cortical surface of fossil mammals from endocranial casts.

The results of the entire study are summarized in Figs. 2 and 3 and in Table 1 (*5*). The wallaby and kangaroo deviate most from the straight (undrawn) trend lines in Figs. 2 and 3. A tentative explanation may be found in the fact that these two specimens had been born and raised in captivity.

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5. A more extensive paper on this subject, considering all its aspects in greater detail, with a statistical analysis of the data, will be submitted for publication elsewhere.
6. Supported by PHS grant NB 7104.

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Energy Flux and Membrane Synthesis in Photosynthetic Bacteria

Abstract. *Synthesis of the energy-converting membrane complex of the photosynthetic purple bacterium* Rhodopseudomonas capsulata *during growth under different conditions of energy flux was studied by examining the disorganizing effects of polymyxin B, with or without lysozyme, on integrity of the cell envelope. Cells growing with a limited supply of energy show an elevated bacteriochlorophyll content and increased resistance to breakdown of the "permeability barrier" by these agents. It seems that purple bacteria respond to energy restriction by preferentially synthesizing excess bacteriochlorophyll-membrane which, in effect, toughens the cell integument.*

The purple photosynthetic bacteria may prove to be particularly useful for analysis of mechanisms involved in regulation of energy-converting membrane synthesis. In these organisms the photochemical energy-conversion (photophosphorylation) system is localized in the plasma membrane and its extensions into the cytoplasm (*1, 2*). Bacteriochlorophyll (BChl) is essential for photophosphorylation, and the BChl contents of cells grown under different conditions provides an index of membrane synthesis and, probably, of differentiation. Limitation of the energy supply during anaerobic photosynthetic growth influences formation of BChl and the energy-converting membranes, and this can be achieved without changing the chemical composition of the growth medium by simply altering the light intensity or by using intermittent illumination. When cells growing in continuous bright light are placed in dim light, or intermittent bright light, the growth rate decreases markedly (*3–5*). Such slowly growing cells have an elevated content of BChl [relative to protein content or dry mass (*3, 5*)], and electron micrographs of thin sections indicate a substantially increased quantity of intracytoplasmic membrane (*6, 7*). In other words, it seems that cells of purple bacteria respond to decrease in the average energy flux by producing more light-harvesting membrane or energy-converting membrane, or both. It has been reported (*7, 8*) that the "additional" membrane has the same BChl content as the "original" membrane, but there is contrary evidence (*2, 9*). The ambiguity on this important point may have resulted, in part, from inadequacy of fractionation procedures, and it is also possible that not all the species of photosynthetic bacteria examined show the same pattern (*2*). Phospholipid is generally considered to be uniquely associated with membranes [but see (*10*)], and it is frequently assumed that membrane composition remains constant in this respect. In *Rhodopseudomonas palustris,* phospholipid and BChl contents may indeed change in constant proportion as light intensity is varied (*2*), but this seems not to be true for other photosynthetic bacteria [(*2, 11, 12*); see also relevant observations in (*10*)]. The difficulties in reconciling available analytical chemical data with ultrastructural observations led us at one point to question the physical reality of additional membrane seen by electron microscopy in cells growing slowly because of a restriction on the supply of energy. In this report, independent evidence is presented for the existence of such membrane.

The approach was based on the use of agents that specifically disorganize constituents of the membrane and cell envelope. We reasoned initially that cells with extensive membranous invaginations should show slower leakage of soluble intracellular enzymes, such as inorganic pyrophosphatase, when attacked with a given limited quantity of polymyxin B. This cyclic polypeptide antibiotic reacts with lipid components of bacterial membrane structures (*13*), produces bleblike extrusions on the surface of certain Gram-negative bacteria (*14*), and can induce gross changes in morphology of photosynthetic bacteria (*1, 15*). Addition of polymyxin B to suspensions of *Rhodopseudomonas capsulata* cells leads to appearance of pyrophosphatase activity as illustrated in

