Our proposed mechanism for the gastric generation of hydrogen ions supports Rehm's concept of an electrogenic chloride pump (*13*) as the primary cell, with the ion-selective membrane model supplying acid and base to complete the circuit.

HARRY P. GREGOR
JESSE M. BERKOWITZ
Departments of Chemistry and *Chemical Engineering, Polytechnic Institute of Brooklyn, Brooklyn, New York*

References and Notes

1. H. P. Gregor and M. A. Peterson, *J. Phys. Chem.* **68**, 2201 (1964).
2. A. Bethe and T. Toropoff, *Z. Physik Chem.* **88**, 686 (1914); **89**, 597 (1915).
3. H. P. Gregor and I. M. Miller, *J. Amer. Chem. Soc.* **86**, 5689 (1964).
4. L. Onsager, *J. Chem. Phys.* **2**, 599 (1934).
5. A. H. James, *Physiology of Gastric Secretion* (Arnold, London, 1957).
6. W. S. Rehm and W. H. Dennis, *Metabolic Aspects of Transport Across Cell Membranes*, Q. R. Murphy, Ed. (Univ. of Wisconsin Press, Madison, 1957), pp. 303–330; C. A. M. Hogben, *Amer. J. Digest. Diseases* **4**, 184 (1959).
7. H. P. Gregor, in preparation.
8. E. E. Crane, R. E. Davis, N. M. Longmuir, *Biochem. J.* **43**, 321 (1948); C. A. M. Hogben, *Proc. Nat. Acad. Sci. U.S.* **37**, 393 (1951).
9. W. S. Rehm, L. E. Hokin, F. E. Coy, F. J. Bajandas, T. P. de Graffenried, *Amer. J. Physiol.* **164**, 187 (1951).
10. W. S. Rehm, *Amer. J. Digest. Diseases* **4**, 194 (1959).
11. ———, *Amer. J. Physiol.* **144**, 115 (1945).
12. ———, *ibid.* **185**, 325 (1956).
13. ———, T. L. Davis, C. Chandler, E. Gohmann, A. Bashirelaki, *ibid.* **204**, 233 (1963).
14. R. E. Davies and A. G. Ogston, *Biochem. J.* **46**, 324 (1950).
15. J. B. Harris and I. S. Edelman, *Amer. J. Physiol.* **206**, 769 (1964).
16. H. H. Ussing and K. Zerahn, *Acta Physiol. Scand.* **23**, 110 (1951).
17. J. M. Berkowitz and H. P. Gregor, in preparation.
18. H. W. Davenport, *J. Physiol.* **97**, 32 (1939).
19. H. D. Janowitz, H. Colcher, F. Hollander, *Amer. J. Physiol.* **171**, 325 (1952).
20. R. C. Ingraham and M. B. Visscher, *J. Gen. Physiol.* **18**, 695 (1935).
21. Supported by a grant from the NSF and a grant from the Office of Saline Water, U.S. Department of Interior. J.M.B. also received a NIH training grant 2A-5126.

29 April 1965

Alternative Forms of Triosephosphate Dehydrogenase in Chromatium

Abstract. *Triosephosphate dehydrogenase was purified extensively from the obligately phototrophic bacterium* Chromatium. *Enzyme prepared from photolithotrophically grown cells differed in several properties from enzyme prepared from photoorganotrophically grown cells. Either form of the enzyme could be transformed in vitro to the other by mild oxidation or reduction, which effected both Michaelis constants and reactive —SH contents of the proteins.*

Numerous studies on the intracellular and phylogenetic distribution of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)-linked triosephosphate dehydrogenase (TPD) lend evidence to the hypothesis that the NADP enzyme functions primarily in photosynthesis (*1–3*). However, the photosynthetic bacteria apparently lack NADP-linked TPD activity (*4*). We have studied the properties of the NAD-dependent TPD of the obligately phototrophic bacterium *Chromatium* in order to clarify more fully the function of the NAD-linked TPD in bacterial photosynthesis.

Chromatium, strain D was grown either photolithotrophically with CO_2 as the sole source of carbon and $Na_2S_2O_3$ as reductant, or photoorganotrophically with sodium malate as source of both carbon and reductant (*5*). Cells were harvested by centrifugation, washed once, and suspended in 0.10*M* potassium-phosphate buffer at *p*H 8.4. The cells were disrupted by high-frequency sound, and whole cells and debris were removed by high-speed centrifugation. Most of the TPD activity was then precipitated by $(NH_4)_2SO_4$ between 0.50 and 0.80 saturation. This precipitate was suspended in the phosphate buffer and a particle-bound pigment fraction was removed by centrifugation at 144,000*g* for 90 minutes. The supernatant fluid was then passed through a 150-ml column (2.8 by 25.0 cm) of Sephadex G-200. The active eluant fraction was brought to 0.50 saturation with $(NH_4)_2SO_4$, and crystallization of TPD was effected. This product gave a monodispersed peak in the analytical ultracentrifuge, although most preparations showed a marked tendency to dissociate. A sharp symmetrical peak of activity was eluted from Sephadex G-200. The purified enzyme migrated as a single protein band during electrophoresis on acrylamide gel. The specific activity of twice recrystallized enzyme was no higher than that of the Sephadex eluate and the latter was used as a source of enzyme in most experiments.

