SOLUBILIZATION OF INSOLUBLE MATTER IN NATURE*

I. THE PART PLAYED BY SALTS OF ADENOSINETRIPHOSPHATE

by

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> "In der Betrachtung umfassender Naturerscheinungen haben wir keinen Masstab mehr für das, was wir gewohnt sind, klein oder gross zu nennen . . . Was in einem begrenzten Raum kaum bemerkbar ist, erscheint in einem unbegrenzten unfassbar gross."
>
> J. VON LIEBIG, *Die Chemie in ihrer Anwendung auf Agricultur und Physiologie.*

The question of the solubilization of insoluble matter in nature is of fundamental importance for all living beings. We have drawn attention to this subject in former publications[1,2,3]. It seems desirable at the present stage to test the validity and applicability of the principle involved from the most divers points of view.

Acid and alkaline substances which could effect solution of insoluble inorganic and organic materials hardly occur in nature or at least are not at the disposal of the cell in significant quantities or concentrations. Other mechanisms therefore must exist to solve this essential task of animate nature. Whenever substances of acid or alkaline character are formed in the course of metabolic processes they are normally soon neutralized or adjusted in such a way that conditions adapted to other functions of the cell are maintained. Disregarding some special cases, the physiological hydrogen ion concentration will be found at an average of 10^{-7} with very small deviations at either side. At slight alkalinity to about pH 8, there is no possibility for the solution of insoluble inorganic salts as, *e.g.* $CaCO_3$ or $Ca_3(PO_4)_2$. In the case of deviations into the acid range, salt formation limits the extent to which the solution can progress. This point may be illustrated by imagining the roots of a plant eliminating phosphoric acid in contact with dolomite. The reaction will come to a halt as soon as Ca- and Mg-phosphate are formed. When the salivary glands of the snail Dolium galea[4] secrete a juice which contains 2.7% H_2SO_4 as well as 0.4% free HCl, the ability of these acids to dissolve alkaline earth carbonates will similarly cease as soon as the corresponding neutral salts have been formed. As another example, the behavior of plant[5] or animal[6]

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myrosulfatase and other sulfatases[7] may be cited. These enzymes acting on mustard oil glucosides or other ester sulfate substrates liberate bisulfate as a primary product; in aqueous solution, this is converted into the neutral salt and free H_2SO_4. During carboxylatic degradation of Na pyruvate, Na bicarbonate and thence, through loss of CO_2, Na_2CO_3 is formed[8] which may bring a difficultly soluble acid into solution. The same applies to NH_4OH derived from urea or amides.

We have described recently another mechanism of solubilization[1,2,3], a mechanism, the effects of which go beyond what takes place when insoluble materials are dissolved by acids or bases. In general the latter reaction comes to a standstill on neutralization. Observations made on a vast number of organic acids and also some inorganic acids have led to the conclusion that exactly at this point of neutralization a special function of the newly formed neutral salts sets in, *i.e., the salts themselves* show a marked power of solubilization. This unexpected phenomenon, which proved impressive in the case of triphosphates[9] as well as salts of nucleic acids and nucleotides[2], applies to a wide variety of substances[1,2,3], including adenosine triphosphates.

The reason for the choice of ATP salts as the subject of a more extensive investigation is twofold: the exceptional importance of ATP and its wide distribution in all cells.

Among the most recently reported effects of ATP only some will be mentioned: the findings of BARKER AND LIPMANN[10] bear on the metabolism of propionibacterium pentosaceum and the phosphorylation of such substances as arabinose, glycerol and erythritol; facts were discovered regarding the role of ATP in peptide bond formation[11] and the part played by ATP in the cytoplasmic state, division and development of the sea urchin egg was investigated[12] as well as its role in STERN AND OCHOA's enzymatic synthesis of citric acid by condensation of acetate and oxaloacetate[13], and the very rapid resynthesis of ATP in embryonic development[14]. Disaggregation of dissolved actomyosin[15], competition with inorganic triphosphate in the hexokinase system[16], the rôle of ATP in the formation of acetylcholine[17] (NACHMANSOHN) and the participation of ATP in fatty acid oxidation[18] were other subjects treated.

