The Inhibition of Mitochondrial Adenosine Triphosphatase

THE EFFECTS OF ALKALI-METAL IONS

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Previous studies reported from this and other Laboratories have shown that isolated mitochondria are able to maintain concentration gradients of Na^+ and K^+ ions. When incubated in media containing an oxidizable substrate and ATP, mitochondria from liver (Macfarlane & Spencer, 1953; Spector, 1953; Price, Fonnesu & Davies, 1956), kidney (Bartley & Davies, 1954; Ulrich, 1961), heart and skeletal muscle (Ulrich, 1959, 1960) accumulate K^+ ions against a concentration gradient. Whether this also holds for Na^+ ions is uncertain, since some investigators have reported an increased uptake of Na^+ ions during metabolic activity (Macfarlane & Spencer, 1953; Bartley & Davies, 1954; Price *et al.* 1956), but others have been unable to demonstrate this (Spector, 1953; Jackson & Pace, 1956; Ulrich, 1959, 1961). So far, however, no conclusive evidence has been obtained that either Na^+ or K^+ ions are transported by mitochondria against both a chemical and an electrical gradient.

Recent studies of the activation of membrane adenosine triphosphatase by Na^+ and K^+ ions indicate that this enzyme may play a role in the transport of these cations across cell membranes. It has been demonstrated that Na^+ and K^+ ions can increase adenosine-triphosphatase activity in microsomes from peripheral nerve (Skou, 1957, 1960) and brain (Järnefelt, 1961), and from membrane preparations of erythrocytes (Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1961), kidney cortex (Whittam & Wheeler, 1961), and *Escherichia coli* (Solomon, 1962). These studies have shown (see, for example, Post *et al.* 1960) that there is a good correlation between the active transport of Na^+ and K^+ ions, increased adenosine-triphosphatase activity, and the inhibition by ouabain of both active transport and the activation of adenosine triphosphatase. Further, Glynn (1962) and Whittam (1962*a, b*) have demonstrated that the adenosine triphosphatase of erythrocyte stroma is stimulated asymmetrically by Na^+ and K^+ ions: Na^+ ions stimulate the enzyme from the inside and K^+ ions stimulate it from the outside, but only in the presence of the other ion on the opposite side of the membrane.

Since mitochondrial adenosine triphosphatase is concentrated in the membrane (Kielley & Kielley,

1953; Siekevitz, Löw, Ernster & Linberg, 1958), a study was undertaken to determine whether the accumulation of Na^+ and K^+ ions by mitochondria could be correlated directly with changes in mitochondrial adenosine triphosphatase. In a preliminary note Ulrich (1962) reported that, when Na^+ and K^+ ions were added to suspensions of mitochondrial membranes, adenosine-triphosphatase activity was increased if the osmolarity of the incubation medium was kept constant with choline chloride. Although these experiments were repeated many times, the results were not consistent. The Na^+ or K^+ ions sometimes caused activation, inhibition, or no change in adenosine-triphosphatase activity, despite rigidly controlled conditions both in the isolation of the mitochondrial membranes and in the assay of adenosine triphosphatase. However, when Na^+ or K^+ ions were added to mitochondrial-membrane suspensions without attempting to maintain a constant osmolarity, both ions caused a significant and consistent decrease in enzyme activity. The experiments reported in the present paper demonstrate that mitochondrial adenosine triphosphatase is inhibited by a variety of alkali-metal ions and that this inhibition is affected also by the anions present.

EXPERIMENTAL

Preparation of mitochondrial membranes. Mitochondria were isolated from the livers of fed male albino Sprague-Dawley rats, weighing 200–300 g. (Holtzmann and Co., Wis., U.S.A.), after decapitation of the animals. The rest of the procedures for obtaining the mitochondria were similar to those described by Ulrich (1961) for the isolation of rabbit-kidney mitochondria.

Mitochondrial membranes were obtained by a modification of the method described by Watson & Siekevitz (1956). The twice-washed mitochondria from 48 g. of liver were suspended with sufficient 50 mM-tris-acetate buffer, pH 7.4 (Myers & Slater, 1957), to give 18 ml. of suspension. The suspension was mixed rapidly with 2 ml. of 3% (w/v) deoxycholic acid. The deoxycholate was prepared by adding solid tris to an aqueous suspension of the acid until it was in solution and then adjusting the pH to 7.6–7.7 by slowly adding 5*N*-HCl. The suspension of disrupted mitochondria was centrifuged for 20 min. at 25 000*g*_{av} in the no. 40 rotor of the Spinco model L ultracentrifuge. The resulting supernatant was divided approximately equally into four tubes and 50 mM-tris-acetate buffer, pH 7.4, was

