though occasional unidentifiable structures were present (*12*). Pancreatic deoxyribonuclease, which can digest nuclear DNA almost entirely even in the presence of mitochondria (*16*), inhibited the incorporation only 10 to 15 percent. Preliminary incubation of the mitochondria alone in strongly hypertonic sucrose for more than short periods of time (1.5*M* for 90 minutes), a procedure which alters mitochondrial structure but would not be expected to affect nuclear fragments or free DNA polymerase, prevented the incorporation entirely. (The incorporation process is stable to such prior incubation when isotonic sucrose is used.) Finally, when the changes in buoyant density of DNA upon denaturation and renaturation are followed by CsCl isopycnic centrifugation (Fig. 1), the isolated mitochondrial DNA, in contrast to the results of control experiments with nuclear DNA (not shown), is renaturable after heat denaturation, and the radioactivity peaks remain coincident with the DNA (OD 260 mμ) peaks both after denaturation and upon renaturation (Fig. 1). Ability to renature has been considered a criterion for distinguishing between mitochondrial and nuclear DNA (*11, 17*).

In the light of these findings, we believe that mitochondria from rat liver cells not only contain a unique DNA (*18*), but that the mitochondria themselves also contain a mechanism for incorporating nucleotides into this DNA. Whether this incorporation reflects the presence of a mechanism for DNA replication, a repair process, or some as yet unknown phenomenon is a problem for future study.

Note added in proof: After this report was submitted, we learned that others, using mitochondrial systems, have obtained incorporation of DNA precursors into DNA (*20, 21*); these workers, however, have not yet established that the labeled DNA is, in fact, mitochondrial DNA.

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References and Notes

1. I. H. Herskowitz, *Genetics* (Little, Brown, Boston, 1965), p. 378; R. Sager in *Proceedings of the XI International Congress of Genetics*, S. J. Geerts, Ed. (Pergamon Press, London, 1963), vol. 3, p. 579.
2. I. H. Herskowitz, *Genetics* (Little, Brown, Boston, 1965), p. 378; K. D. Munkres, N. H. Giles, M. E. Case, *Arch. Biochem. Biophys.* **109**, 397 (1965).
3. D. J. Luck, *J. Cell Biol.* **16**, 483 (1963); *Proc. Nat. Acad. Sci. U.S.* **49**, 233 (1963).
4. M. M. K. Nass and S. Nass, *J. Cell Biol.* **19**, 593 (1963); D. J. L. Luck and E. Reich, *Proc. Nat. Acad. Sci. U.S.* **52**, 931 (1964); G. Kalf, *Biochemistry* **3**, 1702 (1964).
5. J. R. McClean, G. L. Cohn, I. K. Brandt, M. V. Simpson, *J. Biol. Chem.* **233**, 657 (1958); G. Kalf, *Biochemistry* **3**, 1702 (1964); D. J. L. Luck and E. Reich, *Proc. Nat. Acad. Sci. U.S.* **52**, 931 (1964).
6. M. V. Simpson, *Ann. Rev. Biochem.* **31**, 333 (1962).
7. Abbreviations are TTP, thymidine-5′-triphosphate; dATP, deoxyadenosine-5′-triphosphate; dGTP, deoxyguanosine-5′-triphosphate; 3′-TMP, thymidine-3′-monophosphate; OD, optical density.
8. W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.* **183**, 123 (1950).
9. J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).
10. M. Rabinowitz, J. Sinclair, L. DeSalle, R. Haselkorn, H. H. Swift, *Proc. Nat. Acad. Sci. U.S.* **53**, 1126 (1965).
11. G. Corneo, C. Moore, D. R. Sanadi, L. I. Grossman, J. Marmur, *Science* **151**, 687 (1966).
12. We thank Dr. E. S. Canellakis for advice on the end-group analysis of DNA, Dr. E. Gantt for investigation of the homogeneity of the mitochondrial preparation by electron microscopy, and Dr. G. E. Palade for examining some of the electron micrographs.
13. R. Markham and J. D. Smith, *Biochem. J.* **52**, 552 (1952).
14. P. Parsons and M. V. Simpson, in preparation.
15. W. H. Elliot, *Biochem. J.* **86**, 562 (1963).
16. S. Nass, M. M. K. Nass, U. Hennix, *Biochim. Biophys. Acta* **95**, 426 (1965).
17. P. Borst and G. J. C. M. Ruttenberg, *ibid.* **114**, 645 (1966).
18. W. C. Schneider and E. L. Kuff, *Proc. Nat. Acad. Sci. U.S.* **54**, 1650 (1965).
19. D. Billen and R. Hewitt, *Anal. Biochem.* **15**, 177 (1966).
20. D. Haldar, K. Freeman, T. S. Work, *Nature* **211**, 9 (1966).
21. E. Wintersberger, *Biochem. Biophys. Res. Commun.* **25**, 1 (1966).
22. Supported by a grant from the USPHS (AM 07124-04). P.P. is a postdoctoral fellow of the USPHS.
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7 November 1966

