly reducing the phosphorylating activity required to support the concentration gradient.

For the computer simulation of sugar phosphate levels, the principle used was that if the V_{max} for phosphorylation and dephosphorylation are closely similar, then the steady state for a recycling system depends on the degrees of saturation and the respective K_m values. Although this stratagem can be used to give a fair representation of the experimental values in computer simulations there may be several other factors controlling these variables under physiological conditions [10].

The K_m values used were also arbitrary and, in any case, would be different for different sugars and sugar phosphates so that the steady-state values would also vary with the sugar being used. The kinase K_{mp} (1.3 mM) used in the present work was somewhat higher than the K_m values given for deoxyglucose (0.6–0.86 mM) in other tissues [9], but computer simulations can readily be obtained using such lower values. However, it should be emphasized that the accumulation of a free sugar under the dynamic diffusion barrier hypothesis depends on the rate of phosphorylation of that sugar in the funnel complexes, and is not primarily affected by the rates of phosphatase activity, or the intracellular levels of sugar phosphate. There can be no accumulation by this mechanism of any sugar which is not phosphorylated.

A sugar which is accumulated would not induce the uphill transfer of a competitive sugar, since its concentration in the foyers where competition at the inner transporter site occurs will always be lower than that in the cytoplasm and close to the outside concentration. This property of the dynamic diffusion barrier hypothesis also explains why the counter-current formula (Widdas [18]) used by Foley, Cushman and Salans [19] suggested that glucose only equilibrated across the

adipocyte membrane. As a sugar which is phosphorylated, glucose should be accumulated, at least potentially, just like 2-deoxyglucose, and this has been assumed throughout the discussion.

Conclusion

Although there are difficulties in this hypothesis as well as in the earlier hypotheses which have been put forward to explain how adipocytes are able to accumulate certain sugars against the concentration gradient, it has the practical merit of offering a simple basis for writing a computer program to simulate experimental results.

The hypothesis also pinpoints structural and thermodynamic considerations worthy of further study; if these can be resolved the concept of the dynamic diffusion barrier mechanism, and the recycling of free sugar and sugar phosphate which it involves, may come to be viewed as an adaptation with several facets of biochemical and physiological significance as well as interest.

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