In naturally occuring systems and under experimentally produced conditions ATP comes in close contact with an enormous number of substances. We therefore considered it desirable to investigate the effects of salts of ATP on the solubility of insoluble or difficultly soluble substances in a series of various combinations.

EXPERIMENTAL

Solutions of sodium- and ammonium adenosinetriphosphate were used. Soluble salts of ATP with organic bases behave mostly the same way. ATP can be obtained in almost 100% purity according to the directions of WAGNER-JAUREGG[19], BAILEY[20] and COHN AND CARTER[21]. Commercially available preparations were found adequate, however, especially the product of Pabst Laboratories, Milwaukee, Wis., which in the fresh state does not contain any inorganic ortho- or pyro-phosphate but solely an admixture of 5% ADP in 95% pure ATP. Free ATP or its acid sodium salt is dissolved in ice water, cooled down to incipient freezing in an ice-salt mixture and then brought to pH 7.5 with precooled *M* NaOH or *M* NH_4OH, respectively. The solutions applied contained 10 g ATP/100 ml, (*M*/5). Fresh solutions have to be prepared for each series of experiments. In the dark at $-4°$ C solutions are stable for 10 hrs. (Their instability and sensitivity to light is well known[20,21,22].)

The insoluble materials investigated as "substrates" in the solubilization experiments included salts of common and of rare elements; the organic substances were chosen from several physiologically important classes (proteins, protamines, nucleic acids, alkaloids, mercaptans). Difficultly soluble salts made in the test tube or formed under the conditions of special physiological experiments (fluorophosphates, molybdates, tungstates, phosphomolybdates, phosphotungstates, azides, selenites, ferrocyanides, cobalticyanides, iodates) as well as those encountered during biochemical analyses (Ca-oxalate, NH_4-Mg-phosphate) were thought of interest and investigated under varying conditions.

Whenever a precipitate can be dissolved it is of course equally feasible to prevent precipitation

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by previous addition of the solubilizing agent (ATPate). In some cases only the latter effect, *i.e.*, prevention of precipitates can be established.

In their general reactivity salts of ATP preparations of highest purity are not different from those of less purity.

ATP itself also forms several difficultly soluble salts

We shall report the solubilization of Ba- and Ca-ATPate at pH = or > 7.5 in salts of meta-, pyro- or tri-phosphoric acid, glycerophosphates, ribo- and desoxyribonucleates, citrates and various ammonium salts. Only a few examples are presented to show this related effect of arbitrarily selected typical solubilizing agents in cases where the ATP-salt is the substrate. The solubilizing agents occuring in nature as such or in combination with other substances are extremely numerous and their character most varied.

The most significant results of our experiments are tabulated below.

All the experiments indicated in the tables were carried out according to the following schemes:

A. *Solubilization of precipitates*

Example: $CaCO_3$ *substrate*; *required amount ATPate 2 ml*; *pH 7.5*

0.1 ml $M/10$ Na_2CO_3 are added to 0.1 ml $M/10$ $CaCl_2$ directly in a centrifuge tube.

The freshly formed precipitate is centrifuged with several portions of wash water. When 2 ml $M/5$ Na-ATPate, adjusted to pH 7.5, are added to the washed precipitate a clear solution results on stirring with a glass rod.

In all cases the pH is tested. If in the course of the reaction it falls below 7.5, the experiment must be repeated with an ATPate solution which has been adjusted accordingly.

To determine the stability of the clear solution obtained in a more alkaline range, alkali hydroxide or carbonate is added. The use of buffers is disapproved since secondary changes may take place owing to interaction with the constituents of the buffer.

