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 ($\mu$mole 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 $\mu$g/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*

**References and Notes**

1. A. L. Tuttle and H. Gest, *Proc. Nat. Acad. Sci. U.S.* **45**, 1261 (1959); D. M. Geller, in *Bacterial Photosynthesis*, H. Gest, A. San Pietro, L. P. Vernon, Eds. (Antioch Press, Yellow Springs, Ohio, 1963), p. 161; A. Thore, D. L. Keister, A. San Pietro, *Arch. Mikrobiol.* **67**, 378 (1969).
2. G. Cohen-Bazire, W. R. Sistrom, R. Y. Stanier, *J. Cell Comp. Physiol.* **49**, 25 (1957); G. Drews, *Arch. Mikrobiol.* **51**, 186 (1965).
3. J. Yamashita, S. Yoshimura, Y. Matuo, T. Horio, *Biochim. Biophys. Acta* **143**, 154 (1967).
4. A. Baccarini-Melandri, H. Gest, A. San Pietro, *J. Biol. Chem.* **245**, 1224 (1970).
5. B. A. Melandri, A. Baccarini-Melandri, H. Gest, A. San Pietro, in *Colloquium on Bioenergetics: Energy Transduction in Respiration and Photosynthesis* (Pugnochiuso, Italy, in press); A. Baccarini-Melandri and B. A. Melandri, in *Methods in Enzymology*, A. San Pietro, Ed. (Academic Press, New York, 1971), vol. 23, p. 556.
6. M. Avron, *Biochim. Biophys. Acta* **40**, 257 (1960).
7. G. Drews, H.-H. Lampe, R. Ladwig, *Arch. Mikrobiol.* **65**, 12 (1969); J.-H. Klemme and H. G. Schlegel, *ibid.* **68**, 326 (1969).
8. Supported by grants GB 7333X from NSF (to H.G.) and GM 16314 from NIH (to A.S.P.). We thank Prof. Felix Haurowitz and Dr. Sarah Zimmerman, Department of Chemistry, Indiana University, for advice and help in connection with preparation of the antibody to P coupling factor, and Carol Stahl for technical assistance.
* Present address: Istituto Botanico, Universita di Bologna, Bologna, Italy.

13 May 1971; revised 17 June 1971

## 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-

pared from 3-day cultures by freeze-thawing three times, sonication at 9 kcycle for 2 minutes, and filtration through a Millipore filter (pore size, 0.45 $\mu$). Portions of the lysates were plated onto secondary cultivations of chick embryo cells which, after 1 hour at 37°C in an atmosphere humidified with $CO_2$, were supplemented with liquid medium (2 percent calf serum in Gibco 199), or overlaid with hard agar (1 : 1 mixture of 4 percent calf serum in double-strength Gibco 199 and 1.8 percent Difco agar). Portions were also plated on bacterial (Difco stock culture agar), mycoplasma (Difco Bacto PPLO agar plus 1 percent Difco Bacto PPLO serum fraction), or axenic amoeba media.

Whole EG amoebas, when added to monolayer cultures of chick embryo cells, did not multiply and overgrow the cultures as had been observed in a different system of amoeba and cultured cells (*8*), but they did elicit a cytopathic response in the chick cells within 4 to 6 days, in both liquid and solid medium.

Lysates of the amoebas also initiated a cytopathic response of the chick cells within a 4- to 6-day period. Portions of the amoeba lysates plated on bacterial, mycoplasma, and amoeba media showed no growth of bacteria, mycoplasmas, or amoebas for incubation periods of up to 3 weeks. This observation indicates that the cytopathic response in the chick cells was not due to contamination of the lysates by viable amoebas or microorganisms.

The media from the cultures showing cytopathic effects (after freeze-thawing) initiated similar cytopathic responses in other preparations of chick cells. The material has been carried through seven passages on chick cells, each passage representing a 1 : 10 dilution. The cytopathic response, hence, was not simply the result of a toxin from the original amoeba lysate, but rather a material capable of growth in the chick cells.

