carrier-mediated kinetics. The present data certainly can be reconciled with such a suggestion.

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1 R. WHITTAM, *Transport and Diffusion in Red Blood Cells*, Williams and Wilkins, Baltimore, 1964, Ch. X.
2 M. H. JACOBS, *Biol. Bull.*, 107 (1954) 314.
3 H. DAVSON AND J. M. REINER, *J. Cellular Comp. Physiol.*, 20 (1942) 325.
4 H. DAVSON, *A Textbook of General Physiology*, 3rd Ed., Little, Brown and Co., Boston, 1964, p. 325
5 W. D. LOVE AND G. E. BURCH, *Proc. Soc. Exptl. Biol. Med.*, 82 (1953) 131.
6 A. OMACHI, *Science*, 145 (1964) 1 449.
7 A. OMACHI, *The Physiologist*, 8 (1965) 246.
8 D. C. TOSTESON, *Acta Physiol. Scand.*, 46 (1959) 19.
9 M. H. JACOBS, *Cold Spring Harbor Symp. Quant. Biol.*, 8 (1940) 30.
10 M. H. JACOBS AND A. K. PARPART, *Biol. Bull.*, 77 (1939) 318.
11 M. H. JACOBS AND D. R. STEWART, *J. Gen. Physiol.*, 25 (1942) 539.
12 A. K. PARPART, *Cold Spring Harbor Symp. Quant. Biol.*, 8 (1940) 25.
13 R. C. MAWE, *J. Cellular Comp. Physiol.*, 47 (1956) 177.
14 M. H. JACOBS AND D. R. STEWART, *J. Cell. Comp. Physiol.*, 30 (1947) 79.
15 R. EDELBERG, *J. Cellular Comp. Physiol.*, 40 (1952) 529.
16 H. LUCKNER, *Pflüg. Arch.*, 250 (1948) 303 (quoted in DAVSON).
17 F. R. HUNTER, *Am. Zool.*, 6 (1966) 603.
18 P. G. LEFEVRE AND G. F. MCGINNISS, *J. Gen. Physiol.*, 44 (1960) 87.

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Investigation into the permeability of yeast cells to phosphate

Some doubt exists in the literature as to whether yeast cells are permeable to phosphate. Several authors have found that phosphate does not escape or only slowly escapes from yeast cells[3,4,6]. LEGGETT AND OLSEN[5], however, hold that yeast cells are completely permeable to phosphate. The free space for phosphate was about 80% of the cell volume. A rapid release of phosphate occurred from cells previously loaded with ^{32}P, provided they were washed with a solution containing unlabelled phosphate. One of us[1] has also observed a rapid outflow of phosphate from yeast cells, also suggesting that the free space available for phosphate in these cells is relatively extensive.

Cells of *Saccharomyces cerevisiae*, Hansen Delft II, which have a low phosphate content, were aerated in 0.1 M sodium succinate buffer (pH 4.5) at 25° for one day in order to exhaust the internal substrate. No bacterial contamination occurred under our experimental conditions. Determinations of the free space of phosphate ($^{32}P_i$) were conducted according to the method of CONWAY AND DOWNEY[2]. Corrections for intercellular water were made with the help of [*carboxy*-^{14}C]dextran having an

average molecular weight of 73 000. The concentrations of the radioactive compounds were determined on dried samples with an end-window Geiger-Müller tube.

The S value in Table I (see Eqn. 1) is used for expressing the magnitude of the intercellular space available to phosphate or dextran:

$$S = \frac{c_0 - c_m}{c_m} - w \qquad (1)$$

c_0 is the original concentration of solute in the solution and c_m is the concentration after mixing the solution in the proportion 1 l of solution to 1 kg of yeast. w is a correction factor for changes in the osmotic value of the medium (see CONWAY AND DOWNEY[2]).

$$R = \frac{(S_1 - S_2)}{(1 - S_2)} \qquad (2)$$

The R value (see Eqn. 2) is a measure of the space in the yeast that is accessible to the solute investigated, expressed in l/kg yeast. S_1 represents the S value for phosphate and S_2 the S value for dextran.

The S value for phosphate in the presence of 2 mM 2,4-dinitrophenol did not change significantly on increasing the phosphate concentration from 0.001 M to 0.2 M. This indicates that phosphate is not adsorbed to a significant extent by the yeast cells. The other experiments were carried out in 0.1 M phosphate. Variation of dextran concentration between 0.2 to 3% had no effect upon the S value for this compound. The R value for phosphate is very small. Therefore the space of the cell which is accessible to phosphate is extremely limited and the cell membrane is not permeable to this anion. Since LEGGETT AND OLSEN carried out their determinations in the absence of 2,4-dinitrophenol, we have also determined the S values without this inhibitor and found no significant differences. Active absorption did not appreciably influence the results obtained, since the S values found after different times of incubation did not differ significantly.

