[12] Hanson, L. Å., and I. Berggård, *Clin. Chim. Acta*, **7**, 828 (1962).
[13] Stevenson, G. T., *J. Clin. Invest.*, **41**, 1190 (1962).
[14] Burtin, P., L. Hartmann, R. Fauvert, and P. Grabar, *Rev. franc. études clin. et biol.*, **1**, 17 (1956).
[15] Korngold, L., and R. Lipari, *Cancer*, **9**, 262 (1956).
[16] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[17] Müller-Eberhard, H. J., *Scand. J. Clin. and Lab. Invest.*, **12**, 33 (1960).
[18] Berggård, I., *Arkiv Kemi*, **18**, 291 (1962).
[19] Berggård, I., *Arkiv Kemi*, **18**, 315 (1962).
[20] Flodin, P., *Dextran Gels and Their Application in Gel Filtration* (Uppsala: Pharmacia, 1962).
[21] Dische, Z., and L. B. Shettles, *J. Biol. Chem.*, **175**, 595 (1948).
[22] Kunkel, H. G., and R. Trautman, in *Electrophoresis*, ed. M. Bier (New York: Academic Press, 1959), p. 225.
[23] Fleischman, J. B., R. H. Pain, and R. R. Porter, *Arch. Biochem. Biophys.*, Suppl. 1, 174 (1962).
[24] Porter, R. R., *Biochem. J.*, **73**, 119 (1959).
[25] Edelman, G. M., J. F. Heremans, M.-Th. Heremans, and H. G. Kunkel, *J. Exptl. Med.*, **112**, 203 (1960).
[26] Scheidegger, J. J., *Intern. Arch. Allergy Appl. Immunol.*, **7**, 103 (1955).
[27] Yphantis, D. A., American Chemical Society, 140th meeting, Chicago, *Abstracts of Papers*, 1961, p. 1c.
[28] Gally, J. A. and G. M. Edelman, *Biochim. Biophys. Acta*, **60**, 499 (1962).
[29] Webb, T., B. Rose, and A. H. Sehon, *Can. J. Biochem. Physiol.*, **36**, 1159 (1958).
[30] Motulsky, A. G., *Nature*, **178**, 1055 (1956).
[31] Jones, R. T., W. A. Schroeder, J. E. Balog, and J. R. Vinograd, *J. Am. Chem. Soc.*, **81**, 3161 (1959).
[32] Ingram, V. M., and A. O. W. Stretton, *Nature*, **184**, 1903 (1959).
[33] Edelman, G. M., and B. Benacerraf, these PROCEEDINGS, **48**, 1035 (1962).
[34] Franěk, F., I. Řiha, and J. Šterzl, *Nature*, **189**, 1020 (1961).
[35] Edelman, G. M., B. Benacerraf, Z. Ovary, and M. D. Poulik, these PROCEEDINGS, **47**, 1751 (1961).
[36] Remington, J. S., E. Merler, A. M. Lerner, D. Gitlin, and M. Finland, *Nature*, **194**, 407 (1962).

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*BACTERIAL PHOTOPHOSPHORYLATION: REGULATION BY REDOX BALANCE**

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Pigmented particles derived from photosynthetic bacteria catalyze light-dependent synthesis of ATP[1] from ADP and P_i and also manifest a number of light-stimulated oxidation-reduction reactions. The *in vitro* photophosphorylation process first demonstrated by Frenkel[2] occurs readily in the absence of external electron acceptors and, in further contrast with oxidative phosphorylation, does not require addition of electron donors in significant quantity. The over-all reaction is generally interpreted to represent phosphorylation coupled with a closed circuit transfer of photochemically-generated electrons, or hydrogen atoms, through an electron transfer chain to an oxidant (presumably oxidized bacteriochlorophyll),

which is also created by the photoexcitation. Antimycin A is a potent inhibitor of the light-dependent ATP synthesis, which is commonly referred to as "cyclic photophosphorylation."

