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THE MECHANISM OF THE UPTAKE OF SUGARS BY THE RAT HEART AND THE ACTION OF INSULIN ON THIS MECHANISM

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The possibility that the action of insulin involves the permeability of muscle cells to sugars (reviewed by Levine & Goldstein, 1955) has led to a number of investigations on the kinetics of sugar uptake by muscles. Some of these have been interpreted as showing that sugar uptake follows first-order kinetics (Kipnis & Cori, 1957; Resnick & Hechter, 1957). This is in disagreement with the observations on erythrocytes (reviewed by Bowyer, 1957) and with some studies on muscle preparations (reviewed by Park, Reinwein, Henderson, Cadenas & Morgan, 1959). This paper presents some findings on the time course of the uptake of two non-metabolized sugars by a perfused rat heart preparation in which special care has been taken to ensure adequacy of perfusion, stability of sugar permeability and freedom from endogenous insulin action (Zachariah, 1961). It is shown that these results are consistent with a carrier-type mechanism for the transport of sugars. The effects of insulin on the kinetics of the carrier mechanism suggest new interpretations of the nature of insulin action.

Theoretical

Two characteristics of sugar uptake processes that need to be explained by any model of the transport mechanism are of special significance in the choice of a kinetic model: (i) The uptakes of certain sugars apparently reach equilibrium even when a considerable concentration gradient may still be present across the cell membrane. (ii) The relation between the rate of sugar uptake and external sugar concentration is, in the case of many sugars, not linear but of the Michaelis–Menten type. It has been repeatedly suggested that (i) is due to the presence of intracellular barriers to sugar entry in the absence of insulin (Kipnis & Cori, 1957; Morgan, Randle & Regan, 1959). But the observations that the 'volume of distribution' of the sugar is inversely related to its external concentration, as

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can be deduced from the observations of Wick & Drury (1953), Drury & Wick (1954) and Morgan & Park (1958) and that the volumes of distribution of different sugars under identical conditions vary widely (Helmreich & Cori, 1957; Norman, Menozzi, Reid, Lester & Hechter, 1959) make it difficult to maintain a simple concept of an intracellular barrier responsive to insulin. The second characteristic points to a mechanism other than simple diffusion.

A simple concept which can explain these two phenomena as well as many other characteristics of sugar uptake is the carrier hypothesis (Widdas, 1952, 1954). In a simplified version of this model (Bowyer, 1957), the sugar can be described as traversing the membrane in combination with a component of the membrane, the carrier. The carrier may be assumed to move across the membrane by a process equivalent to diffusion at a rate independent of whether it is in combination with the sugar or not. The velocity constants for the formation and dissociation of the sugar–carrier complex may be taken to be the same on the two surfaces of the membrane. In such a model the rate of transport of the sugar would depend primarily on the difference between the concentrations of the sugar–carrier complex at the two boundaries of the membrane.

TABLE 1. Effect of alterations in the equilibrium constant of the dissociation of sugar-carrier complex on the rate of sugar uptake

Apparent equilibrium constant 'M' (mM)	Concn. of sugar-carrier complex		$SC_o - SC_i$ (mM)
	Outer surface SC_o (mM)	Inner surface SC_i (mM)	
0·1	0·9967	0·8571	0·1396
1·0	0·9677	0·3750	0·5927
10·0	0·7500	0·0566	0·6934
100·0	0·2308	0·0060	0·2248

Sugar concentration at the outer surface, 30 mM; sugar concentration at the inner surface, 0·6 mM. Total amount of carrier at each surface is assumed to be equal to 1 mM. SC_o = fraction of available carrier combined with sugar at outer membrane surface; SC_i = fraction of available carrier combined with sugar at inner membrane surface.

A low concentration gradient of the sugar–carrier complex across the membrane can result from a very low or a very high affinity of the carrier for the sugar. This is best illustrated by considering an example. Taking it that the carrier is equally distributed between outer and inner parts of the membrane and that the fractional saturation of carrier is given by the ratio $S/(S+K)$, where S = sugar concn., and K = equilibrium constant of the sugar–carrier reaction, then the state of affairs at a given intracellular concentration can be illustrated by the example in Table 1.