added to give a final volume of approx. 10 ml. in each tube. This was done to minimize contamination of the membrane pellets by deoxycholate. The supernatants were centrifuged for 1 hr. at 105 000g and the resulting pellets (membrane material) were rinsed three times with 50 mM-tris-acetate buffer, pH 7.4, and the pooled pellets then suspended with the buffer. The protein content of the membrane suspension was determined immediately by the biuret method (Gornall, Bardawill & David, 1949), a standard protein solution being used for the construction of the calibration curve. 50 mM-Tris-acetate buffer, pH 7.4, was added to the suspension to give a protein concentration of 6 mg./ml., and 1.6 ml. portions of the suspension were placed in 8 ml. test tubes, frozen with a mixture of acetone and solid CO₂, and stored at -20°.

So that the relative adenosine-triphosphatase activities of the various membrane suspensions can be compared in different experiments, the results presented, both in the Tables and the Figures, include the preparation used (numbered consecutively in the order of preparation from 1 to 6).

Assay of adenosine triphosphatase. In the first part of this study, ATP (tris salt) from the Sigma Chemical Co., St Louis, Mo., U.S.A., was used. Later, ATP (disodium salt) (either from Sigma or Pabst Laboratories, Milwaukee, Wis., U.S.A.) was converted into the tris form as follows, all operations being carried out at 0-4°. ATP (1.5 g.) was dissolved in 4 ml. of water and added to 10 g. of analytical grade cation-exchange resin AG 50W (X 8; 100-200 mesh; H⁺ form) obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. The resin had been washed previously six or seven times with 10 ml. of water. The mixture of resin and ATP was stirred continuously for 15 min., filtered on a sintered-glass funnel, and the resin washed three or four times with 2 ml. of water. The pH of the ATP was adjusted rapidly to approx. 7.4 with solid tris and its concentration determined by measuring the ultraviolet absorption at 259 mμ. The solution, after being diluted, usually to 75 mM with 50 mM-tris-acetate buffer, pH 7.4, was divided into several portions and kept at -20°. In the 2.5 ml. incubation medium, the ATP (tris salt) contributed approx. 0.4 mM-Na⁺ ion (assayed by internal-standard flame photometry). No ultraviolet-absorbing spots more polar than ATP could be detected when the nucleotide was chromatographed in a descending manner on Whatman no. 1 filter paper for 18 hr. at 24-25° with 0.1 M-sodium phosphate, pH 6.8-ammonium sulphate-propan-1-ol (50:30:1, by vol.). When a sample of ATP was chromatographed on Dowex 1 (200-400 mesh) by the method of Cohn & Carter, (1950), only trace amounts of ADP and no AMP could be detected.

Incubations were carried out in duplicate in 8 ml. Pyrex test tubes. ATP (0.1 ml.) at 0° was added to 2.3 ml. of the incubation medium at the beginning of a 10 min. equilibration period. The medium usually contained MgCl₂ (3 mM final concn.), ATP (tris salt) (3 mM, initial concn.), tris-acetate buffer, pH 7.4 (50 mM), and other additions according to the experiment. A few minutes before the start of the equilibration period, 1.6 ml. of 50 mM-tris-acetate buffer, pH 7.4, was added to 1.6 ml. of the frozen membrane suspension that had been thawed completely 1-2 min. before the end of the equilibration period. The reaction was started by the addition of 0.1 ml. of the membrane suspension (0.3 mg. of protein) at 0°.

Incubations were performed at 23° for 15 min. with occasional stirring. The reaction was stopped by the addition of 1 ml. of 25% (w/v) trichloroacetic acid at 0° and the tubes were cooled immediately in a mixture of ice and water. In those experiments in which ATP, ADP and AMP were determined by ultraviolet absorption at 259 mμ after separation by column chromatography (Cohn & Carter, 1950), the reaction was stopped by placing the tubes in a mixture of acetone and solid CO₂. The solutions were transferred to cold 15 ml. polyethylene centrifuge tubes and centrifuged at 0° to sediment the proteins. Samples (0.5 or 1.0 ml.) of the supernatant solution were used to determine the liberated inorganic phosphate by the method of Fiske & Subbarow (1925). To correct for non-enzymic release of inorganic phosphate from ATP, duplicate tubes were incubated without membrane suspension in all experiments and suitable corrections made for the spontaneous hydrolysis of ATP. When the membrane suspension was incubated without ATP, no inorganic phosphate could be detected in the incubation medium.

