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Table I. Estimated Percentage Contribution of the Relaxation Mechanisms to the T_1 of H_2O Solution at 31 °C of the Two Metaphosphates (0.1 M in Phosphate) According to the Diffusion Model

Phosphorus-31 Spin-Lattice Relaxation. 2. Inorganic Ring and Chain Phosphates

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Abstract: The ^{31}P spin-lattice relaxation in dilute solutions of the cyclic metaphosphate anions exhibiting 3–8 phosphorus atoms per molecule is found to be independent of pH but to vary with the ring size and the choice of cation in either H_2O or D_2O . On the other hand, the end- and middle-group relaxation times in dilute solutions of the shorter-chain phosphate anions exhibit complicated oscillatory variations with pH variations which are sufficiently large so as to mask other effects. The ^{31}P spin-lattice relaxation in a dilute H_2O or D_2O solution of a very long chain phosphate is again pH independent, so that the effects of changing the counterion can readily be determined. The relaxation values for the cyclic and long-chain phosphates in dilute aqueous solution are quantitatively interpreted in terms of solvent ^1H – ^{31}P and intramolecular ^{31}P – ^{31}P dipole–dipole relaxation as well as spin-rotation relaxation. Preliminary data are presented for the adenosine di- and triphosphates; and acid-dissociation constants are calculated for the pyro- and tripolyphosphoric acids from the variations of the ^{31}P chemical shift (and J_{PP} coupling constant) with pH.

Although the condensed phosphates¹ and their derivatives make up one of the more important families of chemical compounds (with broad significance to biochemistry² as well as many industrial applications²), little has been reported^{3–5} on their spin-lattice relaxation behavior. In the work presented here, we have studied the spin-lattice relaxation of the cyclic metaphosphates,⁶ ranging from the trimeric anion, $\text{P}_3\text{O}_9^{3-}$, through the octameric anion, $\text{P}_8\text{O}_{24}^{8-}$, with attention being given to the effect of various singly charged cations and pH variations in both water and deuterium oxide. Similar information was also obtained for the di-, tri-, and tetrapolyphosphates as well as for a very long chain polyphosphate.¹ In addition, data are also presented for the adenosine di- and triphosphates.

With recent wide acceptance of Fourier-transform nuclear magnetic resonance, we expect that ^{31}P spin-lattice relaxation measurements will find wide use as a probe^{4,7} into biological processes, especially since recent studies^{8–11} show the feasibility of carrying out ^{31}P NMR assays of discreet molecular species within living tissues. The information presented herein should be of considerable value as a reference standard for such probe studies.

Experimental Section

NMR Measurements. A Varian XL-100-15 multinuclear spectrometer was employed with an external heteronuclear lock on ^2H coming from D_2O in an inner concentric tube. The system includes a TT-100 Fourier-transform unit from Nicolet Technology Corporation, Inc., using a data system having 20K of 20-bit word storage. The sample temperature in the probe was maintained at 31 ± 1 °C and measurements of the ^{31}P spin-lattice relaxation times were accomplished by the inversion-recovery method^{12,13} under conditions where a 90° pulse for the ^{31}P nuclei was provided in 63 μs . The value of τ in the $180^\circ - \tau - 90^\circ$ sequences was chosen to bracket the inversion point at which $T_1 = \tau/\ln 2$. The reported T_1 values were all finally computed by hand from a plot of $\ln(I_\infty - I_\tau)$ vs. τ , where I is the intensity for the peak. In a previous study, we found that the standard error in the measurements carried out by this procedure was in the range of 5–8% of the T_1 value for the concentration and range of relaxation times measured here.

^{31}P chemical shifts are reported relative to the usual¹⁴ standard¹⁵ of 85% orthophosphoric acid, with positive chemical shifts being associated with increasing field strengths as is customary. The spectrometer frequency for ^{31}P was 40.5 MHz (^1H , 100.0 MHz).