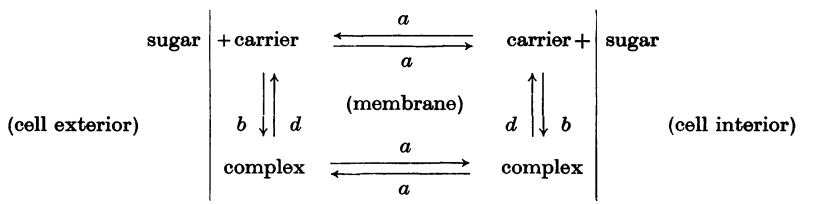
It will be clear from this Table that at intracellular concentrations which are low relatively to the external one, the carrier at the inner border of

the membrane may be so nearly saturated, provided that the equilibrium constant is low enough, that further sugar penetration must be very slow. Increase in equilibrium constant, though it reduces the fractional saturation at the outer border of the membrane appreciably, has a much more marked effect on saturation at the inner border, and the sugar–carrier complex concentration gradient is markedly increased. When the equilibrium constant is still further increased the fractional saturation of carrier at the outer border is further decreased markedly and this decrease can more than offset further diminution in saturation at the inner border, thus diminishing the sugar–carrier complex concentration gradient.

If sugar penetration conforms to the carrier model, insulin could increase penetration in one of four ways: by (*a*) increasing the amount of available carrier, (*b*) increasing the mobility of the carrier–complex in the membrane, (*c*) increasing affinity of carrier for sugar if the carrier has a low affinity, (*d*) decreasing affinity of carrier for sugar if the carrier has a very high affinity.

Test of the applicability of the carrier hypothesis and exploration of the mode of action of insulin can therefore be made by following the time course of penetration of sugars, and by endeavouring to find values for the parameters of penetration which will throw light on any changes caused by insulin in carrier concentration and affinity.

The general nature of the model proposed by Widdas is shown in the following scheme:



The rate of penetration of sugar into the cell according to this model is given by Bowyer (1957) as:

$$\text{Transfer rate} = \frac{Kad}{2b} \cdot \frac{C_1 - C_2}{C_1C_2 + \left(\frac{a+d}{b}\right)(C_1+C_2) + \left(\frac{a+d}{b}\right)^2 \frac{a^2}{b^2}}, \qquad \text{(i)}$$

where K = the sum of the concentrations of carrier at the two surfaces,
C_1 = concentration of sugar at the external surface,
C_2 = concentration of sugar at the internal surface.
a = transfer constant of carrier (free or combined) in the membrane,
b = velocity constant of sugar–carrier complex formation, and
d = velocity constant of sugar–carrier complex dissociation.

This can be rearranged and integrated if it is assumed that a^2/b^2 is negligible. In fact it can be shown that in our conditions, provided that a is appreciably smaller than d, no significant error is introduced by neglecting this term. Since d is the velocity constant of dissociation of the complex and a that of transfer across the membrane, such a relation of the two constants seems likely.

When the external sugar concentration is constant and equal to C_1 and the initial internal concentration is zero, the integrated equation is:

$$(C_1+M)^2 \ln \frac{1}{1-f} - C_1(C_1+M)f = Nt, \qquad \text{(ii)}$$

$$M = \frac{a+d}{b}, \quad N = \frac{Kad}{2b} \quad \text{and} \quad f = C_2/C_1.$$

Thus M is the apparent dissociation constant of the sugar–carrier complex and N may be called a membrane constant. When C_1, M and N are constant, changes in t will produce proportionate changes in the left-hand side of equation (ii). Thus if f values for the same concentration of sugar are determined at different values of t (i.e. the time course of sugar uptake), it is possible to estimate the values of M and N.