Phosphate Uptake in an Obligately Marine Fungus: A Specific Requirement for Sodium

Abstract. *Phosphate uptake in the obligately marine fungus* Thraustochytrium roseum *is maximally stimulated by sodium chloride in a range of concentrations (0.2 to 0.4 molar) similar to those commonly encountered in littoral habitats. The effectiveness of sodium chloride for phosphate transport extends beyond its osmotic function and can be attributed specifically to sodium. Increases in respiration in the presence of the salt can be ascribed primarily to an osmotic effect.*

Sea water or NaCl is required for the culture of many marine bacteria and fungi (*1, 2*). Recent studies suggest that the growth requirement for NaCl is specific for these organisms and may be independent of its osmotic function (*3*). Payne (*4*) related the importance of sodium to its function in facilitating substrate penetration, and MacLeod *et al.* (*5*) provided evidence of its role in the oxidation of exogenous substrate. They showed that those substrates supporting optimal growth were also those whose oxidation was stimulated by sodium. Experiments with cell-free extracts have been unsuccessful since NaCl was not related to the activation of specific enzymes (*5*). Attention has therefore focused upon the possible role of NaCl in transport phenomena. Using substrate analogs that could not be metabolized, Drapeau and MacLeod (*6*) concluded that, in marine bacteria, the primary function of sodium was to permit the entry of these compounds into the cell. In view of these findings, *Thraustochytrium roseum*, an obligately marine, nonfilamentous phycomycete (*2*), seemed uniquely suited for examination of the effect of NaCl on other processes such as ion transport. The utility of this fungus lies in its high degree of endogenous respiration, which is unaffected by exogenous substrates (*7*). Consequently, the influence of NaCl on ion transport could be studied in the absence of external energy sources, which might influence ion uptake.

Thalli of *T. roseum* were grown for 4 days at 20°C in a chemically defined medium (*8*). The cultures were maintained on a reciprocal shaker under diffuse incandescent light (8×10^4 erg/cm^2). Cells from cultures in the log phase of growth were centrifuged and resuspended in the same volume

Table 1. Influence of cations and sucrose on phosphate and oxygen uptake by *Thraustochytrium roseum*. The reaction mixture contained: tris-HCl (0.1*M*, *p*H 7.8), KH_2PO_4 (0.001*M*, *p*H 7.8), cells (210 μg of protein per milliliter), and salts as indicated. Oxygen uptake was linear during the 4-hour experimental period.

Additions	Concentration (mole/liter)	Phosphate uptake (μmole/2 hr)	Phosphate uptake (μmole/4 hr)	Oxygen uptake (μmole/1 hr)
None		0.00	0.07	6.52
LiCl		0.09	0.11	5.45
NaCl	0.2	0.32	1.01	9.38
KCl	0.2	0.04	0.10	8.04
RbCl	0.2	0.00	0.00	6.92
$MgCl_2$	0.067	0.23	0.40	8.22
Sucrose	0.2	0.00	0.27	8.08
Sucrose	0.4	0.09	0.26	6.83

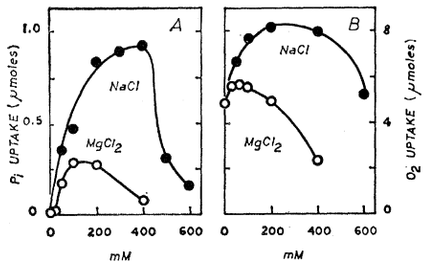

Fig. 1. Effect of various concentrations of NaCl and $MgCl_2$ on respiration and phosphate uptake by *Thraustochytrium roseum*. The reaction mixture was identical to that described in Table 1 except for variations in the concentration of NaCl and $MgCl_2$.

of medium lacking a phosphate source. The vessels were replaced on the shaker, and cells were harvested after two days. These phosphate-depleted cells were centrifuged, washed once in [tris (hydroxymethyl)aminomethane] tris-HCl buffer (0.1*M*, *p*H 7.8) and used immediately in tests for phosphate uptake. Reaction mixtures were centrifuged at 4°C at appropriate times, and the disappearance of inorganic phosphate in the supernatants was measured by the method of Fiske and Subbarow (*9*). Oxygen uptake was determined by conventional manometric techniques (*10*), and protein was measured in the manner described by Lowry *et al.* (*11*).

Initial investigations were concerned with the relative effect of monovalent cations on phosphate uptake (Table 1). There was no phosphate uptake in the presence of tris-HCl buffer alone or with monovalent cations other than sodium. Since magnesium and calcium are important mineral constituents of seawater, their possible influence was also examined. At an ionic strength (0.2) equivalent to that of the NaCl used, $MgCl_2$ was approximately 40 percent as effective as NaCl in promoting phosphate uptake. Because the use of $CaCl_2$ at that ionic strength would precipitate phosphate, lower concentrations (1.0 m*M*) were used, and no phosphate uptake occurred. Combinations of NaCl and $MgCl_2$ and of NaCl and $CaCl_2$ did not produce any synergistic effects with respect to phosphate uptake. The use of sucrose revealed that, under the experimental conditions, phosphate uptake is only partially a reflection of osmotic pressure. In the presence of sucrose, which did not serve as the sole source of carbon for growth (*2*), phosphate uptake was only 26 percent of that with sodium. Except for LiCl, all the salts and sucrose stimulated respiration, though to different extents (Table 1). Sodium chloride was most marked in promoting oxygen uptake; KCl enhanced oxygen consumption 53 percent as much as NaCl.