Sample Preparation. The solutions of sodium and potassium hydroxides used in preparing and neutralizing the various phosphates

were each prepared in duplicate from two fresh bottles of the anhydrous compound obtained from different manufacturers. This preparation consisted of decanting the supernatant liquid from a saturated solution before diluting to the desired strengths. The chosen bottles were selected so as to give in the saturated solution only a small amount of carbonate precipitate exhibiting a clean white color. The tetramethylammonium hydroxide was freshly prepared by passing a solution of the twice recrystallized chloride through a well-washed column of Dowex-1 resin in the hydroxide form, followed by concentration in a rotary evaporator. Aged preparations of this base often exhibit an ESR signal and are then found to induce shortening of phosphate T_1 values.

The long-chain polyphosphate¹⁶ ($\bar{n} = 100.8$) and the cyclic metaphosphates⁶ were prepared as their anhydrous sodium salts by procedures previously described. A number of different commercial preparations of adenosine di- and triphosphates obtained from various manufacturers were purified by means of a previously described⁶ column-chromatography technique, employing diethylaminoethyl-cellulose as the exchange resin. The three different samples of sodium tripolyphosphate that were studied came from different sources: one was prepared by repeated precipitation of the hexahydrate from aqueous solution, another was a purified sample obtained from Dr. E. J. Griffith of the Monsanto Co., and the third was an old Monsanto pilot-plant sample which by chemical analysis seemed free of transition-metal impurities. Similarly, two samples of tetrasodium pyrophosphate from different sources were employed.

The various desired phosphate salt samples were prepared through the use of ion-exchange chromatography by passing appropriate solutions of the phosphates in water through a small (1 × 10 cm) Dowex-50 ion-exchange column in the proton form and immediately titrating the effluent with the hydroxide of the desired cation to the appropriate pH.⁶ This procedure, expeditiously carried out at 24 °C, has been shown^{6,17} not to degrade the phosphates to any measurable extent. Since the affinity of the Dowex-50 resin increases greatly with cationic charge, the column would be expected to be efficient in removing trace transition-metal ions.

Evidence that this cation-exchange preparation of the various phosphates has led to an effective elimination of relaxation-contributing impurities has been obtained for 0.033 M solutions of sodium tripolyphosphate and of sodium adenosine triphosphate with the pH adjusted to 7.0 and 10.0. In the case of the tripolyphosphate anion, each of the three preparations exhibited different T_1 values, but, after passage through the ion-exchange column and pH adjustment, they all gave the same T_1 value, which was somewhat longer than the largest T_1 of the original samples. A similar finding resulted when the same test was applied to the five commercial samples of adenosine triphosphate, one of which exhibited a considerably smaller T_1 than that of the purified materials from the exchange columns. This test was also used on the two samples of pyrophosphate and to select the source of sodium or of potassium hydroxide leading to the highest T_1 .

before the ion-exchange step for a phosphate made from the base and a clean batch of H_3PO_4 .

Results and Interpretation

Cyclic Metaphosphates. Over the pH range of 4 to 12, the spin-lattice relaxation time of any of the cyclic metaphosphates with either tetramethylammonium, sodium, or potassium as the counterion (as measured in either a H_2O or D_2O solution which was 0.1 M in phosphorus) was found to be independent of pH and of concentration as long as the concentration was less than 0.2 M in total phosphorus. There was, however, a considerable difference in relaxation time when going from one counterion to another and upon changing from H_2O to D_2O as the solvent. The variation of the observed T_1 values with ring size for solutions in H_2O or D_2O which were 0.1 M in phosphorus are shown in Figure 1 for the tetramethylammonium salts at pH 11. Note that T_1 , as measured in either H_2O or D_2O , varies in a strictly logarithmic manner with the number of phosphorus atoms in the metaphosphate ring. Furthermore, the two straight lines in Figure 1 intersect, so that the spin-lattice relaxation time for the octametaphosphate in D_2O is less than the value found in H_2O and this difference presumably becomes more pronounced for the larger cyclic anions. The experimental data for the relaxation of the tetramethylammonium metaphosphates are given with high precision by the following two equations which refer to D_2O and H_2O solutions, respectively, where n_P is the number of phosphorus atoms per metaphosphate ring and T_1 is the spin-lattice relaxation time in seconds.