One interesting aspect of the carrier hypothesis is that it accounts for a phenomenon which is difficult to explain in any other way, namely that the movement of a sugar in one direction can be influenced by the movement of another sugar using the same transport mechanism in the opposite direction. This was first pointed out by Widdas (1952) and has been demonstrated in erythrocytes (Park, Post, Kalaman, Wright, Johnson & Morgan, 1956), and in the rat heart (Morgan & Park, 1958). One implication of this is that the same sugar under appropriate conditions can act either as an inhibitor or as accelerator of the uptake of another sugar. This paradoxical effect may be explained as follows: The uptake of sugar by the carrier mechanism can be described as the algebraic sum of the independent processes of influx and efflux of the sugar. Thus any factor which tends to inhibit the influx more than the efflux would tend to produce a reduction in the net uptake of sugar. This is the effect observed when a mixture of a test sugar and a competing sugar is added to the external medium. On the other hand, any factor which tends to produce a preferential inhibition of efflux would tend to cause an acceleration of the net uptake of sugar. Such an effect can be produced by a competing sugar, if it is present in about the same or greater concentration inside the cell as on the outside before the test sugar is added to the medium. In such a situation, in the early stages of the uptake of the test sugar its efflux will be inhibited much more than its influx because the ratio of the inhibitor

to the test sugar is much greater on the inside than on the outside. A demonstration of this effect would add to the evidence in favour of the carrier hypothesis.

METHODS

The technique of perfusion of the heart, the modified Krebs-bicarbonate perfusion medium containing serum protein, and the method of estimating the sugars have been described elsewhere (Zachariah, 1961).

All hearts were perfused for 30 min with a medium lacking the sugar to be studied and then transferred to a second apparatus filled with medium containing this sugar in a standard concentration (30 mM). Hearts were then perfused for 15, 25, 35, 45 or 60 min, and fractional penetration into the cell water was then determined.

The preliminary period of perfusion with the sugar-free medium is referred to as 'pre-perfusion' and the period of subsequent perfusion with the sugar-containing medium as 'perfusion'.

Insulin (crystalline, British Drug Houses, Batch No. 2189), when used, was present in the media for both perfusion and pre-perfusion in a concentration of 0·2 m-u./ml.

It has been shown that under the conditions of the present experiments the intracellular water content of the cell is constant and has the mean value of 2·450 ml./g dry weight (Zachariah, 1961). On this basis the cell-water content, extracellular space and fractional penetration of cell water by sugar were calculated from the dry weight of the tissue by methods described previously.

RESULTS

Time course of permeation of the heart by pentoses

The number of determinations made for the estimation of extent of permeation at any one time varied between 4 and 8. The means and standard errors are given in Table 2, and the closest-fit curves of the form of equation (ii) are shown in Figs. 1 and 2.

TABLE 2. Time course of penetration of L-arabinose and D-xylose into the intracellular water of the perfused rat heart in the presence and absence of insulin (0·2 m-u./ml.)

Length of perfusion (min)	L-arabinose		D-xylose	
	No insulin	Insulin	No insulin	Insulin
15	17·04±2·91 (6)	38·59±5·03 (5)	22·43±2·14 (5)	46·12±5·49 (4)
25	16·25±1·90 (8)	45·99±4·01 (6)	27·42±2·53 (5)	57·46±3·00 (4)
35	22·33±3·72 (7)	59·34±7·92 (5)	30·91±2·70 (5)	73·33±2·80 (5)
45	25·31±2·61 (7)	70·44±5·47 (5)	34·23±3·76 (5)	—
60	25·58±2·96 (8)	73·80±5·57 (6)	40·24±3·61 (5)	80·00±4·17 (5)

The figures give the intracellular concentration as percentage of the extracellular concentration (30 mM) with the standard errors of the mean and the numbers of observations in each set.

The values of the constants M and N (eqn. ii) corresponding to these fitted curves are given in Table 3. It will be seen that in the absence of insulin the values of M, the half-saturation concentration, are very low compared with the concentration of pentose used (30 mM), and that in the presence of insulin they are much higher, i.e. there is apparently a fall in the affinity of the carrier for the pentose.