The TPD activity was measured in the reductive direction from 1,3-diphosphoglyceric acid to glyceraldehyde 3-phosphate (G-3-P) by the method of Heber, Pon, and Heber (*3*) except phosphoglyceric acid kinase was added in at least twofold excess in all cases. Activity in the oxidative reaction from G-3-P to 1,3-diphosphoglyceric acid was measured as described (*2*).

Under conditions of saturation, the assay showed that enzyme from either photolithotrophically or photoorganotrophically grown cells had a *p*H optimum between 8.3 and 8.5. The specific activities of the enzyme from both sources were equal. The molecular weights, as judged by ultracentrifugation and elution from Sephadex G-100 and G-200 columns (*6*), were 120,000, which is identical to that of TPD prepared from other sources. The enzymes also had similar Michaelis constants (K_m) for NAD and for NADH (Table 1). The enzyme from photolithotrophically grown cells, however, had affinities three times higher for G-3-P and 1,3-diphosphoglyceric acid than did the enzyme prepared from photoorganotrophically grown cells. These observations led to the question of whether *Chromatium* contains two different NAD-specific TPD's or a single TPD, the properties of which are controlled by growth conditions and the metabolic requirements of the cell. Since growth medium containing $Na_2S_2O_3$ and CO_2 has a lower redox potential than does the sodium malate medium, the effect of mild oxidation and reduction on the properties of purified TPD from *Chromatium* were examined.

The TPD prepared from photoorganotrophically grown cells was reduced by treatment with 0.10*M* sodium ascorbate for 1 hour. The TPD from photolithotrophically grown cells was oxidized by overnight dialysis against 0.10*M* potassium phosphate buffer, *p*H 8.0, which removed the cysteine used to protect the enzyme. Reduction of the TPD from cells grown on sodium malate reduced the K_m for 1,3-diphosphoglyceric acid from $10^{-2}M$ to $4 \times 10^{-3}M$ (Table 1), approximately equal to the enzyme prepared from cells grown on CO_2. Conversely, oxidation of the enzyme prepared from cells grown on CO_2 raised the K_m for the 1,3-diphosphoglyceric acid to 9×10^{-3}, a value approximately equal to enzyme from cells grown on sodium malate.

The number of active —SH groups

Table 1. Properties of triosephosphate dehydrogenase from *Chromatium*. Purified enzyme was oxidized or reduced as described in text. The K_m is an average based on five independent experiments. DiPGA is used as an abbreviation for 1,3-diphosphoglyceric acid.

Source of enzyme	K_m				Moles —SH group per mole enzyme
	DiPGA	NAD	G-3-P	NADH	
Untreated CO_2*	3×10^{-3}	5×10^{-5}	1.7×10^{-4}	3.0×10^{-6}	4.2
Oxidized CO_2	9×10^{-3}	6.5×10^{-5}	5.0×10^{-4}	4.0×10^{-6}	2.4
Untreated mal†	1×10^{-2}				2.4
Reduced mal	4.0×10^{-3}				3.3

* CO_2 refers to cells that were grown photolithotrophically. † Mal refers to cells that were grown photoorganotrophically.

per protein molecule was measured by the nitroprusside reaction (*7*) in both treated and untreated TPD's from both sources of cells (Table 1). The untreated enzyme from cells grown on CO_2 contained 4.2 reactive —SH groups per molecule, and the untreated enzyme from cells grown on malate contained 2.4 —SH groups per protein molecule—with the assumption of 100 percent purity of the preparations. Oxidation of the former, which increased the K_m threefold, also reduced the number of reactive —SH groups to 2.4. Conversely, reduction of the latter enzyme, which reduced the K_m, raised the number of reactive —SH groups to 3.3. All changes in K_m and in reactive —SH content were reversible.

