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CHANGES IN THE PHOSPHATIDE PATTERN OF YEAST CELLS IN RELATION TO ACTIVE CARBOHYDRATE TRANSPORT

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SUMMARY

1. In recent papers experimental evidence has been presented indicating a relationship between polyphosphate and active transport of glucose in yeast cells. This is expressed in the hypothesis that, during active transport of glucose, a carrier–phosphate–glucose complex is formed.

2. Studies on galactose uptake in a yeast mutant g_1G_2, which is defective in galactose metabolism, show that during active transport of galactose a concomitant conversion of phosphatidylglycerolphosphate to phosphatidylglycerol occurs. The same conversion was found during the uptake of several amino acids.

3. Preloading mutant g_1G_2 cells with radioactive phosphate shows that the increase of radioactivity in phosphatidylglycerolphosphate starts after a considerable lag time. This lag time is approximately the same for polyphosphate, which suggests the reaction:

polyphosphate + phosphatidylglycerol → phosphatidylglycerolphosphate

4. This led to the formulation of the following hypothesis: phosphatidylglycerolphosphate is an intermediate in the transfer of a phosphate moiety from polyphosphate to a carrier–phosphate–substrate complex and the reaction phosphatidylglycerolphosphate→ phosphatidylglycerol is important for active transport in general.

INTRODUCTION

Experiments by VAN STEVENINCK[1] and by VAN STEVENINCK AND BOOIJ[2] have demonstrated that a relation exists between active glucose transport and the presence of polyphosphate in baker's yeast. Part of the polyphosphate is present on the exterior of the yeast cells. In monoiodoacetate-inhibited yeast the uptake of glucose is correlated with the disappearance of phosphate groups from the exterior of the cell. This phenomenon suggests that the uptake of glucose is coupled with a phosphorylation.

As phosphorylated derivatives of glucose are not taken up by the yeast cell[3] it is improbable that a simple phosphorylation of hexose can be the explanation of the phenomenon. The presence of phosphatases at the outside of the membrane[4] would also highly reduce the probability of the presence of free phosphorylated glucose.

These considerations led VAN STEVENINCK to the hypothesis that glucose will be coupled to a membrane constituent by phosphorylation or that a membrane constituent will be phosphorylated before the binding to glucose. After this reaction the hypothetical combination membrane constituent–phosphate–glucose is transported to the inside of the cell, where glucose or a phosphorylated derivative of glucose is split off. This unknown membrane constituent could accordingly be called the glucose carrier.

The aim of this investigation is to establish whether the uptake of a sugar by yeast cells is correlated with a change in the lipid pattern of the yeast cell, especially as regards the phosphate turnover in the lipids.

Preliminary testing with baker's yeast Strain CBS 1172 and glucose as a substrate revealed some difficulties to be encountered in the experimental approach of specific transport reactions.

Glucose, which is used up in metabolic reactions by baker's yeast, causes so many changes in the lipid pattern of the yeast cell that the consequences, if any, of a specific transport mechanism cannot be distinguished separately. It could be argued that, to prevent the changes in the lipid pattern caused by metabolic reactions, either non-metabolizable sugars such as sorbose[5,6] or galactose[7] (in uninduced cells) must be used or, with the use of glucose as a substrate, the cells must be poisoned with monoiodoacetate to prevent glycolysis[6]. The changes caused by metabolic reactions only can be established with the use of ethanol as a substrate.

However, the transport of non-metabolizable sugars in yeast is a "carrier-mediated facilitated diffusion" and not an active transport[8,9]. The uptake of galactose and sorbose in baker's yeast therefore is not associated with a phosphorylation reaction and changes in the phosphatide pattern are not to be expected and were not found.

The action of iodoacetate is an inhibition of glycolysis, thereby allowing free glucose to accumulate in the cell. But in addition, the metabolic support for active transport is removed, so that the only possible transfer of sugar is *via* the facilitated diffusion system[8]. Again, changes in the phosphatide pattern are not to be expected and were not found.

The changes in the lipid pattern with the use of ethanol as a substrate are approximately the same as with glucose. However, the conclusion that the changes, with ethanol as well as with glucose, are caused by metabolic reactions only, is premature, because the results do not exclude the existence of an additional effect of transport and metabolic reactions in the case of glucose.

