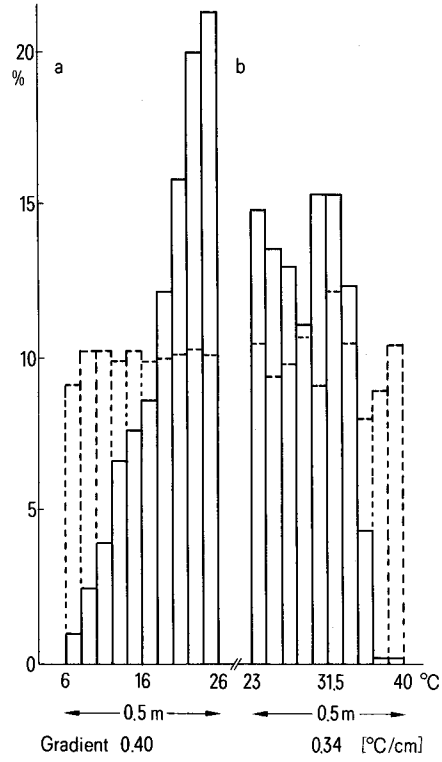
gradient from +6 to 26 °C (0.4 °C/cm) and 23–40 °C (0.34 °C/cm), respectively, was set up within 20 min (initial homogeneous temperature 24 °C, rate of temperature change: 1.5 °C/min), all the groups migrated very slowly in the direction of the temperature preferendum. The solid line columns of the diagram show the distribution of the groups on the observation plane 2 h after the temperature gradient was set. At ambient temperatures above 38 °C, the groups of *C. campylum* dispersed, but not at lower temperatures.



Dispersion of groups of *Colpidium campylum* in the observation vessel (1 single trial out of 10 experiments). Dotted lines: dispersion in a homogeneous temperature field (24 °C) 2 h after starting the experiment. The total number of groups (a, 396; b, 297, each 1–6 mm ∅) is set as 100%. Solid lines: Dispersion 2 h after setting up the temperature gradient (a, +6–26 °C, b, 23–40 °C, number of groups in a: 172, in b: 177). The groups had moved in the direction of the preferred temperature range, but not all had already reached it. This took place after about 9 h, when all individuals were aggregated in 1 or 2 large groups within the preferred temperature range of 24–28 °C.

During migration these ciliate groups show a characteristic structure: A straight front of motionless individuals is formed in the direction of the preferendum. At the opposite end the ciliates are free-swimming, but never lose contact to the group. These individuals show a tendency to swim over the group in the direction of the preferred temperature and settle down at the front.

In the case of extremely large groups (∅ 10 mm), macroscopically speaking, it seems as if the group which may be regarded as a compact mass, protracts and retracts 'pseudopodia' in all directions. If one of them lies in the direction of the thermopreferendum, the whole group will follow. Obviously, this phenomenon can be interpreted as a system of exploration.

The locomotion of the whole group is much slower than that of the isolated individuals in the capillary; nevertheless the orientation of the whole group during migration is precise. High temperature sensitivity must be assumed in the individual given such a little temperature change per time unit[5]. Under our experimental conditions, it takes a group 2–3 h to migrate over a distance of 10 cm. After a sufficient time (about 9 h) all groups reach the thermopreferendum and form one or 2 large aggregations within that zone.

The results of 15 experiments indicate that isolated individuals of *Colpidium campylum* are able to find their thermopreferendum in a temperature gradient within a few min. Within a group, the social tendency prevents the single members from giving up contact with the other individuals, thus working against a rapid search for a more favourable environment. This does not hold true for ambient temperatures above 38 °C. In this case the avoiding tendency is predominant.

This behaviour in *C. campylum* is neither related to sexuality, food absorption, nor to abiotic factors such as light, oxygen, etc., but to the social disposition of the animals. A different kind of social behaviour has been discovered in *Colpidium colpoda*[6].

1 To whom reprint requests should be addressed.
2 C. Aylmer and A.H. Reisner, J. gen. Microbiol. *67*, 57 (1971).
3 W. Rose, Z. Tierpsychol. *21*, 257 (1963).
4 H. Kersken, Verh. dt. zool. Ges. *1977*, 253.
5 K. Tawada and H. Miyamoto, J. Protozool. *20*, 289 (1973).
6 H. Kersken, in preparation.

Mg^{2+}-ATPase defective mutant of *Escherichia coli* and thiamine transport

T. Nishimune and R. Hayashi[1]

Department of Microbiology, Yamaguchi University School of Medicine, Ube, Yamaguchi-ken (Japan 755), 12 January 1979

Summary. Mg^{2+}-ATPase deficient mutant of *Escherichia coli* showed an evident dependency of thiamine uptake on the oxidative metabolism of glucose, whereas the parent strain did not. In both cells, this uptake was completely inhibited by H^+ conductors.