As a control identically prepared precipitates are digested with 2 ml distilled water. Under these conditions the $CaCO_3$ does not go into solution. Cases where more soluble substrates are solubilized to a significant degree in these control experiments, so that the effect of the ATP salt is not striking, are not included in the tables.

As a rule washing of the precipitates as indicated above can be omitted. The neutral salts formed during the precipitation reactions (NaCl, Na_2SO_4, $NaNO_3$ etc.) do not affect the results obtained. At the small concentrations used the changes in solubility sometimes observed in the case of ammonium salts (see p. 659) are not significant.

The term "soluble" in the tabulated results refers to solution at room temperature within a reasonable time. Where "solubility on heating" is indicated, slight warming to 50–60° C will usually suffice. With constant shaking the same phenomena may be observed after standing at 20–25° C for a longer period of time.

Since all reactions are carried out at pH > 7 it may be assumed for simplicity's sake that normal salts are formed in all cases, in particular normal phosphates, borates and silicates.

B. *Complete inhibition of precipitation*

Example: $BaSO_4$ *substrate, required amount ATPate 0.5 ml*

To 0.1 ml $M/100$ Na_2SO_4 0.5 ml $M/5$ ATPate is added, then 0.1 ml $M/100$ $BaCl_2$. A clear solution results. (In the particular case cited the solution is stable for many hours at any pH. To precipitate $BaSO_4$ prolonged boiling with HCl to hydrolyze ATP is required.)

Control experiments are carried out in the same way with 0.5 ml water replacing the ATPate. $BaSO_4$ precipitates almost at once.

The following *abbreviations* are used in the tables:

ppt. = precipitate
inh. = inhibition
t = turbid
cl = clear
sol. = solution

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TABLE I

EFFECT OF ADENOSINE TRIPHOSPHATE ON THE SOLUBILITY OF DIFFICULTLY SOLUBLE INORGANIC COMPOUNDS

Substrate (i)	*ml ATPate required* (ii)	*pH of sol.*	*Results*	*Remarks*
$Mg(NH_4)PO_4$	2	7.5–8.5	sol.	remains clear on heating
$MgSiO_3$ (iii)	2	7.5–8.5	sol.	remains clear on heating
$Mg(PF_6)_2$	1	7.5–8.5	sol.	
$CaCO_3$	2	7.5–8.5	sol.	
$Ca_3(PO_4)_2$	2	7.5–8.5	sol.	
$Ca(COO)_2$	2	7.5	Na-ATP sol. NH_4-ATP inh.	remains clear > 3 hrs same result at pH 8
CaB_4O_7	1	7.5	sol.	same result at pH 8
$CaSiO_3$ (iii)	1	7.5–9	sol.	
$ZnSiO_3$ (iii)	1	7.5–9	sol.	
$ZnSeO_3$	1	7.5–9	sol.	
$CdCO_3$	4	6.5	inh.	same result at pH 7.5
$Cd_3(PO_4)_2$	0.8	6.5	inh.	same result at pH 7.5
$BaSO_4$ (iv)	0.5	any	inh.	(v)
HgN_3	4	8	inh.	slightly t at pH 6
Hg_3PO_4	4	6	sol.	same result at pH 7.5
$Hg_3(PO_4)_2$	4	7.5	inh.	
$2HgO.HgCl_2$ (vi)	0.5	7.5	inh.	
$Pb(COO)_2$ (vii)	4	6	inh.	same result at pH 7.5 slight turbidity, increasing on standing
$PbCO_3$ (vii)	4	7.2	inh.	becomes t after 5 min
$Pb_3(PO_4)_2$ (vii)	4	7.5	inh.	t at first, becomes cl. on shaking, t again on long standing
$Pb(PF_6)_2$ (vii)	3	7.5	sol.	
$AlPO_4$	2	6	inh.	same result at pH 7.5 almost cl., becomes t on standing
$CrPO_4$	2	7.5	inh.	same result at pH 8 remains cl. on heating
$Pr_2[(COO)_2]_3$ (viii)	4	7.5	inh.	slightly t. No effect at higher pH
UO_2HPO_4 (ix)	4	7.5	inh.	becomes cl. on shaking
$CuCO_3$	1	7.5	sol.	same result at pH 9
$Cu_3(PO_4)_2$	1	7.5	sol.	becomes cl. on shaking and standing; same result at pH 8
$MnCO_3$	2	7.5	sol.	same result at pH 8.5
$Mn_3(PO_4)_2$	2	7.5	sol.	same result at pH 8.5
$FeCO_3$	2	7.5	inh.	becomes t on standing
$Fe_3(PO_4)_2$	2	6	inh.	same result at pH 7.5 becomes t on standing
$CoCO_3$	1	7.5	sol.	
$Co_3(PO_4)_2$	1	7.5	sol.	
$CoSeO_3$	1	7.5	sol.	
$NiCO_3$	1	7.5	sol.	almost cl. at first, clears completely on standing
$Ni_3(PO_4)_2$	2	7.5	sol.	almost cl.
$Hg_3Co(CN)_6$	2	6	inh.	same result at pH 7.5 slightly t
$Hg_3[Co(CN)_6]_2$	2.5	6	inh.	same result at pH 7.5
$Mn_3[Co(CN)_6]_2$	1.3	7.5	inh.	
$Fe_3[Co(CN)_6]_2$	1.3	7.5	inh.	
$Co_3[Co(CN)_6]_2$	2.5	6	inh.	same result at pH 7.5