Table 2. Influence of sodium salts on phosphate and oxygen uptake by *Thraustochytrium roseum*. The reaction mixture contained: tris-HCl (0.1*M*, *p*H 7.8), KH_2PO_4 (0.001*M*, *p*H 7.8), cells (165 μg of protein per milliliter), and salts (0.2*M*) as indicated. Oxygen uptake was linear during the 4-hour experimental period.

Additions	Phosphate uptake (μmole/4 hr)	Oxygen uptake (μmole/1 hr)
NaCl	0.48	7.32
NaBr	0.44	7.58
NaI	0.50	7.72
NaF	0.00	3.61
$NaNO_3$	0.29	6.92
Na_2SO_4	0.36	7.72
None	0.05	5.12

Since $MgCl_2$ also increased phosphate uptake, though to a lesser degree than NaCl (Table 1), a determination was made of the effects of different concentrations of these cations (Fig. 1). Phosphate uptake varies linearly with increase in the concentration of NaCl up to 200 m*M* with no marked increase over the wide range of 200 to 400 m*M*. This range corresponds to concentrations of NaCl encountered in littoral waters. The decline in phosphate uptake with further increases in the concentration of NaCl is attributable to unfavorable osmotic conditions since sucrose at comparable osmotic pressures was also inhibitory. Maximal enhancement of phosphate uptake in the presence of $MgCl_2$ was only 30 percent of that achieved with NaCl. Sucrose and $MgCl_2$ at equal osmolarities gave the same response; this fact suggests that the action of $MgCl_2$ was primarily osmotic and points further to the functional specificity of NaCl for phosphate uptake.

Endogenous respiration occurred in the absence of any cations (Fig. 1B). Sodium chloride enhanced oxygen uptake; so did $MgCl_2$, but to a minor extent. The inhibitory action of these salts at high concentrations may also be due to osmotic phenomena.

To determine whether the stimulation of phosphate uptake by NaCl was attributable to the cation, we tested the influence of various sodium salts (Table 2) and found that the effect was due to the presence of sodium. Compared to the halides, $NaNO_3$ and Na_2SO_4 were less effective, and this may be due to an effect of the anions per se or to the higher ionic strength of solutions of the latter compound. Previous experiments revealed inhibition of phosphate uptake at high ionic strengths. The results with NaF are not unexpected in view of the established role of fluoride as a metabolic inhibitor.

All sodium salts increased respiration to approximately the same extent as NaCl. However, it was surprising that respiration in the presence of NaF was 50 percent of that in the presence of NaCl.

In this organism the requirement for sodium is not limited to its osmotic function. The nutritional requirement for sodium can now be viewed, at least in part, as a consequence of its role in facilitating ion transport. This phenomenon is reminiscent of sodium-stimulated substrate uptake in marine bacteria, shown by Drapeau and MacLeod (*6*). Although NaCl always stimulated endogenous respiration, this effect could be duplicated by a variety of other substances that do not stimulate phosphate uptake. This indicates that there is no necessary direct relationship between enhancement of respiration and of phosphate uptake. These data suggest an experimental approach for relating metabolism to the ecology of marine microorganisms. We have also studied other factors involved in phosphate utilization (*12*).

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References and Notes

1. R. A. MacLeod, *Bact. Rev.* **29**, 9 (1965); H. S. Vishniac, *Limnol. Oceanogr.* **5**, 362 (1960).
2. S. Goldstein, *Mycologia* **55**, 799 (1963).
3. R. A. MacLeod and E. Onofrey, *J. Cell. Comp. Physiol.* **50**, 389 (195 S. Goldstein and M. Belsky, *Amer. J. Bot.* **51**, 72 (1964).
4. W. J. Payne, *J. Bacteriol.* **80**, 696 (1960).
5. R. A. MacLeod, C. A. Claridge, A. Hori, J. F. Murray, *J. Biol. Chem.* **232**, 829 (1958).
6. G. R. Drapeau and R. A. MacLeod, *Biochem. Biophys. Res. Commun.* **12**, 111 (1963).
7. M. M. Belsky and S. Goldstein, *Arch. Mikrobiol.* **49**, 375 (1964).
8. S. Goldstein and L. Moriber, *ibid.* **53**, 1 (1966).
9. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* **66**, 375 (1925).
10. W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Manometric Techniques* (Burgess, Minneapolis, ed. 3, 1957).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
12. P. A. Siegenthaler, M. M. Belsky, S. Goldstein, M. Menna, *J. Bacteriol.*, in press.
13. Partially supported by NSF grant GB-3340 and the City University of New York.
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14 July 1966