INORGANIC POLYPHOSPHATE METABOLISM IN *CHLOROBIUM THIOSULFATOPHILUM*

D. E. HUGHES[1], S. F. CONTI, AND R. C. FULLER

Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire

Received for publication 5 October 1962

ABSTRACT

HUGHES, D. E. (Dartmouth Medical School, Hanover, N.H.), S. F. CONTI, AND R. C. FULLER. Inorganic polyphosphate metabolism in *Chlorobium thiosulfatophilum.* J. Bacteriol. **85**:577–584. 1963.—Cells of the obligate phototroph *Chlorobium thiosulfatophilum,* when grown on a normal concentration of inorganic phosphate, accumulated large intracellular metachromatic granules identified as polymetaphosphate. Inorganic phosphate was released from polymetaphosphate by cell-free extracts. This release was adenosine diphosphate-dependent and light-independent. When cells were subcultured through serial transfer in the absence of inorganic phosphate, the production of polymetaphosphate granules was almost completely stopped. After the serial transfer, these cells showed little or no endogenous release of inorganic phosphate or release from added polymetaphosphate. Using such polymetaphosphate-free cells, it has been possible to demonstrate photosynthetic phosphorylation by the naturally occurring photosynthetic macromolecules isolated from this organism. These particles, of about 100 A in diameter with a molecular weight of approximately 1.5 million, are by far the simplest functional naturally occurring photosynthetic electron-transport units thus far described.

The internal structure of the green sulfur bacterium *Chlorobium thiosulfatophilum* is unusual among the photosynthetic microorganisms in that vesicular or lamellar structures normally associated with photosynthetic bacteria cannot be observed by electron microscopy of cells in various stages of growth (Bergeron and Fuller, 1961). Ultrathin sections illustrate a granular cytoplasm. Pigmented particles (approximately 150 A diam) can be isolated from cell-free extracts and shown to contain the bulk of the chlorophyll and other components normally associated with photophosphorylation (Hulcher and Conti, 1960). When these particles were incubated with adenosine diphosphate (ADP) in the light, photophosphorylation was not detected but there was a rapid release of inorganic phosphate which was independent of light and dependent on the addition of ADP. It seemed possible that this phosphate release was due to the breakdown of high-polymer inorganic polyphosphate, since sections as well as dried-cell preparations often showed large (0.1 to 0.15 μ) spherical, electron-dense granules similar to those found in the corynebacteria and mycobacteria and known to be associated with polyphosphate (Hughes and Muhammed, 1962). In this paper, it is shown that the latter granules in *Chlorobium* are also associated with polyphosphate. Experiments on the metabolism of polyphosphate and photophosphorylation by the isolated pigmented particle fraction are also reported.

[1] On leave as Visiting Professor of Microbiology from the Cell Metabolism Unit, Medical Research Council, University of Oxford.

MATERIALS AND METHODS

Growth and handling of microorganism. Chlorobium was grown on the medium of Larsen (1953) in glass-stoppered bottles immersed in water at 30 C and illuminated from the side by tungsten-filament lamps. Some growth experiments were carried out in a 7-liter fermentor (New Brunswick Scientific Co.) containing 5 liters of medium and gassed continuously with tank nitrogen passed through a vanadous sulfate-zinc mixture to remove traces of O_2.

Growth was measured in a Lumetron colorimeter (model 401A) using a 625-mμ filter. Optical density readings were correlated with dry weight, determined after washing the cells in water and drying to constant weight at 110 C in an air stream. In some experiments, total nitrogen was also estimated by the micro-Kjeldahl method.

It is noteworthy that no single method of measuring cell density is sufficiently accurate, owing to variable amounts of elemental sulfur deposited into the medium. After growth, the cells were collected by centrifugation and washed in either 0.9% NaCl-water, or 1.0 M tris(hydroxymethyl)aminomethane (tris) buffer containing 0.4 M glucose. Cell-free extracts were prepared in a Hughes (1951) press, French press (Milner, Lawrence, and French, 1950), or an MSE (Measuring and Scientific Equipment) 50-w, 20-kc ultrasonic disintegrator (Hughes, 1961). Further details of the method of treatment and fractionation of the extracts are stated below.