Although many studies have been reported which account for the energy requirement in the uptake of thiamine by microbial cells[2–7], the roles of intracellular ATP and H^+ gradients across the cell membrane have not been established. This paper describes the possibility of participation by an activated membrane state in a shock sensitive system of thiamine uptake.

Results and discussion. The table shows characteristics of the 2 types of mutants which were obtained. In the strain 19-1 the activity levels of Mg^{2+}-ATPase were within the normal variation of nonenzymatic dephosphorylation of ATP during the assay. The strain 16-1 was isolated as a low glycolytic activity mutant. The levels of glycolytic H^+ production and O_2 consumption were about 25% or less in

comparison to those of the parent cell. However, the Mg^{2+}-ATPase activity per mg protein was appreciably higher than that of the parent. In 19-1, on the other hand, the aerobic and anaerobic H^+ production from glucose, together with the O_2 consumption rate, showed no significant decrease from the values measured in the parent. In the uptake of thiamine by the parent cell, it was extremely difficult to demonstrate the dependency of the reaction on the aerobic respiration. No decrease in the uptake activity could usually be observed in the presence of CN^- or under N_2 atmosphere, even when the concentration of CN^- could inhibit the O_2 consumption completely and immediately (figure, 70-23). Arsenate ion, on the other hand, inhibited the uptake almost completely, indicating the participation of ATP as an energy source in the uptake reaction. Dinitrophenol (DNP) and trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP) were used as H^+ conductors[12]. As shown in the figure, they markedly inhibited the uptake activity indicating that the concentration gradient of H^+ across the membrane was necessary for the active transport of thiamine by *E. coli* 70-23.

Although the participation in the energy supply by membrane Mg^{2+}-ATPase could not be denied completely (remaining ATPase activity in the mutant 19-1), the possibility of the compensation in the energy supply from the respiratory chain was clearly suggested by the inhibitory effects of CN^- and N_2 atmosphere in 19-1 (approx. 50% inhibition, figure, 19-1). This mutant depends upon pathways other than oxidative phosphorylation for its ATP synthesis. Hence, the intracellular ATP was assumed to keep its level in N_2 atmosphere. Under both conditions, complete inhibition of the uptake activity was not observed. This finding, together with the result that $HAsO_4^{2-}$ can almost completely inhibit the uptake of thiamine in 19-1, is quite significant. Thus the incomplete inhibition of anaerobiosis or CN^- would be interpreted to suggest that the intracellular ATP can be utilized for thiamine uptake in a manner which does not rely upon the membrane Mg^{2+}-ATPase.

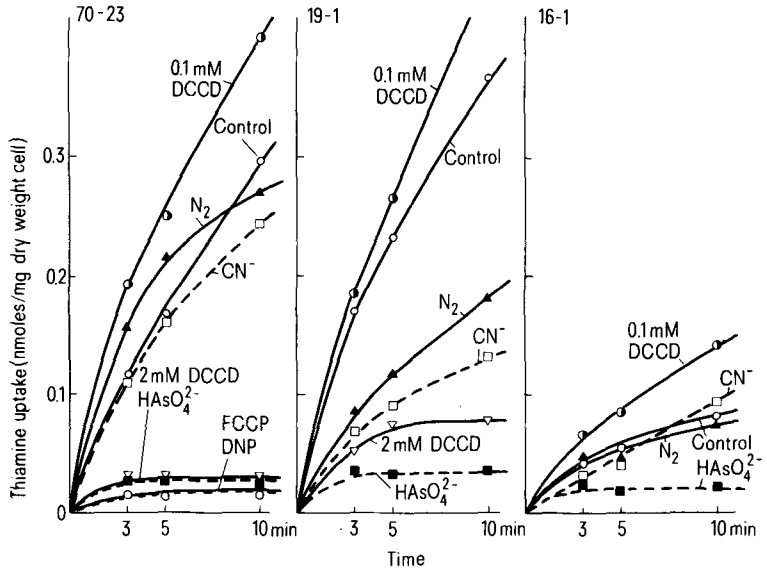
In the strain 16-1, just as in the parent strain 70-23, the inhibitory effect of CN^- or anaerobiosis could not be observed. Therefore, the dependency of uptake upon respiratory chain could be demonstrated only when the cell was defective of the Mg^{2+}-ATPase. Although the membrane Mg^{2+}-ATPase has a higher activity in 16-1, the uptake activity was about $\frac{1}{3}$ of the parent. The plausible reason for this decreased uptake is the low activity (about $\frac{1}{4}$ of the parent) in both the respiration and the glycolytic ATP synthesis (H^+ production).

