second; these will be made available through the NASA Data Center at Greenbelt, Maryland.

In the matter of very large motions the Bruce and Palomar surveys together have now produced between 1900 and 2000 new motions larger than 0.5 arc second annually, while from all other sources some 1400 have become known. Including the data obtained from the hand-blinked plates, we have now published data on some 5000 new white dwarfs and degenerate stars, or more than 90 percent of those known. Similarly, we have found and published data on some 3000 stars of low luminosity—those which are indicated, statistically, to be of less than one-thousandth of the sun's luminosity. In this matter the plates of the Palomar 48-inch Schmidt telescope are unique, for from all other sources barely 30 such stars have been announced.

Although a very large part of the operational expense of our machine lies in the computer costs, still the overall costs in terms of results achieved are remarkably low. This is very important these days when, because of dwindling federal support for science, we are all of us becoming cost conscious. When I did the Bruce survey on Harvard plates it produced some 80,000 new motions at a total cost of $180,000, or $2.25 per new motion (in 1930–1950 dollars). From the hand-blinked Palomar plates I published some 65,000 new motions against a total cost of $275,000 in National Science Foundation grants, or $4.25 per new motion (1962–1972 dollars). The total operational cost of our machine has been $280,000 to date (NASA and NSF); hence the 26,000 new large motions published have cost a little under $11 apiece (1972–1974 dollars), while if we add in the 250,000 smaller motions the average cost comes down to just about $1 (1972–1974 dollars). The only remotely comparable stellar motion survey which has now been in operation for about 18 years has produced fewer than 6000 new motions for a total cost of over $300,000, or well over $50 per new motion (1956–1974 dollars).

Even if we amortized the entire cost of constructing our machine, $787,000, in our present operations—that is, if we retired our machine tomorrow and put it in storage—we would still have produced 26,000 new large motions at a cost of $1,067,000, or $41 per new motion (even this is much better than our only competitor), and counting the 250,000 smaller motions (all of them new) the cost would be less than $4 per new motion.

We are now approaching the end of our operations on the Palomar survey plates, and we can justifiably claim that the machine has proved to be fantastically and superlatively successful.

Personally I would consider it a great pity if the machine were now retired—"pickled," and put in storage. A machine as versatile as this could be quite easily adapted to a host of other programs, and I for one would welcome any and all applications for such use from other scientists.

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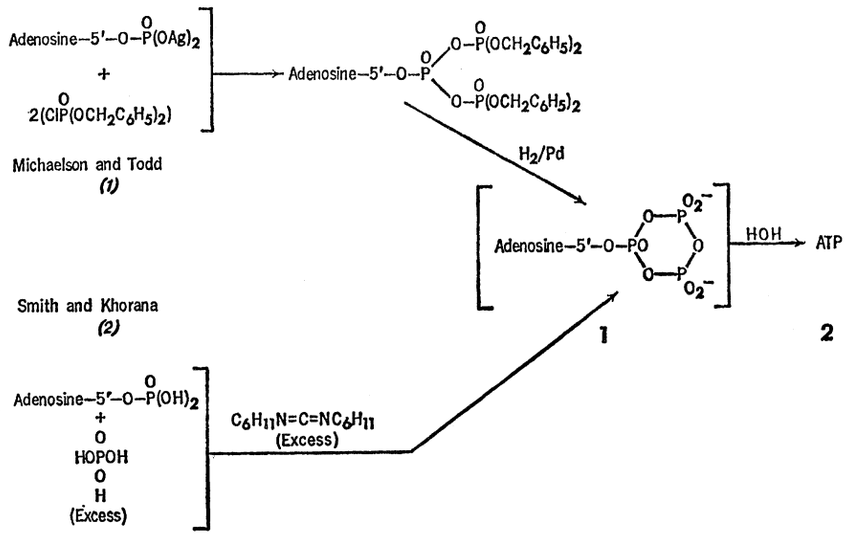
Cyclization of the Phosphate Side Chain of Adenosine Triphosphate: Formation of Monoadenosine 5′-Trimetaphosphate