Chemical estimations. Phosphate was estimated by the method of Fiske and SubbaRow (1925), and the optical density measured in a Lumetron colorimeter, a Bausch & Lomb spectrophotometer (Spectronic 20 model), or a Zeiss spectrophotometer at 650 mμ. Chromatography of the adenine phosphates was carried out essentially as described by Krebs and Hems (1953). Radioactive counts were obtained on aluminum planchets in a Nuclear-Chicago automatic counter or on paper with a thin end-window counter (Fuller, 1956). Radioautographs were prepared on Ansco nonscreen X-ray film.

Phosphate distribution in extracts was measured on cells washed twice with distilled water. Trichloroacetic acid (2 volumes of 10%) was then added, and the cells were shaken for 1 hr. After centrifugation, the cells were extracted once with 5% trichloroacetic acid and then twice with 10% trichloroacetic acid. Phosphorus estimations were carried out as described above.

When orthophosphate release from cells was determined (Table 2), each tube contained: extract, 0.5 ml; tris buffer (pH 7.0), 50 μmoles; and, where indicated, ADP, 2 μmoles; polyphosphate, 50 μmoles; in a total volume of 3.0 ml. After incubation at 25 C, a 0.5-ml sample was pipetted into 1.0 ml of 5% trichloroacetic acid, and phosphorus was estimated in the clear supernatant after centrifugation.

When P^{32}-labeled ortho- or polyphosphate was fed to cells and the formation of adenosine triphosphate $(ATP)^{32}$ analyzed, the following procedure was used (see Table 3). Polyphosphate and orthophosphate were adjusted to 0.1 M and to give 1,458 and 1,438 counts per min per 0.01 ml, respectively. The *Chlorobium* extract was prepared by crushing the cells in a Hughes press, deoxyribonuclease treatment, and removal of cell debris by centrifugation at 6,000 × *g* for 5 min. Each reaction tube contained: extract, 0.5 ml; 0.5 M tris buffer (pH 7.2), 0.5 ml; 0.01 M $MgCl_2$, 0.5 ml; and, where indicated, 0.05 M ADP, 0.5 ml; 0.1 M polyphosphate or orthophosphate, 0.2 ml; in a total volume of 0.5 ml. Samples (1.0 ml) were taken at 0 min and 6 hr, and 1 ml of 5% albumin and 0.2 ml of 20% trichloroacetic acid were added. The precipitate was removed by centrifugation, and 0.1-ml samples of the supernatant were chromatographed on paper and counted.

Metachromasy measurements were made on a Cary 14 spectrophotometer. Each cuvette contained: polyphosphate, 5 μmoles; and toluidine blue, 0.1 ml of a solution containing 0.02 mg/ml. Total volume was 3.0 ml.

Photosynthetic phosphorylation was measured anaerobically (N_2) in the light and dark in Warburg vessels containing 0.8 ml of purified particles from *Chlorobium*; 10 μmoles of $MgCl_2$; 10 μmoles of KH_2PO_4; 100 μmoles of tris buffer (pH 7.0); 7.5 μmoles of ADP; 0.5 ml of hexokinase (Sigma Chemical Co.), and water to a total volume of 2.5 ml. To stop the reaction, 0.5 ml of 10% trichloroacetic acid was added, and the precipitate removed by centrifugation. Phosphate was estimated as described. Rates of phosphate uptake are expressed as μmoles of orthophosphate esterified per hr per mg of chlorophyll.

Materials. Unless otherwise stated, all materials were commercial products and not purified further. Potassium Kurrol's salts and sodium Graham's salt were gifts from J. R. Van Vazer (Monsanto Chemical Co.). Synthetic polyphosphate labeled with P^{32} was prepared as described by Muhammed, Rodgers, and Hughes (1959).

Electron microscopy. Whole cells were prepared by the blot-dry method on carbon-covered copper grids. Electron microscopy was done with a Bendix-Akashi Tronscope (model TRS-50).