Lysates from amoebas grown in axenic media and in media containing bacteria, and amoebas which had $EG_S$ and which had not $EG_B$ been grown in medium containing chick embryo extract, all produced the cytopathic condition in the cultured chick cells. The material initiating the cytopathic response is therefore associated with the amoebas, and not with the growth medium used.

The appearance of the cytopathic change in the chick cells occurred within a 12- to 24-hour interval in the cultures in 4 to 6 days after the addition of the amoeba lysates, or the passaged material, and involved 90 to 95 percent of the cells in the culture. The beginning of the cytopathic change was characterized by the presence of numerous rounded floating cells with a corresponding reduction in the numbers of fibroblasts that adhered to the dish (Fig. 1). Within an additional day the elongated fibroblasts disappeared altogether, leaving remnants of crenated, ruptured, and degenerate cells. The appearance of the degenerate cytopathic cultures underwent no further change for periods up to 3 weeks.

During the time that the fluids from the cytopathic cells were being passaged in chick cells, it was observed that some of the inoculated cultures contained mycoplasmas, as confirmed by growth on PPLO agar (*9*) and microscopic observation. *Mycoplasma* is not thought to be the causative agent for the cytopathic responses observed in the chick cells because (i) not all of the samples producing the cytopathology contained mycoplasma when portions were cultured on PPLO agar; (ii) the cytopathology progressed in cultures treated with 1000 $\mu$g of kanamycin sulfate per milliliter (Kantrex Bristol Laboratories), a substance which reduced the number of mycoplasmas present by 90 percent or more, as determined by the number of colony formations on PPLO agar; and (iii) chick cells inoculated with $3 \times 10^3$ mycoplasmas per plate showed no evidence of the cytopathology described during 9 days of incubation, although the mycoplasmas increased to $4 \times 10^7$ per plate.

End-point dilutions of the material responsible for the cytopathic response were made in 35-mm petri plates on secondary chick embryo cells in liquid medium. For the most part, cytopathic responses occurred in plates inoculated with the lower dilutions ($10^{-2}$ to $10^{-4}$) of the amoeba lysates. Freshly prepared passaged material occasionally yielded responses at a dilution of $10^{-6}$. Reassay of the passaged material after storage at 2°C or at −40°C for several weeks, however, yielded responses only in the lower dilutions ($10^{-2}$ to $10^{-3}$), although reassay of the amoeba lysates showed that they had maintained their