Existence of a second type of bacterial photophosphorylation, dependent on light-stimulated *net* electron transfer between added electron donor and acceptor systems, has recently been claimed by Nozaki, Tagawa, and Arnon.[3] According to their interpretation, pigmented particles catalyze an antimycin-resistant photophosphorylation coupled with "noncyclic" electron flow between ascorbate + DCPIP (electron donor) and NAD (acceptor); NAD is presumably reduced by electrons ejected by photoexcitation of bacteriochlorophyll and the oxidized pigment is restored to its original state by electrons from ascorbate + DCPIP. We have recently suggested[4] that a more plausible explanation of the light-stimulation of certain oxidation-reduction reactions in these organisms (or particles obtained therefrom) is the promotion of "dark" reactions by energy-rich intermediates produced through the interaction of light with the photochemical apparatus, i.e., energy-dependent electron transfer of the kind observed by Chance and Hollunger[5] in mammalian mitochondria. In this communication, we summarize evidence supporting the view that the apparent "noncyclic" phosphorylation reported by Nozaki *et al.*[3] is in reality cyclic photophosphorylation, which is antimycin-resistant due to the ability of DCPIP to effect a "by-pass" in light-induced cyclic electron transfer. In addition, the results confirm and extend the observation, due mainly to Kamen and colleagues,[6, 7] that optimal activity of the cyclic photophosphorylation system in isolated pigmented particles is dependent on maintenance of a suitable redox potential.

Materials and Methods.—Growth of bacteria: Rhodospirillum rubrum (strain Sl) was grown photosynthetically in a synthetic medium containing succinic acid (6 gm/liter), and L-glutamic acid (1 gm/liter) as the sole nitrogen source. Other ingredients of the medium and the general procedures used in growing the cultures were as described by Ormerod *et al.*,[8] except that a gas phase was not used.

Preparation of pigmented particles: Cells were harvested after 62–65 hr of growth, washed twice with 0.005 *M* potassium phosphate buffer pH 7.6, and ground[9] with alumina (Alcoa A-301). The disrupted cells were extracted with the phosphate buffer noted, and alumina, unbroken cells, and large subcellular fragments removed by low-speed centrifugations. Pigmented particles were collected from the supernatant fluid by centrifugation at 59,000 × *g* for 30–45 min. The particles were washed twice and stored in concentrated suspension in the phosphate buffer, under an atmosphere of H_2, in an ice bath. All operations during preparation of the cell-free extracts and particle suspensions were carried out at 0°–4°C.

Incubation conditions and analytical methods: The experimental suspensions were placed in Warburg vessels which were shaken in a bath equipped with a glass bottom. Illumination was provided, from below, by 50-watt reflector flood lamps. For most of the experiments, as noted, a sheet of red cellophane (total cutoff of light <555 mμ) was inserted between the lamps and the glass window. The light intensity, without the red filer, was 1500 foot-candles as measured with a General Electric light meter. Dark controls were simultaneously run in a shielded portion of the same bath.

Light-induced phosphorylation (LIP) of adenosine diphosphate was determined by measuring the disappearance of P_i in the presence of Mg^{++}, hexokinase, and glucose; ADP and hexokinase were added to the other components at zero time. To terminate the reactions, trichloroacetic acid was added to give a final concentration of 3.5%. P_i in the deproteinized fluids was determined by the method of Taussky and Shorr[10] and the results expressed as Light-Dark differences. Control experiments showed that addition of the various supplements used (ascorbate, DCPIP, etc.) did not induce phosphorylation in darkness.

