alter filterability, it seemed possible that a shape or volume change might be occurring in parallel with the alterations in deformability. Hematocrit and microscopic observation showed no change in mean cell volume or cell shape under conditions that alter filterability.

These data show that red cell suspensions are responsive to several vasoactive substances known to have effects on the circulation in vivo at similar concentrations. The response of red cells to PGE_2 is of interest in that it is the most sensitive response to this compound reported to date; this suggests that red cells may be one of the primary receptors for prostaglandin stimuli.

Burton and Weed (*6*, *7*), among others, have pointed out the importance of deformability in the microcirculation; and Weed (*7*) has proposed that deformability governs the destruction of erythrocytes by the spleen. Leblond *et al.* (*8*) have recently proposed that release of young erythrocytes by the marrow is also a function of deformability. It is becoming clear that deformability is a functional characteristic of the red cell which varies with cell age and metabolic condition. Our report shows that deformability is subject to alteration by hormonal stimuli. This in turn indicates that the red cell must be considered for a role in the moment-to-moment control of the circulation, and that hormonal stimuli may play a role in destruction of red blood cells.

JAMES E. ALLEN
HOWARD RASMUSSEN
Department of Biochemistry, University of Pennsylvania School of Medicine, Philadelphia 19104

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Interchangeability of Phosphorylation Coupling Factors in Photosynthetic and Respiratory Energy Conversion

Abstract. *The nonsulfur purple photosynthetic bacterium* Rhodopseudomonas capsulata *can obtain energy for growth either by anaerobic photophosphorylation or dark oxidative (aerobic) phosphorylation. Successful resolution of phosphorylation coupling factors from energy-converting membranes of this bacterium permitted tests for reciprocal function of such protein factors in oxidative- and photophosphorylation processes. Evidence was obtained for the interchangeability of coupling factor preparations from dark-grown and photosynthetically grown cells in both kinds of energy conversion.*

In eucaryotic cells, photosynthetic and respiratory phosphorylation processes are generally catalyzed by different kinds of membrane systems that are spatially separated in the form of discrete organelles (chloroplasts and mitochondria) which are believed to be semiautonomous in character. On the other hand, the procaryotic nonsulfur purple bacteria, which can obtain the energy for growth by either photosynthetic or (dark) respiratory phosphorylation, do not contain subcellular organelles, and a considerable body of evidence indicates that in these organisms the cytoplasmic membrane or its extensions, or both, must be the locus of both kinds of energy conversion (*1*). When typical bacteria with these capacities are transferred from photosynthetic to heterotrophic conditions, or vice versa, appropriate differentiation of the energy-converting membrane system ensues (*2*). The possibility that common components may function in the photosynthetic and respiratory phosphorylation processes of such organisms has been discussed [for example, see (*3*) and Thore *et al.* (*1*)], but until now there has been no compelling evidence in support of this notion. In this report, we present results showing that phosphorylation coupling factor preparations derived from heterotrophically and photosynthetically grown cells of *Rhodopseudomonas capsulata* (strain St. Louis; American Type Culture Collection No. 23782) can link either oxidative or light-dependent electron transport to phosphorylation of adenosine diphosphate (ADP).

Phosphorylating membrane preparations were obtained from cells grown either photosynthetically (anaerobically) or aerobically in darkness, in a synthetic medium (*4*) containing DL-malate and $(NH_4)_2SO_4$ as the respective carbon and nitrogen sources. In the following, the designation P is used for membrane vesicles, or derived components, from cells grown photosynthetically, and A for corresponding preparations from bacteria cultivated aerobically in darkness. Membrane vesicles, or fragments, will be referred to as particles. The P particles were prepared as described previously (*4*). The P coupling factor was extracted from acetone powders of such particles, and purified by procedures described elsewhere (*5*). For certain experiments, P particles depleted of P coupling factor (that is, "uncoupled" P particles) were required, and these were prepared by sonication of the particles in the presence of 1 m*M* ethylenediaminetetraacetate [EDTA; for details, see (*4*)]. The procedure used for obtaining A particles was very similar to that employed for isolation of P particles. In brief, cells grown aerobically in darkness were suspended in 0.05*M* glycylglycine buffer (*p*H 7.2), containing 0.4*M* sucrose, and disrupted in a French pressure cell. Residual intact cells and large debris were removed from the extracts by a low-speed centrifugation and A particles then were collected by centrifugation at 250,000*g* for 1 hour. These were ordinarily resuspended in the glycylglycine plus sucrose buffer, supplemented with 10 m*M* $MgCl_2$ and bovine serum albumin (2 mg/ml). Alternatively, to prepare uncoupled A particles, the sedimented particles were resuspended in 1 m*M* EDTA and the cold suspension was subjected to three short bursts (30 seconds each) of sonic energy from a Biosonik oscillator (Bronwill Scientific); the treated particles were collected by centrifugation at 250,000*g* for 1 hour and resuspended in the buffer of glycylglycine plus sucrose plus bovine serum albumin ($MgCl_2$ omitted). For measurement of phosphorylation, a hexokinase plus glucose "trap" was employed to convert [^{32}P]ATP, produced