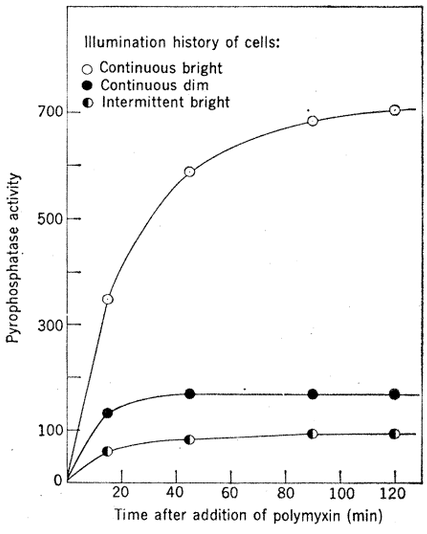

Fig. 1. Evocation of inorganic pyrophosphatase activity in *Rhodopseudomonas capsulata* cell suspensions by polymyxin B. *Rhodopseudomonas capsulata* (American Type Culture Collection No. 23782) was grown with Lumiline lamps as the light source, as described earlier (*5*). Continuous bright light, intensity of 6000 lu/m^2; continuous dim, 440 lu/m^2; intermittent bright, 6000 lu/m^2, on and off at 30-second intervals. In each instance, cells from 10 ml of culture were harvested when the density was approximately 390 μg of dry weight per milliliter (during logarithmic growth). The bacteria were washed with and resuspended in 5 ml of 0.05*M* tris(hydroxymethyl)aminomethane-HCl buffer, *p*H 9.0. At zero time, 5 ml of polymyxin B sulfate (Mann Research Laboratories, Inc., New York; 125 μg/ml in the buffer noted) were added from a syringe, with rapid mixing. Suspensions were incubated at 30°C and 0.1 ml samples removed at intervals (with an automatic pipette) for enzyme activity assay. Each sample was placed in 0.9 ml of solution containing: $Na_4P_2O_7$, 6 μmole; $MgCl_2$, 6 μmole; and tris-HCl buffer (*p*H 9) 22.5 μmole. Following incubation at 30°C for 5 minutes, 1 ml of cold 10 percent trichloroacetic acid was added and inorganic phosphate determined in a suitable portion by a modification of the Fiske-SubbaRow method (*19*). Pyrophosphatase activity is expressed as micrograms of phosphorus liberated (as P_i) per hour per 0.1 ml of suspension.

Fig. 1. The kinetics with which enzyme activity level increases in the suspensions clearly differ for cells grown in continuous saturating light as compared with those grown either in continuous dim light or intermittent bright light. Cells growing rapidly in continuous bright light have a BChl content of about 0.65 percent of their dry weight, while the level is approximately twice as high in organisms grown in the other illumination regimens specified (*5*). It is evident that cells of low BChl (that is, presumably minimal membrane) content are particularly susceptible to the activity-inducing effect of the antibiotic.

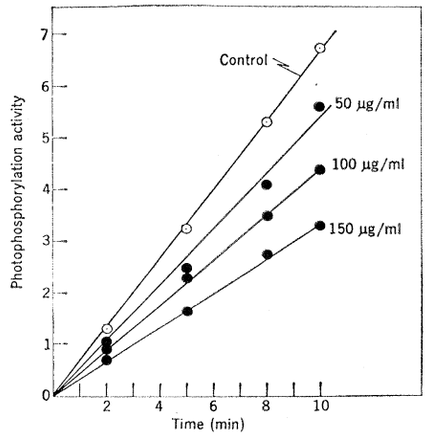

Fig. 2. Effect of polymyxin on photophosphorylation catalyzed by pigmented particles from *Rhodopseudomonas capsulata*. Cells grown in high-intensity light were washed and resuspended in 0.05*M* glycylglycine buffer (*p*H 7.2) and disrupted in a French pressure cell. Large debris was removed from the extract by a low-speed centrifugation, and pigmented particles were then collected by centrifugation at 100,000*g* for 90 minutes. The particles were washed with the buffer and resuspended in buffer plus glycerol (1:1, by volume). Reaction mixtures contained, per milliliter: adenosine diphosphate, 2 μmole; $^{32}P_i$, 10 μmole; $MgCl_2$, 10 μmole; glucose, 33 μmole; sulfate-free hexokinase, in excess; glycylglycine buffer (*p*H 8.0), 33 μmole. Each mixture was placed in a special glass vessel (designed by Dr. R. Togasaki, Botany Department, Indiana University) which was continuously flushed with argon. After 8 minutes of gassing, the pigmented particles were introduced (20 μg of BChl per milliliter, final concentration) and immediately thereafter, polymyxin B as indicated. Two minutes later (zero time), illumination (> 9700 lu/m^2) was begun with a 150-watt reflector flood lamp. Samples were withdrawn at intervals, deproteinized with trichloroacetic acid, and organic ^{32}P (glucose-6-phosphate) was determined as described by Avron (*20*). Photophosphorylation activity is expressed as micromoles of glucose-6-phosphate produced per milligram of BChl (at 28°C).