Bacteriochlorophyll, in the pigmented particles, was determined by the method of Cohen-Bazire *et al.*[11]

Results.—Effects of reducing and oxidizing agents on LIP: Earlier studies[2, 12] with washed pigmented particles from *R. rubrum* disclosed that addition of very small quantities of certain organic substrates (e.g., succinate, lactate, α-ketoglutarate, $NADH_2$) caused activation of LIP under the usual conditions of anaerobic assay. This substrate effect has been frequently attributed to removal, by dehydrogenase action, of inhibitory traces of contaminating oxygen. On the other hand, the activation of LIP by reducing agents has been ascribed[6, 7, 13] to establishment of an optimal redox potential of one or more participating electron carriers. Our experiments support the conclusion that the effects of oxidizable organic substrates are primarily concerned with adjustment of the redox potential to a region required for maximal LIP. The redox potential can apparently also be shifted to this region by molecular hydrogen, as indicated by the data of Table 1.

TABLE 1

EFFECTS OF AIR AND HYDROGEN ON LIGHT-INDUCED PHOSPHORYLATION

Gas phase	P_i utilized (μmoles)
Argon	1.7
Helium	1.9
Hydrogen	3.9
Air	0

The reaction mixtures contained in a final volume of 3.0 ml: Tris buffer, pH 8.0, 100 μmoles; $MgSO_4$, 5 μmoles; K_2HPO_4, 10.2 μmoles; ADP, 0.5 μmole; glucose, 30 μmoles; hexokinase, 1 mg; particles, equivalent to 0.12 mg bacteriochlorophyll. Temperature, 30°C; incubation time, 15 min.

Under an atmosphere of H_2, LIP is significantly stimulated in comparison with the activity observed under argon or helium. Furthermore, the stimulatory effect of H_2 is catalytic in the sense that it is exerted in the absence of manometrically detectable utilization of H_2. The particulate preparations used in the present studies contain an active hydrogenase which is capable of oxidizing H_2 with a variety of electron acceptors (e.g., PMS, DCPIP, *p*-benzoquinone, ferricyanide).[4, 14] In view of the evidence[7] that *c*-type cytochromes act as electron carriers in LIP, it is particularly noteworthy that *R. rubrum* particles can also reduce mammalian cytochrome *c* through hydrogenase action.[15]

It appears that the degree of inhibition of LIP by oxygen is dependent on the vigor of aeration and can be significantly diminished by the presence of metabolizable substrates[16, 17] or reducing agents.[7] In our experience, with well-shaken reaction mixtures, virtually complete inhibition is observed upon aerobic incubation when reducing substances are not added (Table 1).

Displacement of the redox potential; "over-reduction" and "over-oxidation": If a critical redox range is required for optimal LIP, it is evident that appreciable displacement of the potential should eventually lead to inhibition. LIP by *R. rubrum* particles is, in fact, inhibited when certain "exogenous" electron donors (e.g., succinate, $NADH_2$, ascorbate) are present in excess.[7, 17] Inhibition by "over-reduction" is particularly marked if the experimental system also contains a redox dye (PMS or DCPIP) which can readily interact with components of the photochemical electron transfer chain. Thus, partially reduced PMS is an activator of LIP, but the dye becomes a strong inhibitor when it is maintained in the reduced state by an appropriate reducing agent.[12] These and similar results ob-

served with *Chromatium* particles[6] indicate that efficient LIP is dependent on maintenance of redox components of the phosphorylating system at particular oxidation-reduction potentials.

The experiment summarized in Table 2 illustrates the effect of over-reduction

TABLE 2

INHIBITION OF LIGHT-INDUCED PHOSPHORYLATION BY OVER-REDUCTION WITH H_2

Additions	Gas phase	P_i utilized (μmoles)
None	A	5.0
Ascorbate (0.2 μmole)	A	5.7
Ascorbate + DCPIP (0.2 μmole)	A	7.9
None	H_2	7.6
Ascorbate (0.2 μmole)	H_2	7.3
Ascorbate + DCPIP (0.2 μmole)	H_2	3.3