It seems evident that baker's yeast Strain CBS 1172 cannot be used to establish the changes in the phosphatides caused by active transport reactions only.

However, yeast mutants have been found which are defective in the various stages of galactose uptake and metabolism. In the g_1 form the mutant is defective in the ability to produce galactokinase and in the g_2 form the mutant is defective in the ability to produce the active transport system (permease) for galactose[10,11]. In the form g_1G_2 the transport system can be induced but not the galactokinase. Before induction[8] galactose enters the cell slowly but is not metabolized. After induction, the transport rate of galactose increases 10-fold and free galactose accumulates in the cell. On induction the transport system for galactose changes from a facilitated diffusion to an active transport. The mutant g_1G_2 therefore enables us to determine the changes in the lipid pattern caused by active transport reactions only.

MATERIALS AND METHODS

Yeast

The yeast mutant, genetically defined as g_1G_2, which is defective in the ability to produce galactokinase, was obtained from the collection of DE ROBICHON-SZULMAJSTER[10]. This mutant was cultured aerobically at 27° during 48 h on a medium of the following composition: 0.3 % Bacto yeast extract, 0.3 % Bacto malt extract, 0.5 % Bacto peptone, 0.1 % Bacto tryptone, 0.005 % L-histidine, 0.003 % L-tryptophan and 1 % glucose in water.

After harvesting in a Sharples centrifuge the mutant was washed three times with distilled water. Adaptation to galactose was brought about by incubating the mutant cells (aerobically at 27°) in a normal culture medium without Bacto malt extract and in which the glucose was replaced by galactose. After induction the cells were washed several times with distilled water. The yeast was then starved in distilled water with aeration and subsequently washed again twice.

The Warburg method was used to insure that none of the g_1G_2 cultures utilized galactose. The active transport of galactose in the induced cultures was measured according to the method of DE ROBICHON-SZULMAJSTER[10,12].

Labeling of the phospholipids with radioactive phosphorus was carried out by incubating a 10 % aq. suspension of 30 g yeast with 2 mC sodium [^{32}P]orthophosphate at 27°, during 2 h, with aeration. The yeast was washed again 3 times before use in the experiments.

Extraction of lipids

The extraction of lipids from whole yeast cells according to the method of FOLCH, LEES AND SLOANE-STANLEY[13] resulted in very low lipid yields. The method of HARRISON AND TREVELEAN[14] in which yeast cells, prior to extraction, were broken by shaking a thick aqueous suspension of the yeast several hours with glass beads resulted in a non-reproducible formation of lysocompounds.

A method for the extraction of lipids from whole yeast cells with neutral solvents, which is not complete but which leads to reproducible results in regard to the ratio of the extracted lipids, is described below.

Five g (wet weight) of yeast are stirred 90 min at room temperature in 160 ml of methanol. After filtration the residue is extracted 90 min with 160 ml of a chloroform–methanol mixture (1:1, v/v). After filtration the residue is once more extracted 90 min with 160 ml of chloroform. To the combined filtrates chloroform is added until a final ratio of chloroform:methanol (2:1, v/v) is reached. Non-lipid contaminants are removed, according to the method of FOLCH, LEES AND SLOANE-STANLEY[13] with a 0.1 M KCl soln. The lipid layer is evaporated to dryness at 50° under vacuum–nitrogen and the residue is dissolved in isoamylalcohol–benzene (1:1, v/v) to a final concentration of 4 %, w/v.

Paper chromatography

For the paper chromatographic separation of the lipids a slit-feeding apparatus[15] is used. The separation is accomplished on formaldehyde-impregnated paper, according to the procedure of HÖRHAMMER, WAGNER AND RICHTER[16], with butanol–acetic acid–water. A chromatogram is shown in Fig. 1.

The identity of phosphatidylglycerol was established by comparison with a synthetic phosphatidylglycerol*. The chromatographic behaviour of the intact phospholipid and of the products formed by mild alkaline hydrolysis, performed according to the method of DAWSON[17] and FERRARI AND BENSON[18], was identical for the natural and synthetic phospholipids.