Abstract. *Monoadenosine 5′-trimetaphosphate has been prepared from adenosine 5′-triphosphate by a carbodiimide-mediated condensation. The molecule was characterized by ^{31}P nuclear magnetic resonance, and its ^{31}P spectrum was simulated through the assumption of a three-phosphorus spin system. The molecule is highly reactive and is rapidly converted to adenosine triphosphate upon contact with water.*

The adenosine 5′-monoester of trimetaphosphate (**1**), a molecule which can be pictured as arising from adenosine triphosphate (ATP, **2**) by an intramolecular condensation between the α and γ groups of the tripolyphosphate side chain, has been considered as a possible intermediate in reactions involving ATP.

In 1949 Michelson and Todd (*1*) obtained ATP by reacting dibenzyl phosphochloridate with the disilver salt of adenosine 5′-monophosphate and then removing the benzyl groups by hydrogenation. They concluded that a reaction which produced **1** as an intermediate occurred at some stage, probably during the debenzylation, and that hydrolysis of **1** at the esterified phosphate position then took place to produce the linear triphosphate derivative, ATP.

Smith and Khorana (*2*), in studies on the chemical synthesis of nucleoside 5′-diphosphates and 5′-triphosphates, found that the triphosphates were formed as the major products when nucleoside 5′-monophosphates were condensed with excess orthophosphoric acid in anhydrous pyridine with use of excess amounts of dicyclohexylcarbodiimide. This was interpreted as taking place through the formation of the trimetaphosphate esters (**1**, in the case of adenosine) which were hydrolyzed to the corresponding linear tripolyphos-



phates upon the addition of water during the preparative procedures.

Michelson and Todd (*1*) raised the question of whether **1** plays a role in biological systems associated with ATP. This was based in part on an earlier suggestion by Lipmann (*3*) that the formation of trimetaphosphate rings and certain of their derivatives "might well have a bearing on the problem of the utilization of 'energy rich' phosphate bonds in muscular contraction" (*1*).

No direct evidence for the existence of **1** has been presented. We now report the detection, through use of ^{31}P nuclear magnetic resonance (NMR), of a cyclic derivative of ATP. Evidence is presented that this compound corresponds to structure 1. It is pictured as arising through the reaction of the tri-*n*-butylammonium salt of ATP with excess dicyclohexylcarbodiimide in anhydrous pyridine solution as formulated.

The tri-*n*-butylammonium salt of ATP was prepared by immediately mixing an aqueous solution of the free acid (obtained by passing 0.3 mmole of the sodium salt through a column of Dowex 50 H^+ ion-exchange resin) with tri-*n*-butylamine (1.2 mmole) in acetone. The ammonium salt was dried under reduced pressure at 25°C, through successive evaporations of 50 percent benzene in acetone. The resulting glassy syrup was then dissolved in 3 ml of anhydrous pyridine and subjected to ^{31}P NMR analysis (*4, 5*) (Fig. 1, top spectrum). The final pyridine solution could also be prepared by immediately mixing the aqueous free acid from the column with tri-*n*-butylamine in pyridine followed by successive evaporations of anhydrous pyridine. In either case the anhydrous pyridine solutions did not show measurable amounts of phosphorus-containing impurities upon ^{31}P NMR analysis.

After ^{31}P NMR analysis, the solution was treated with 1.8 mmole of molten dicyclohexylcarbodiimide in a single addition at 25°C. (Diisopropylcarbodiimide may also be used with equivalent results.) The reaction proceeded rapidly with the nearly instantaneous precipitation of the urea and without noticeable evolution of heat. After 15 minutes, the reaction mixture was filtered directly into an NMR tube; the center spectrum of Fig. 1 was obtained from the resulting clear solution. Immediate hydrolysis of a portion of this reaction mixture with water (*2*) yielded essentially pure ATP as determined by ^{31}P NMR and thin-layer chromatography (*6*).