$$\log (T_1)_{\text{D}_2\text{O}} = -0.1262n_P + \log 133.4 \quad (1)$$

$$\log (T_1)_{\text{H}_2\text{O}} = -0.0419n_P + \log 31.0 \quad (2)$$

By comparing a spin-lattice relaxation time obtained in deuterium oxide with an equivalent one obtained in water, it is possible to factor out the relaxation attributable to the dipole-dipole interaction between the hydrogen atoms of the aqueous solvent and the ^{31}P nuclei under observation. From the standard equation for the dipole-dipole interaction^{18,19} and the fact that an observed $(1/T_1)$ is made up of the sum of the various $(1/T_1)$ contributions, we obtained the following equation:

$$\frac{(1/T_1)_\text{H} - (1/T_1)_x}{(1/T_1)_\text{D} - (1/T_1)_x} = \frac{3\gamma_\text{H}^2}{4(2\gamma_\text{D}^2)} = 15.91 \quad (3)$$

where $(1/T_1)_\text{H}$ and $(1/T_1)_\text{D}$ refer to the observed ^{31}P relaxation in the H_2O solution and in the D_2O solution, respectively, and $(T_1)_x$ represent the intramolecular spin-lattice relaxation time in a hypothetical aqueous solvent which contributes no dipole-dipole relaxation to the phosphorus nuclei. As indicated by the dotted line in Figure 1 the variation of $(T_1)_x$ with molecular size for the cyclic tetramethylammonium metaphosphates is represented by eq 4.

$$\log (T_1)_x = -0.1358n_P + \log 157.4 \quad (4)$$

When sodium or potassium is substituted for tetramethylammonium as the counterion, the resulting plots of the type of Figure 1 are also straight lines with different slopes and intercepts than are found in the case of the tetramethylammonium salts. The equations for the spin-lattice relaxation times of the cyclic potassium metaphosphates are given in eq 5-7 for solutions in D_2O , H_2O , and a hypothetical non-spin-active water, respectively.

$$\log (T_1)_{\text{D}_2\text{O}} = -0.0866n_P + \log 85.1 \quad (5)$$

$$\log (T_1)_{\text{H}_2\text{O}} = -0.0076n_P + \log 24.9 \quad (6)$$

$$\log (T_1)_x = -0.0825n_P + \log 83.0 \quad (7)$$

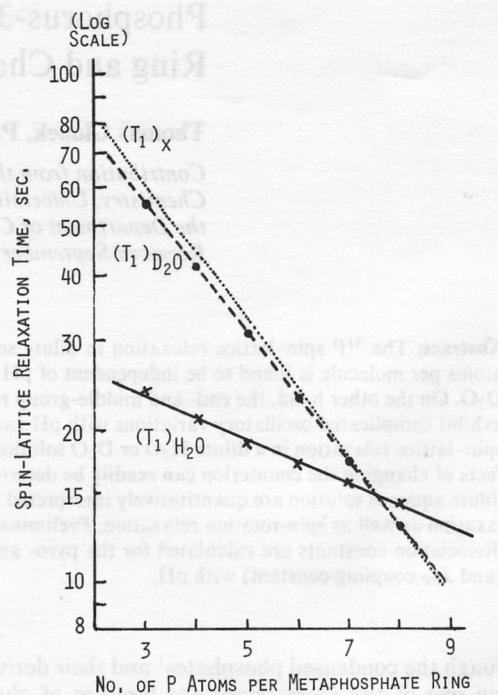


Figure 1. Variation at 31 °C of the ^{31}P spin-lattice relaxation time with molecular size for the tetramethylammonium cyclic metaphosphates at a concentration of 0.1 M in phosphorus. The solid line refers to H_2O as solvent, the dashed line to D_2O , and the dotted line to $(T_1)_x$ value.