TABLE I (continued)

Substrate (i)	*ml ATPate required* (ii)	*pH of sol.*	*Results*	*Remarks*
$Ni_3[Co(CN)_6]_2$	1.3	7.5	inh.	
$Co[Fe_2(CN)_6]_2$	2	6	inh.	same result at pH 7.5
$Fe_4[Fe(CN)_6]_3$ (Prussian Blue)	large excess	7	sol.	blue violet solution, decolorized to light green on heating
$Fe(SCN)_3$	1.5	acid or 8	sol.	decolorized on heating, remains colorless on adding NH_4OH to pH 8.5. No precipitation of $Fe(OH)_3$
$HgIO_3$	2	5.5	sol.	t on standing
$Pb(IO_3)_2$	4	7.5	inh.	t on standing
$AgIO_3$	2	7.5	sol.	t on standing
$Cu(IO_3)_2$	2	7.5	sol.	remains cl. $>$ 2 hrs
$Ba(IO_3)_2$	2	7.5	inh.	t on standing
$(UO_2)S$	4	7.5	inh.	
$Zn(C_7H_4NS_2)_2$ (x)	4	7.5	inh.	t on standing
$Fe(C_7H_4NS_2)_2$ (x)	4	7.5	inh.	t on standing
$Fe(C_7H_4NS_2)_3$ (x)	4	7.5	inh.	not completely cl.
$Co(C_7H_4NS_2)_2$ (x)	4	7.5	inh.	t on standing
$Ni(C_7H_4NS_2)_2$ (x)	4	7.5	inh.	t on standing

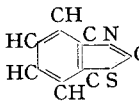
(i) The substrates are prepared by precipitation of 0.1 ml $M/10$ metal salts solutions.
(ii) $M/5$ Na- or NH_4-ATP solutions at pH 7.5 are used. Unless the contrary is indicated both salts behave in the same way.
(iii) Prepared by precipitation of easily soluble Na silicate which in turn was made from $SiCl_4$ or $Si(OC_2H_5)_4$ and the equivalent amount of NaOH.
(iv) $M/100$ solutions were used in this case. For experimental conditions see p. 656.
(v) There may be a connection between the solubilization of $BaSO_4$ and the findings fo ALBAUM *et ass.*[25] who were unable to remove all Ba^{++} ions with Na_2SO_4 from the atypical ATP they isolated from mung beans.
(vi) This substrate is the insoluble mercuric oxychloride obtained in glittering violet or purple leaflets on precipitation of $HgCl_2$ with Na_2CO_3.
(vii) NH_4-ATP proved greatly superior to Na-ATP for the solubilization of Pb salts.
(viii) $PrCl_3$ is precipitated by NH_4-ATP. Therefore $PrCl_3$ is added last in this case.
(ix) 1 ml Na-ATP forms a precipitate with 0.05 ml $M/10$ $UO_2(NO_3)_2$, but on shaking this precipitate goes into solution.
(x) Because it is odorless 2-mercaptobenzothiazole  has been preferred for these experiments.