When both ascorbate and DCPIP were used, they were mixed in a side-arm of the vessel while the latter was being gassed. The additions were introduced at zero time. Other experimental conditions as in Table 1, except that the particles contained 0.11 mg bacteriochlorophyll and the incubation time was 30 min.

on LIP. In the control series, under argon, 6.6×10^{-5} M ascorbate accelerated LIP slightly while a pronounced stimulation was caused by addition of DCPIP, exactly titrated with the same concentration of ascorbate. LIP by *Chromatium* particles is similarly activated by ascorbate + DCPIP mixtures.[6]

With H_2 as the gas phase, LIP proceeded at the "activated" rate in the absence of other adjuvants. Addition of ascorbate alone had little effect, but the ascorbate + DCPIP mixture was inhibitory. These results can be explained by assuming that, in the presence of H_2, the hydrogenase tends to keep the dye in the reduced state and, thereby, shifts the over-all redox potential of the particles to an unfavorable (reduced) position. In the absence of H_2, the dye would presumably remain in a more oxidized state, particularly because the particles catalyze a rapid anaerobic photooxidation of reduced DCPIP with endogenous electron acceptors.[18] It is evident that in systems containing DCPIP exactly titrated with ascorbate, the "extra" reducing effect of H_2 creates a redox situation comparable to that existing when DCPIP + excess ascorbate are present under an inert gas. Accordingly, we have found that if the ascorbate/DCPIP ratio exceeds 2, LIP under argon is inhibited.

The extent of inhibition of LIP by over-reduction with H_2 is, within limits, dependent on the hydrogenase content of the system or on the activity of the enzyme under the particular conditions used (see discussion of Table 6). This can be readily demonstrated by increasing the enzyme level with an exogenous source of hydrogenase as shown in Table 3.

TABLE 3

OVER-REDUCTION OF THE PHOTOPHOSPHORYLATION SYSTEM BY AN EXOGENOUS HYDROGENASE

Additions	Gas phase	P_i utilized (μmoles)
None	H_2	5.2
DCPIP (0.2 μmole)	H_2	5.5
Escherichia coli particles (protein $\equiv$ 300 μg)	H_2	3.2
E. coli particles + DCPIP	H_2	0.5
E. coli particles + DCPIP + fumarate (20 μmoles)	H_2	1.8
None	A	4.1
E. coli particles	A	4.2

The dye, when used, was reduced in darkness with H_2 by the hydrogenase(s) prior to initiation of the LIP reaction. Hydrogenase-containing particles from *E. coli* were prepared as described in ref. 19. Other conditions as in Table 1, except that the *R. rubrum* particles contained 0.13 mg bacteriochlorophyll and the reaction time was 20 min in red light.

Ascorbate was not added in the trials summarized in Table 3, and the results therefore reflect the effects of reducing activity primarily due to hydrogenase. Addition of only DCPIP to the *R. rubrum* particles had a negligible effect on LIP in this particular experiment, but presence of the dye frequently enhances the activity. In the absence of the dye, the particulate *Escherichia coli* hydrogenase caused an appreciable inhibition of LIP; separate tests showed complete inhibition when the quantity of *E. coli* particles was increased 2.5 fold. It can be seen that LIP was not suppressed by the *E. coli* particles when an argon atmosphere was used. Accordingly, it seems probable that the remarkable inhibition observed under H_2 is caused by over-reduction of electron carriers of the *R. rubrum* LIP system by the *E. coli* hydrogenase. When both DCPIP and the exogenous hydrogenase were present, LIP was almost completely suppressed and inclusion of fumarate in the reaction mixture led to a definite relief of the inhibition; the mechanism of this type of reversal is discussed below.

It might be expected[6] that extensive displacement of the redox potential by "over-oxidation" would also cause inactivation of the LIP system, and the inhibitory effects of oxidized dyes,[12] ferricyanide,[20] and oxygen can be rationalized on this basis.