Under an aerobic condition, the membrane bound Mg^{2+}-ATPase inhibitor N,N'-dicyclohexylcarbodiimide

Characteristics of membrane Mg^{2+}-ATPase defective mutant. Mg^{2+}-ATPase was assayed as follows: 1.0 ml of the reaction mixture contained 100 mM Tris HCl (pH 9.1), 2 mM $MgCl_2$, 4 mM ATP, and the sample. When Ca^{2+} was substituted for Mg^{2+}, the ATPase activity was detected at approximately 20–25% of the Mg^{2+} levels. After 10-min preincubation at 37 °C, the reaction was started with the addition of ATP and terminated with 1.0 ml of cold 20% trichloroacetic acid. The inorganic phosphorous was measured by the method of Baginski et al.[10]. ATPase-less mutants of *E. coli* 70-23 were selected, after treatment with N-methyl-N'-nitro-N-nitrosoguanidine, on the defect of utilization of succinate, malate, and D-lactate[11]. Utilization of substrates for growth was determined by the growth test on a minimal agar plate containing each substrate of 0.2% of glucose or pyruvate or D-lactate, or 0.5% of glycerol or succinate or malate.

	Escherichia coli		
	70-23	19-1	16-1
Proton production* (H^+ nmole/mg/min)	150	170	37
Proton production in the presence of 2 mM KCN (H^+ nmole/mg/min)	190	180	48
O_2 consumption** (ngatom/mg/min)	650 ± 100	630 ± 100	150 ± 50
Mg^{2+}-ATPase activity*** (Pi nmole/mg/min)	650	40	1300
Support of growth as a sole carbon source			
Glucose	+	+	+
Glycerol	+	+	−
Pyruvate	+	+	−
Succinate	+	−	−
D-lactate	+	−	−
Malate	+	−	−

* Approximately 250 µg dry weight/ml cell suspension in 2 mM Tris HCl (pH 7.4) containing 44 µM glucose was kept at 37 °C with gentle stirring. ** Cell suspension, 250 µg/ml, in 0.17 M potassium phosphate buffer (pH 7.2) supplemented with 0.4% glucose was used for the measurement. *** Washed cell was suspended in 33 mM Tris HCl (pH 7.4) to 5 mg/ml and sonicated for 3 min by 10 KHz sonifier at 4 °C. Intact cells and large fragments were spun down at 10,000 × g for 5 min and discarded.



Control experiments received the same solvent. Inhibitors were added into the reaction mixture before the start of preincubation. The period of contact of DCCD with the cell was about 1 h at 25 °C and 10 min at 37 °C. Concentration of inhibitors were 10 mM $NaHAsO_4$, 2 mM KCN, 2 mM DNP, and 50 µM FCCP.

Thiamine uptake by *E. coli* mutants in the presence of inhibitors. The uptake activity was measured according to a previous method[8,9] but the concentration of thiamine and bacterial cells were 3×10^{-8} M and ca. 50 µg/ml, respectively, throughout this study. The cells were prepared as described previously[8]. DCCD and DNP were in C_2H_5OH and FCCP was in dimethylsulfoxide.

(DCCD)[13] had practically no effect on thiamine uptake. A slight activation by 0.1 mM DCCD may probably be explained by the prevention of dissipation of the electrical potential through the membrane[14]. When the concentration was raised to 2 mM, an apparently nonspecific inhibitory effect could be observed similar to the reported case of aerobic proline uptake[15]. $HAsO_4^{2-}$ is known to decrease the intracellular ATP level[15]. As shown in all 3 strains in the figure, 10 mM Na_2HAsO_4 could completely inhibit the uptake of thiamine.

The fact that the Mg^{2+}-ATPase-less mutant of *E. coli* did not show any lowered thiamine uptake aerobically, and also the finding that about 50% of the activity was inhibited by anaerobic conditions suggests that the H^+ gradient across the membrane can be established by this ATPase-less mutant (probably defective in the F_1 fraction). The possibility is also supported by the fact that H^+ gradient has been detected in Mg^{2+}-ATPase defective mutants of *E. coli*[14,16]. The uptake activity shown above is expressed as the total amount of accumulated thiamine. The step of phosphorylation does not exert any serious influence on the rate of uptake because about the same level of uptake is observed in both *E. coli* 70-23 and its phosphorylation defective mutants[17].