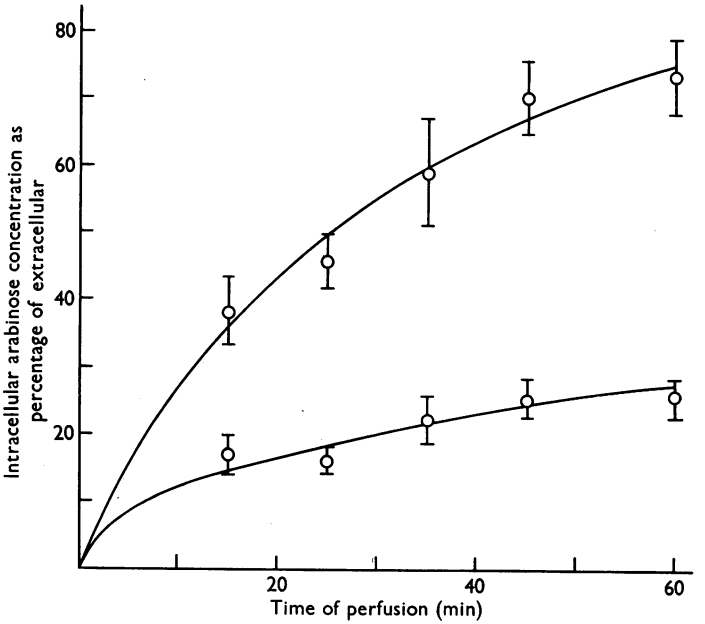


Fig. 1. The time course of penetration of L-arabinose (30 mM) into the cells of the heart. The vertical bars represent ± the standard error of the mean. The upper curve refers to the insulin experiments. The insulin concentration was 0·2 m-u./ml.

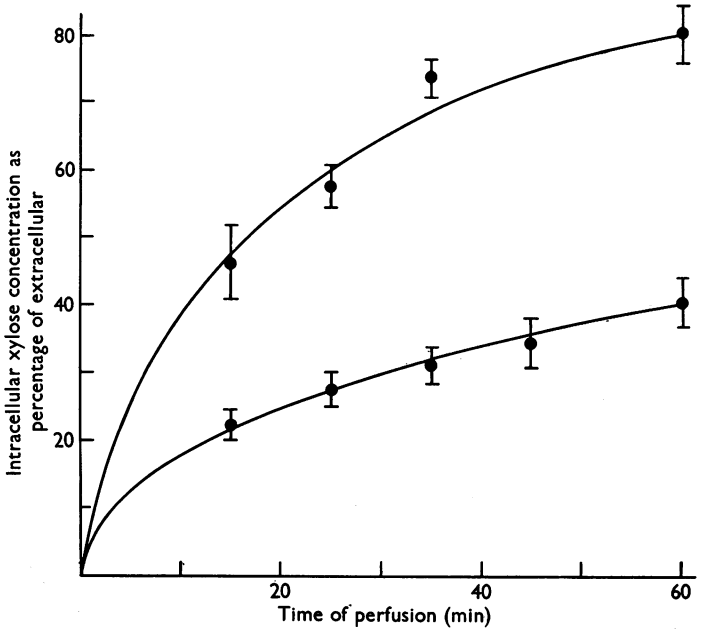


Fig. 2. The time course of penetration of D-xylose (30 mM) into the cells of the heart. The vertical bars represent ± S.E. of the mean. The upper curve refers to the insulin experiments. The insulin concentration was 0·2 m-u./ml.

Effect of glucose on the penetration of pentoses into the heart. Hearts were perfused for 30 min with protein–pyruvate medium and then perfused for 35 min with similar medium with added pentose (30 mM) and glucose (10 mM). The effect of this treatment on the percentage permeation of the hearts by pentose is shown in Table 4. There is no doubt that glucose exerts a profound inhibitory effect on permeation.

TABLE 3. Estimated values of 'M' and 'N'

		L-arabinose	D-xylose
No insulin	M (mM)	0·06	0·21
	N (mM².min^{-1})	0·71	1·71
	N/M (min.mM^{-1})	11·8	8·1
Insulin	M (mM)	25·0	6·6
(0·2 m-u./ml.)	N (mM².min^{-1})	50·3	22·8
	N/M (min.mM^{-1})	2·0	3·5

These values provide the best fit of the observations on the time course of sugar uptake to the descriptive equation for transport by the carrier mechanism given as equation (ii) on p. .