Antimycin-sensitivity of LIP: The LIP activity of *R. rubrum* particles, in the absence of dyes or other adjuvants, is very sensitive to inhibition by antimycin A.[12] Supplementation with partially reduced PMS, however, accelerates LIP activity and also confers antimycin-resistance on the system. These observations suggest[12] that when the oxidation-reduction potential of the system is properly adjusted, PMS can act as a redox carrier which by-passes a rate-limiting antimycin-sensitive step. The results of Table 4 show that under an atmosphere of H_2, the dye DCPIP also catalyzes antimycin-resistant LIP.

TABLE 4

ANTIMYCIN-RESISTANCE OF LIGHT-INDUCED PHOSPHORYLATION IN THE PRESENCE OF DCPIP

Additions	P_i utilized (μmoles)
None	9.9
Antimycin A (10 μg)	0
DCPIP (0.2 μmole)	10.6
DCPIP + antimycin A	7.8

Gas phase, H_2. Other conditions as in Table 3.

The foregoing results, and those of similar experiments with particles from malate-grown cells, indicate that PMS and DCPIP interact with the LIP system in essentially the same way. Both dyes are readily reduced by the particulate *R. rubrum* hydrogenase,[4] and the similarity of their behavior is further illustrated by the experimental results of Table 5.

TABLE 5

OVER-REDUCTION OF THE PHOTOPHOSPHORYLATION SYSTEM IN THE PRESENCE OF PMS

Additions	P_i utilized (μmoles)
None	6.0
Antimycin A (10 μg)	0.7
PMS (1 μmole)	5.1
PMS + antimycin A	3.4
PMS + ascorbate (1 μmole)	0.2

Experimental conditions as in Table 4.

With only PMS added, LIP was partially suppressed, presumably due to slightly adverse displacement of the redox potential resulting from hydrogenase activity; the phosphorylation was, as expected, largely antimycin-resistant. The simultaneous presence of H_2, PMS, and a low concentration of ascorbate led to essentially complete inhibition of LIP. These results indicate that the *R. rubrum* particles become easily over-reduced when either PMS or DCPIP (see Table 2) plus an equivalent amount of ascorbate are present together with H_2. It would appear, furthermore, that under conditions of over-reduction, the dyes cannot effectively catalyze the antimycin-resistant by-pass discussed earlier.

Adjustment of the redox potential by added reductants and oxidants: The LIP system of isolated pigmented particles evidently is weakly "poised," i.e., shows comparatively low resistance to displacement of the redox potential.[21] Shift of the potential to the reduced side, and its attendant effects on LIP, should therefore be easily reversed by addition of oxidants. Vernon and Ash[17] have accordingly shown that fumarate or NAD (plus pyruvate and lactic dehydrogenase) can prevent the over-reduction effect of excess succinate or ascorbate + DCPIP on LIP by *R. rubrum* particles. Concurrently, a *net* light-stimulated electron flow between added reductant (e.g., ascorbate + DCPIP) and oxidant (fumarate or NAD) can also be demonstrated. Similarly, if a sufficiently high concentration of particles is used, light-stimulated consumption of H_2 occurs with fumarate as the ultimate electron acceptor when DCPIP or certain other redox mediators of relatively high potential are present.[4] Nozaki *et al.*[3] have concluded that the occurrence of antimycin-resistant phosphorylation during net electron transfer between ascorbate + DCPIP and NAD constitutes evidence for the existence of a "noncyclic" photophosphorylation mechanism, i.e., a phosphorylation coupled with the light-stimulated oxidation-reduction reaction noted. The data of Table 6, however, show that antimycin-resistant phosphorylation, *dependent on the presence of both reductant and oxidant*, can occur in the absence of net electron flow.

