

presented here on the Ivory Coast tektites do not clarify the significance of this isochron.

However, the plotted points for the Ivory Coast tektites lie essentially on the Birrimian-age isochron of 1.97×10^9 years. Only three of the tektite points fall exactly on the isochron; the others all occur, at varying distances, slightly to the left of the line. This selective scatter to the left of the line can be ascribed to fractional volatilization of Rb relative to Sr during the fusion process which formed the tektites. Several recent studies suggest that selective volatilization of Rb relative to Sr took place during the fusion process that produced tektites (13) and, further, that the SiO₂ content decreases (hence the density increases) with increasing volatilization (14). The tektites listed in Table 1 (in order of increasing specific gravity) could be considered, therefore, as being listed roughly in order of increasing degree of volatilization and increasing loss of rubidium relative to strontium. There is an inverse correlation of rubidium with density, and a direct correlation of density with the distance of the points to the left of the line. That is, the samples at the top of the table, which according to the referenced studies should have suffered the least fractionation, lie on the line, while samples with increasing density (that is, less SiO₂ and more volatilization) fall, in general, further to the left of the isochron.

The age relationships presented here for the Ivory Coast tektites and the Birrimian rocks support the hypothesis of terrestrial origin for these tektites. The probability that these tektites, if extraterrestrial, would have just the right combination of rubidium, strontium, and strontium isotopic composition to lie on the isochron of the country rock on which they fell seems rather remote. Because much of the basement of West Africa is approximately 2×10^9 years old, this age value does not point unequivocally to the Bosumtwi crater as their place of formation. However, chemical similarities between the Ivory Coast tektites and glass from the Bosumtwi crater support this hypothesis of common origin. Gentner *et al.* (7) report the close similarity between the Bosumtwi crater glass and the Ivory Coast tektites for seven trace elements. Our Rb, Sr, and strontium isotopic analyses support this close similarity between the two types of materials. In addition, preliminary

chemical analyses of these samples show a close similarity for the major elements examined: Fe, Ti, Ca, Mg, K, Na, and Mn (15). The concentrations of all these elements, except Ca, in the one piece of Bosumtwi glass available, lie within the rather restricted range of the 11 analyzed Ivory Coast tektites.

These three lines of evidence—the Rb-Sr age correlation (age of parent material), the K-Ar age correlation (age of fusion), and the chemical correlation—strongly support the hypothesis that the Ivory Coast tektites are related to the phenomenon that formed the Bosumtwi crater.

Note added in proof. An independent Rb-Sr isotopic study of three Ivory Coast tektites and five Bosumtwi crater glasses has recently been completed by Lippolt and Wasserburg (*Z. Naturforsch.*, in press). Their data and conclusions are essentially in agreement with this paper.

C. C. SCHNETZLER

*Goddard Space Flight Center,
Greenbelt, Maryland*

W. H. PINSON

P. M. HURLEY

*Department of Geology and
Geophysics, Massachusetts Institute
of Technology, Cambridge*

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16. We are indebted to the following for donating samples: John Saul and Elliot Miller, of Saul Miller, Consulting Geologists; E. C. T. Chao of the U.S.G.S.; R. M. Walker and R. L. Fleischer of the General Electric Research Laboratory; and the Société de Développement Minéral de la Côte d'Ivoire. Financial aid for this research was furnished in part by NASA through the Center for Space Research at the Massachusetts Institute of Technology and in part by the U.S. Atomic Energy Commission. Field work for the collection of the tektites in the Ivory Coast by John Saul and Elliott Miller was sponsored by the National Geographic Society. We also thank W. R. Shields of the National Bureau of Standards for the use of mass spectrometers, H. H. Thomas, M. L. Bottino, and P. D. Fullagar for aid in analytical work, and members of the GSFC Geochemistry Laboratory for helpful discussions.

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Imidonitrogen in *Chlorella* "Polyphosphate"

Abstract. "Polyphosphate" and fragments isolated from acid hydrolysis of polyphosphate have an infrared absorption band at 1400 cm^{-1} , which is characteristic of imidodiphosphate linkages. Complete hydrolysis of purified "polyphosphate" releases 1 to 2 moles of phosphate per mole of ammonia. The polymer must contain subunits which are cyclic and which contain both imidodiphosphate linkages and phosphate anhydride linkages.