TABLE II

EFFECT OF Na-ATP ON DIFFICULTLY SOLUBLE INORGANIC TRIPHOSPHATES

Substrate	*pH*	*Results*	*Remarks*
$Zn_5(P_3O_{10})_2$	7.5	sol.	same result at pH 8
$Cd_5(P_3O_{10})_2$	7.5	sol.	same result at pH 8.5
$(UO_2)_5(P_3O_{10})_2$	7.5	sol.	same result at pH 8.5
$Mn_5(P_3O_{10})_2$	7.5	sol.	same result at pH 8.5

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TABLE III

EFFECT OF ADENOSINE TRIPHOSPHATES ON THE SOLUBILITY OF DIFFICULTLY SOLUBLE ALKALOID, PROTAMINE AND PROTEIN COMPOUNDS

Precipitate	*M/5 ATP salt used*	*Results*	*Remarks*
Brucine Cd iodide	NH_4	sol.	
Brucine desoxyribonucleate	NH_4	sol.	
Nicotine Hg iodide KI	NH_4	sol.	
Strychnine Hg iodide KI	NH_4	sol.	on heating
Protamine ribonucleate (i)	NH_4	sol.	specially on slight heating
Protamine desoxyribonucleate (i)	NH_4	sol.	specially on slight heating
Ovalbumin ribonucleate (ii)	NH_4	sol.	pH 5–8
Ovalbumin desoxyribonucleate (ii)	NH_4	sol.	pH 5–8
Ovalbumin + free HPO_3 (ii)	Na	sol.	
Ovalbumin + hydrogenferrocyanic acid (ii)	NH_4	sol.	
Ovalbumin + HgI_2.KI (ii)	NH_4	sol.	

(i) Protamine sulfate preparations of the Paul Lewis Laboratories, Milwaukee, Wis. and of E. R. Squibb & Sons were used. The former is reported to be prepared from spermatozoa of *oncorhynchus tschawytscha* (quinnat salmon). We wish to thank both companies for putting the material at our disposal.

(ii) The analogous *bovine-plasma-albumin* compounds are solubilized in exactly the same way.

TABLE IV

SUBSTANCES NOT SOLUBILIZED TO A SIGNIFICANT EXTENT BY Na-ATP OR NH_4-ATP UNDER THE GIVEN EXPERIMENTAL CONDITIONS

$Al(OH)_3$	$BaMoO_4$	$BaWO_4$	$Be_3(PO_4)_2$	$Cu_2Fe(CN)_6$
$FePO_4$	$Nd(OH)_3$	$Pb(N_3)_2$	$Th(OH)_4$	$Th(IO_3)_4$
$Ti(OH)_4$	Tl_2S	$(UO_2)_2Fe(CN)_6$		

Brucine phosphotungstate
Brucine phosphomolybdate
Nicotine tannate
Strychnine ribonucleate

General remarks to Table V

The observations tabulated below show the reverse effect of that treated heretofore. Just as the soluble adenosinetriphosphates affect the solubility of a number of "substrates", the difficultly soluble salts of ATP are brought into solution at neutral or slightly alkaline pH by the solubilizing effect of various salt solutions.

The reciprocal solubilizations of related substances seem especially remarkable. Table V should be compared with Table II. While the Zn, Mn and uranyl triphosphates were shown to be soluble in Na-ATP, the Ba, Ca and Zn salts of ATP are brought into solution by Na-triphosphate.