The same information is presented in eq 8-10 for the cyclic sodium metaphosphates.

$$\log (T_1)_{\text{D}_2\text{O}} = -0.0938n_P + \log 88.3 \quad (8)$$

$$\log (T_1)_{\text{H}_2\text{O}} = -0.0610n_P + \log 43.9 \quad (9)$$

$$\log (T_1)_x = -0.0960n_P + \log 93.7 \quad (10)$$

Note that the intercept terms in eq 1, 2, and 4-10 correspond to the spin-lattice relaxation of a hypothetical cyclic metaphosphate anion having no phosphorus atoms (i.e., of zero molecular weight).

In these dilute solutions, the value of $(1/T_1)_x$ ought to be dominated by two relaxation mechanisms: dipole-dipole interactions between the phosphorus atoms of a given cyclic metaphosphate anion and the spin rotation of this molecular anion, remembering that this latter relaxation mechanism will not only depend on the gyration of the molecule as a whole but will also be partly due to segmental motions within the molecule, with the segmental-motion contribution becoming relatively more important with increasing molecular size. The other two standard relaxation mechanisms that might contribute to $(1/T_1)_x$ are based on scalar spin-spin coupling and chemical-shift anisotropy. However, by putting appropriate values into the equations for these relaxation mechanisms, it was found that their contributions are insignificant.

The ^{31}P - ^{31}P dipole-dipole relaxation for a pair of phosphorus atoms is given by the expression

$$1/T_1 = \frac{3}{2}(\hbar^2\gamma^4 b^6 \tau_d^2) = 2.293 \times 10^{-38}(\tau_d/b^6)^2 \quad (11)$$

where γ_P is the magnetogyric ratio of phosphorus and b is the distance separating the two phosphorus atoms. The respective correlation time, τ_d , may be roughly estimated from the following relationship:

$$\tau_d = 4\pi\eta a^3/3kT \quad (12)$$

with $\tau_d = (1 \times 10^{-12})a_\text{A}^3$ for the effective molecular diameter, a_A , in Å.

X-ray data on several different crystalline tri- and tetra-

Table I. Estimated Percentage Contribution of the Predominate Relaxation Mechanisms to the ^{31}P Spin-Lattice Relaxation in H_2O Solution at 31 °C of the Tetramethylammonium Cyclic Metaphosphates (0.1 M in Phosphorus), Assuming a Debye Diffusion Model

n_P^a	Dipole-dipole		
	Due to H_2O	$^{31}\text{P}-^{31}\text{P}$	Spin rotation
3	61	15	24
4	53	22	25
5	42	31	27
6	25	42	33
7	6	57	37
8	0	63	37

^a n_P is the number of phosphorus atoms per cyclic molecule.

metaphosphates show that the distance, b_{PP} , between neighboring phosphorus atoms in the metaphosphate ring is about 2.90 Å. Using this number and reasonable values for the molecular diameter, and allowing for interaction of a given phosphorus with other more distant phosphorus atoms in addition to its pair of nearest neighbors, the $1/T_1$ contribution due to dipole-dipole interaction between phosphorus atoms was estimated. By subtracting this from $(1/T_1)_x$, the value of $1/T_1$ attributable to spin rotation was approximated. This value was then used to estimate the product of the moment of inertia of the molecule, I , and the averaged spin-rotation coupling constant, \bar{C} , using^{20,21}

$$(1/T_1)_{SR} = (I^2 \bar{C}^2 / 3\hbar^2)(1/\tau_d) \quad (13)$$

The product, $I\bar{C}$, obtained from these approximate calculations was found to increase a little over threefold when going from the trimetaphosphate to the octametaphosphate as might be expected. According to these rough calculations, the total spin-lattice relaxation for the variously sized tetramethylammonium metaphosphates in 0.1 M aqueous solution is brought about by the proportionate contributions from the three contributing relaxation mechanisms shown in Table I.