RESULTS

Extraction and identification of polyphosphate. A high proportion of cells of *Chlorobium* grown for 2 to 6 days were found to have from one to three electron-dense granules measuring from 0.1 to 0.15 μ in diameter. These persisted after extraction with 5% trichloroacetic acid but were found to disappear, leaving a less dense area, after extraction with 10% trichloroacetic acid

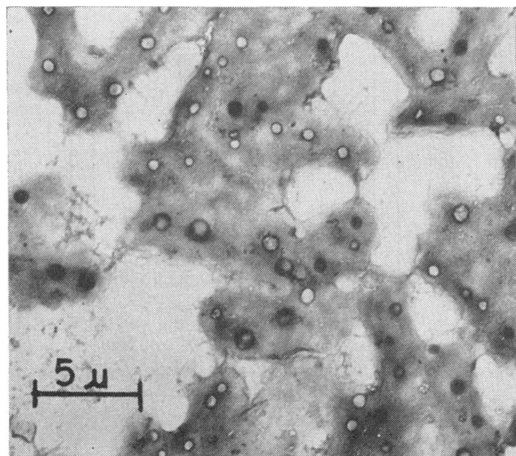


FIG. 1. *Electron micrograph of whole cells of Chlorobium after extraction with 10% trichloroacetic acid.*

(Fig. 1). This behavior is similar to that of the metachromatic granules in *Corynebacterium xerosis* (Hughes and Muhammed, 1962), where the 10% trichloroacetic acid extracts but not the 5% extracts were found to contain polyphosphate. The electron-dense granules in *Chlorobium* were also associated with metachromasy of toluidine and polychrome methylene blue, but this reaction was too indefinite to follow changes after extraction with trichloroacetic acid. In thin sections, the electron-dense granules were found to vaporize slowly at high beam intensities. This again supports the suggested presence of polyphosphate in the granules.

A number of bottles containing from 3 to 12 g of cells grown for varying lengths of time, with all cells showing a high degree of metachromasy as well as large numbers of electron-dense granules, were extracted twice with five volumes of 5% trichloroacetic acid and then with five volumes of 10% trichloroacetic acid. The extracts were adjusted to pH 6.8 with KOH and then to pH 3.5 by the addition of 3 M acetate buffer. The phosphates were precipitated with an excess of 1.0 M $BaCl_2$. After standing at 0 to 2 C overnight, the precipitate was collected by centrifugation and washed twice in 0.05 M acetate buffer (pH 3.5). The barium salts were decomposed either by trituration in 0.1 M H_2SO_4 or with Dowex 50 (Na^+ form), and the supernatants obtained by centrifugation were adjusted to standard volumes. Inorganic orthophosphate and

TABLE 1. *Phosphate distribution in extracts of Chlorobium**

Age of cells	Amt	Inorganic phosphate (μmoles/mg of N)			
		5% TCA†		10% TCA†	
		A	B	A	B
days	*mg (dry wt)/ml*				
4	175	1.2	1.3	0.8	1.6
6	185	1.6	1.9	1.0	2.9

* Column A, after 5 min of hydrolysis; column B, after 7 min of hydrolysis.

† Trichloroacetic acid.

orthophosphate released after 7 min of hydrolysis were estimated.

As shown in Table 1, the 10% trichloroacetic acid extracts contained the bulk of 7-min hydrolyzable phosphate which, under similar extraction conditions, was found to arise mainly from long-chain inorganic polyphosphate (Muhammed, Rodgers, and Hughes, 1959). The bulk of the hydrolyzable phosphate in the 10% extract remained after dialysis for 1 week against water or a variety of buffers and salt solutions. The dialyzed 10% extract, when mixed with toluidine blue, showed similar spectral changes to those of similar concentrations of synthetic polyphosphate [Kurrol's salt $(KPO_3)_n$; Fig. 2]. It is clear from these experiments that cells of *Chlorobium,* containing the large electron-dense granules, may also contain up to two-thirds of their phosphate in the form of polyphosphate. The apparent changes in structure produced in the electron microscope and by 10% trichloroacetic acid extraction are strong presumptive evidence that the polyphosphate is located in the granules. When extracted with trichloroacetic acid, this polyphosphate appeared to be chemically similar to Graham's or Kurrol's salt.

Growth experiments. The formation of polyphosphate was followed during growth by extraction of the cells successively with 5 and 10% trichloroacetic acid, barium precipitation at pH 3.5, and estimation of the 7-min hydrolyzable phosphate as previously described. In the first experiments, 1-liter bottles were inoculated from a 3-day-old culture, and the cells were collected at intervals. There were considerable differences in the amount of polyphosphate in the cells; in general, the older cells contained higher concentrations (Table 1).

