$$\langle a_{\alpha\beta}(u_k)D^\alpha u_k, D^\beta v\rangle = \langle D^\alpha u_k, \bar{a}_{\alpha\beta}(u_k)D^\beta v\rangle,$$

where $D^\alpha u_k$ converges strongly to $D^\alpha u$ in $L^2(\Omega)$. Furthermore, $\bar{a}_{\alpha\beta}(u_k)D^\beta v$ converges strongly to $\bar{a}_{\alpha\beta}(u)D^\beta v$ in $L^2(\Omega)$ by dominated convergence. Hence, each term in the sum for $a(u_k, v)$ converges to the corresponding term in the sum for $a(u, v)$, $(G(u_k), v)_m \to (G(u), v)_m$ for each v in V, and the proof of Lemma 3 is complete.

Proof of Theorem 1: By Lemma 2, it suffices to show that the mapping G is one-to-one and onto V. By Lemmas 3 and 1, G is demicontinuous and satisfies the inequality (2.4) of Theorem 4 of Section 2. By that Theorem, G is one-to-one, onto, and has a continuous inverse. QED

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*PHOTOSYNTHETIC PHOSPHORYLATION CATALYZED BY FACTORS ISOLATED FROM PHOTOSYNTHETIC ORGANISMS**,†

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Photosynthetic organisms are of prime importance to life in that they can convert electromagnetic energy into useful chemical energy. It is generally accepted that adenosine triphosphate† is one of the initial stable products of the energy conversion mechanism. Equation 1 summarizes the over-all reaction whereby electromagnetic energy is converted to chemical energy in the form of ATP.

$$\text{ADP} + \text{Pi} \xrightarrow[\substack{\text{Chromatophores}\\ \text{or Chloroplasts}}]{\text{Light}} \text{ATP} \qquad (1)$$

This energy conversion process, which doubtless is multicomponent in nature, is commonly referred to as photosynthetic phosphorylation or photophosphorylation. In view of the importance of this process in cellular metabolism and physiology, it is of considerable interest to determine the naturally occurring components involved in photophosphorylation.

Subsequent to the initial observations of photophosphorylation in bacteria by Frenkel[1] and in higher plants by Arnon *et al.*,[2] a variety of redox substances have been shown to catalyze photophosphorylation.[3] However, many of these substances do not occur in nature. On the other hand, the pyridine nucleotides catalyze a

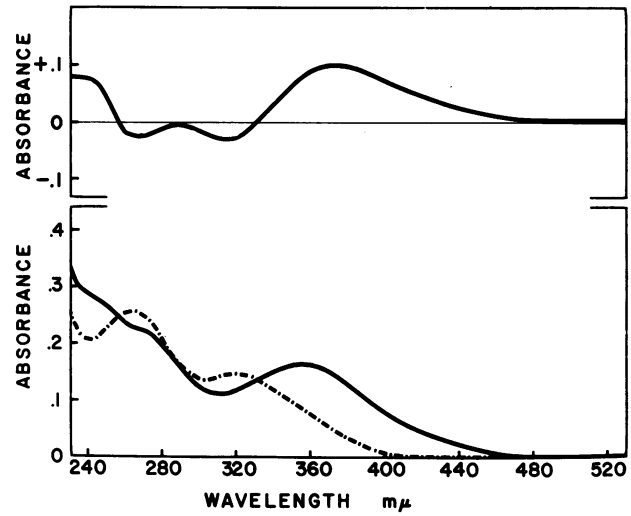

FIG. 1.—Lower portion is absorption spectra of the active factor isolated from spinach chloroplasts. Solid line, factor in 0.1 *N* NaOH; broken line, in 0.1 *N* HCl. Upper portion: difference spectrum.

physiological photophosphorylation in the presence of photosynthetic pyridine nucleotide reductase.[4, 5]

In a search for the components involved in photophosphorylation by spinach chloroplast fragments, we observed that ATP production in the presence of NADP was stimulated much more than was NADP reduction. Further study revealed that this additional ATP production was catalyzed by a water-soluble, heat-stable factor(s) which was quite active in the absence of other exogenous redox substances. Evidence is presented that this factor(s) occurs in all types of photosynthetic organisms. Furthermore, the factor from one type of organism catalyzes photo-