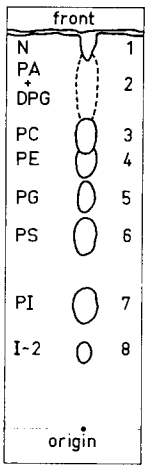

Fig. 1. Paper chromatographic separation of phospholipids on formaldehyde-impregnated paper. N, neutral fats and free fatty acids; PA, phosphatidic acid; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; I-2, an unidentified inositide.

Spot No. 7 is a mixture of two phospholipids: phosphatidylinositol and phosphatidylglycerolphosphate. Phosphatidylinositol prepared from green peas according to the procedure of ANSELL AND HAWTHORNE[19] yields a spot with the R_F of spot No. 7. Mild alkaline hydrolysis of phosphatidylinositol and of the eluate of spot No. 7 yields, in both cases, glycerylphosphorylinositol. However, the latter hydrolysis yields several other products as well. Besides small amounts of glycerolphosphate and inositolphosphate, a product is formed which could be identified as glycerylphosphorylglycerylphosphate[20], the deacylation product of phosphatidylglycerolphosphate.

Conclusive evidence for the identification of phosphatidylglycerolphosphate was obtained with phospholipase C (EC 3.1.4.3.) from *Bacillus cereus*[20]. This breakdown yields phosphatidylglycerolphosphate in addition to 1,2-diglyceride. The chromatographic behaviour of the former product was identical to that of the second compound in spot No. 7.

Quantitative estimation of phospholipids

The quantitative estimation of phospholipids was established by dividing an uncoloured part of the paper chromatogram, by means of a coloured guiding strip, into parts, each of which contained one phospholipid. The different phospholipids were

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eluted from the chromatogram and phosphorus was determined according to the method of CHEN, TORIBARA AND WARNER[21].

For those phospholipids which are not clearly separated by the paper chromatographic method described above, *e.g.* phosphatidylinositol and phosphatidylglycerolphosphate, the quantitative estimation was performed after a column chromatographic separation on silica gel, according to the method of HANAHAN, DITTMER AND WARASHINA[22]. With the use of chloroform–methanol elution mixtures, in which the methanol concentration is slowly increasing, phosphatidylglycerolphosphate leaves the column well before phosphatidylinositol.

Radioactive phosphorus was estimated by immersing a strip of the paper chromatogram, containing one phospholipid, in a scintillation fluid of the following composition:

500 ml isoamylalcohol, 500 ml benzene, 4 g 2,5-diphenyloxazole (PPO) and 0.2 g 1,4-bis-(5-phenyloxazolyl-2)-benzene (POPOP). Counts/min were measured with the Unilux liquid scintillation counter of Nuclear Chicago.

RESULTS

Estimation of the phospholipids of yeast mutant g_1G_2

In Table I quantitative estimates of the phosphatides of mutant g_1G_2 cells are given. Before this estimation the cells were suspended in water only, without the addition of any substrate, comparable to the blank in our experiments.

TABLE I

PHOSPHOLIPID COMPOSITION OF YEAST MUTANT g_1G_2

Phosphatides	*% total lipid-phosphate*
Phosphatidic acid + diphosphatidylglycerol	5.3
Phosphatidylcholine	45.2
Phosphatidylethanolamine	16.7
Phosphatidylglycerol	2.5
Phosphatidylserine	9.3
Phosphatidylinositol + phosphatidylglycerolphosphate	19.8
Unidentified inositide	1.2

Labeling of the phosphatides of mutant g_1G_2

30 g mutant g_1G_2 cells were, after induction into galactose, suspended in 300 ml distilled water to which 2 mC [^{32}P]orthophosphate were added. The suspension was aerated at 25° and the incorporation of ^{32}P in the phosphatides was determined as a function of time. The results for several phosphatides are shown in Fig. 2.

The incorporation of ^{32}P into phosphatidylglycerolphosphate, in contrast to other phospholipids, shows a considerable lag time. The increase of the per cent radioactivity in phosphatidylglycerolphosphate and a concomitant decrease in phosphatidylglycerol starts after about 25 min, whereas the total amount of the two compounds combined remains constant.

The per cent radioactivity of all other phospholipids, also of those not shown in Fig. 2 remains approximately constant after 5 min of incorporation time.