1 Acknowledgment. We are indebted to Miss M. Abe for her excellent technical assistance.
2 Z. Suzuoki, J. Biochem. *42*, 27 (1955).
3 H.Y. Neujahr, Acta chem. scand. *20*, 771 (1966).
4 T. Kawasaki, I. Miyata, K. Esaki and Y. Nose, Archs Biochem. Biophys. *131*, 223 (1969).
5 T. Nishimune, D. Miura and R. Hayashi, Vitamins *47*, 211 (1973).
6 T. Kawasaki and K. Yamada, Biochem. biophys. Res. Commun. *47*, 465 (1972).
7 A. Iwashima, H. Nishino and Y. Nose, Biochim. biophys. Acta *330*, 222 (1973).
8 T. Nishimune and R. Hayashi, Biochim. biophys. Acta *244*, 573 (1971).
9 T. Nishimune and R. Hayashi, Biochim. biophys. Acta *328*, 124 (1973).
10 E. Baginski and B. Zak, Clin. chim. Acta *5*, 834 (1960).
11 R.D. Simoni and M.K. Shallenberger, Proc. natl Acad. Sci. USA *69*, 2663 (1972).
12 A. Finkelstein, Biochim. biophys. Acta *205*, 1 (1970).
13 F.M. Harold and J.R. Baarda, J. biol. Chem. *244*, 2261 (1969).
14 K. Altendorf and F.M. Harold, J. biol. Chem. *249*, 4587 (1974).
15 W.L. Klein and P.D. Boyer, J. biol. Chem. *247*, 7257 (1972).
16 B.P. Rosen, Biochem. biophys. Res. Commun. *53*, 1289 (1973).
17 H. Nakayama and R. Hayashi, J. Bact. *118*, 32 (1974).

The effects of two neutral polymers on the geometry and deformability of the human erythrocyte[1]

L.S. Sewchand, S. Leuchter, R.E. Lovlin, J.S. Beck and S. Rowlands

Faculty of Medicine, University of Calgary, Calgary, Alberta (Canada T2N 1N4), 18 January 1979

Summary. Polyvinylpyrrolidone and dextran decrease cellular deformability. Changes in volume do not wholly account for the changes which imply a stiffening of the plasma membrane. The effects differ from those induced by charged macromolecules.

Adsorption of macromolecules onto the human red cell membrane (RCM) causes changes in cellular area and volume[2], in cellular deformability[3,4] and in cellular aggregation[5-7]. 2 neutral polymers, polyvinylpyrrolidone (PVP) and dextran (Dx) are readily adsorbed onto the RCM[8-10], and induce aggregation[10-12]. This study indicates that they also change cell geometry and deformability.

Methods. Cells from a finger-prick were suspended (v/v concentration ~1% to minimize rouleau formation) in Tris-HCl-buffered Ringer solution (pH 7.40±0.02, 310±2 mOsm) containing PVP (360,000 daltons) or Dx (70,000 daltons). Cells falling towards a glass coverslip on an inverted microscope with oil immersion optics were photographed when seen on-edge and the magnification was measured by a stage micrometer. Using the criterion of Ponder[13,2] the profile of each cell was drawn on an enlarged photograph. With the method recently described by Beck[14], the volume and area of each cell was calculated from measurements of diameter and of maximum and minimum thickness. Deformability is operationally defined by our method[15] which is to measure the pressure, averaged over at least 50 cells, needed to suck a cell within 1 sec into a micropipette of diameter approximately 2 µm. The treated cells are standardized against untreated cells from a control subject whose cells, in turn, have been standardized against a small normal population as described by Schachar et al.[15]. For all measurements differences between mean values were assessed by Student's t-test and regarded as significant for $p < 0.01$. In the next section each quoted result is accompanied by the SE of the mean value.

Results and discussion. For each of a number of concentrations of the 2 polymers, between 60 and 160 cells were photographed, their profiles measured and their areas and volumes calculated[14]. Up to a concentration of PVP of 3 $g \cdot l^{-1}$ none of the changes in the measured or calculated values reached statistical significance by our above-stated criterion. At a concentration of 5 $g \cdot l^{-1}$ the mean minimum thickness of the cells had increased from a control value of 1.30±0.010 µm to 1.47±0.012 µm, a change of 13%. Maximum thickness increased 3.6%, from 2.51±0.012 µm to 2.60±0.021 µm while volume increased 6.1% from 104.2±1.1 µm to 110.6±1.7 µm. Changes in diameter and area of the cells did not reach statistical significance, nor did variation in any of the values as the concentration of PVP was further increased from 5 $g \cdot l^{-1}$ to 15 $g \cdot l^{-1}$.

Dx at 5 $g \cdot l^{-1}$ caused a similar increase in cellular volume (8.5%) from a control value of 104.2±1.1 μm^3 to 113.1±1.8 μm^3, but the changes in maximum and minimum thicknesses of the cells differed markedly from those in PVP. The change in minimum thickness (2.1%) did not reach statistical significance while maximum thickness increased from 2.51±0.012 µm to 2.79±0.016 µm a change of 11%. As concentration of Dx was increased 10-fold there were no further significant changes. The increase in cell volume in