In experiment I (at 30°C), the inhibitory over-reduction effect of H_2 + ascorbate + DCPIP was completely reversed by addition of fumarate. In fact, there was a definite stimulation of LIP as compared with the "cyclic" photophosphorylation control (i.e., the trial with no additions). LIP in the "restored" system was, furthermore, quite resistant to inhibition by antimycin A. It is of importance to note that appreciable net electron transfer did not occur in the "complete" system, i.e., 8.5 μmoles of P_i were esterified in the presence of only 0.2 μmole of reducing agent and H_2 consumption was not detectable.

Experiment II was conducted at 20°C in view of Vernon's observation[18] that this temperature is optimal for NAD-dependent photooxidation of reduced DCPIP by *R. rubrum* particles. At 20°C, the *R. rubrum* hydrogenase is practically inactive and inhibition of LIP by over-reduction was consequently not observed. Under the redox conditions obtaining with ascorbate + DCPIP present, the LIP activity was somewhat enhanced and was completely resistant to antimycin. Further addition of a small quantity of NAD caused pronounced stimulation of LIP and this system also showed a higher rate of phosphorylation in the presence of antimycin than the "cyclic" control. Basically similar results have been obtained in experiments using a trapping system for $NADH_2$ (viz., pyruvate and

dialyzed lactic dehydrogenase). As in experiment I, there was no appreciable net oxidation-reduction under the conditions employed for experiment II.

TABLE 6

ACTIVATION OF ANTIMYCIN-RESISTANT CYCLIC PHOTOPHOSPHORYLATION BY DCPIP AND "REDOX BUFFERS"

Experiment	Temperature	Additions	P_i utilized (μmoles)
I	30°C	None	7.5
		Antimycin A (10 μg)	0.7
		Ascorbate (0.2 μmole) + DCPIP (0.2 μmole)	0.7
		Ascorbate + DCPIP + fumarate (10 μmoles)	8.5
		Ascorbate + DCPIP + fumarate + antimycin A	4.4
II	20°C	None	4.4
		Antimycin A (10 μg)	0
		Ascorbate (0.2 μmole) + DCPIP (0.2 μmole)	5.3
		Ascorbate + DCPIP + antimycin A	5.0
		Ascorbate + DCPIP + NAD (1 μmole)	7.5
		Ascorbate + DCPIP + NAD + antimycin A	5.1

In experiment I, the particles contained 0.11 mg bacteriochlorophyll; in experiment II, 0.20 mg. Other conditions as in Table 4, except that the incubation time was 30 min.

The results shown in Table 6 again emphasize the ability of DCPIP to catalyze an antimycin-resistant by-pass in the LIP mechanism when the redox potential is suitably adjusted. We conclude that the apparent "requirement" for both (added) reductants and oxidants can be explained by their action in establishing a redox environment which permits efficient operation of cyclic LIP.

Discussion and Summary.—The present results, together with those of earlier investigations,[6, 7, 12, 13, 17] provide strong evidence for the conclusion that an optimal oxidation-reduction potential is required for maximal coupling of light-activated electron transport to phosphorylation processes. This prominent characteristic and the marked sensitivity of isolated particles to displacement of the redox potential imply that either the photochemical apparatus, *in vivo*,[23] can be maintained within a restricted redox range, or that the rate of photophosphorylation is normally subject to metabolic regulation by changes in the temporal concentrations of certain physiological reductants and oxidants. In this connection, it is of interest that extensive shift of the redox potential, with consequent inhibition of phosphorylation, can apparently be induced in intact cells of *R. rubrum* by addition of low concentrations of certain redox dyes; PMS, thionine and benzyl viologen cause inhibition of photometabolism and, under these conditions, the cells resort to a "dark" fermentation of endogenous reserves (with the formation of fatty acids) even though they are under continuous illumination.[27] This striking metabolic effect, which is also caused by antimycin A, indicates that photophosphorylation activity inhibits fermentation and, in this sense, the induced transition could be said to represent a "photosynthetic Pasteur effect."