Figure 1 shows the ^{31}P NMR spectra obtained from the pyridine solution of ATP (**2**) before (top spectrum) and after (center spectrum) the addition of the dicyclohexylcarbodiimide condensing agent. Both spectra were taken while the protons of the adenosyl moiety were being decoupled from the phosphorus atoms of the polyphosphate side chain. The region shown encompasses the end phosphate group and middle phosphate group portions of the ^{31}P NMR resonance band (*5*). In the top spectrum, the doublet centered at 11 ppm arises from the γ (chain terminal) end phosphate grouping of the ATP tripolyphosphate chain. The doublet at 12 ppm arises from the α (ester) end phosphate grouping. In the absence of ^1H decoupling, the α end group resonance multiplet is split by the two 5′-protons of the adenosyl moiety and appears as a doublet of triplets (*7*, p. 13). The multiplet at 24 ppm arises from the β middle phosphate group. This multiplet appears as a triplet in Fig. 1 because of the sweep conditions used and the near-identical coupling constants between the middle phosphate group and the two different end phosphate groupings. Actually the resonance multiplet is a pair of overlapping doublets (*7*, p. 13). A spectrum covering the entire phosphate region showed only the end and middle phosphate resonances appearing in Fig. 1.

The center spectrum was obtained from the reaction mixture 15 minutes after the addition of dicyclohexylcarbodiimide to the solution giving the top spectrum. The end phosphate group resonances from ATP have essentially disappeared, and the middle group triplet has been replaced by a multiplicity of middle phosphate resonances. The multiplet extending to higher fields from 2 ppm, to a first approximation, exhibits the characteristic features of a classical ab_2 NMR resonance system (*7*, p. 14; *8*, p. 15, spectrum 2-4), where two of the three nuclei are nearly equivalent, and shift differences between the *a* and *b* forms are small in relation to the value of the coupling constant. However, upon refined analysis (*8*, p. 21 and p. 54, spectrum 3B-2; *9–11*) (bottom computer-simulated spectrum in Fig. 1) this system was determined to be of type *abc* with 43.5 and 59.0 hz separating the *a* and *b* and *a* and *c* resonance groupings, respectively, while 15.5 hz separated the