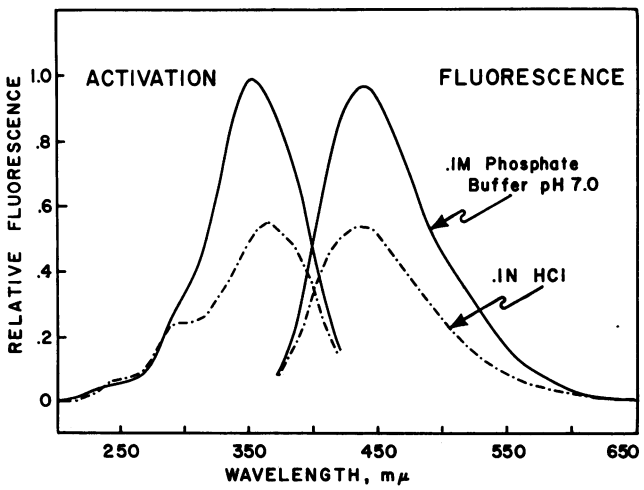

FIG. 2.—Activation and fluorescence spectra of the active factor from spinach chloroplasts, 0.2 mg/ml. Slit arrangement No. 3; meter multiplier setting 0.03; sensitivity 50.

phosphorylation by either higher plant chloroplasts or bacterial chromatophores. It is proposed that the factor(s) be named phosphodoxin, e.g., spinach phosphodoxin or *Rhodospirillum rubrum* phosphodoxin.

Methods.—*Rhodospirillum rubrum* chromatophores were prepared as described by Vernon and Ash,[6] and bacteriochlorophyll was determined by the method of Van Niel and Arnold.[7] The method of Anderson and Fuller[8] was used for the preparation of Chromatium chromatophores and the determination of bacteriochlorophyll. Spinach chloroplast fragments were prepared by isolation of intact chloroplasts in 0.35 *M* NaCl and 0.02 *M* Tris, pH 7.6, and then suspending the intact chloroplasts in 0.035 *M* NaCl and 0.002 *M* Tris pH 7.6. ATP was determined routinely as ATP^{32} by the method of Nielson and Lehninger[9] as modified by Avron.[10] In some experiments ATP was determined spectrophotometrically with glucose, hexokinase, NADP, and Zwischenferment. Reaction mixtures for assaying the photoproduction of ATP^{32} contained the following components in μmoles: Tris-HCl buffer, pH 7.5 or 7.8, 48; $MgCl_2$, 2; ADP, 1; Pi + P^{32} (containing 0.5–1 μc), 1; chlorophyll or bacteriochlorophyll, less than 30 μg; and water to a total volume of 1 ml. Reaction mixtures were illuminated laterally in 1 cm cuvettes at 2,500 ft-c at room temperature (19–20°C). When anaerobic conditions were employed, Warburg vessels were illuminated at 1,500 ft-c at 20°C. The vessels were flushed for 5 min with nitrogen or argon prior to illumination and throughout the illumination period to maintain anaerobic conditions. The vessels were shaken slowly throughout the gassing and illuminating procedure. Absorption spectra were obtained with a Cary Model 14 recording spectrophotometer. Fluorescence and activation spectra were obtained with an Aminco-Bowman spectrophotofluorometer.

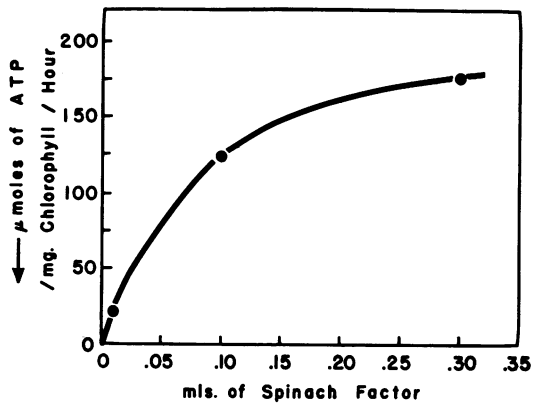

FIG. 3.—Effect of concentration of the active factor isolated from spinach chloroplasts on photophosphorylation of fragmented spinach chloroplasts.