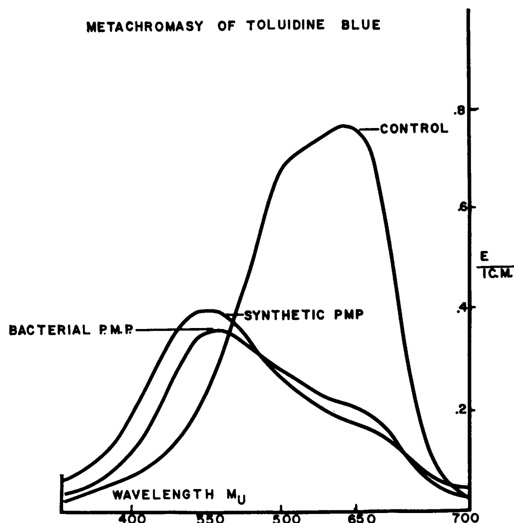


FIG. 2. *Metachromasy of polyphosphate isolated from Chlorobium compared with synthetic Graham's salt and control with orthophosphate.*

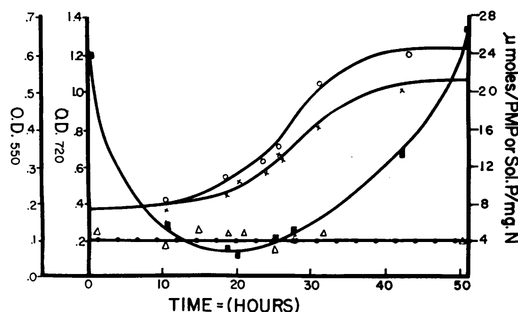


FIG. 3. *Production of polymetaphosphate during growth of Chlorobium. Symbols:* ■ = *polyphosphate;* △ = *orthophosphate;* ○ = *optical density at 720 mμ; and* × = *optical density at 550 mμ.*

Experiments to follow polyphosphate changes during growth were carried out in a fermentor. As shown in Fig. 3, polyphosphate concentration in the cells falls at first and then rises rapidly during the early logarithmic and stationary phase of growth. It was difficult, however, to control by this means the production of large amounts of cells containing low concentrations of polyphosphate, as the period during which the cells contained low concentrations of polyphosphate was relatively short. When the cells were grown on a phosphate-free medium, the polyphosphate level of the cells decreased after the first subculture, and after the third they contained very little

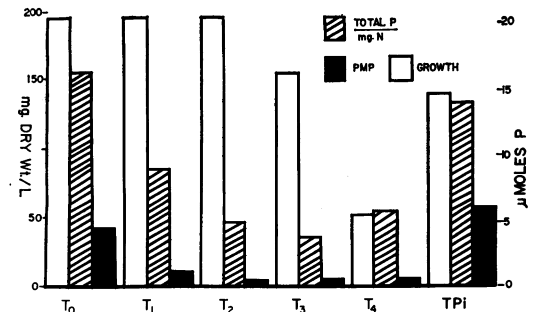


FIG. 4. *Serial transfers of Chlorobium in phosphate-deficient medium.* T_1, T_2, T_3, *and* T_4 *indicate four serial transfers.* TPi *cells were* T_4 *cells inoculated into normal phosphate medium.*

polyphosphate, although the yield of cells was almost as high as in medium containing the usual amount of phosphate. Upon the fourth subculture, growth was reduced and cells were morphologically abnormal. At this stage, they contained no detectable polyphosphate, and the total phosphate content was also reduced. The orthophosphate content of the medium was estimated (Fig. 4); the results suggest that cells grow normally in a concentration of 0.05 M phosphate, but below this level growth is inhibited. Cells from the fourth serial subculture grew normally when transferred to fresh medium containing phosphate. The changes in distribution of electron-dense granules were also followed in this experiment. Granule disappearance paralleled polyphosphate disappearance (Fig. 5), thus lending further support to the suggestion that the bulk of the polyphosphate is located in the granules.