The Ba- and Ca-salts of ATP were prepared according to the directions of BARRENSCHEEN AND FILZ[26] taking into account the supplementary data of KERR[27] and BIELSCHOWSKY[28].

Ba-ATP is also solubilized by NH_4-oxalate, NH_4Cl, neutral Na-molybdate and Na-borate. Even after standing at room temperature (25° C) for 10 days no hydrolysis of Ba-ATP in NH_4Cl to orthophosphate can be detected; typical tests with magnesia mixture or molybdate in dilute HNO_3 in the cold do not precipitate orthophosphate from Ba-ATP in this solution.

The solubility of the very difficultly soluble Ba-ATP in ammonium salts is analogous to the identical behavior of alkaline earth and other insoluble salts of D-fructose-1,6-diphosphoric acid, D(—)-3-phosphoglyceric acid, inositol-hexaphosphate[1] and to the complete inhibition of the precipitation of Ca-, Sr- and Ba-glycero-phosphates at boiling temperature in the presence of ammonium salts. The examples cited seem another manifestation of a remarkable phenomenon illustrated in previous publications[29].

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TABLE V

EFFECT OF VARIOUS SOLUBILIZING AGENTS ON THE SOLUBILITY OF DIFFICULTLY SOLUBLE ADENOSINE TRIPHOSPHATES

ATP salt	*mg*	*Solubilizing agent*	*ml*	*pH*	*Results*	*Remarks*
Ba	50	*M* $NaPO_3$	1	7.5 or >	sol.	
Ba	50	*M* $K_4P_2O_7$	excess	7.5–8.5	slightly sol.	
Ba	50	*M*/2 $Na_5P_3O_{10}$	3	7.5	sol.	
Ba	50	*M* monoethanolamine metaphosphate	3	7.5–9	insol.	
Ba	50	*M* Na-glycerophosphate	4	7.5	sol.	t at pH 8
Ba	50	5% Na-ribonucleate	excess	7.5–8.5	sol.	more readily sol. on heating
			2	8	inh. (i)	
Ba	50	6% Na-desoxyribonucleate	excess	7.5	difficultly sol.	
			excess	8	more readily sol.	
			3	7–8	inh. (ii)	
Ba	50	5% Na-phytate	2.5		insol.	
Ba	50	*M* K-citrate	2.5	7–9	very sol.	
Ba	50	*M* K-tartrate	2.5	7–9	very sol.	
Ba	50	*M* Na-malate	3	7–9	sol.	
Ba	50	2 *M* NH_4Cl	4	7–9	sol. on heating	Addition of alcohol gives flocculent precipitate
Ba	50	2 *M* NH_4NO_3	4	7–9	sol.	reprecipitated by alcohol
Ba	50	2 *M* NH_4SCN	4	7–9	very sol.	reprecipitated by alcohol
Ca	50	*M* $NaPO_3$	1	8	sol.	
Ca	50	*M* $K_4P_2O_7$	1	9	sol.	
Ca	50	*M*/2 $Na_5P_3O_{10}$	0.5	8	sol.	
Ca	50	*M* monoethanolamine metaphosphate	1	7.5	sol.	On addition of NH_4OH to pH 9 the solution solidifies to a transparent gel
Ca	50	*M* Na-glycerophosphate	2.5	7.5	sol.	
Ca	50	5% Na-ribonucleate	2	every	sol.	in the cold or on heating
Ca	50	6% Na-desoxyribonucleate	2	every	sol.	slowly
Ca	50	5% Na-phytate	4	7–8	sol.	difficultly sol.
Ca	50	*M* Na-pyruvate	3	8	sol.	difficultly sol.
Ca	50	2 *M* NH_4Cl	2.5	7.5	sol.	can be precipitated by alcohol
Ca	50	2 *M* NH_4SCN	2.5	7.5	sol.	can be precipitated by alcohol
Zn	50	*M*/2 $Na_5P_3O_{10}$	1.5	7–7.3	very sol.	
In (iii)	50	*M* $NaPO_3$	2	7.5	sol.	almost completely
In (iii)	50	*M* $K_4P_2O_7$	2	9	sol.	almost completely
La (iv)	50	*M* $NaPO_3$	2	7.5	sol.	
La (iv)	50	*M* $K_4P_2O_7$	2	9	sol.	
La (iv)	50	5% Na-ribonucleate	3	7.5	almost sol. on heating	
La (iv)	50	5% Na-ribonucleate	2	6	inh. (v)	no effect at pH 7.5