Our experiments indicate that, under certain conditions, DCPIP can act as an efficient electron carrier which by-passes an antimycin-sensitive step, or region, of the cyclic photophosphorylation system of isolated particles. This activity of the dye is abolished, however, if it is maintained primarily in the reduced state by an excess of reducing agent (either ascorbate or ascorbate + H_2). "Over-reduction" (or "over-oxidation") results in inhibition of phosphorylation, presumably due to severe displacement of the redox potential of one or more components of the electron transfer chain and, in systems containing DCPIP, to a

requirement for a particular steady-state ratio of oxidized to reduced dye for effective carrier action. The dye PMS also enables particles from *R. rubrum* to catalyze an antimycin-resistant photophosphorylation and, from all indications, both PMS and DCPIP affect the phosphorylation system in the same manner.

Photophosphorylation by isolated particles is activated by suitably adjusting the redox potential with mild reducing systems; this can be readily achieved through hydrogenase action or by addition of ascorbate alone. The inhibition caused by "over-reduction" with external electron donors can be effectively reversed by supplementation with certain electron acceptors, e.g., fumarate or NAD. Restoration of photophosphorylation by this means can occur *in the absence of net electron transfer* between the exogenous donor and acceptor systems. It seems reasonable to conclude that addition of the electron acceptor has the primary effect of adjusting the redox potential to an electrochemical range which is optimal for the operation of cyclic photophosphorylation. If dyes such as PMS or DCPIP are present under favorable redox conditions, the phosphorylation becomes resistant to antimycin A, apparently because the antibiotic-sensitive step is circumvented. The postulated[3] existence of "noncyclic" photophosphorylation is ultimately based on the observation of antimycin-resistant phosphorylation in experimental systems containing DCPIP as well as added reductants and oxidants. It is evident from the results described that the criterion of antimycin-resistance does not necessarily provide an adequate basis for establishing the occurrence of a process of this kind.

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[1] Abbreviations used: LIP, light-induced phosphorylation; ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate; NAD, nicotinamide adenine dinucleotide; $NADH_2$, reduced NAD; DCPIP, 2,6-dichlorophenolindophenol; PMS, N-methyl phenazonium methosulfate; Tris, tris(hydroxymethyl)aminomethane.

[2] Frenkel, A. W., *J. Amer. Chem. Soc.*, **76**, 5568 (1954); *J. Biol. Chem.*, **222**, 823 (1956).

[3] Nozaki, M., K. Tagawa, and D. I. Arnon, these PROCEEDINGS, **47**, 1334 (1961).

[4] Bose, S. K., and H. Gest, *Nature*, **195**, 1168 (1962).

[5] Chance, B., and G. Hollunger, *J. Biol. Chem.*, **236**, 1534 (1961).

[6] Newton, J. W., and M. D. Kamen, *Biochim. Biophys. Acta*, **25**, 462 (1957).

[7] Horio, T., and M. D. Kamen, *Biochemistry*, **1**, 144 (1962).

[8] Ormerod, J. G., K. S. Ormerod, and H. Gest, *Arch. Biochem. Biophys.*, **94**, 449 (1961).

[9] Grinding under an atmosphere of argon did not appear to significantly alter the LIP activity of the isolated particles.

[10] Taussky, H. H., and E. Shorr, *J. Biol. Chem.*, **202**, 675 (1953).

[11] Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier, *J. Cell. Comp. Physiol.*, **49**, 25 (1957).

[12] Geller, D. M., and F. Lipmann, *J. Biol. Chem.*, **235**, 2478 (1960).

[13] Frenkel, A. W., in *Research in Photosynthesis*, ed. H. Gaffron (New York: Interscience, 1957), p. 303.

[14] Bose, S. K., H. Gest, and J. G. Ormerod, *J. Biol. Chem.*, **236**, PC 13 (1961).

[15] Frenkel, A. W., and K. Cost, *Nature*, **195**, 1171 (1962).

[16] Frenkel, A. W., in *The Photochemical Apparatus, Its Structure and Function*, Brookhaven Symposia in Biology, No. 11 (1959), p. 276.