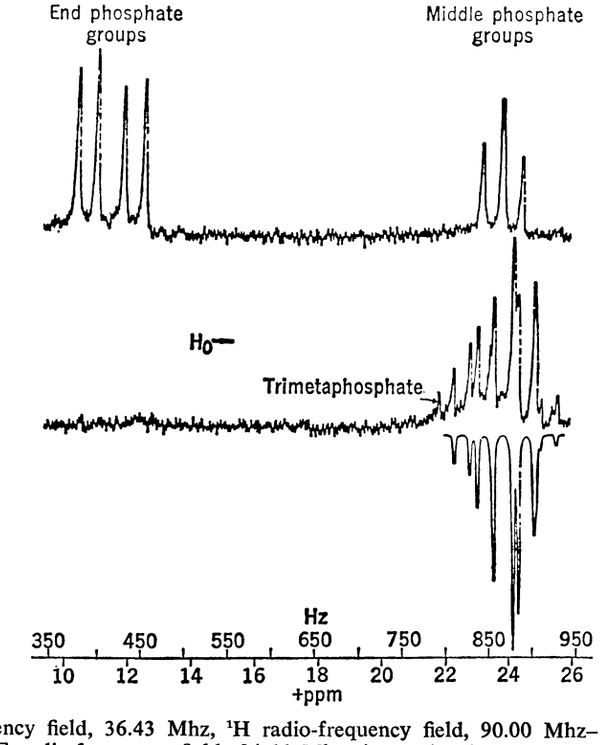


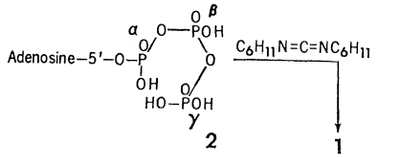
Fig. 1. Actual and theoretical ^{31}P NMR spectra. (Top spectrum) Tri-*n*-butylammonium ATP in pyridine; $\delta_\alpha = 12.2$ ppm (446 hz); $\delta_\beta = 10.8$ ppm (392 hz); $\delta_\gamma = 23.8$ ppm (865 hz); $J_{\alpha\beta} = 24.0$ hz; $J_{\gamma\beta} = 21.8$ hz; ($J_{\alpha\text{-}5'CH_2} = 5.7$ hz). (Center spectrum) Monoadenosine 5′-trimetaphosphate in pyridine, actual spectrum. (Bottom spectrum) A computer-simulated (*9, 11*) *abc* phosphate spin system; $\delta_a = 22.9$ ppm (837.0 hz); $\delta_b = 24.2$ ppm (880.5 hz); $\delta_c = 24.6$ ppm (896.0 hz); $J_{ab} = 23.5$ hz; $J_{ac} = 22.9$ hz; $J_{bc} = 23.7$ hz; these parameters were obtained through iterative procedures (*8, 10*). The experimental spectra were recorded while the proton resonance band was being strongly irradiated to effect complete decoupling of protons, and signal-averaging processes were employed; ^{31}P radio-frequency field, 36.43 Mhz, ^1H radio-frequency field, 90.00 Mhz–broad band modulated, ^{19}F radio-frequency field, 84.66 Mhz; internal reference, C_6F_6. Chemical shifts are given relative to external 85 percent orthophosphoric acid (*4*). The center spectrum contains a small amount of trimetaphosphate.

b and *c* groupings. The four major resonance lines of the multiplet lying to lower fields arise from the *a* portion; these appear as a complicated, but nonetheless analyzable, 12-line pattern if the spectrum is obtained in the absence of ^1H decoupling. The resonances lying at higher fields are not affected by ^1H decoupling. The signals (Fig. 1) were the only ^{31}P resonances observed and the *abc* multiplet is consistent with the cyclic, trimetaphosphate ester form, **1**, of ATP. In **1** all the phosphate groupings are of the middle phosphate type with one of them bonded to an ester function which bears methylene protons. The *a* portion of the spectrum is interpreted as arising from the esterified middle phosphate of structure **1** (derived from the α-phosphate of ATP, **2**). The *bc* portion is interpreted as arising from the ionized phosphates of structure **1** (derived from the β and γ phosphates of ATP).

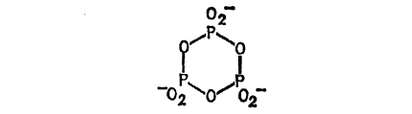
The nonequivalence of the ionized phosphates of **1** is not immediately obvious and deserves some explanation. The adenosyl moiety contains optically active centers, and this, coupled with the tetrahedral arrangement of groupings about the ester phophorus atom, bestows the property of dissymmetry on the molecule. This renders the two ionized phosphates nonequivalent. It is likely that a certain amount of interaction between the two rings of adenosine trimetaphosphate contributes to this nonequivalence. This interaction is sterically favored, and the magnitude of the expected interaction should be significant since the linkages in condensed phosphates possess considerable π-bond character (*12*).

The center spectrum shows the presence of a small amount of trimetaphosphate (**3**). Over a period of several hours the signal from **3** increased while the *abc* multiplet decreased proportionately. Thus **3** could arise as a result of the removal of the adenosyl grouping of structure **1** by a component (or components) of the reaction mixture: the carbodiimide, the corresponding urea, or the pyridine solvent (*13*). Hydrolysis of the reaction mixture producing the middle spectrum gave ATP as the sole detectable adenosine phosphate derivative and a small amount of trimetaphosphate. The formation of ATP is compatible with the hydrolysis of structure **1** at the ester phosphate position.