The normal pathway for the incorporation of ^{32}P in the phospholipids[23,24] leads *via* compounds such as ADP, ATP, CDP and CTP. BORST PAUWELS, LOEF AND HAVINGA[25] showed that the labeling of these intermediates reaches a maximum in a very short time (10–60 sec). A lag time of 25 min for the increase of radioactivity in phosphatidylglycerolphosphate suggests the existence of a donor of the terminal phosphate moiety in phosphatidylglycerolphosphate, other than those mentioned above.

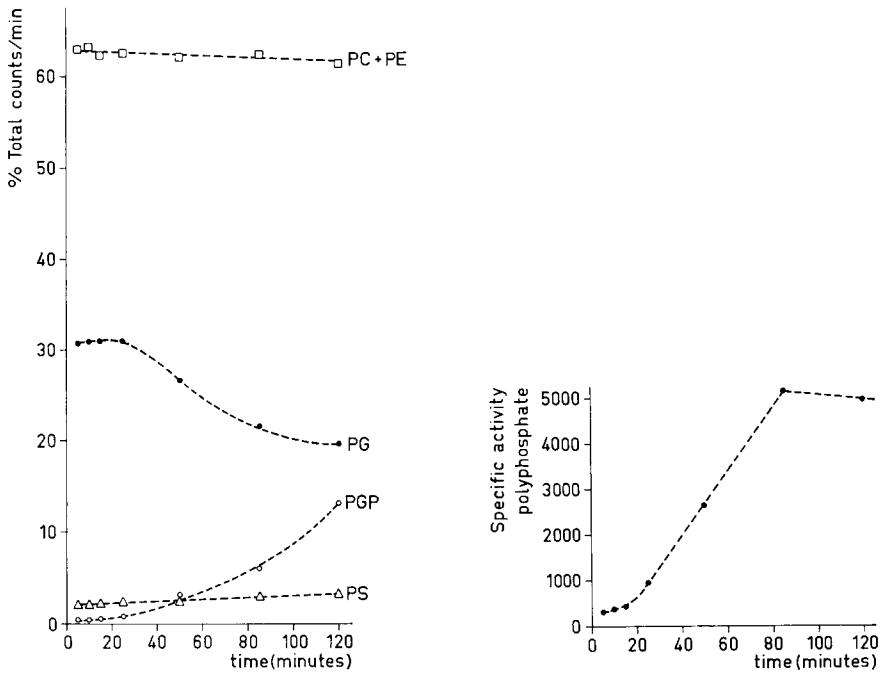

Fig. 2. Time curve of the incorporation of ^{32}P in several phosphatides of the yeast mutant g_1G_2. For abbreviations see Fig. 1. PGP, phosphatidylglycerolphosphate.

Fig. 3. Specific activity of polyphosphates from mutant g_1G_2 cells as a function of duration of exposure to ^{32}P.

Polyphosphates

In experiments on the incorporation of ^{32}P in polyphosphates, LOHMANN[26] demonstrated that a group of low-polymer polyphosphates incorporated ^{32}P after a lag time of approx. 30 min.

Extraction of polyphosphates, according to the method of VAN STEVENINCK[1], from mutant g_1G_2 cells shows a time curve for the incorporation of ^{32}P as demonstrated in Fig. 3. A considerable increase of radioactivity in polyphosphates is found after 25 min of incorporation time. This is comparable to the lag time for the increase of per cent radioactivity in phosphatidylglycerolphosphate. The concomitant decrease of per cent radioactivity in phosphatidylglycerol (see Fig. 2) suggests the existence of an equilibrium between phosphatidylglycerol and phosphatidylglycerolphosphate, an equilibrium in which polyphosphate can participate as a donor of the phosphate moiety.

Changes in the lipid pattern during active transport

Five g of labeled mutant g_1G_2 cells (2 h incubation with ^{32}P), adapted to galactose, were suspended in 50 ml distilled water. A second aliquot of 5 g of yeast, treated in the same manner, was suspended in 50 ml 1 % aqueous galactose solution. Both suspensions were aerated at 25°. After 2 min the cells were separated from the supernatant by centrifugation and suspended in methanol. The result of a subsequent analysis of the phospholipids is shown in Table II.