The polyphosphate in *Chlorella* is not a simple phosphate anhydride polymer (1-3); nor is it, because of its stability in water, one of the many polymers containing phosphorus which are unstable in water. The properties of trimetaphosphoimidate (4) prompted a study to determine whether metaphosphoimidate groups occur within the material of high molecular weight commonly designated as polyphosphate. Imidodiphosphate linkages have not been reported in natural products.

Sodium trimetaphosphoimidate was synthesized (5) in order to study its properties in more detail. Its specific metachromasy (2) is 0.3 compared to 0.0 for trimetaphosphate. Thus metaphosphoimidates are more metachromatic than metaphosphates. This is significant because *Chlorella* polyphosphates have been reported to have very high specific metachromasies, which could not be explained if the polyphosphate contained only anhydride linkages (2). Trimetaphosphoimidate re-

leased 30 percent of its phosphorus as orthophosphate when treated for 7 minutes with 1*N* hydrochloric acid at 100°C. (This release may be abbreviated as follows: $\Delta_7P = 30$ percent.) A phosphate anhydride is completely hydrolyzed under these conditions ($\Delta_7P = 100$ percent). Purified *Chlorella* polyphosphate is intermediate ($\Delta_7P = 85$ percent) (3). When trimetaphosphoimidate is refluxed for 1 hour in 18*N* sulfuric acid, it releases all of its nitrogen as ammonia and all of its phosphorus as orthophosphate.

Trimetaphosphoimidate was hydrolyzed in 1*N* HClO₄ (15 minutes at 100°C), neutralized with KOH, and chromatographed on a column containing Dowex-1-formate resin (3). Four fractions were resolved. The first fraction (61 percent of the total phosphorus applied to the column) was orthophosphate. The second fraction (19 percent of the total phosphorus) was eluted in the diphosphate position and was a mixture of pyrophosphate and imidodiphosphate ($\Delta_7P = 69$ percent). The third fraction (8 percent of the total phosphorus) was eluted in the triphosphate position and was mostly imidotriphosphate, a known (4) major intermediate of acid hydrolysis ($\Delta_7P = 50$ percent). The fourth fraction (12 percent of the total phosphorus) was either unaltered trimetaphosphoimidate or, more likely, the product formed by the substitution of one trimetaphosphoimidate nitrogen by oxygen ($\Delta_7P = 10$ percent).

Trimetaphosphoimidate was also hydrolyzed in 0.5*N* KOH (18 hours at 38°C) neutralized with HClO₄, and chromatographed on a column containing Dowex-1-formate resin in the same manner. Only three fractions were resolved. The first fraction (4 percent of the total phosphorus applied to the column) was orthophosphate. The second fraction (60 percent of the total phosphorus) was eluted in the diphosphate region and was mostly imidodiphosphate ($\Delta_7P = 10$ percent). The third fraction (36 percent of the total phosphorus) was eluted in the triphosphate region and was mostly imidotriphosphate ($\Delta_7P = 50$ percent).

Thus hydrolysis in 1*N* acid at 100°C for 15 minutes of trimetaphosphoimidate releases orthophosphate and di- and triphosphates which are more resistant to acid hydrolysis than pyro- and tripolyphosphate. Under these conditions *Chlorella* polyphosphate releases 95 percent orthophosphate and 5 percent di- and triphosphates which are

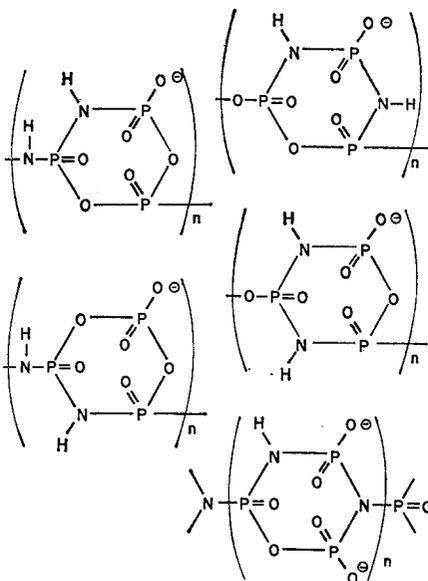


Fig. 1. Some possible repeating units in the structure of *Chlorella* "polyphosphate."