Phosphate release by cell-free extracts. Cell-free extracts were prepared from cells, grown in the usual way, by crushing in the Hughes press. After crushing, the cells were suspended in four volumes of 0.05 M tris buffer (pH 7.0), homogenized in a Kontes-type glass homogenizer, and treated for 30 min at 0 to 2 C with deoxyribonuclease. The extract was then divided into two portions, one of which was centrifuged for 10 min at 6,000 × g. The extract and the supernatant after centrifugation were then tested for endogenous release of phosphate on incubation at 25 C in the light or dark, and for the release of phosphate from added polyphosphate. Under a variety of conditions, negligible phosphate release was found with either extract unless ADP was also

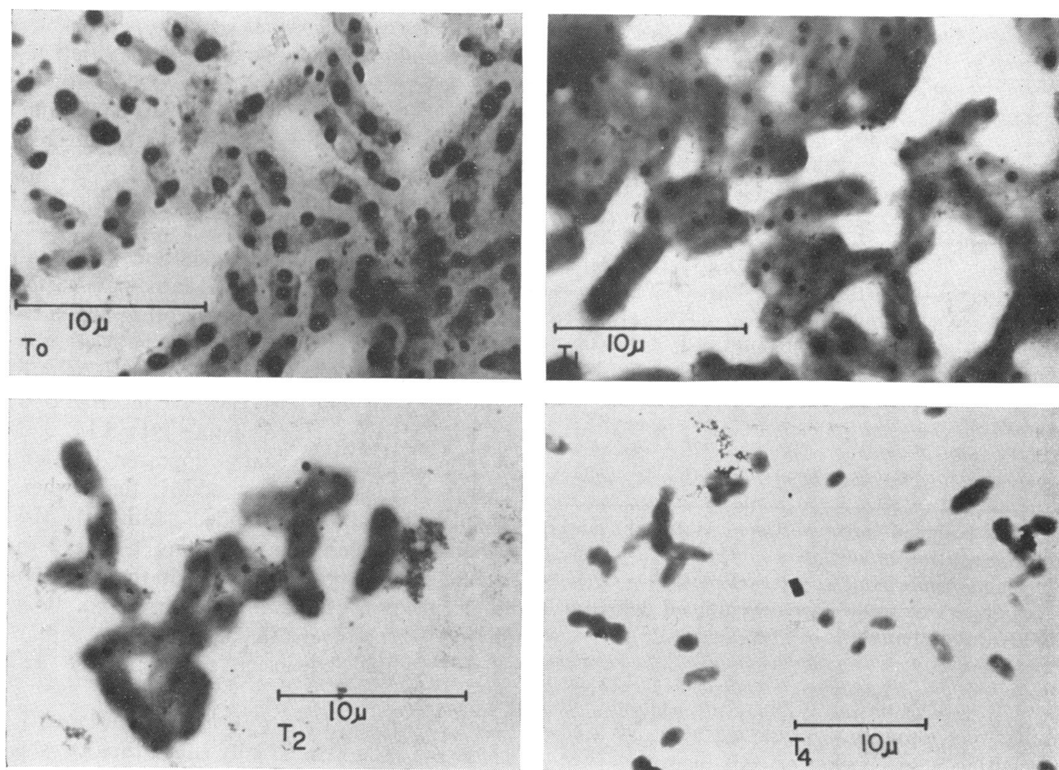


FIG. 5. *Effect of phosphate deficiency on granule size and distribution. Samples of cells from the experiment shown in Fig. 4 were mounted by the blot-dry method, washed in water, and examined without further treatment.*

added (Table 2). In the presence of ADP, endogenous phosphate release was linear for up to 3 hr, after which the rate decreased progressively. The addition of synthetic polyphosphate (Graham's salt) extended the linear rate of phosphate release but did not increase the initial rate. There was no stimulation by added Mg^{++}, Mn^{++}, or ethylenediaminetetraacetate.

A similar phosphate release was found with particulate fractions prepared by high-speed centrifugation in experiments designed to measure photophosphorylation. In such preparations, phosphate release was independent of light and remained linear for up to 2 hr. There was no phosphate release when the preparation was boiled for 2 min.