TABLE II

THE INFLUENCE OF THE UPTAKE OF GALACTOSE ON THE PHOSPHATIDES OF YEAST MUTANT g_1G_2

Phosphatides	*% total lipid-phosphate*		*% total counts/min*	
	Blank	*Galactose*	*Blank*	*Galactose*
Phosphatidic acid + diphosphatidylglycerol	5.3	5.5	4.9	5.4 (5.6)
Phosphatidylcholine + phosphatidylethanolamine	61.5	62.1	46.5	53.6 (53.5)
Phosphatidylglycerol	2.8	4.6	19.1	26.8
Phosphatidylserine	9.1	9.3	3.9	4.5 (4.5)
Phosphatidylinositol + phosphatidylglycerolphosphate	20.0	17.3	24.8	8.7
Unidentified inositide	1.3	1.2	0.8	1.0 (0.9)

The decrease of the per cent radioactivity of phosphatidylinositol *plus* phosphatidylglycerolphosphate is not in agreement with the decrease in per cent total lipid-phosphate. This indicates that the mixture phosphatidylinositol *plus* phosphatidylglycerolphosphate contains a relatively large amount of one compound (phosphatidylinositol) with a small specific activity and a small amount of a second compound (phosphatidylglycerolphosphate) with a large specific activity. Column chromatographic separation of phosphatidylinositol and phosphatidylglycerolphosphate reveals no difference in the total amount of phosphatidylinositol and in the per cent radioactivity of phosphatidylinositol before and after the uptake of galactose. Thus, during active transport of galactose, the amount of phosphatidylglycerolphosphate decreases. The concomitant increase in the amount of phosphatidylglycerol suggests a direct conversion of phosphatidylglycerolphosphate to phosphatidylglycerol during active transport.

The figures in brackets in the per cent radioactivity column in Table II are calculated from the figures in the blank by increasing them in proportion to the difference between the changes in the per cent radioactivity of phosphatidylglycerolphosphate and phosphatidylglycerol, *viz.* 8.4 %. From the agreement between the calculated figures and the ones actually found it is evident that the phospholipids, other than phosphatidylglycerolphosphate and phosphatidylglycerol, do not show significant changes under the described experimental conditions.

Reproducibility

The figures for the normal phospholipid composition of mutant g_1G_2 cells, as shown in Table I, fluctuate very slightly ($\pm$ 0.2) when different yeast cultures are used in the experiments. However, notwithstanding the many precautions which

were taken to standardize the experimental conditions, the incorporation of ^{32}P into the phospholipids and the magnitude of the changes in the lipid pattern during active transport show considerable fluctuations for different cultures.

In our experiments, the incorporation of ^{32}P into phosphatidylinositol and phosphatidylglycerolphosphate varies from 46 % to 8 % and the decrease in per cent radioactivity of phosphatidylglycerolphosphate during active transport varies from 16 % to 5 %. It is possible that these fluctuations are caused by small changes in the different enzymatic processes involved in lipid synthesis and exchange reactions. However, in all experiments with mutant g_1G_2 cells during active transport of galactose, there was a decrease in the amount of phosphatidylglycerolphosphate and a concomitant increase in the amount of phosphatidylglycerol. The figures in Table II are figures derived from one experiment, chosen to represent a series of similar experiments.

In the discussion section of this paper the possible function of phosphatidylglycerolphosphate and phosphatidylglycerol in an active transport mechanism is postulated. It is argued that these two phospholipids, apart from their presence in a reaction chain as proposed by KENNEDY[24], form an integral part of an active transport system. In this respect it is reasonable to assume that, although in all experiments the changes in phosphatidylglycerolphosphate and phosphatidylglycerol are in the appropriate direction, the magnitude of the changes fluctuates in different experiments.

The relation between active transport and the reaction phosphatidylglycerolphosphate → phosphatidylglycerol

It is to be expected that yeast mutant g_1g_2, in which the active transport system for galactose cannot be induced, will not show the reaction phosphatidylglycerolphosphate → phosphatidylglycerol described for mutant g_1G_2. Experiments with mutant g_1g_2 proved this to be the case.

Mutant g_1G_2, in which active galactose transport can be induced, shows a strong difference before and after induction. Before induction the characteristic phospholipid reaction does not occur after addition of galactose.