from $^{32}P_i$, to [^{32}P]glucose-6-phosphate; after deproteinization with trichloroacetic acid (TCA), organic ^{32}P was separated and determined by the method of Avron (*6*) ([^{32}P]ATP and $^{32}P_i$ are designations for ^{32}P-labeled adenosine triphosphate and orthophosphate, respectively).

Uncoupling of A particles as described did not affect the rates of O_2 consumption with succinate or reduced nicotinamideadenine dinucleotide (NADH) as substrates, but the capacity for oxidative phosphorylation was almost entirely abolished. Figure 1 shows the activity of purified P coupling factor in restoring the phosphorylation capability to uncoupled A particles. The O_2 consumption rates (measured polarographically with a Yellow Springs Instrument Co. oxygen meter, model 51; expressed in terms of electron equivalents) were not appreciably affected by addition of the coupling factor. Similarly, addition of the phosphorylation substrates or chemical uncouplers of phosphorylation had little effect on the respiratory rates. Other experiments indicated that roughly comparable quantities of P coupling factor are required to restore half the maximal reconstituted activity levels in this oxidative phosphorylation system and in photophosphorylation [in the latter case, P coupling factor is added to uncoupled P particles and the incubation mixtures illuminated, and so forth, as described elsewhere (*4*)]. From the data of Table 1, it is apparent that the oxidative phosphorylation activity reconstituted by addition of P coupling factor to uncoupled A particle shows sensitivities to oligomycin and cyanidecarbonyltrifluoromethoxyphenylhydrazone (FCCP) similar to those observed with untreated (that is, coupled) A particles.

Further evidence for close similarity of the P and A coupling factor preparations was provided by immunochemical tests. Purified P coupling factor was used as an antigen for preparation of an antiserum (from rabbits), from which a partially purified γ-globulin fraction was obtained. This fraction was found to inhibit the photophosphorylation, adenosine triphosphatase, and $^{32}P_i$—ATP exchange activities of coupled P particles (nonimmune γ-globulins, however, did not significantly affect these reactions). The experiments summarized in Table 2 show that the γ-globulin fraction also effectively in-

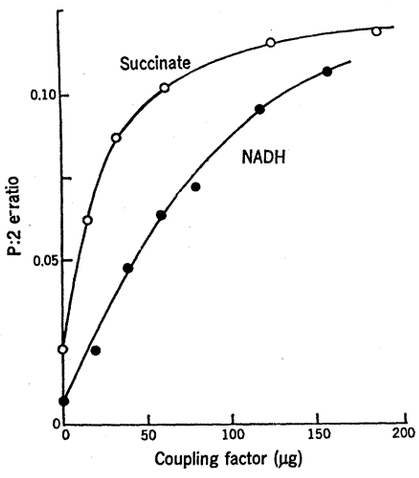

Fig. 1. Reconstitution of oxidative phosphorylation in uncoupled particles derived from cells grown aerobically in the dark, by addition of "photosynthetic" coupling factor. The assay mixtures for phosphorylation contained, in a final volume of 1.5 ml: glycylglycine buffer (*p*H 8), 100 μmole; glucose, 25 μmole; sulfate-free hexokinase, 25 units; ADP, 3 μmole; $MgCl_2$, 15 μmole; EDTA, 1.5 μmole; bovine serum albumin, 2 mg; sodium succinate, 30 μmole or NADH, 9 μmole; [^{32}P]Na_2HPO_4 (1.5×10^5 count/min per micromole), 15 μmole. Purified "photosynthetic" (P) coupling factor was preincubated with uncoupled "aerobic" (A) particles (0.6 mg protein) for 17 minutes at 30°C in the dark in the presence of all the components indicated except sodium succinate, NADH, and $^{32}P_i$. Succinate or NADH was added and the mixtures were shaken for 3 minutes. Phosphorylation was then initiated by addition of $^{32}P_i$; after 7 minutes, reaction was terminated by adding 0.2 ml of ice-cold 50 percent TCA. Organic ^{32}P was determined as noted in the text. For measurements of O_2 uptake (calculated as electron equivalents), assay mixtures were prepared as specified above except that ADP, $^{32}P_i$, and the glucose plus hexokinase trap were omitted.