There was little or no endogenous phosphate release in the presence or absence of ADP from extracts of cells subcultured three or four times successively on phosphate-free medium. Similarly, there was no release of phosphate from added polyphosphate in these extracts when tested

TABLE 2. *Orthophosphate release from polyphosphate by extracts of Chlorobium**

Expt	Addition	Orthophosphate formed (μmoles)				
		0.5 hr	1 hr	2 hr	6 hr	18 hr
A	None	—	—	0.16	0.22	—
	ADP	—	—	6.5	8.3	—
B	None	0	0	—	1.5	1.5
	ADP	3.9	7.1	—	27.6	32.0
	ADP + polyphosphate	4.4	9.0	—	27.0	44.6
C	None	0	3.0	—	3.2	3.2
	ADP	10.0	17.0	—	67.0	101.0
	ADP	18.0	35.0	—	17.0	67.0

* Procedures: experiment A, crude Hughes press extract; experiment B, Hughes press extract after centrifugation at 6,000 × *g* for 10 min; experiment C, ultrasonic extract after centrifugation at 10,000 × *g* for 15 min.

TABLE 3. *Phosphate metabolism in Chlorobium extracts**

Reagent added	Reaction time	Spot "x"	ATP	ADP
	hr		*counts/min*	*counts/min*
Expt A†				
ADP, poly-P^{32}	0	—	197	1,720
	6	949	3,913	12,990
ADP, ortho-P^{32}	0	0	0	436
	6	328	603	6,974
Poly-P^{32}	6	521	652	1,250
Ortho-P^{32}	6	637	809	5,067
Expt B‡				
ADP, poly-P^{32}	6	696	2,179	290
ADP, ortho-P^{32}	6	899	1,615	283

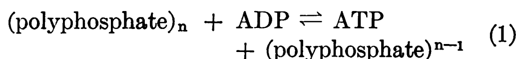
* For reaction mixture, see Materials and Methods section of text.

† Whole crude Hughes extract.

‡ Hughes extract after removing cell debris at 6,000 × *g* for 20 min.

under a variety of conditions. Almost identical results were found with crude extracts prepared by treating a suspension of cells [one part (wet wt) cells and four parts water] with a large probe for 4 min in an MSE disintegrator (50 w, 20 kc) and with an extract prepared in a French press.

Mechanism of orthophosphate formation in cell-free extracts. The ADP-dependent formation of phosphate from polyphosphate in cell-free extracts might be due to the presence of an enzyme similar to that described in *Escherichia coli* which carried out reaction 1 together with an adenosine triphosphatase (2; Kornberg, Kornberg, and Simms, 1956).



$$(\text{polyphosphate})_n + \text{ADP} \rightleftharpoons \text{ATP} + (\text{polyphosphate})^{n-1} \quad (1)$$

$$\text{ATP} \rightarrow \text{ADP} + \text{Pi} \quad (2)$$

To test this, extracts were incubated with ADP and polymetaphosphate (PMP) uniformly labeled with P^{32}, with the expectation that both the ATP and inorganic phosphate (Pi) formed would be labeled. After the reaction was terminated, the excess of labeled polyphosphate was removed by the addition of bovine serum albumin and trichloroacetic acid, and the extracts were neutralized and chromatographed on Whatman no. 4 paper. The orthophosphate was separated by ascending chromatography in isopropyl ether-formic acid, and the nucleotides developed with isobutyric acid-ammonia according to Krebs and Hems (1953). Nucleotide spots were detected with an ultraviolet lamp, and radioactivity was estimated by making radioautographs and counting the spots with an end-window Geiger counter. Orthophosphate-P^{32} was formed from polyphosphate-P^{32} and also incorporated into spots corresponding to ATP, ADP, and one other unidentified ultraviolet-absorbing spot (Table 3). It was also found, however, that labeled orthophosphate incubated with the extracts was incorporated into ADP and to a lesser extent into ATP and the other spot. More counts appeared in ATP when polyphosphate was added than when orthophosphate-P^{32} was added. Although the mechanism proposed in reactions 1 and 2 is feasible, further proof must await the isolation and purification of the enzymes involved. It is noteworthy, however, that all extracts so far tested exhibited adenosine triphosphatase activity.