(i) Precipitation can be inhibited by adding 0.02 ml *M*/5 Na-ATP to 2 ml Na-ribonucleate before further addition of 0.02 ml 25% Ba-acetate. The resulting solution remains clear on warming and on standing. Control experiments with 4 ml water instead of the ribonucleate give the normal precipitate.

(ii) Inhibition is tested as under (i). The same order of addition to 3 ml desoxyribonucleate gives a solution which remains clear on warming and standing.

(iii) This substrate is made by precipitation of *M*/10 $In_2(SO_4)_3$ with *M*/5 Na-ATP. The thick white precipitate obtained remains insoluble in excess Na-ATP, even on heating.

(iv) Obtained by precipitation of *M*/10 $LaCl_3$ with *M*/5 Na-ATP.

(v) *cf.* (i) and (ii). To 2 ml Na-ribonucleate 0.5 ml *M*/5 Na-ATP are added, then 0.5 ml *M*/10 $LaCl_3$. The solution becomes clear after a short time, even in the cold.

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TABLE VI

EFFECT OF VARIOUS SOLUBILIZING AGENTS ON PRECIPITATES OBTAINED BY INTERACTION OF PROTAMINE SULFATE AND $M/5$ ADENOSINE TRIPHOSPHATES

ATP salt reacting with protamine	*Solubilizing agent*	*pH*	*Results*	*Remarks*
Na	M $NaPO_3$	7.5	sol.	on heating
Na	M $K_4P_2O_7$	8	sol.	in the cold
Na	5% Na-ribonucleate	7.5	sol.	in the cold (i)
NH_4	$M/5$ NH_4 ATP (excess)	7.5	sol.	on slight heating

(i) On warming the precipitate reappears, but is brought back into solution on cooling.

DISCUSSION

Qualitative experiments have shown that similar tests with analogous results could be conducted with a considerably larger number of "substrates". The data reported in this paper, however, impressively demonstrate the extent and range of solubilizing power of ATP salts. Water insoluble inorganic salts such as carbonates, phosphates, fluorophosphates*, silicates, borates, iodates, azides, cobalticyanides and ferrocyanides are brought into solution. The solubility of Ca-oxalate, NH_4Mg phosphate and Mg fluorophosphate seems worthy of special mention. The precipitation of other salts, *e.g.*, $BaSO_4$, sulfides, mercaptides and azides, is inhibited by the presence of ATP salts. Similar effects are exerted on the solubility of difficulty soluble compounds of alkaloids, proteins and protamines with typical precipitants incl. nucleates.

Both the solubilizing agent and the substrates described are important naturally occuring substances. The reported phenomena are observed in aqueous solutions at pH = or > 7.5. In this respect the behavior of the adenosinetriphosphates resembles that of ribo- and desoxyribonucleates. The solubilizing power of the ATPates may not be as marked but it is superior to that of simple nucleotides. Analogies to the specific effects of inorganic triphosphate are also clearly indicated.