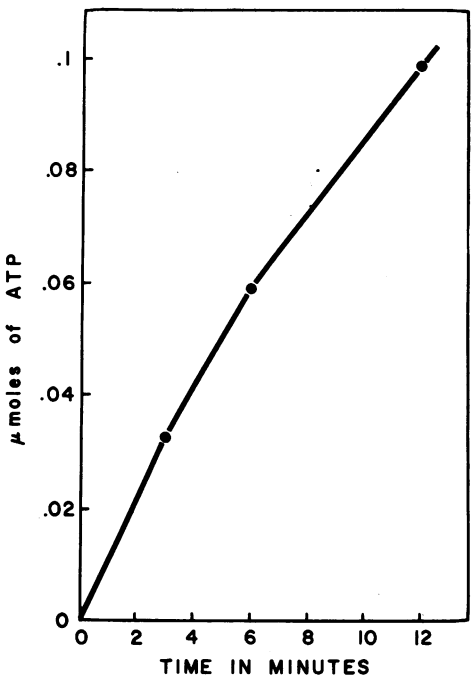

FIG. 4.—Proportionality with time of photophosphorylation of fragmented spinach chloroplasts in the presence of the factor.

Results.—Acetone powder of intact spinach chloroplasts was used as the source

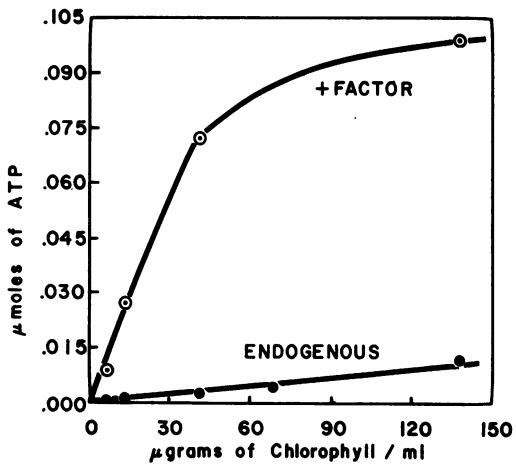

FIG. 5.—Effect of chlorophyll concentration on photophosphorylation of fragmented spinach chloroplasts.

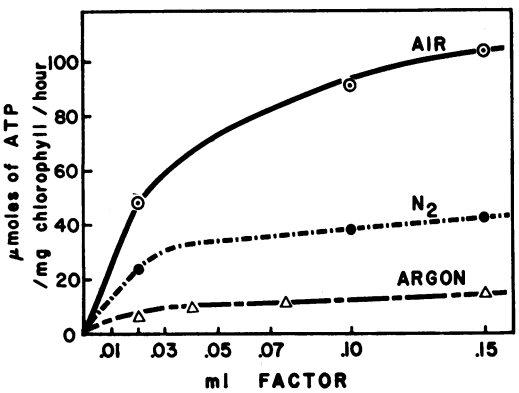

FIG. 6.—Effect of anaerobic conditions on photophosphorylation of fragmented spinach chloroplasts in the presence of the factor.

of the factor. The factor was isolated by suspending the acetone powder in water and heating. After cooling, the suspension was centrifuged and the pellet discarded. Following acetone fractionation (−20°C) of the supernatant, the preparation was chromatographed on Whatman 3MM paper using n-propanol:H_2O:1% NH_4OH (3:1:2). The active fraction, which had an R_f of 0.47 in this solvent system, was eluted with water, and the eluted material was taken to dryness *in vacuo* and the residue extracted with ethanol. When the ethanol solution was allowed to sit at −20°C overnight, a precipitate formed, which was washed with ethanol and dissolved in water.

Absorption spectra of the active factor isolated from spinach chloroplasts are presented in the lower portion of Figure 1. There is a pronounced pH dependent shift in the absorption spectrum. The upper portion of Figure 1 shows the difference spectrum in 0.1 *N* HCl versus 0.1 *N* NaOH. This pH dependence is readily visible in that an acid solution of the factor is nearly colorless, whereas an alkaline solution is yellow.

Fluorescence and activation spectra of the active factor are given in Figure 2. A pronounced fluorescence peak was observed at 440 mμ, and this was activated maximally by irradiation at 358 mμ. The activation and fluorescence spectra (Fig. 2) indicate that the absorption maximum at 358 mμ observed in the alkaline absorption spectrum (Fig. 1) is connected with the fluorescence. The intensity of both the activation and fluorescence spectra (Fig. 2) is pH dependent in a fashion similar to the absorption spectrum (Fig. 1).