Light-dependent Pi uptake by cell-free extracts. Chlorobium cells were serially subcultured to the T_3 stage (Fig. 4) to reduce their polyphosphate content, harvested and washed in water, and finally suspended in 8 ml of 0.1 M tris, 0.4 M

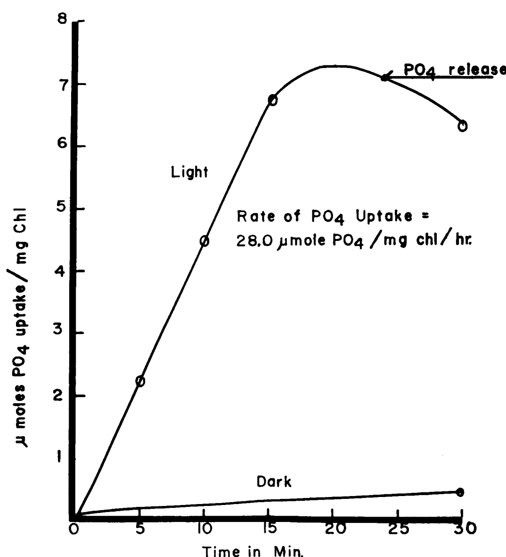


FIG. 6. *Phosphorus uptake by a particulate fraction from Chlorobium.*

glucose buffer (pH 7.0). The suspension was treated for 4 min with a large probe of an MSE disintegrator, then centrifuged at 6,000 × *g* for 15 min to remove cell walls and other debris. The supernatant was centrifuged for 1 hr at 198,000 × *g*, and the dense green pellet resuspended in the tris-glucose buffer by a 10-sec burst of sonication. This cell-free extract consisted predominantly of small pigmented particles measuring 100 to 150 A in diameter and sedimenting at 50 Svedberg units (Bergeron and Fuller, 1961). The extract was incubated in the light and in the dark with ADP, $MgCl_2$, hexokinase, and glucose. There was negligible phosphate release either in the dark or light for a period of up to 20 min. In the light, there was phosphate disappearance which proceeded linearly in this particular experiment for a period of 20 min, after which there was some phosphate release (Fig. 6). This experiment is the first demonstration of photosynthetic phosphorylation by a naturally occurring nonvesicular particle of such small size.

Discussion

The presence of metachromatic granules is widespread among bacteria and, in some cases, for instance in the corynebacteria (Sall, Mudd, and Takagi, 1958), mycobacteria (Winder and Denneny, 1957), and *Aerobacter* (Smith, Wilkinson, and Duguid, 1954) there is strong presumptive evidence that they contain large amounts of long-chain polyphosphate (Hughes and Muhammed, 1962). Up to the present time, however, it has not been possible to isolate the granules from other cell constituents, and the composition of the granules is uncertain. Widra (1959) suggested from histochemical studies that the granules have a complex structure in which ribonucleoprotein, lipid, and polyphosphates are combined.

The present experiments show that in *Chlorobium* the occurrence and disappearance of polyphosphates, metachromatic, and electron-dense granules parallel one another. The behavior on extraction with trichloroacetic acid and in an electron microscope further supports the idea that the bulk of the polyphosphate is in the granule. The fact that phosphate is released on incubation of the washed granules with ADP also suggests that sufficient polyphosphate remains insoluble or is attached to the granules or some other solid material not removed by the washing procedure.

It is still not clear whether polyphosphate can serve as a phosphagen or represents a phosphate store which cells accumulate under abnormal conditions, such as when growth is inhibited and energy production is carried on in excess of growth requirements (Harold, 1962). In *Chlorobium*, it was shown here that growth may continue normally when polyphosphate formation is almost completely suppressed by phosphate depletion. Under these conditions, the cells also appear to release larger amounts of sulfur into the medium. In *C. xerosis* (Hughes and Muhammed, 1962), polyphosphate formation was also suppressed, even in high phosphate concentrations, when the cells were growing rapidly; it was suggested that the enzyme polyphosphatase played a role in the depletion of polyphosphate under these conditions. It is noteworthy, therefore, that *Chlorobium*, which apparently does not form a polyphosphatase, continues to form polyphosphate during the logarithmic and stationary stage of growth, suggesting that, under the conditions of growth used in these experiments, excess ATP is formed by photophosphorylation. Phosphate depletion was the most effective way of depleting cells of polyphosphate. When this was done, there was no phosphate release on incubation of the particulate cell-free preparation, and good rates of photophosphorylation could be observed.