TABLE I

S VALUES FOR PHOSPHATE (S_1) AND DEXTRAN (S_2) AND R VALUE FOR PHOSPHATE FOUND UNDER DIFFERENT CONDITIONS AT pH 4.5 AND 25°

S.E. is the standard error; n, the number of determinations.

Time (min)	*No dinitrophenol*			*2 mM dinitrophenol*							
	S_1	*S.E.*	n	S_1	*S.E.*	n	R	*S.E.*	S_2	*S.E.*	n
1	0.241			0.252	± 0.009	4	0.020	± 0.021	0.237	± 0.010	3
10	0.270										
20	0.237										
Mean value	0.249	± 0.010	3								

LEGGETT AND OLSEN exhausted their cells from internal substrate by washing them with acid. Moreover, they worked at pH 4.0 instead of at pH 4.5 and did not add succinate buffer, but only phosphate. It is seen in Table II that the S values increase with time under these conditions in contrast to the results with non-acid-

washed cells. This increase was inhibited by 2,4-dinitrophenol. Paper-chromatographic separation of 2% trichloroacetic acid extracts obtained after incubating the yeast for 60 min with radioactive phosphate without 2,4-dinitrophenol showed that a large part of the phosphate was incorporated into other phosphate compounds. The S_1 value for orthophosphate alone was of the same order of magnitude as that found for total phosphate in the presence of 2,4-dinitrophenol. S_1 values for a strain of yeast present in our laboratory (*S. cerevisiae* Hansen Gebrüder Mayer), which is grown under the conditions described by LEGGETT AND OLSEN[5], are also given in Table II. These values also appeared to be low, even in the absence of 2,4-dinitrophenol. According to CONWAY AND DOWNEY[2] the space in yeast accessible to small molecules is about 8% of the total cell volume; being nearly equal to the volume of

TABLE II

S VALUES FOR PHOSPHATE (S_1) FOUND WITH ACID-WASHED YEAST AS A FUNCTION OF TIME IN THE PRESENCE OR ABSENCE OF 2 mM 2,4-DINITROPHENOL

The yeast cells were washed for 2 h in 0.001 M HCl and five times with distilled water. The *S* values were determined in 0.1 M potassium phosphate (pH 4.0) at 25°. S.E. is the standard error; *n*, the number of determinations.

Time (min)	*S. cerevisiae Hansen Delft II*						*S. cerevisiae Hansen Gebrüder Mayer*					
	− Dinitrophenol			*+ Dinitrophenol*			*− Dinitrophenol*			*+ Dinitrophenol*		
	S_1	*S.E.*	*n*	S_1	*S.E.*	*n*	S_1	*S.E.*	*n*	S_1	*S.E.*	*n*
2	0.322	0.013	3	0.283	0.003	3				0.237	0.010	5
5							0.240	0.007	4			
10	0.362	0.019	3	0.280	0.009	2						
30	0.549	0.055	3	0.258	0.002	3						
60	0.625	0.137	2	0.286	0.006	3	0.270	0.016	6			
	0.334*											

* S_1 value calculated for $^{32}P_i$ (see also text).

the cell wall. However, the present results indicate that the free space available to phosphate is apparently smaller than this 8%, suggesting that only a small part of the wall is accessible to phosphate.

Our results show that the plasmalemma of the yeast is not permeable to phosphate. This might indicate that, in accordance with the views of GOODMAN AND ROTHSTEIN[3], phosphate is transferred by an active process occurring at the cell membrane .The above-mentioned phosphate release[1] from the cell must also be due to an active process and cannot be explained by merely passive diffusion according to our present results.

In referring to earlier literature, LEGGETT AND OLSEN hypothesized that the pronounced permeability they have observed to phosphate and also to sulphate and bromide may be traced to the similarities of yeast to slightly vacuolated root-tip cells, cells which are very permeable to inorganic ions. Electron micrographs of cross sections made from our yeast showed that these cells were not largely vacuolated either. Therefore our results indicate that the permeability of slightly vacuolated cells to inorganic ions may not be universal.

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1 G. W. F. H. BORST PAUWELS, *J. Cellular Comp. Physiol.*, 69 (1967) 241.
2 E. J. CONWAY AND M. DOWNEY, *Biochem. J.*, 47 (1950) 347.
3 J. GOODMAN AND A. ROTHSTEIN, *J. Gen. Physiol.*, 40 (1957) 915.
4 G. HEVESY, K. LINDERSTRØM-LANG AND N. NIELSEN, *Nature*, 140 (1937) 725.
5 J. E. LEGGETT AND R. A. OLSEN, *Plant Physiol.*, 39 (1964) 387.
6 P. A. SWENSON, *J. Cellular Comp. Physiol.*, 56 (1960) 77.

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