Many photosynthetic organisms were examined for a heat-stable, water-soluble factor which would catalyze photophosphorylation. The organisms tested and the rates of photochemical ATP production by fragmented spinach chloroplasts in the presence of the factor isolated from each organism are shown in Table 1. No significance is attached to the rate of ATP production observed in the presence of the factor isolated from each organism, except that a higher rate indicates that the factor has been further purified from that specific organism. Since all of these

activities were measured with fragmented spinach chloroplasts, it is evident that the organism from which the factor was isolated had little effect on the ability of the factor to catalyze photophosphorylation by chloroplasts. For example, factor(s) made from algae or a flagellate or photosynthetic bacteria are active with spinach chloroplasts. In addition, the factor made from spinach chloroplasts stimulates photophosphorylation by chromatophores from Chromatium and *Rhodospirillum rubrum*.[11]

TABLE 1

ORGANISMS CONTAINING A FACTOR WHICH STIMULATES PHOTOSYNTHETIC PHOSPHORYLATION

Source		μmoles of ATP/mg chlorophyll/hr*,†
Spinach chloroplasts		196
Spinach leaves		180
Phormidium luridum	Whole cells	49
Chlorococcum wimmeri	Whole cells	25
Chlamydomonas reinhardi	Whole cells, + strain	25
Tribonema aequale	Whole cells	27
Euglena gracilis	Whole cells	25
Chlorella pyrenoidosa	Whole cells	69
Rhodospirillum rubrum	Chromatophores	54
Rhodospirillum rubrum	Whole cells	50
Chromatium, strain D	Chromatophores	56
Chromatium, strain D	Whole cells	115

* The endogenous rate in these experiments varied from 0.5 to 3 μmoles of ATP formed per mg chlorophyll per hr.

† All preparations were assayed with spinach chloroplast fragments.

The effect of the concentration of factor isolated from spinach on photophosphorylation by fragmented spinach chloroplasts is shown in Figure 3. The reaction is linear with time for about 5 min (Fig. 4) and with chlorophyll concentration up to 40 μgrams of chlorophyll per ml (Fig. 5). Anaerobic conditions inhibited the reaction about 90 per cent with argon as the gaseous phase; with nitrogen only about 60 per cent inhibition was observed (Fig. 6). The possibility exists that the nitrogen contained a small quantity of oxygen which was sufficient to oxidize some component of the reaction mixture.

Rhodospirillum rubrum chromatophores support photophosphorylation in the absence of exogenous redox substances.[1] The data presented in Table 2 indicate that this endogenous photophosphorylation decays rapidly upon storage of the chromatophores. Furthermore, the factor isolated from *Rhodospirillum rubrum* stimulates ATP production by a fairly constant amount regardless of the endogenous rate of photophosphorylation (Table 2, last two columns). The time course

TABLE 2

STIMULATION BY FACTOR OF PHOTOSYNTHETIC PHOSPHORYLATION BY *Rhodospirillum rubrum* CHROMATOPHORES

Days after preparing chromatophores*	μmoles of ATP per mg bacteriochlorophyll per hr		
	Endogenous	Plus factor	Plus factor minus endogenous
0	81	193	112
3	68	206	138
4	15	146	131
5	17	139	122
6	28	168	140
10	15	126	111
20	21	149	128

* Chromatophores suspended in 0.1 *M* Tris, pH 7.8 plus 10% sucrose. Stored under vacuum and argon at 4°C.

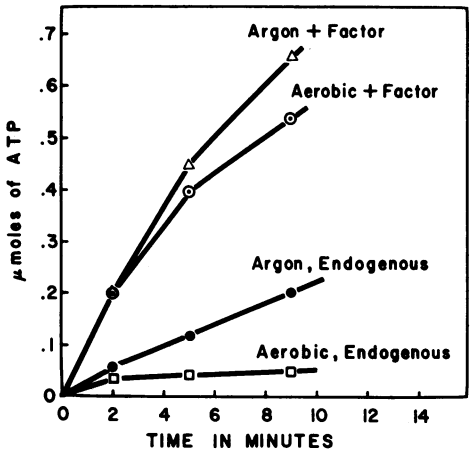

FIG. 7.—Effect of anaerobic conditions and time on photophosphorylation of *Rhodospirillum rubrum* chromatophores in the absence and presence of the factor isolated from *Rhodospirillum rubrum*.