TABLE 4. Inhibition of pentose uptake by glucose

Pentose (30 mM)	Insulin (0·2 m-u./ml.)	Percentage penetration of cell water by pentose in 35 min*: Without glucose (A)	With glucose (10 mM) (B)	$A-B$	$100\frac{A-B}{A}$
L-Arabinose	Nil	22·33 ± 3·72 (7)	8·95 ± 1·72 (4)	13·38	59·9
	+	59·34 ± 7·92 (5)	42·96 ± 7·19 (5)	16·38	27·6
D-Xylose	Nil	30·91 ± 2·70 (5)	23·63 ± 3·01 (6)	7·28	23·6
	+	73·33 + 2·80 (5)	60·83 + 4·69 (5)	12·50	17·0

* Mean ± S.E. of mean (no. of observations).

Effect of galactose on permeation. One series of experiments with L-arabinose was made in the same way as the glucose experiments, using 10 mM galactose. The mean percentage penetration of the intracellular water in 35 min was 18·44 ± 2·82 (5) as compared with the corresponding figure without galactose (Table 1) of 22·33 ± 3·72 (7).

In a second series of experiments the hearts were pre-perfused with 10 mM galactose and then perfused for 35 min with 30 mM L-arabinose and 10 mM galactose. The mean percentage penetration of the pentose was 24·62 ± 3·57 (5).

There is here an indication that galactose can either accelerate or slow down penetration of the pentose, depending on the way in which it is presented to the heart.

DISCUSSION

The time course of sugar penetration found in these experiments does not conform to first-order kinetics. Figure 3 is a plot of ln $1/(1-f)$

against time of the results of Table 2. Although there is an approximately linear relation in all cases, no one of the lines goes through the origin, and the intercepts are far too large to be ascribed to experimental error. For first-order relations the lines must pass through the origin.

It has previously been suggested (Bronk & Fisher, 1957) that the time course of sugar penetration could be accounted for on the hypothesis that uniformly slow diffusion occurred throughout the cell contents. A curve describing this sort of process has been presented by Crank (1956) and it can be shown that the present data do in fact conform to such a curve.

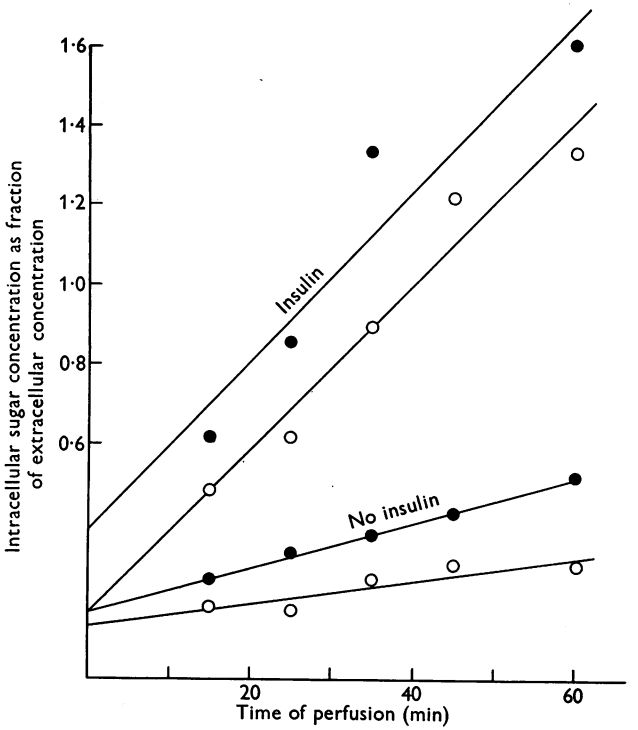


Fig. 3. The lack of correspondence of sugar penetration with first-order kinetics. ○ = L-arabinose; ● = D-xylose.

However, the relationship described in equation (ii) is indistinguishable from the Crank curve in the range of values of M and N applicable to our data.