Using the same values of a_A and b_{PP} for the sodium and potassium cyclic metaphosphates as were employed for the equivalent tetramethylammonium salts leads, of course, to the same values for the $1/T_1$ contribution due to $^{31}\text{P}-^{31}\text{P}$ dipole-dipole relaxation for any of these counterions. By subtracting this value from the respective $(1/T_1)_x$, the spin-rotation contribution, $(1/T_1)_{SR}$, to the overall relaxation may be estimated. For the tetramethylammonium counterion, it is found that $(T_1)_{SR}$ decreases gradually from 101 s for the trimetaphosphate to 35 s for the octametaphosphate; whereas for the potassium counterion, there is a gradual increase from 65 to 108 s when going from the tri- to the octametaphosphate anion. On the other hand, there is not much change when sodium is the counterion, since in this case the value of $(T_1)_{SR}$ drops from 69 s for the trimetaphosphate to 65 s for the hexametaphosphate and increases back to 73 s for the octametaphosphate. We interpret these findings to mean that, in the presence of the tetramethylammonium cation, the spin-rotation relaxation mechanism includes an increasing amount of segmental motion within the molecule as the ring size is increased but that, in the presence of the potassium counterion, the molecule is stiffened (probably into the more expanded conformations for the larger cyclics) so that increase in molecular size leads to an increase in $(T_1)_{SR}$. The sodium counterion seems to have an effect intermediate between that of the tetramethylammonium and the potassium ion. These necessarily speculative conclusions are in agreement with prior interpretations of ^{31}P chemical-shift data on the cyclic metaphosphates in terms of conformational differences.⁶

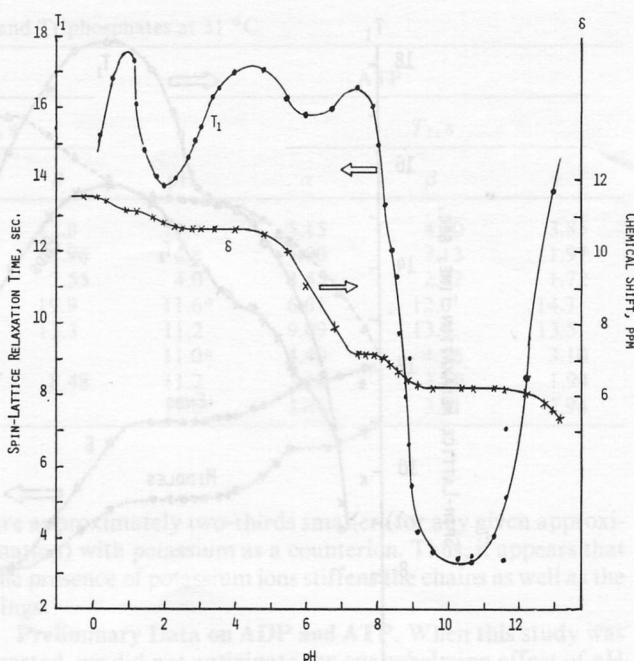


Figure 2. Variation with pH at 31 °C of some ^{31}P NMR parameters of 0.05 M pyrophosphoric acid upon titration with sodium hydroxide. The dots represent the experimental T_1 values and the crosses the respective measured chemical shifts.

Chain Phosphates. Unlike the cyclic metaphosphate for which the line widths are always very narrow and the T_1 values do not vary from pH 4 to 12, it is found for the shorter chain phosphates that both the spin-lattice relaxation time and the line width vary greatly with the pH, exhibiting several maxima and minima over the entire pH range. This means that in some pH regions, where the value of T_1 changes precipitously, it is extremely difficult to obtain reproducible spin-lattice relaxation times. Therefore, comparisons of the effects of other variables, such as the kind of counterion employed or the solvent used, may be extremely difficult.

In Figure 2, the change with pH of the ^{31}P spin-lattice relaxation time measured in an aqueous solution (0.1 M in P) of pyrophosphoric acid, with sodium as the counterion, is reported as a function of pH. An oscillatory variation of the spin-lattice relaxation with pH (as observed for the pyrophosphate anion in this figure) is also found in the case²² of orthophosphoric acid titrated with tetramethylammonium hydroxide. However, the minima for the pyrophosphoric acid do not appear as close to the pK_a values as was the case for the orthophosphoric acid. The pK_a values for the solutions for which data are reported in Figure 2 may be obtained from the ^{31}P NMR chemical-shift values, which are also presented in this figure. Thus, the apparent acid-dissociation constants for 0.05 M pyrophosphoric acid (with sodium as the counterion) were read from the halfway points between the plateaus in the curve of chemical shift given in Figure 2. They correspond to the following: $pK_1 = 0.5$, $pK_2 = 1.8$, $pK_3 = 6.2$, and $pK_4 = 8.6$. These dissociation constants should be compared with the ones reported²⁷ for 0.01 M pyrophosphoric acid (with tetramethylammonium as the counterion) in the presence of 1.0 M tetramethylammonium bromide, for which the values of pK_2 through pK_4 were found to be 1.75, 5.98, and 8.74.