Furthermore it was shown that this reaction can be demonstrated when another substrate, e.g. tryptophan or glycine, is added to mutant g_1G_2. It may be expected that the reaction phosphatidylglycerolphosphate → phosphatidylglycerol is typical for many types of active transport in yeast cells.

DISCUSSION

Experiments with mutant g_1G_2 of *Saccharomyces cerevisiae*, preloaded with ^{32}P and in which active galactose transport is induced, show a characteristic change in the lipid pattern. Upon addition of galactose to the cells phosphatidylglycerolphosphate disappears partly and the amount of phosphatidylglycerol increases at the same time. As galactose is not metabolized, this reaction must be related to the transport mechanism.

The reverse reaction is observed when labeled phosphate is taken up by the cell in the absence of substrate. In this case, after a lag time of 25–30 min, two phenomena occur practically simultaneously: the label appears in polyphosphate and the per cent radioactivity of phosphatidylglycerol decreases whilst that of phosphatidyl-

glycerolphosphate increases. This suggests that polyphosphate acts as a phosphate donor to phosphatidylglycerol.

These results may be summarized in the following equation:

$$\text{Phosphatidylglycerolphosphate} \underset{\text{polyphosphate}}{\overset{\text{active transport}}{\rightleftharpoons}} \text{phosphatidylglycerol}$$

The question arises of how these phenomena should be interpreted. At first sight it seems attractive to suppose that phosphatidylglycerol acts as a carrier for galactose transport. This idea would tally with VAN STEVENINCK'S hypothesis[1,2] in the following way. Polyphosphate donates phosphate to phosphatidylglycerol at the outside of the membrane. The resulting complex (phosphatidylglycerol–phosphate–substrate) passes through the membrane and is split into two or three components. However, this hypothesis cannot be true as according to this view the amount of phosphatidylglycerol would either decrease or remain the same, while the amount of phosphatidylglycerolphosphate would increase. Our experiments show the opposite to be true during galactose transport by mutant g_1G_2.

In trying to reconcile the hypothesis and the experimental results the following idea might be entertained. Suppose that in active transport phosphatidylglycerol is removed from the equilibrium expressed in the scheme proposed by KENNEDY AND WEISS[23] and KENNEDY[24]:

Phosphatidic acid $\rightleftarrows$ phosphatidylglycerolphosphate $\rightleftarrows$ phosphatidylglycerol $\rightleftarrows$ diphosphatidylglycerol

The membrane might, on the exterior, compensate for this loss by making phosphatidylglycerol from phosphatidylglycerolphosphate. Our experiments would then be the result of this compensation reaction. A further complication would be that phosphate ions would be freed as a consequence of the compensation reaction. However, the extensive literature on active carbohydrate transport has never shown that phosphate ions appear in the medium as a result of this transport.

Moreover, it would be surprising that amino acids and sugars would make use of the same carrier. Experiments by EGAN AND MORSE[27] on the uptake of carbohydrates by *Staphylococcus aureus* suggest the presence of one carrier for various sugars. The specificity shown in the process of active uptake is in this case ascribed to specific permeases needed for the formation of the carrier–substrate complex. There is no indication, however, that amino acids might be transported with the aid of a carbohydrate carrier. The large difference in chemical structure between these two substrates does not make this hypothesis attractive.

The same might be said concerning the idea that phosphatidylglycerolphosphate would be the carrier ready for use. Moreover, this supposition conflicts with the simultaneous phosphorylation and substrate binding found by VAN STEVENINCK[1] and by VAN STEVENINCK AND BOOIJ[2].

In general, another type of objection may be raised concerning the view that substances like phosphatidylglycerol or phosphatidylglycerolphosphate would act as carriers in active transport. LEFEVRE[28] deduced from experiments on the uptake of sugars by erythrocytes that the cells have a maximum of $5 \cdot 10^5$ transport sites per cell. The maximum transport rate of glucose by erythrocytes is a few times higher than that in yeast. Neglecting this difference one may compute that the amount of

phosphatide-like carrier (estimated mol.wt. 800) would be 6 μg per g yeast at most. The amount of phosphatidylglycerol and phosphatidylglycerolphosphate per g yeast is, however, 20 to 50 times as high.