[17] Vernon, L. P., and O. K. Ash, *J. Biol. Chem.*, **235**, 2721 (1960).

[18] Vernon, L. P., *Plant and Cell Physiol.*, Japan, in press.

[19] Gest, H., in *Phosphorus Metabolism*, ed. W. D. McElroy and B. Glass (Baltimore: Johns Hopkins Press, 1952), vol. 2, p. 522.

[20] Bové, J., C. Bové, F. R. Whatley, and D. I. Arnon, presented by D. I. Arnon at the Ninth International Botanical Congress, Montreal, Canada (August, 1959).

[21] The term "poise," as applied to oxidation-reduction phenomena, has been frequently used incorrectly as denoting the position or value of the potential of a redox system. The verb, *poise*, in the sense of "to balance, to keep in equilibrium" was originally used by Clark[22] as follows: "A solution may be said to be poised when it tends to resist change in E_h on addition of an oxidizing or reducing agent."

[22] Clark, W. M., *Public Health Reports (U.S.)*, **38**, 443 (1923); Reprint No. 823; *Oxidation-Reduction Potentials of Organic Systems* (Baltimore: Williams and Wilkins Co., 1960), p. 145.

[23] Several types of evidence[24, 25] indicate that the photochemical apparatus of *R. rubrum* is normally integrated with the cytoplasmic membrane, or membranous extensions penetrating the cytoplasm. Disengagement of the pigment system, with its associated electron transfer carriers, from the native cellular matrix might be expected[6, 26] to cause a number of changes, including disturbance of redox balance.

[24] Tuttle, A. L., and H. Gest, these PROCEEDINGS, **45**, 1261 (1959).

[25] Giesbrecht, P., and G. Drews, *Arch. Mikrobiol.*, **43**, 152 (1962).

[26] Gest, H., and M. D. Kamen, in *Encyclopedia of Plant Physiology*, ed. W. Ruhland (Berlin-Göttingen-Heidelberg: Springer-Verlag, 1960), vol. V/2, p. 568.

[27] Gest, H., J. G. Ormerod, and K. S. Ormerod, *Arch. Biochem. Biophys.*, **97**, 21 (1962).

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*A COMPARISON OF CLOSTRIDIAL FERREDOXINS**

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Mortenson, Valentine, and Carnahan[1] have recently isolated a non-heme iron-containing protein from *Clostridium pasteurianum* that is required for the formation of acetyl phosphate and hydrogen from pyruvate by extracts of that organism. They have named this protein "ferredoxin". Valentine, Jackson, and Wolfe[2] have reported that ferredoxin is also necessary for hydrogen formation in the fermentation of xanthine by *Micrococcus lactilyticus*. Tagawa and Arnon[3] subsequently crystallized *C. pasteurianum* ferredoxin and found it to have a molecular weight of about 12,000, 10 atoms of iron per molecule, and a redox potential of -418 mV at pH 7.11. Ferredoxin was also shown to be reversibly oxidized and reduced and to be similar to the "photosynthetic pyridine nucleotide reductase" originally isolated from spinach by San Pietro and Lang.[4]

Because of our interest in the metabolism of pyruvate by *Clostridium acidi-urici* and *Clostridium cylindrosporum*,[5] we examined these purine-fermenting organisms for ferredoxin and found them to contain relatively high concentrations of this protein. In the present communication, the isolation of crystalline ferredoxin from *C. acidi-urici* is described and its properties are compared with those of crystalline ferredoxin isolated from *C. pasteurianum*.

Materials and Methods.—Organisms: C. acidi-uridi[6] was grown on uric acid as previously described.[7] *Clostridium butyricum* was grown on glucose as described by Wolfe and O'Kane,[8] and *C. pasteurianum* was grown on sucrose with nitrogen gas as the sole nitrogen source as described by Carnahan and Castle.[9]