We attribute the oscillations of the ^{31}P spin-lattice relaxation time over an extensive pH range, as observed for the ortho-, pyro-, and tripolyphosphate anions, to a previously unreported relaxation mechanism. Thus, the minima found²² at the pK_a values for the dilute aqueous solution of the orthophosphate anion with various proportions of hydrogen and tetramethylammonium cations as the counterions is thought

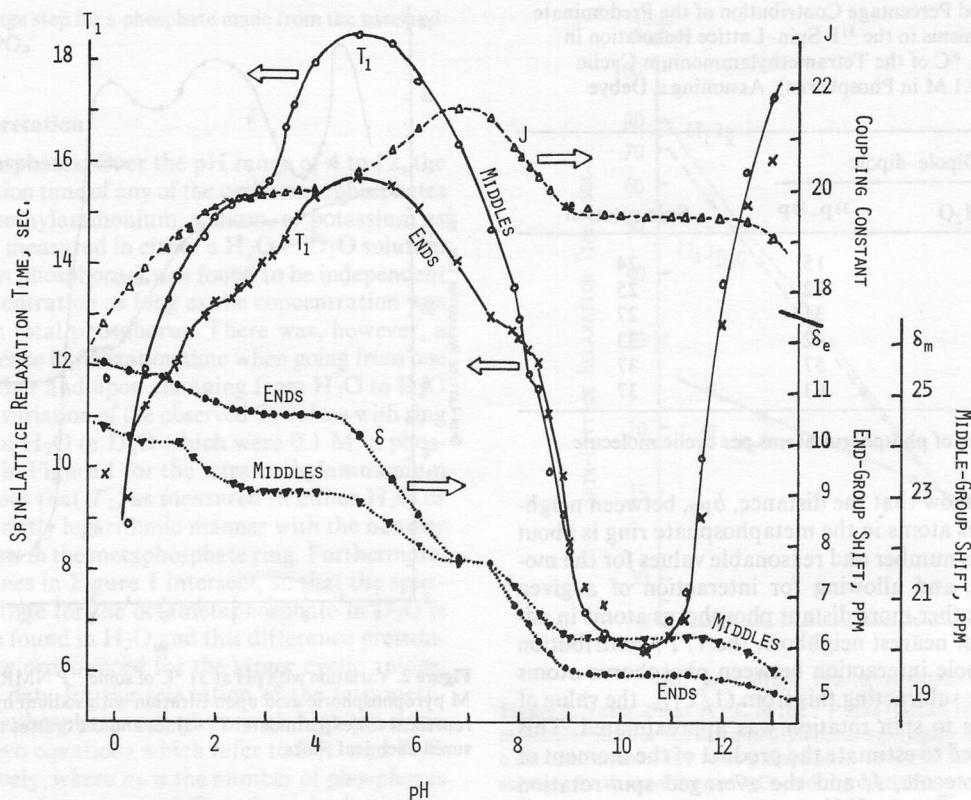


Figure 3. Variation with pH at 31 °C of some ^{31}P NMR parameters of 0.033 M tripolyphosphoric acid upon titration with sodium hydroxide. The dashed line shows the chief coupling constant, J ; the dotted lines give the chemical shift for the end and middle groups, as labeled; the solid lines present the T_1 values for the end and middle groups, also as labeled.