The symmetrical diesterified hexametaphosphate (**4**), which would arise

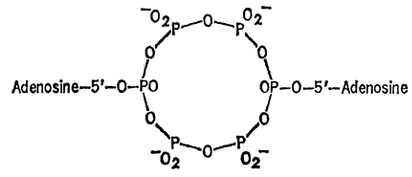


2



1 + $H_{11}C_6N(H)C(=O)NC_6H_{11}$

3



4

through two intermolecular condensations between the respective α and γ phosphates of two molecules of ATP, also contains only middle phosphate groups. Moreover, as in **1**, the ratio of the esterified- to the ionized-middle groups is 2 : 1, and each esterified group is attached to two apparently identical ionized groups. Indeed any higher cyclic polymer of ATP (tri-, tetra-, and other) produced in this manner would show these same relationships. It seems unlikely that **4** would produce ATP and trimetaphosphate as the only detectable phosphorus-containing hydrolysis products since one of the possible modes of cleavage at the two reactive ester positions would produce pyrophosphate and P^1,P^4-diadenosine 5′-tetrapolyphosphate. It is also difficult to see how trimetaphosphate would readily arise from this molecule in the anhydrous pyridine reaction mixture. Finally, it seems unlikely that the complicated spin-spin interactions which would be operative within such a molecule would give rise to the *abc* NMR pattern observed even if the average conformation of the molecule in solution rendered it completely symmetrical. The iterative analysis of the spectral lines is not consistent with a hexa- or higher phosphorus spin system.

Accordingly, we suggest that the molecule giving rise to the middle spectrum of Fig. 1 corresponds to the adenosine monoester of trimetaphosphate, structure **1**.

From the evidence presented it appears to us that molecules such as **1** very probably are intermediates in many nonbiological reactions that involve the side chains of the naturally occurring nucleoside polyphosphates. It also seems entirely possible that they may play a role in certain of the biological processes involving the parent compounds. If this proves to be the case it is likely that they will be found only in a nonaqueous milieu, such as the lipoid portions of cell organelles, or perhaps in intimate association with enzyme proteins. In view of the properties of such molecules the usual methods employed for the preparation of cellular fractions would probably lead to their rapid conversion to ATP. It appears that the free energy for the hydrolysis of **1** to ATP may be comparable to that for the hydrolysis of ATP to adenosine diphosphate plus orthophosphate; certainly ATP becomes the only detectable form present upon the addition of water. In this context, according to the early notation of Lipmann (*3*), this molecule seems to represent a kind of "super high-energy phosphate" analogous to the branched inorganic phosphates as previously described (*14*). Moreover, in striking contrast to the low rate of hydrolytic cleavage of ATP at neutral *p*H, the hydrolysis of this molecule to produce ATP is extremely rapid.

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15. This work was supported by the General Research Support grant awarded to the University of Illinois College of Medicine, a grant from the Research Board of the Graduate College, University of Illinois at the Medical Center, and PHS grant 11702. We thank S. Brudno and A. Kilburn of the Research Resources Laboratory–Computer Center for their assistance in the computer analysis aspect of this work.

22 January 1974

Visual Fields of Cats with Cortical and Tectal Lesions

Absrtact. *When cats were tested for visual field perimetry, the field of vision for each eye separately was from 45° contralateral to 90° ipsilateral. After either bilateral occipitotemporal lesions (with a split of the tectal commissure) or bilateral area 17, 18, and 19 lesions, the cats could see with each eye only from the midline to 90° ipsilateral. A cat that became nearly totally blind as a result of bilateral occipitotemporal decortication had a subsequent tectal split which enabled it to see with each eye from the midline to 90° ipsilateral.*

Sprague reported that cats with a large, unilateral, occipitotemporal cortex ablation develop a stable hemianopia (*1*), but that in such cats considerable visually guided behavior was restored for the previously blind hemifields after either an ablation of the superior colliculus contralateral to the cortical lesion or a transection of the commissure of the superior colliculus (*2*). As a tentative explanation for this remarkable phenomenon, Sprague suggested (i) that the midbrain subserves certain aspects of visually guided behavior; (ii) that each colliculus receives a facilitatory input from the ipsilateral cortex and a balancing inhibitory input from the contralateral colliculus via the collicular commissure; and (iii) that as a result of the imbalance caused by the cortical lesion, the function of the ipsilateral colliculus is inhibited. Under these conditions, the colliculus is nonfunctional for visually guided behavior, and this results in the hemianopia. Function is returned by destroying either the other colliculus or the collicular commissure.