Discrimination between the slow-diffusion and carrier hypotheses can, however, be effected. The slow-diffusion hypothesis cannot explain the competition between pentoses and glucose or galactose which has been demonstrated in these experiments and in those of Morgan & Park (1958). Further, the experiments in which galactose presented to the outside of

the cell has an inhibitory effect, whilst galactose presented simultaneously to both sides of the cell membrane has an acceleratory effect, suggest strongly that the determinants of permeation are in the cell membrane itself.

On the other hand, all these effects are precisely what is to be expected on the carrier hypothesis. We are therefore of the opinion that the conformity of the observations to equation (ii), together with the competition and acceleration effects, make a *prima facie* case for the carrier hypothesis.

A decision between the hypotheses could be made on an experimental basis. If intracellular concentration is expressed as a fraction of extracellular concentration the Crank curve should be of the same form whatever the concentration of sugar added to the perfusate. On the other hand, as inspection of equation (ii) will show, on the carrier hypothesis, the form of the curve for any given pair of values of M and N will be markedly dependent on the external sugar concentration. Experiments to test the effect of change of concentration of sugar on the time course of penetration are in progress.

The effects of galactose on pentose penetration

It will be seen that the two effects of galactose which have been described are of small magnitude, and not very firmly established. A probable reason for this is that the initial favourable state for the observation of these effects would, on the carrier hypothesis, rapidly disappear. If galactose is initially wholly outside the cells it can compete with the pentose only by combining preferentially with carrier, and so will penetrate the cells and reduce the effective affinity for pentose of the carrier at the inner border of the membrane, thereby increasing the concentration difference of pentose-carrier complex across the membrane and offsetting, at least in part, the inhibitory effect of the external galactose. If galactose is initially in equal concentration on both sides of the membrane, i.e. if galactose flux in both directions is equal, the presentation of pentose to the outer border of the cell membrane will inhibit galactose influx and bring about a net loss of galactose from the cell. This sort of effect has already been described by Morgan & Park (1958) for hearts equilibrated with 3-methylglucose and then perfused with glucose and 3-methylglucose. This effect will result in rapid diminution of the initial acceleratory effect of galactose. The finding of conditions in which pentose penetrations of adequate extent for exact measurement can take place in very short times is a prerequisite for the full establishment of the dual effects of a competing sugar.

The effect of glucose on pentose penetration

It is not possible to interpret these data in any detail. Since glucose entering the cells will be metabolized in large part, and since the pentose will alter the rate of entry, a complex situation results. The important point is that glucose does inhibit the penetration of the two pentoses studied both in the presence and absence of insulin, and there is therefore ground for supposing that the pentose penetration process is also the process responsible for the penetration of glucose into the cells. The common sensitivity of these processes to insulin suggests that the mode of action of insulin on pentose penetration which we propose to deduce from our observations is also the mode of action of insulin on glucose penetration.

The significance of the parameters of the carrier equation

The values of the constants M and N in equation (ii) which correspond to the curves fitted to the data in Fig. 1 are given in Table 3. The constant M is the Michaelis constant of combination of carrier with sugar. It will be seen that, in the absence of insulin, this constant is very much lower than the external sugar concentration used. When insulin is present this constant rises markedly. Thus, if the carrier hypothesis is valid, one effect of insulin must be to lower markedly the affinity of the carrier for the sugar being transported. As has already been pointed out (p. 74), a carrier with very high affinity for its substrate will be inefficient, and its efficiency will be increased by fall of affinity, within limits.

Insulin appears to have this effect, and this effect would in itself be adequate to account for its action. Before discussing further implications of this interpretation of our results, it is necessary to look at the changes brought about by insulin in the second parameter, N, of equation (ii).

The parameter N is equal to $Kad/2b$, where K measures the content of carrier in the membrane, and a, b and d are the velocity constants respectively of movement of carrier across the membrane, of association of sugar and carrier, and of dissociation of the sugar-carrier complex. The assumption made in the introduction that the term a^2/b^2 in equation (i) is negligible is tantamount to the assumption that a is negligible in relation to d. This implies that $M = (a+d)/b$ is negligibly different from d/b. Thus N/M is negligibly different from $Ka/2$.