to result from a rapid oscillation of a given phosphate molecule-ion back and forth between the more and less protonated forms corresponding to the anionic species which bracket the particular pK_a value, with the concomitant hydrogen transfer probably occurring via a Grotthuss-like chain process. Considering all three Cartesian components of the fluctuating magnetic field, the general relationship²³ for the relaxation rate may be solved to give the following equation for the effect of such chemical exchange modulating the chemical shift and hence affording a contribution, $(1/T_1)_{\text{cs}}$, to the spin-lattice relaxation.

$$\left(\frac{1}{T_1}\right)_{\text{cs}} = \frac{2\pi^2\Delta\nu^2}{3} \frac{\tau_0}{1 + \omega_0^2\tau_0^2} \quad (14)$$

where $\Delta\nu$ is the total frequency difference between the two chemical shifts, τ_0 is the characteristic time of the exchange, and ω_0 is the nuclear-resonance frequency. An equally plausible mechanism for a chemical exchange contribution to the spin-lattice relaxation lies in the modulation of the spin-rotation part of the relaxation process, a modulation due to different spin-rotation values for the two exchanging chemical forms.

The fact that the minima seen for the pyro- and tripolyphosphate anions do not correspond well with the pK_a values is probably due to the fact that these anions associate with sodium as well as with hydrogen cations so that the modulation of the chemical shift involves transferrals of both of these cations.

The variation with pH of the observed spin-lattice relaxation of the tripolyphosphate anion, at a concentration corresponding to 0.1 M in P with sodium as the counterion, is presented in Figure 3. The T_1 values given in this figure are also seen to change in an oscillatory manner with pH. As might be expected, the two terminal groups, e, of the tripolyphosphate anion exhibit a different spin-lattice relaxation time than does the middle group, m, although they vary in the same general

Table II. Estimation of the Apparent Ionization Constants of Tripolyphosphoric Acid (with Sodium as Counterion) at 0.033 M

Source (^{31}P NMR data)	pK_1	pK_2	pK_3	pK_4	pK_5
End-group chemical shift	~0	~0	1.6	5.6	8.1
Middle-group chemical shift	~0	~0	1.8	5.5	8.2
Coupling constant				5.6	8.0
Literature ^a	(0.5)	1.15	2.04	5.69	8.56

^a From ref 26, 0.01 M acid (with tetramethylammonium as counterion) in 1.0 M tetramethylammonium bromide.

Table III. Middle-Phosphate-Group ^{31}P Spin-Lattice Relaxation Times for a Centapolypophosphate Mixture (0.1 M in P) at 31 °C

Cation	Solvent	T_1 , s	$(n_P)_{\text{eff}}$
$(\text{CH}_3)_4\text{N}^+$	H_2O	5.08	18.8
	D_2O	7.70	9.82
	x	7.97	9.54
K^+	H_2O	10.9	47.2
	D_2O	11.6	10.0
	x	11.7	10.3
Na^+	H_2O	3.02	19.1
	D_2O	6.96	11.8
	x	7.61	11.4

manner with changing pH. The end groups, which might be expected to have more freedom of motion than the middle group, consistently exhibit a somewhat shorter relaxation time. Note that both the pyro- and tripolyphosphate anions exhibit their smallest ^{31}P spin-lattice relaxation time in the neighborhood of pH 10.

Table IV. ^{31}P Spin-Lattice Relaxation Times for 0.1 M Adenosine Di- and Triphosphates at 31 °C

Cation	Solvent	pH	ADP		ATP			
			α	β	pH	α	β	γ
$(\text{CH}_3)_4\text{N}^+$	H_2O	11.3	9.17	12.8	11.1	5.15	4.50	3.85
		6.8	8.72	8.96	6.8	5.00	2.13	1.97
		4.0	7.41	7.55	4.0	3.55	2.02	1.72
	D_2O	11.6 ^a	10.3	19.9	11.6 ^a	6.61	12.0	14.7
		11.2	11.7	13.3	11.2	9.09	13.3	13.5
	D_2O				11.0 ^a	4.40	4.56	3.10
K^+	H_2O				11.2	3.10	3.10	1.94
	D_2O				11.6 ^a	3.67	3.91	2.94

^a Directly measured pD.