Although the pyrophosphatase of *R. capsulata* is a soluble enzyme, control experiments showed that only a small fraction of the total enzyme activity observed in such experiments can be found in the suspending medium (approximately 10 percent at 15 minutes, with cells grown in continuous bright light). Thus, the primary effect of polymyxin is to make the cells permeable to the substrate of the enzyme, namely, magnesium pyrophosphate (*16*). It should be noted that cells grown under the several conditions specified contain somewhat different levels of pyrophosphatase activity. For example, extracts, obtained by means of a French pressure cell, of bacteria grown in continuous saturating light typically show activities of approximately 36 μg of phosphorus released per hour [in the form of orthophosphate (P_i)] per microgram of protein; activity in comparable extracts from cells grown in the intermittent light regimen is definitely lower, namely, about 23 μg of phosphorus per hour per microgram of protein. Despite this difference, it is clear that there is considerably less "permeability barrier" (to be overcome by the particular concentration of polymyxin used) offered by the cell envelope and derivative membranes of "low-BChl cells" grown in continuous saturating light. Do photochemical regions of the membrane contribute to the permeability barrier under consideration? That they probably do is suggested by the results of experiments that tested the effect of polymyxin on photophosphorylation catalyzed by pigmented particles, obtained by shearing cells (membranes) in a French pressure cell. Polymyxin inhibits photophosphorylation significantly even when added at a concentration of 5 μg per microgram of BChl (Fig. 2) (in the experiments of Fig. 1, the concentrations used were in the range of approximately 14 to 27 μg per microgram of BChl).

Certain Gram-negative bacteria are very susceptible to lysis by the combined action of polymyxin and lysozyme (*17*). The photosynthetic bacterium *Rhodospirillum rubrum* shows this behavior (*1*), and *R. capsulata* was also found to be extremely sensitive to this combination, to the extent that in experiments with polymyxin alone the possibility of contamination with lysozyme from salivary spray makes the use of automatic pipettes advisable. The synergistic action of polymyxin and lysozyme, which leads to rapid and extensive disorganization of the cell envelope complex, is quite fast even at 0°C, as shown in Fig. 3. Comparison of cells grown in continuous and intermittent light shows that the kinetics and extents of the decrease of turbidity are consistent with the conclusion that per unit of dry weight there is considerably more membrane material in the slower-growing BChl-rich cells. In contrast with the results observed with polymyxin alone, at 30°C most of the pyrophosphatase activity is found in the suspending medium within 5 minutes after the addition of polymyxin plus lysozyme. It is also noteworthy that the pigmented membranous structures remaining after treatment with polymyxin plus lysozyme sediment at relatively low centrifugal force [observed also with *R. rubrum* (*1*)].

The present findings indicate that there is an inverse relation between growth rate (divisions per hour) and quantity of membrane per unit of dry weight. We suggested earlier (*5*) that a relation of this kind reflects operation of a regulatory system designed to cope with diminishing supply of energy. If under conditions of energy stress (for example, in dim light) chemical control

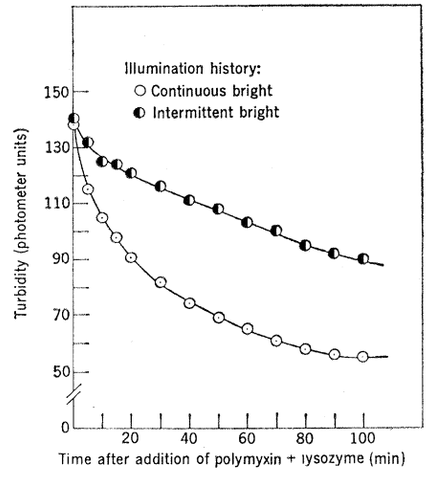

Fig. 3. Lysis of intact cells of *Rhodopseudomonas capsulata* by polymyxin plus lysozyme. Cell suspensions were prepared as indicated in Fig. 1, except that the organisms were harvested at a density of about 440 μg of dry weight per milliliter and cells from 5 ml of medium were resuspended in 4 ml of buffer. At zero time, polymyxin and lysozyme were added (in 1 ml) to give final concentrations of 50 μg/ml and 1.2 μg/ml, respectively. The suspensions were incubated at 0°C, and turbidity measurements made with a Klett-Summerson photometer equipped with a No. 66 filter. In the absence of polymyxin plus lysozyme, virtually no change in turbidity was observed over the time period shown.

signals dictate preferential synthesis of additional energy-converting machinery, formation of other cell components essential for rapid growth [such as ribosomes (*18*)] would be inhibited and, inevitably, the growth rate would be decelerated. A regulatory system performing in this fashion should be characterized as a partially compensatory control mechanism rather than as a finely tuned regulation device. It seems likely that in addition to changes in quantity, changes in composition of energy-converting membranes must occur under different nutritional conditions to permit economic use of the energy and material resources available for biosynthesis. It is hoped that polymyxin and related antibiotics will serve as sensitive reagents for defining such alterations.