Table 1. Effects of inhibitors on oxidative phosphorylation activity of "native" and "reconstituted" A particles. Assay conditions were as given in the legend of Fig. 1. Succinate oxidase activities of the "native" and "reconstituted" A particles were 5.7 and 7.5 μmole of O_2 per hour per milligram of protein, respectively; with NADH, the activities were 9.3 and 11.9, respectively. These activities were not affected by addition of coupling factor, oligomycin, or FCCP. The uncoupled A particles used for the reconstitution experiments showed P : 2*e* ratios of 0.04 with succinate and 0.03 with NADH.

Additions	P : 2*e* values			
	"Native" particles		"Reconstituted" particles*	
	Observed	Relative	Observed	Relative
Sodium succinate	0.17	1.00	0.12	1.00
+ oligomycin (1.33 μg/ml)	0.00	0.00	0.00	0.00
+ FCCP ($5 \times 10^{-6}M$)	0.10	0.59	0.06	0.50
+ FCCP ($1 \times 10^{-5}M$)	0.07	0.41	0.03	0.25
+ FCCP ($2 \times 10^{-5}M$)	0.00	0.00	0.00	0.00
NADH	0.20	1.00	0.14	1.00
+ oligomycin (1.33 μg/ml)	0.00	0.00	0.00	0.00
+ FCCP ($5 \times 10^{-6}M$)	0.10	0.50	0.09	0.65
+ FCCP ($1 \times 10^{-5}M$)	0.04	0.20	0.05	0.36
+ FCCP ($2 \times 10^{-5}M$)	0.00	0.00	0.00	0.00

* In the reconstitution experiments, uncoupled A particles (0.58 mg of protein) were preincubated with P coupling factor (0.13 mg of protein) for 20 minutes, inhibitors (dissolved in a small volume of ethanol) were then added, and immediately afterward the reactions were initiated by addition of $^{32}P_i$.

Table 2. Inhibition of oxidative phosphorylation activity of A particles by antibody for P coupling factor. An antiserum was obtained from rabbits injected with purified P coupling factor, and the γ-globulin fraction of the serum precipitated by 30 to 50 percent saturation with $(NH_4)_2SO_4$ was used. For each determination, antibody (where indicated) and A particles (0.53 mg of protein) in a volume of 0.2 ml were preincubated in an ice bath for 20 minutes, the phosphorylation substrates, and so forth, were added, and the assay was performed as described in the legend of Fig. 1. "P_i esterified" and "O_2 uptake" are given in terms of micromoles per hour per milligram of A particle protein.

γ-Globulin fraction added (mg)	Oxidative phosphorylation activity					
	Succinate-dependent			NADH-dependent		
	P_i esterified	O_2 uptake	P : 2*e*	P_i esterified	O_2 uptake	P : 2*e*
0.0	1.9	6.3	0.15	5.2	7.8	0.33
1.1	0.9	6.1	0.07	3.7	7.9	0.23
2.1	0.5	6.0	0.04	2.7	8.0	0.17
2.6	0.5	6.2	0.04	2.3	7.9	0.14
3.7	0.2	6.1	0.02	1.9	7.8	0.12

hibited the oxidative phosphorylation activities of A particles with both succinate and NADH serving as substrates. The rates of O_2 consumption were unaffected by the presence of antibody, and this constitutes further evidence that the coupling factor is concerned with a late stage in the actual phosphorylation of ADP, and not with electron transfer processes that are the driving force for phosphorylation.

The coupling factor from A particles has not been purified yet, but the experiments of Table 3 demonstrate that sonication of A particles in the presence of EDTA releases a protein (or proteins) that can restore photophosphorylation activity to uncoupled P particles. We regard these results as the reciprocal of those already presented which show that P coupling factor restores oxidative phosphorylation activity to uncoupled A particles. In separate experiments, not detailed here, the expected reconstitution of oxidative phosphorylation in A particles by A coupling factor was readily demonstrable.