These results indicate that *Chromatium* many contain a single NAD-dependent TPD, the physical and chemical properties of which are controlled by the chemical environment of the cells. Whereas, in higher plants, the NAD-dependent TPD probably functions primarily in the direction of glycolysis and the NADP-dependent TPD functions primarily in the photosynthetic Calvin cycle, the NAD-dependent TPD of *Chromatium* functions in the synthetic direction during both lithotrophic and organotrophic growth conditions. During photolithotrophic growth, TPD must support synthesis of C_6 compounds and support the function of a Calvin cycle (*5*). Organotrophic growth results in a tenfold reduction of Calvin-cycle activity (*5*) and, under such conditions, TPD activity would be necessary only for the production of compounds for carbon storage and for glycolysis. In an organism with small intracellular pools of both 1,3-diphosphoglyceric acid and G-3-P (*5*), and a high content of TPD with a relatively low affinity for these substrates, a small change in K_m could easily control the rate of the TPD reaction. Such a control mechanism might be based on direct, environmentally caused, changes in the tertiary structure of the protein molecule. These results are interesting in view of the results of Horecker and Cremona (*8*) who found that two —SH groups might play a role in the regulation of activity of fructose 1,6-diphosphate aldolase and in view of the work of several authors demonstrating the reversible loss of antibody activity produced by reduction of S—S linkages (*9*).

Regulation of TPD activity in *Chromatium* by such a mechanism would provide control of a key reductive reaction in photosynthesis and would be rapid in both directions since the *de novo* synthesis of a protein is not required.

G. A. HUDOCK*
D. B. MELLIN
R. C. FULLER

Department of Microbiology,
Dartmouth Medical School,
Hanover, New Hampshire

References and Notes

1. M. Gibbs, *Nature* **170**, 164 (1952); R. H. Hageman and D. T. Arnon, *Arch. Biochem. Biophys.* **57**, 421 (1955); G. A. Hudock and R. C. Fuller, *Plant Physiol.*, in press.
2. R. C. Fuller and M. Gibbs, *Plant Physiol.* **34**, 324 (1959).
3. U. Heber, N. G. Pon, M. Heber, *ibid.* **38**, 355 (1963).
4. R. M. Smillie and R. C. Fuller, *Biochem. Biophys. Res. Commun.* **3**, 368 (1960).
5. R. C. Fuller, R. M. Smillie, E. C. Sisler, H. L. Kornberg, *J. Biol. Chem.* **236**, 2140 (1961).
6. P. Andrews, *Biochem. J.* **91**, 222 (1964).
7. R. R. Grunert and P. H. Phillips, *Arch. Biochem.* **30**, 217 (1955).
8. B. L. Horecker and T. Cremona, *Science* **148**, 665 (1965).
9. P. L. Whitney and C. Tanford, *Proc. Nat. Acad. Sci. U.S.* **53**, 524 (1965); E. Haber, *ibid.* **52**, 1099 (1964).
10. Supported in part by GB 2631 from the National Science Foundation, and in part by a traineeship 5TL GM 961-03 (to G.A.H.) from the National Institute of General Medical Sciences.
* Present address: Indiana University, Department of Zoology, Bloomington.

1 July 1965

Protein Synthesis in Enucleated Eggs of Rana pipiens

Abstract. *Tritiated leucine of high specific activity was injected into enucleated* Rana pipiens *eggs. After 6 hours, there was significant incorporation of the label into protein. The amount of incorporation in these eggs was as great as that in fertilized, normally developing eggs.*

Many kinds of experiments have shown that early development in the amphibian egg can occur in the absence of obvious nuclear control. For example, partially cleaved blastulae have been obtained by fertilizing frogs' eggs with heavily irradiated sperm and then mechanically removing the egg nucleus (*1*). Likewise, development to the blastula or gastrula stage occurs in the presence of lethal hybrid nuclei (*2*), and in the presence of nuclei with very abnormal chromosomal complements (*3*). However, neither the nature of the synthetic processes occurring during early development nor the importance of nuclear participation in the control of these processes is clearly understood.

Early investigations had suggested that very little, if any, net RNA, DNA, or protein synthesis occurs before gastrulation (*4*), but recent reports have indicated that RNA synthesis (*5, 6*), and possibly protein synthesis (*5, 7*), does take place during cleavage stages.

Tiedemann and Tiedemann (*8*) have presented evidence for protein synthesis in anucleate halves of ligated *Triton* eggs, indicating that protein synthesis can occur in the absence of a nucleus. Further evidence for the nonparticipation of DNA in the control of early synthesis was reported by Brachet *et al.* (*7*) who showed that inhibition by actinomycin D of DNA-directed RNA synthesis had little effect on cleavage even when the antibiotic was injected directly into fertilized eggs. On the other hand, injection of puromycin, an inhibitor of protein synthesis, effectively stopped cleavage shortly after treatment. These results, although somewhat indirect, suggest that protein synthesis which does occur during early development is not necessarily subject to direct nuclear control. The following experiments provide direct evidence for the synthesis of protein in enucleated eggs of the leopard frog, *Rana pipiens*.

Ovulation was induced in mature