relatively acid resistant (3). Alkaline hydrolyzates of both trimetaphosphoimidate and *Chlorella* polyphosphate contain only a few percent of orthophosphate and a large amount of di- and triphosphates. In the case of polyphosphate these are predominantly pyro- and tripolyphosphate. In the case of trimetaphosphoimidate they are predominantly imidodiphosphate and imidotriphosphate.

Chlorella polyphosphate was treated with venom phosphodiesterase and it was then chromatographed on a column of diethylaminoethylcellulose resin to remove the polyphosphate from the RNA components. These polyphosphate fractions and the di- and triphosphates isolated from acid hydrolyzates of polyphosphate by column chromatography on Dowex-1-formate resin were analyzed on a Perkin-Elmer infrared spectrophotometer, model 521. Samples were prepared by the potassium bromide pellet technique (2). Sodium trimetaphosphoimidate; hydrolytic fractions obtained from trimetaphosphoimidate such as imidodiphosphate and imidotriphosphate; ortho-, pyro- and tripolyphosphate; yeast RNA; Graham's salt, a synthetic true polyphosphate (5); and reagent blanks were analyzed in the same way. All compounds which contained imidodiphosphate linkages, and only these compounds, exhibited an absorption at 1400 cm^{-1} . Intact *Chlorella* polyphosphate and the acid-resistant di- and triphosphates derived from it also had an absorption band at 1400 cm^{-1} .

Purified fractions of *Chlorella* poly-

phosphate were also assayed for ammonia nitrogen which was released by refluxing one hour in 18*N* sulfuric acid. The released ammonia was determined by a modified Nessler reaction (6) after micro-Kjeldahl distillation. The ratio of moles of phosphate per mole of ammonia released varied from one to two in a variety of fractions tested.

Thus *Chlorella* polyphosphate, as isolated, contains both phosphate anhydride and imidodiphosphate bonding. However, oxygen is easily substituted for nitrogen in polyphosphoimidates yielding mixed bonding or even true polyphosphates. A certain amount of this probably happens during isolation since this replacement takes place in water from pH 1 to 14 (4, 7). Also, cyclic groups or at least branching must occur in the polymer, since both linear polyphosphoimidates and polyphosphates are stable to alkaline hydrolysis (5, 7). In contrast cyclic (meta) phosphate anhydrides or phosphoimidates are cleaved at alkaline pH (4). The phosphorus atoms at which branching occurs must have at least one imido-linkage, since a phosphate trianhydride is immediately hydrolyzed in the presence of water (5). Not enough is known about the stabilities of other potential branching combinations to make further conclusions. Some possible repeating units in the structure of *Chlorella* polyphosphate are shown in Fig. 1. In such a polymer the ratio of phosphorus to nitrogen would be 1.5. Variations, which are not excluded by my data, include eight-membered rings, higher or lower ratios of phosphorus to nitrogen, the presence of mono-, di-, or triphosphate connections between rings, or branching brought about by connecting more than two rings to some rings. Upon alkaline hydrolysis such polymers might be expected to cleave within each repeating unit to open the ring and between units to yield low-molecular-weight products, but little orthophosphate. The composition of the hydrolyzate would depend upon slight, but as yet unknown, differences in the stability of the bonds in each unit. Acid hydrolysis of such polymers would cleave the anhydride linkages quickly and would carry out two competitive reactions at the imidodiphosphate sites, substitution of oxygen for nitrogen or cleavage (4). Anhydride bonds produced by oxygen substitutions would then be cleaved. Any amido groups produced by cleavage would be quickly hydrolyzed in acid to give ammonia. Thus

hydrolysis would be expected to yield a large amount of orthophosphate and small amounts of imidodiphosphate and imidotriphosphate. Polymers of the type shown in Fig. 1 would be highly metachromatic due to the presence of few secondary phosphate ionizations, which inhibit metachromasy (8). They might also exhibit a completely different affinity for RNA than a true polyphosphate, thus resembling the naturally occurring "polyphosphate."