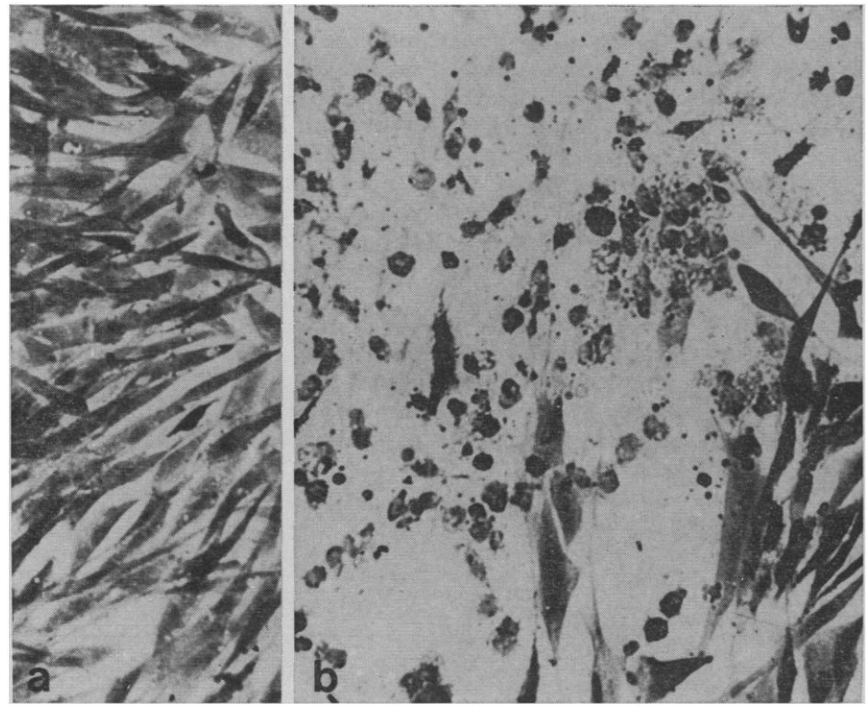


Fig. 1. Areas of cultures of secondary chick embroyo fibroblasts: (a) uninoculated control; (b) 4 days after inoculation with 0.5 ml of amoeba lysate in 4.5 ml of medium (2 percent calf serum in Gibco 199). The right side shows cells similar in appearance to the cells of the uninoculated control cultures; the left and center show the shrunken, rounded, and ruptured cytopathic cells. Air-dried; stained with May-Grumwald Giemsa stain (× 500).

cytopathic capacity for more than 5 months' storage at −40°C.

Plaques induced by lysate material are formed on cultures of chick embryo cells under hard agar, attaining diameters of up to 1 cm in 3 weeks. Material recovered from the plaques initiates cytopathic responses on chick cells in liquid medium similar to those initiated by the original amoeba lysates.

The presence of new infectious material in the chick cells was determined by making end-point dilutions of the supernatant and cellular fractions of inoculated cultures sampled at daily intervals. The cells were inoculated with material that was infectious at a $10^{-2}$ dilution for 1 hour at 37°C, thoroughly washed, and placed in medium (2 percent calf serum in Gibco 199). The cells remained indistinguishable from the uninoculated control cells for 4 days following inoculation, and during this time they were noninfectious in both the supernatant and the cellular fractions. On the fifth day after inoculation about 25 percent of the cells were visually cytopathic in areas that spread to include the entire plate by the next day. In direct correlation with the appearance of the cytopathic cells, the supernatant and cellular fractions of the cultures were infectious at dilutions of $10^{-3}$ and $10^{-4}$, respectively, on the fifth day. The infectivity of the supernatant fraction increased somewhat to equal that of the cellular fraction at a $10^{-4}$ dilution by the sixth day.

It is concluded that the EG amoebas contain a material capable of infecting chick embryo cells and causing them to undergo cytopathic changes with the release of more of the infectious material. The material is present in two lines of the amoeba which were separated shortly after the initial isolation of the EG strain and maintained in separate laboratories in media with different growth supplements.

T. H. DUNNEBACKE
*Virus Laboratory, University of California, Berkeley 94720*

F. L. SCHUSTER
*Department of Biology, Brooklyn College, Brooklyn, New York 11210*

### References and Notes

1. F. L. Schuster, *J. Protozool.* **16**, 724 (1969).
2. ——— and T. H. Dunnebacke, *J. Ultrastruct. Res.*, in press.
3. C. G. Butt, C. Baro, R. W. Knorr, *Amer. J. Clin. Pathol.* **50**, 568 (1968).
4. R. J. Duma, H. W. Ferrell, E. C. Nelson, M. M. Jones, *New Engl. J. Med.* **281**, 1315 (1969).
5. S. L. Chang, *J. Parasitol.* **57** (Suppl.), 10 (1971).
6. F. L. Schuster and G. Svilha, *J. Protozool.* **15**, 752 (1968).
7. W. Balamuth, *ibid.* **11** (Suppl.), 19 (1964).
8. T. H. Dunnebacke and R. C. Williams, *Proc. Nat. Acad. Sci. U.S.* **57**, 1363 (1967).
9. L. J. Lewandowski, Virus Laboratory, University of California, Berkeley, personal communication.
10. One of us (F.L.S.) thanks Dr. William Balamuth for the hospitality of his laboratory during the summer of 1970, when much of this work was performed. We also thank Dr. R. C. Williams for his helpful advice and Miss T. Klassen and Mrs. C. Lizarraga for technical assistance. This investigation was supported in part by PHS research grant CA 02245 from the National Cancer Institute, and in part by City University of New York Faculty Research Award 1217.