In addition to the T_1 data, Figure 3 also gives the variation with the pH of the single coupling constant, J_{PP} , and of the ^{31}P chemical shifts of the end and middle groups of the tripolyphosphate anion. Note that the pH variation of these properties exhibits more complexity than was previously reported.²⁴ In the case of the tripolyphosphate anion apparent acid-dissociation constants may be obtained independently from the pH variation of either (1) the end-group chemical shift, (2) the middle-group chemical shift, or (3) the coupling constant. These results are reported in Table II, where they are compared with the literature values²⁵ reported for the tetramethylammonium cation and an ionic strength of 1.0.

Although the values of the ^{31}P spin-lattice relaxation of the end and middle groups of the shorter chain polyphosphates are extremely sensitive to the pH, as exemplified by Figures 2 and 3, this is no longer true for the middle-group ^{31}P nuclei which represent essentially all of the phosphorus atoms in a long-chain polyphosphate. Indeed, with respect to this behavior as well as to its very narrow line width,²⁶ the very long chain polyphosphates seem to be closely related to the cyclic metaphosphates. Since the T_1 values do not change with pH in the range of pH 4–12, the data on the effect of changing cations in either H_2O or D_2O solutions may be handled the same way for a long-chain polyphosphate as for a cyclic metaphosphate, as shown in Table III. By inserting the T_1 values presented in this table into the appropriate equation for the variation for spin-lattice relaxation time of the cyclic metaphosphates (eq 1, 2, 5–10), the effective number of phosphorus atoms, $(n_p)_{\text{eff}}$, in a cyclic metaphosphate having a T_1 value equal to that observed for the very long chain polyphosphate can be obtained. Values of $(n_p)_{\text{eff}}$ are reported in the last column of Table III. On the basis that a very large ring should be indistinguishable from a very long chain, $(n_p)_{\text{eff}}$ should approximate the ring size at which chains and rings become indistinguishable with respect to segmental motion. Note in Table III that these values vary with both the kind of cation and the choice of solvent. The relatively large value of 47 for $(n_p)_{\text{eff}}$ for potassium metaphosphate in water (as compared to 19 for the tetramethylammonium salt in the same solvent) is in accord with the previous conclusion that the potassium ion tends to render the metaphosphate rings rigid, so that a much larger cyclic molecule is needed to achieve a ring flexibility that is commensurate with that of an infinitely long chain.

The problem of interpreting the values of $(1/T_1)_x$ in terms of a ^{31}P – ^{31}P dipole-dipole contribution plus a spin-rotation contribution is made particularly complicated for the long-chain phosphates by the difficulty of estimating the effective value of τ_d and a_A for a “jump” segment of an infinite chain. However, several reasonable approximations have led to values of $(1/T_1)_{\text{SR}}$ in the neighborhood of 0.09 to 0.12 s⁻¹ with the tetramethylammonium counterion, and the values of $(1/T_1)_{\text{SR}}$

are approximately two-thirds smaller (for any given approximation) with potassium as a counterion. Thus, it appears that the presence of potassium ions stiffens the chains as well as the rings.

Preliminary Data on ADP and ATP. When this study was started, we did not anticipate the overwhelming effect of pH changes on the shorter chain polyphosphates, as indicated in Figure 2 and 3. Therefore, T_1 measurements were made on adenosine diphosphate and adenosine triphosphate only at pH values near the equivalence points of the monoprotonated and fully ionized terminal PO_4 group as well as at the intermediate pH. The resulting data are presented in Table IV. Since as yet we have no idea as to the pH profile of the spin-lattice relaxation times of these molecules, it is unwise to carry out a lengthy interpretation of the data of Table IV. However, it is clear that the T_1 values for the adenyl derivatives of pyro- and tripolyphosphate exhibit unusually low values for a chain phosphate. Similar unusually short T_1 values have been noted²⁷ for adenosine monophosphate and its 3',5'-cyclic analogue. This shortening of the T_1 in the adenyl series of phosphate derivatives might well be attributable to association²⁸ in aqueous solution and/or the folding back of the purine ring over the condensed phosphate chain as suggested by Szent-Gyorgyi.²⁹

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