On the basis of these considerations a carrier function of phosphatidylglycerol or phosphatidylglycerolphosphate is highly improbable. Nevertheless, our experiments point to a role of these substances in a process of active transport.

Two main points form the basis of an hypothesis concerning this role: first, polyphosphate is used in active transport[1,2]; secondly, phosphatidylglycerol is transformed to phosphatidylglycerolphosphate with the aid of polyphosphate and phosphatidylglycerolphosphate disappears in the case of active transport.

This leads to the hypothesis that a phosphate monomer from polyphosphate is tranferred to a carrier–phosphate–galactose complex *via* the phosphatidylglycerolphosphate–phosphatidylglycerol system (Fig. 4).

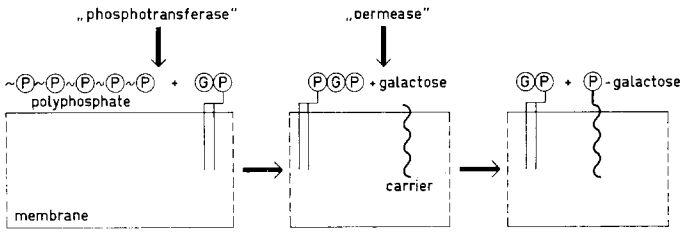

Fig. 4. Proposed scheme of the transfer of a phosphate monomer from polyphosphate to a carrier–phosphate–galactose complex *via* the phosphatidylglycerolphosphate (PGP)–phosphatidylglycerol (PG) system.

This process is a combination of two reactions:

(1) a transfer of a phosphate group from polyphosphate to phosphatidylglycerol, and (2) a transfer of the phosphate group from phosphatidylglycerolphosphate to the carrier and galactose system. In yeast cells the first reaction seems to be a general one, found in several types of active transport. According to MORTON[29] and FISCHER AND STETTEN[30] several hydrolytic enzymes possess a transferase activity. The second reaction is substrate-specific. In our case an induced, galactose-specific, permease is necessary.

The hypothesis given is in accordance with the scheme for galactoside transport in *Escherichia coli*[31], the mechanism of glucoside transport given by OKADA AND HALVORSON[32] and the model of the transport system described by VAN STEVENINCK AND ROTHSTEIN[8]. In the latter model the same carrier is used in facilitated diffusion and active transport of galactose in the yeast mutant g_1G_2. In facilitated diffusion the sugar is transported as a carrier–substrate complex, in active transport a carrier–phosphate–substrate complex is formed. Our experiments demonstrated that in mutant g_1g_2 and in the non-adapted mutant g_1G_2 the reaction phosphatidylglycerolphosphate → phosphatidylglycerol does not occur in the presence of galactose.

The scheme given above may have a more general application (Fig. 5) and can be summarized as follows:

Phosphatidylglycerol + phosphate → phosphatidylglycerolphosphate
Phosphatidylglycerolphosphate + carrier + substrate
→ carrier–phosphate–substrate + phosphatidylglycerol
Carrier–phosphate–substrate → transmembrane transport

It may be suggested that the ATPase system for the uptake of Na^+ and K^+ by erythrocytes[33–35] fits in with our hypothesis. Here it is possible that ATP acts as a phosphate donor. It will be worthwhile to investigate whether the reaction phosphatidylglycerolphosphate → phosphatidylglycerol also takes place in animal tissue.

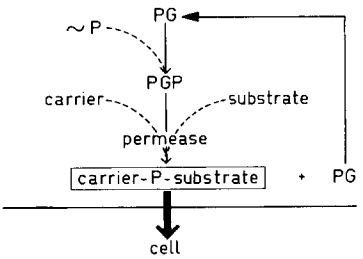

Fig. 5. Generalized hypothesis of the role of the reaction phosphatidylglycerolphosphate (PGP) → phosphatidylglycerol (PG) in active transport.

The difficulties encountered when trying to isolate the ATPase system from erythrocytes (see *e.g.* refs. 35, 36) show that the phenomenon of active transport is integrated with the structure of the membrane. If our hypothesis may be generalized, this is not surprising, as we have shown that two membrane constituents form an integral part of the process of active uptake.

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