12 July 1971

## Potassium Flux: a Common Feature of Albizzia Leaflet Movement Controlled by Phytochrome or Endogenous Rhythm

Abstract. *Leaflets close when potassium moves into dorsal and out of ventral pulvinule motor cells and open when the flux direction is reversed. This is true whether leaflet movement is controlled by an endogenous rhythm or by phytochrome.*

Various leguminous plants, including *Albizzia julibrissin*, have doubly compound leaves with paired leaflets that regularly move toward and away from each other in response to a variety of endogenous and exogenous stimuli. Leaflets usually open in the light and close when darkened, but their movement will persist with a circadian rhythm if plants are kept in constant intensity light or uninterrupted darkness (*1*). During certain parts of the diurnal cycle, nyctinastic closure is controlled by the regulatory pigment phytochrome (*2*, *3*), which also controls many aspects of plant development such as seed germination, photomorphogenesis, and floral initiation (*4*).

Leaflet orientation depends on the relative turgor of motor cells on dorsal and ventral sides of the pulvinule, a thickened organ at the base of the leaflet (*5*). Potassium (K) flux is involved in turgor changes in *Mimosa pudica* motor cells (*6*) and in stomatal guard cells (*7*). The K concentration of the *Albizzia* pulvinule is sufficiently high (about 0.47*N*) that K flux can exert significant osmotic effect. We have reported (*8*) that *Albizzia* leaflets close in the dark when K moves into dorsal motor cells and out of ventral motor cells, and that preirradiation with red light is required for K efflux from ventral cells and for optimum movement of K into dorsal cells. Thus K flux in key pulvinule cells is the basis for phytochrome-controlled nyctinastic leaflet closure. Our study was undertaken to determine whether it is also the basis for endogenously rhythmic leaflet movement.

*Albizzia* plants were grown from seed in the greenhouse and transferred to controlled growth chambers 3 days or more before experiments, to allow time for plants to adjust to new environmental conditions. Growing conditions, experimental procedures, and light sources were as described (*8*).

A typical *Albizzia* leaf contains hundreds of pinnule pairs with similar K content and physiological behavior. We obtained comparative kinetic data on leaflet movement and K flux by measuring the angle between paired leaflets at designated time intervals, and then excising, freezing, and sectioning

Table 1. Leaflet closure in the light and correlated changes in K, the ratio of K to Ca, and the ratio of K to P in dorsal and ventral motor cells. The experimental procedure was the same as that described for Fig. 1, except that leaflet angles were measured and pulvinules were excised during the 12th to 16th hour of the photoperiod only. Data include standard deviations.

| Time (hr) | Angle between leaflets | Ventral | | | Dorsal | | |
|---|---|---|---|---|---|---|---|
| | | K | K/Ca | K/P | K | K/Ca | K/P |
| 12 | 180° | 302 ± 98 | 1.15 ± .38 | 0.35 ± .11 | 215 ± 31 | 0.64 ± .23 | 0.23 ± .05 |
| 13.5 | 110° | 300 ± 45 | 0.96 ± .14 | 0.30 ± .07 | 268 ± 63 | 0.87 ± .17 | 0.30 ± .07 |
| 14.5 | 60° | 214 ± 40 | 0.87 ± .13 | 0.25 ± .06 | 297 ± 45 | 0.99 ± .18 | 0.35 ± .11 |
| 16.5 | 10° | 163 ± 40 | 0.48 ± .11 | 0.14 ± .04 | 344 ± 44 | 0.98 ± .26 | 0.42 ± .20 |