Both the source of Pi and the mechanism of its release from cell-free extracts are uncertain. There was no stimulation of phosphate release by ADP in extracts of polyphosphate-depleted cells, and these extracts did not release orthophosphate from added polyphosphate. In the polyphosphate-rich cells, there was no additional stimulation by added polyphosphate but such an addition led to a higher total phosphorus release. Thus, the direct hydrolysis or breakdown of polyphosphate has not been established with certainty. It may be only tentatively assumed, therefore, that the bulk of the Pi is formed from the polyphosphate by the suggested mechanism in which ADP is phosphorylated by polyphosphate and the terminal bond of ATP is hydrolyzed. This is the simplest hypothesis which fits both the fact that ATP becomes labeled from polyphosphate and the lack of Pi release in the absence of ADP. It is clear, however, that

additional reactions must lead to labeling of both ADP and the unknown ultraviolet-absorbing spot when either labeled orthophosphate or polyphosphate is present in these crude extracts. The precise mechanisms are being explored further with the aid of purified extracts.

The lack of phosphorus release from polyphosphate in depleted cells suggests operation of a control mechanism similar to that observed in *C. xerosis*, where it was found that the enzyme polyphosphatase was inducible and under the control of the polyphosphate or orthophosphate level in the cell.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (GB-76), the U.S. Public Health Service (2G-961), and U.S. Atomic Energy Commission Contract AT(30-1)2801.

LITERATURE CITED

BERGERON, J. A., AND R. C. FULLER. 1961. The photosynthetic macromolecules of *Chlorobium thiosulfatophilum*, p. 307-324. *In* T. W. Goodwin and O. Lindberg [ed.], Biological structure and function, vol. 2. Proc. Intern. Congr. Biochem. Academic Press, Inc., New York.

FISKE, C. H., AND Y. SUBBAROW. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. **66**:375-400.

FULLER, R. C. 1956. Modified end-window counting tube for paper chromatograms. Science **124**:1253.

HAROLD, F. M. 1962. Depletion and replenishment of the inorganic polyphosphate pool in *Neurospora crassa*. J. Bacteriol. **83**:1047-1057.

HUGHES, D. E. 1951. A press for disrupting bacteria and other microorganisms. Brit. J. Exptl. Pathol. **32**:97-109.

HUGHES, D. E. 1961. The disintegration of bacteria and other microorganisms by the M.S.E.-Mullard ultrasonic disintegrator. J. Biochem. Microbiol. Technol. Eng. **3**:405-433.

HUGHES, D. E., AND A. MUHAMMED. 1962. The metabolism of polyphosphate in bacteria. Colloq. Intern. Centre Natl. Rech. Sci. (Paris) **106**:591-602.

HULCHER, F., AND S. F. CONTI. 1960. Cytochromes in chlorophyll-containing particles of *Chromatium* and *Chlorobium thiosulfatophilum*. Biochem. Biophys. Res. Commun. **3**:497-503.

KORNBERG, A., S. R. KORNBERG, AND E. S. SIMMS. 1956. Metaphosphate synthesis by an enzyme from *Escherichia coli*. Biochim. Biophys. Acta **20**:215-227.

KREBS, H. A., AND R. HEMS. 1953. Reactions of adenosine and inosine triphosphates in animal tissues. Biochim. Biophys. Acta **12**:172-180.

LARSEN, H. 1953. On the microbiology and biochemistry of the photosynthetic green sulfur bacteria. Det Kgl Videnskabers selskabs Skrifter, F. Bruns, Trondheim.

MILNER, H. W., N. S. LAWRENCE, AND C. S. FRENCH. 1950. Colloidal dispersion of chlorophyll material. Science **111**:633-634.

MUHAMMED, A., A. RODGERS, AND D. E. HUGHES. 1959. Purification and properties of polymetaphosphatase from *Corynebacterium xerosis*. J. Gen. Microbiol. **20**:482-495.

SALL, T., S. MUDD, AND A. TAKAGI. 1958. Polyphosphate accumulation and utilization as related to synchronized cell division of *Corynebacterium diphtheriae*. J. Bacteriol. **76**:640-645.

SMITH, I. W., J. F. WILKINSON, AND J. P. DUGUID. 1954. Volutin production in Aerobacter aerogenes due to nutrient imbalance. J. Bacteriol. **68**:450-463.

WIDRA, A. 1959. Metachromatic granules of microorganisms. J. Bacteriol. **78**:664-670.

WINDER, F. G., AND J. M. DENNENY. 1957. The metabolism of inorganic polyphosphate in mycobacteria. J. Gen. Microbiol. **17**:573-585.