In view of the particular properties of adenosinetriphosphate and their special functions** in cell metabolism the question arises whether the phenomena of solubilization are in any way connected with the functions of ATP in nature and to what extent its effect on solubility of so many substances has a definite place in the mechanism of biological reactions***. Two distinct spheres may be recognized: On the one hand the precipitation of a number of biologically important substances is prevented or those in an insoluble form are solubilized by ATPates, on the other hand solubilizing agents

* Mg fluorophosphate has gained a special importance through the discovery of WARBURG AND CHRISTIAN[23] that fluoride inhibition of the enolase is due to the complex formation of the enzyme. Apparently Mg fluoride-phosphate complexes can also be formed with other enzymes[24].

** According to WARBURG[30] ATP is probably of importance in the mechanism of photosynthesis. This would indicate that ATP can take part in the degradation of organic matter in nature as well as in its production.

*** We succeeded recently in an experimental biochemical confirmation of this effect by enzymatic procedure. The report will follow.

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which are constituents of all cells prevent the precipitation or effect solubilization of difficultly soluble derivatives of ATP.

The part played by such reactions in processes taking place inside the cell and the interaction with substances found in the cell environment[1,2,3] may be visualized more clearly after consideration of the phenomena described. For an understanding of the principle underlying the new concept one fundamental fact must be taken into account: While the reactants may be present in minute concentrations in any one case, the substances either are not used up at all or else are reproduced continuously in the course of normal metabolism and transported to their sphere of action. The materials considered are all widely distributed. Significant effects may arise and gain importance in accord with the saying of J. VON LIEBIG*:

"In a comprehensive view of the phenomena of nature, we have no scale for that which we are accustomed to name small or great . . . That which is scarcely observable in a confined district appears inconceivably large when regarded in its extension through unlimited space."

SUMMARY

The ability of soluble salts of ATP to solubilize numerous inorganic and organic compounds in neutral or slightly alkaline medium and to keep these compounds in solution is demonstrated. The latter can be natural products or synthetic substances. Difficultly soluble salts of ATP themselves are solubilized at physiological conditions of pH and temperature by other solubilizing agents. Among the latter are normal constituents of cells and products of intermediary metabolism. To these phenomena connected in different ways with ATP might be attributed essentiality as ATP is an omnicellular substance and plays a part in the course of many important biological processes.

RÉSUMÉ

Nous avons démontré que des sels solubles d'ATP sont capables de solubiliser ou de maintenir en solution de nombreux composés inorganiques et organiques en milieu neutre ou légèrement alcalin. Ces composés peuvent être d'origine naturelle ou synthétique. Des sels peu solubles d'ATP sont solubilisés à des conditions physiologiques de pH et de température par d'autres agents solubilisants. Citons parmi ces derniers certains constituants normaux des cellules et des produits du métabolisme intermédiaire. Ces phénomènes, qui sont en rapport, de différentes manières, avec des sels d'ATP peuvent être considérés remarquables, parce que l'ATP est une substance omnicellulaire et qu'il joue un rôle au cours de nombreux processus biologiques importants.

ZUSAMMENFASSUNG

Für lösliche Salze von ATP wird die Fähigkeit nachgewiesen, bei neutraler oder schwach alkalischer Reaktion zahlreiche anorganische und organische Verbindungen in Lösung überzuführen oder zu halten. Letztere können natürlich vorkommende oder künstlich erzeugte Substanzen sein. Schwer lösliche Salze des ATP selber werden unter physiologischen Bedingungen von pH und Temperatur von zahlreichen Solventien in Lösung gebracht. Zu letzteren gehören normale Zellinhaltsstoffe und Produkte des intermediären Stoffwechsels. Die in verschiedener Weise mit Salzen des ATP verknüpften Lösungserscheinungen verdienen Beachtung, weil ATP omnicellulär auftritt and am Ablauf wichtiger biologischer Vorgänge beteiligt ist.

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* JUSTUS VON LIEBIG, *Chemistry in its application to agriculture and physiology*. Fourth Edition, Taylor and Walton, London 1847; edited from the manuscript of the author by LYON PLAYFAIR, Ph.D. and WILLIAM GREGORY, M.D.

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