The present findings constitute the first evidence for functional interchangeability of phosphorylation coupling factors from heterotrophically and photosynthetically grown cells of the same organism, and it can be argued that this potentiality facilitates the observed ready reversibility of adaptation between the two modes of growth. It is known (*7*) that membrane preparations from dark-grown (aerobically) cells of photosynthetic bacteria are capable of catalyzing photophosphorylation at relatively high specific activities (relative to bacteriochlorophyll content). Conversely, membrane fragments from cells grown photosynthetically have (dark) oxidative phosphorylation capacity [Geller (*1*)]. Definitive proof for molecular identity or dissimilarity of the P and A coupling factors will consequently require further investigation, and may be provided by study of interchangeability behavior of the factors during purification.

Table 3. Reconstitution of photophosphorylation activity in uncoupled P particles by an A coupling factor preparation. The A preparation was made as follows. A-type particles were sonicated in the presence of EDTA as in the uncoupling procedure described in the text. The uncoupled A particles were sedimented by high-speed centrifugation and a protein fraction obtained from the supernatant fluid by precipitation with $(NH_4)_2SO_4$ between 30 to 50 percent saturation in the presence of 4 m*M* ATP. The fraction was dissolved in 0.05*M* tris-HCl buffer (*p*H 8), containing 4 m*M* ATP, and the solution was filtered through a Sephadex G-25 column; proteins collected after passage of the void volume were used. Protein content of the preparation was 2 mg/ml. Details of the procedure used in testing for reconstitution of uncoupled P particles by coupling factors have been described previously (*4*).

Preparation A added (ml)	Photophosphorylation activity (μmole of ATP synthesized per hour per milligram of bacteriochlorophyll)
None	12.5
0.05	23.0
0.10	37.0
0.20*	62.5
0.30	70.0

* Addition of oligomycin (1.33 μg/ml) reduced the photophosphorylation activity with this quantity of A preparation to 9.5.

BRUNO A. MELANDRI*
ASSUNTA BACCARINI-MELANDRI*
ANTHONY SAN PIETRO
HOWARD GEST
Departments of Microbiology and *Botany, Indiana University, Bloomington 47401*

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* Present address: Istituto Botanico, Universita di Bologna, Bologna, Italy.

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Infectious Agent from a Free-Living Soil Amoeba, Naegleria gruberi

Abstract. *A subcellular infectious material has been found in a strain of the amoeba* Naegleria gruberi, *strain EG, which is capable of infecting chick embryo cells and causing them to undergo cytopathic changes with the release of more infectious material. The material is present in two lines of the amoeba which were separated shortly after the isolation of the strain and subsequently maintained in separate laboratories.*

The free-living amoebas, *Naegleria gruberi*, strain EG, contain particles that appear by observation in the electron microscope to be virus-like (*1, 2*). It has been suggested that, if these objects do indeed represent virus particles, they may have been introduced into the amoebas during laboratory cultivation, perhaps by way of the chick embryo extract used as a supplement in the growth medium (*1*). The present investigation was designed to determine if the EG amoebas contained a biologically active agent that has characteristics of a virus, and, if so, to determine the relation between its presence and the conditions of cultivation of the amoebas. The experiments have shown that the amoebas contain a subcellular agent that produces a cytopathology in cultured chick embryo cells and that the agent is not associated with the growth medium. An agent in *Naegleria gruberi* presents a potential medical relation with humans, since strains of these amoebas have been isolated from encephalitic victims (*3, 4*) and are considered to be pathogens (*5*).

Two lines of *Naegleria gruberi*, strain EG, were used. One line (EG_S) was cultivated in a medium composed of yeast extract, peptone, and liver supplemented with 5 percent chick embryo extract [axenic culture; (*6*)], or in a medium of yeast extract, peptone, and glucose to which living *Aerobacter aerogenes* was added (*1*). The other line (EG_B) had been maintained axenically in the laboratory of Dr. William Balamuth, Department of Zoology, University of California, Berkeley, in a medium of yeast extract, proteose peptone, and liver supplemented with 1 percent alpha globulin fraction IV-4 (*7*).

Lysates of the amoebas were pre-