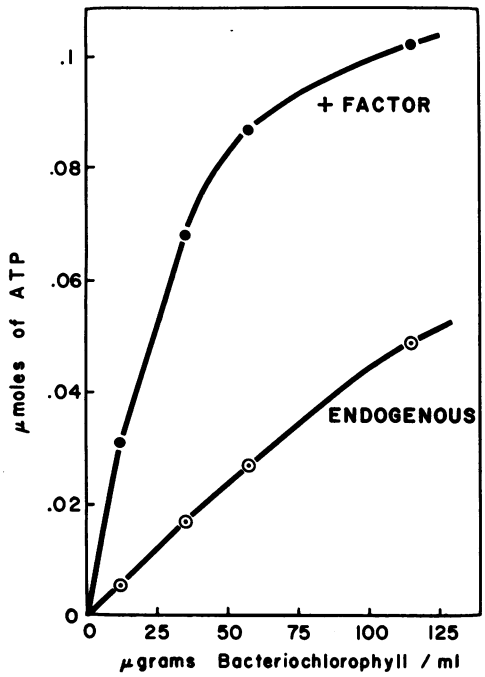

FIG. 8.—Effect of bacteriochlorophyll concentration on photophosphorylation by *Rhodospirillum rubrum* chromatophores.

for both the endogenous and the endogenous plus factor photophosphorylation is given in Figure 7. The effect of bacteriochlorophyll concentration is presented in Figure 8. In contrast to the photophosphorylation in the presence of the factor catalyzed by fragmented spinach chloroplasts under anaerobic conditions (Fig. 6), *Rhodospirillum rubrum* chromatophores in the presence of the factor are unaffected by anaerobic conditions after 2 min of illumination (Fig. 7). The endogenous photophosphorylation shows a marked stimulation under argon (Fig. 7, two lower curves), and this stimulation probably accounts for the slight increase under argon observed in the presence of the factor after 10 min of illumination (Fig. 7, two upper curves).

Discussion.—Several other naturally occurring redox "cofactors," namely, vitamins K_3 and K_5,[12] flavin mononucleotide,[13] "allagochrome,"[14] a "flavone-type" compound,[15] and photosynthetic pyridine nucleotide reductase,[16] have been shown to catalyze photophosphorylation. Therefore, a comparison of the properties of the factor reported herein with these other redox "cofactors" is pertinent. A comparison of the absorption and fluorescence spectra of flavins and vitamins K_3 and K_5[17, 18] with that of the factor (Figs. 1 and 2) indicates that the factor probably is not a flavin or vitamin K_3 or K_5. A further point of dissimilarity is that the factor reported in this paper is insensitive to ultraviolet irradiation even when irradiated for a period of 1 hr.

Habermann[14, 19] presented data which indicate that "allagochrome" has a component with an absorption peak at 673 mμ. This is not observed with the factor described here. Two further differences can be cited. First, "allagochrome" is not heat-stable, and secondly, it contains protein.[14, 19] In contrast, this new factor is heat-stable and devoid of protein. These characteristics also rule out photosynthetic pyridine nucleotide reductase.

Krogmann and Stiller[15] have reported that a "flavone-type" compound isolated from chloroplasts prepared in nonaqueous media catalyzed photophosphorylation. It was of interest, therefore, to determine if the factor was a flavone. The factor does not form lead, ferric, or ferrous salts characteristic of flavones and, furthermore, is insoluble in butanol and ethyl acetate. From a comparison of the absorption spectra of the factor (Fig. 1) with those given by Krogmann and Stiller, it is apparent that their compound and ours are different. It is also pertinent to point out that Chlorella contains the factor (Table 1) and that Chlorella does not contain flavones (Dr. R. Hill, personal communication). This comparison with the characteristics of other reported "cofactors" of photophosphorylation suggests that we have isolated a new, naturally occurring, heat-stable, water-soluble component of the photophosphorylation process.

Summary.—The isolation from photosynthetic organisms and the properties of a naturally occurring, water-soluble, heat-stable factor (phosphodoxin) which catalyzes photosynthetic phosphorylation either by spinach chloroplasts or bacterial chromatophores are presented. Phosphodoxin has been isolated from the following types of photosynthetic organisms: higher plant, algae, flagellate, and bacteria.

The authors are indebted to Mrs. Ferne Lubbers for the preparation of chromatophores, to Dr. T. E. Brown and Mrs. F. L. Richardson for the algal cultures, and to Dr. R. A. Lazzarini for the sample of Euglena.

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† Abbreviations used are: ADP and ATP, adenosine di- and triphosphate; NADP, nicotinamide adenine dinucleotide phosphate.

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