There have been no reported confirmations of this phenomenon which Sprague reported in 1966 and few experimental attempts to understand it further [see (*3*)]. I now report a confirmation and extension of Sprague's finding.

I tested five cats with bilateral cortical lesions. In three (C3, C6, and C7), the lesion included most of the occipitotemporal cortex; in the other two (C10 and C11) it included only areas 17, 18, and most of 19 (Fig. 1). In addition, the collicular commissures were transected at the time of the cortical ablation in C3 and C7, and in a second operation 9 months later in C6. All cats except C6 were killed and perfused with 10 percent formol saline. Their brains were blocked stereotaxically, removed, photographed, and cut coronally in 40-μm sections that were stained alternately with cresyl violet for cell bodies and by the Mahon method for fibers. The lesions in cats C3, C7, C10, and C11 were reconstructed (Fig. 1). Cat C6 is being retained for further study, and I assume tentatively that its lesions are similar to those of C3 and C7 (Fig. 1), particularly since visualization was especially good during the two operations in C6.

I tested all cats pre- and postoperatively, and used previously described methods to study visual placing, visual following of moving objects, and the extent of visual field perimetry (*4*). For the perimetry, each cat was taught to fixate on one object while a second stimulus was rapidly introduced vertically from above into the visual field, and the presence or absence of the cat's orienting response to the second stimulus was noted. Every 15° sector of the visual field was thus tested numerous times, and a percentage of correct orienting responses was calculated. For a control, I computed for each cat the baseline percentage of apparent orienting responses in the absence of a second stimulus. Only regions of the visual field in which the second stimulus evoked a significantly ($P < .001$ on a χ^2 test) higher percentage of orienting than this baseline percentage are considered to be regions visually attended to by the cat. The cats were tested binocularly as well as monocularly by means of a contact occluder over one cornea.

The visual field perimetry data for

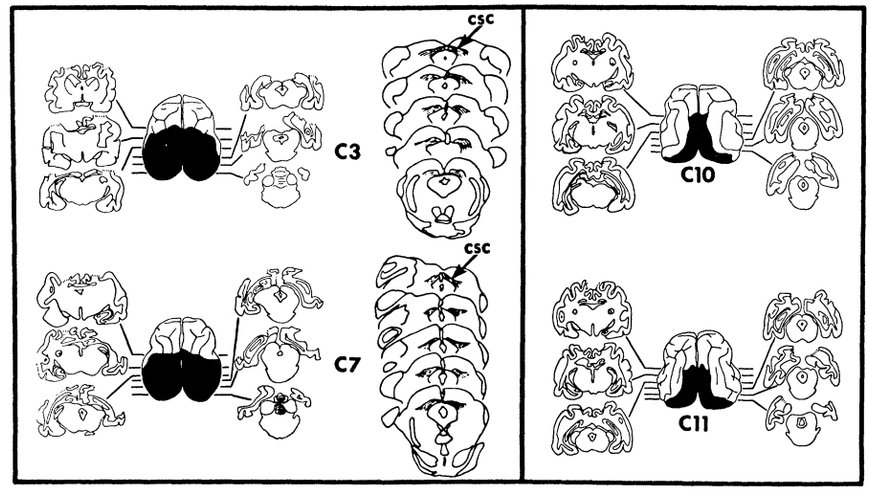

Fig. 1. Reconstruction of lesions. In all four cats, the lateral geniculate nucleus showed retrograde degeneration throughout its extent. For C3 and C7, the cortical lesions involved most of the occipitotemporal cortex including all known visual projection zones of the geniculate and pulvinar and lateral posterior nucleus complex (*11*); the commissure of the superior colliculus (*CSC*) was completely sectioned in each cat except for a few fibers surviving in the posterior quarter of the commissure. For cats C10 and C11, the lesion involved dorsally all of the lateral gyrus and medially all cortex superior to the lower bank of the splenial sulcus; all known visual recipient zones of the geniculate were removed, but the visual projections of the pulvinar and lateral posterior nucleus complex were largely spared (*11*).