DAVID L. CORRELL

Radiation Biology Laboratory,
Smithsonian Institution, Washington

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Hemagglutination by Fava Bean Extract Inhibited by Simple Sugars

Abstract. Hemagglutination by extract of fava bean was inhibited by 5-percent *d*-glucose, *d*-fructose, or maltose, but not by 5-percent *d*-galactose or lactose. Failure to inhibit seems to reflect the presence of a hydroxyl group at the carbon No. 4 position. Hemagglutination was enhanced by dextran of high molecular weight, but not by dextran of low molecular weight. The finding supports the hypothesis that large molecular size explains the enhancement by gum acacia of hemagglutination by fava bean.

Agglutination of normal human erythrocytes by substances other than blood group-specific isoantibodies is well known (1). Inhibition of this hemagglutination by various complex sugars has been reported (2). The effects of simple sugars on hemagglutination by saline extract of fava bean are the subject of this report.

Hemagglutination by fava bean was inhibited by 5-percent *d*-glucose, *d*-fructose, or maltose, but not by 5-percent *d*-galactose or lactose (Table 1). The determining factor appears to reside in the configuration of the No. 4 carbon atom: presence of a hydroxyl group prevents inhibition of hemagglutination. Inhibition by normal

human serum of hemagglutination by fava bean has been reported (3); the concentration of glucose in normal human serum, 0.6 to 1.05 mg ml⁻¹, cannot explain such inhibition. Inhibition was complete with 0.6-, partial with 0.55-, and none with 0.4-percent *d*-glucose. Agglutination disappeared if normal serum or 5-percent *d*-glucose was added after agglutination occurred. The inhibition in vitro by normal serum of hemagglutination by fava bean did not occur in the serum of a child with favism (4). These observations suggest that induced hyperglycemia or transfusion of plasma may have a therapeutic effect in human favism.

Hemagglutination by fava bean is

enhanced by gum acacia (3). Similar studies with dextrans of varying molecular weights (5) show augmented titers (1:640) with dextran of high molecular weight (75,000), but not with dextran of lower molecular weight (41,500). The hypothesis that enhancement of agglutination by gum acacia is due to the gum's molecular size is strengthened. Agglutination by dextrans of high molecular weight also is inhibited by 5-percent *d*-glucose or normal human serum.

CONSUELA B. PERERA

ABRAHAM M. FRUMIN

Departments of Laboratories and
Research, Albert Einstein Medical
Center, Southern Division,
Philadelphia, Pennsylvania 19147

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Encephalitogenic Activity of Bovine Basic Proteins

Abstract. Two basic proteins isolated from bovine white matter in connection with a study of the protein-bound phosphoinositides of central nervous system tissue have been tested for encephalitogenic activity. The biological activity of these proteins, which is equivalent to that of basic encephalitogenic proteins isolated in other laboratories, suggested that they are identical.

The basic protein fraction of central nervous system (CNS) myelin has been investigated intensively because it can induce experimental allergic encephalomyelitis (EAE) in various animals (1). Although the encephalitogen is part of the myelin proteolipid, it is not found in the chloroform-methanol (2:1 mixture) extract of the whole tissue. Its absence from the chloroform-methanol extract of whole tissue is presumably caused by some interaction between the encephalitogenic proteolipid and other constituents of the tissue

Table 1. Effects of simple sugars on hemagglutination by saline extract of fava bean. Degree of agglutination indicated by number of plus signs.

Sugar concentration (%)	Extract titer				
	1:10	1:20	1:40	1:80	1:160
None	++++	++++	+++	++	0
Lactose 5	++++	++++	+++	++	0
<i>d</i> -Galactose 5	++++	++++	+++	++	0
Maltose 5	0	0	0	0	0
<i>d</i> -Fructose 5	0	0	0	0	0
<i>d</i> -Glucose 5	0	0	0	0	0
<i>d</i> -Glucose 0.6	0	0	0	0	0
<i>d</i> -Glucose .55	++	++	0	0	0
<i>d</i> -Glucose .4	+++	+++	++	++	0