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Thermal Radiation in Metabolic Chambers

Abstract. *Emissivities and ratios of surface areas of metabolic chambers and their contents have been usually ignored in studies of the metabolic rates of animals. Failure to take these factors into account can lead to errors in the interpretation of results.*

A wide variety of containers have been used to measure metabolic heat production and the rates of evaporative water loss of animals. Frequently, such chambers have had smooth metallic inner surfaces. Generally, such metallic surfaces have high infrared reflectances and low emissivities (*1*). The exchange of thermal radiation between the animal and the chamber walls is often not considered. If the chamber walls are highly reflective to infrared radiation, the energy reflected back to the animal from the chamber walls may have a significant effect on the energy balance of the animal (*2*).

The exchange of radiant energy between an animal (or plant) and a closed chamber is determined by the surface temperatures and emissivities of the organism and the container walls, their surface areas, and the percentage of each area that "views" the other (the view factor) (*3*, *4*). An equation that describes the theoretical exchange of radiation between an object and its container may be derived in at least two ways. In an intuitive derivation one may imagine two infinite parallel planes with a finite surface between them. The infinite planes represent the container walls and the finite surface represents the organism. We here assume for simplicity that the absorptivity of the animal is perfect, that is, absorptivity is 1. Energy radiated from an animal (designated surface 1) will strike surface 2 (the infinite parallel planes) where a fraction will be absorbed and some will be reflected. The proportion of the reflected energy incident on surface 1 will be determined by the view (shape) factor from surface 2 to surface 1 (F_{21}) (*3*). The rest will pass the animal and be absorbed or reflected by the opposite plane. On each pass of reflected energy, some falls on the animal. The equation describing the transfer of radiant energy from surface 1 to surface 2 is

$$E_{1\to 2} = \alpha_2\epsilon_1\sigma T_1^4 A_1 + \alpha_2[\rho_2(\epsilon_1\sigma T_1^4 A_1) - \rho_2(\epsilon_1\sigma T_1^4 A_1)F_{21}] + \alpha_2\rho_2[\rho_2(\epsilon_1 A_1\sigma T_1^4) - \rho_2(\epsilon_1 A_1\sigma T_1^4)F_{21}] - \alpha_2\rho_2[\rho_2(\epsilon_1 A_1\sigma T_1^4) - \rho_2(\epsilon_1 A_1\sigma T_1^4)F_{21}]F_{21} + \ldots \quad (1)$$

or

$$E_{1\to 2} = \alpha_2\epsilon_1 A_1\sigma T_1^4[1 + \rho_2(1 - F_{21}) + \rho_2^2(1 - F_{21})^2 + \rho_2^3(1 - F_{21})^3 \ldots] \quad (2)$$

where α is the absorptivity, ε is the emissivity, ρ is the reflectivity, σ is the Stefan-Boltzmann constant, T is the surface temperature in °K, and A is the surface area.

Since Eqs. 1 and 2 have the form

$$1 + x + x^2 + x^3 \ldots = 1/(1 - x) \quad (3)$$

then

$$E_{12} = \frac{\alpha_2\epsilon_1 A_1\sigma T_1^4}{1 - \rho_2(1 - F_{21})} \quad (4)$$

In similar fashion the radiant energy transferred from surface 2 to surface 1 is

$$E_{21} = \frac{\alpha_2\epsilon_2 A_2 F_{21}\sigma T_2^4}{1 - \rho_2(1 - F_{21})} \quad (5)$$

Since the net energy transferred by radiation is the difference between Eqs. 4 and 5 and $A_1F_{12} = A_2F_{21}$, $F_{12} = 1$, $\alpha = \varepsilon$, and $\alpha + \rho = 1$ (*5*). Equation 6 is similar to Christiansen's equation (*6*).

$$Q_{12} = \frac{\epsilon_1\epsilon_2 A_1\sigma(T_1^4 - T_2^4)}{1 + (1/\epsilon_2 - 1)(A_1/A_2)} \quad (6)$$

where Q_{12} is the net transfer of energy from surface 1 to surface 2. A more

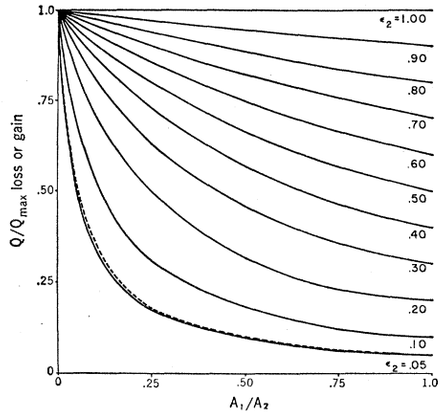

Fig. 1. Ratio of actual net radiant exchange to maximum possible net exchange as a function of area ratios and container emissivities. Maximum net exchange occurs when both surfaces have an emissivity of 1.0. All solid lines are computed on the assumption that the animal surface has an emissivity of 1.0. The dashed line is the difference in the solution at $\epsilon_2 = 0.05$, if $\epsilon_1 = 0.95$ instead of 1.0. The difference is even smaller at higher values of ϵ_2.