It will be seen from Table 3 that insulin increases the value of N, but far less so than that of M, so that N/M is lower in the presence of insulin than in its absence. Thus insulin seems either to decrease the amount of carrier or to decrease the ease with which carrier crosses the membrane.

Both these effects are physical possibilities if one assumes that insulin

affects the carrier by combining with it. If the carrier-insulin complex is much more efficient than free carrier, then the effect will be that, in the presence of insulin, sugar transport will be virtually completely effected by that fraction of the carrier combined with insulin. The effective value of K would therefore decrease. If the carrier is a small molecule, the value of a could be markedly decreased by combination of carrier with insulin. If the carrier were a large molecule, such an effect would be less likely.

This analysis leads to several consequences susceptible to experimental test. The first is that, if the effective carrier, in the presence of any significant amount of insulin, is the insulin-carrier complex, then the value of M should not change with increasing concentrations of insulin. The second is that, if the proportion of the carrier present as insulin-carrier complex rises as the insulin concentration increases, the value of N/M should increase with increasing amounts of insulin to a maximum. The third is that this maximal value of N/M should be equal to the value in the absence of insulin if the ease of movement of the insulin-carrier complex across the membrane is indistinguishable from that of free carrier, and should be less than it if the complex moves less readily than free carrier.

General considerations

It is important to keep in mind that the permeability of one particular kind of cell membrane to a particular species of solute can be altered in different ways. It has been shown in a previous paper (Zachariah, 1961) that in the absence of insulin the permeability of rat heart cells to pentoses rises sharply with time unless an appropriate plasma protein is present in the perfusate. This phenomenon is not susceptible to analysis by the methods used here, since the permeability of such cells is changing continuously. But, since this increase in permeability is brought about by the absence of something from the perfusate, and not by the presence of something, e.g. of insulin, it is presumably of a different nature. This may have a bearing on the claim by Resnick & Hechter (1957) that these are two separable sugar permeation processes in the rat diaphragm. It seems likely therefore that studies of the effect of insulin must be made either in our conditions of stable permeability or in hearts perfused for sufficiently long with protein-free perfusate to attain, if possible, a new stable state of increased permeability.

The point of greatest general importance in this work is that it has become apparent that 'activation' of the permeation mechanism for sugars by insulin can be accounted for by partial 'inhibition' of the carrier. If subsequent work substantiates this proposition, it might prove of value in connexions other than that of reorienting thinking about the nature of insulin action on sugar transport. There seems no reason why

insulin should not combine with carriers in the cell membrane which are specific for solutes other than sugars, and in instances in which the affinities of such carriers for their substrates are low an action of insulin on them similar to that postulated here for the sugar carrier would result in inhibition of penetration of their substrates into the cells.

The relation between carriers and enzymes

The essential feature of a carrier is that it should be capable of combining reversibly with its substrate. If this should be the sole requirement, then any enzyme present in the cell membrane should be able to act as a carrier of its substrate or of its competitive inhibitors. It is even conceivable that an enzyme might transport its substrate without appreciable transformation, especially if the substance of the cell membrane formed an unfavourable medium for co-factors of enzymic action.

It may therefore well be the case that there is no fundamental discrepancy between the view that enzymes play a part in permeation and the view that permeation is a passive process involving specific membrane carriers.

SUMMARY

1. Measurements have been made of the time course of penetration of L-arabinose and D-xylose into the perfused rat heart, using the precautions described by Zachariah (1961) to ensure a period of stable permeability.

2. The effect of a submaximal concentration of insulin (0·2 m-u./ml.) on the time course of penetration of the pentoses has been determined.

3. When glucose is added to the perfusate it reduces the rate of penetration of the pentoses.

4. Galactose added to the perfusate at the same time as the pentose reduces the extent of pentose penetration. If the heart is previously equilibrated with galactose, and pentose is then added to the perfusate, the rate of pentose penetration is increased.

5. These findings are shown to be consistent with the hypothesis that the pentose enters the cells in combination with a specific carrier in the cell membrane.

6. The accelerating effect of insulin on pentose penetration is shown to be explicable in terms of a reduction of the affinity of the carrier for the pentose.

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