Since several of the phosphate determinations were near the limits of sensitivity of the procedure (less than 0.40 μmole of inorganic phosphate/mg. of protein), the duplicate values are presented in the Tables where these are not identical. Considerable errors are involved in determining the percentage change in these experiments, as, for example, in Expt. 2 in Table 5 where CTP was used as a substrate, and in Expt. 3 (b) in Table 7. With the same membrane preparation, control phosphate determinations sometimes differed by as much as 30% when these were less than 0.40 μmole of inorganic phosphate/mg. of protein and determined on different days. However, in no instance was the effect of the alkali-metal ion altered.

Chemicals. All solutions were prepared with distilled water that had been redistilled in Pyrex glassware and then stored in polyethylene containers. With the following exceptions, all reagents were analytical grade. Rubidium chloride, caesium chloride, caesium nitrate and caesium sulphate were the 'purified' grade obtained from the Fisher Scientific Co., Pittsburgh, Pa., U.S.A. 2,4-Dinitrophenol and choline chloride were obtained from the Eastman-Kodak Co., Rochester, N.Y., U.S.A. The sodium salts of ADP, AMP, GTP, ITP, UTP and CTP were obtained from the Sigma Chemical Co. Digitonin ('A grade') was obtained from the California Corp. for Biochemical Research. Deoxycholic acid ('special enzyme grade') was obtained from the Mann Research Laboratories, New York, N.Y., U.S.A.

RESULTS

Effects of temperature and protein concentration. The rate of liberation of inorganic phosphate from ATP at 37° was not linear during the first 15 min. except possibly with 0.02 mg. of protein/ml. (Fig. 1). At 23° the rate was linear only for the first 15-20 min., and 1 mM-ADP caused a 41% inhibition of adenosine-triphosphatase activity and 3 mM-ADP caused a 61% inhibition (cf. Kielley & Kielley, 1953; Cooper, 1958; Siekevitz *et al.* 1958; Pullman, Penefsky, Datta & Racker, 1960).

At 23° the amount of inorganic phosphate formed was proportional to the protein concentration up to 0.12 mg./ml. Pullman *et al.* (1960) also found that, during a 10 min. incubation period at 30°, the activity of purified adenosine triphosphatase, measured in the absence of an ATP-regenerating system, was proportional to the protein concentration up to approx. 0.08 mg. of protein/ml.

Effects of alkali-metal ions. A concentration of 50 mM-potassium chloride inhibited the release of inorganic phosphate from ATP by the mitochondrial-membrane suspension. This inhibition appeared to be significantly greater after 20 min. of incubation, when the rate of phosphate release was no longer linear. When the medium was assayed for ATP, ADP and AMP after incubation of the membrane suspension, no AMP could be detected either in the absence of alkali-metal ions or in the presence of 100 mM-sodium chloride or of 150 mM-lithium chloride.

At a concentration of 10 mM, both potassium chloride and sodium chloride cause a small but significant inhibition of adenosine-triphosphatase activity which increases with increasing concentrations of the salts (Fig. 2). This inhibition appears to be non-specific since, at a concentration of 150 mM, lithium chloride, rubidium chloride, caesium chloride, ammonium chloride or choline chloride inhibits the enzyme to about the same extent (Table 1). However, at isosmolar concentrations, the nitrates are more inhibitory and the sulphates less so than the chlorides. When two salts are added, the combined inhibition is usually somewhat greater than that of the more potent salt (cf. Table 1: Expt. 3).

Effects of magnesium ion and adenosine triphosphate concentrations. When the concentration of Mg^{2+} ions was varied from 0.25 to 12.0 mM, with that of ATP being kept at 3 mM, maximum adenosine-triphosphatase activity occurred when the two compounds were present in a molar ratio of 1:1 (Fig. 3). The decrease in adenosine-triphosphatase activity at high concentrations of Mg^{2+} ions also has been observed by Kielley & Kielley (1953), Myers & Slater (1957), and Cooper (1958). If the concentration of Mg^{2+} ions was kept constant at 3 mM but that of ATP was varied from 0.5 to 12.0 mM, similar results were obtained. In both experiments, 100 mM-potassium chloride inhibited the enzyme at all concentrations of Mg^{2+} ions and ATP. When the ATP concentration was increased, the inhibition of the enzyme by 100 mM-potassium chloride increased from 28 % at 0.5 mM-ATP to 46 % at 6 mM-ATP, and then dropped again to 22 % with 12 mM-ATP. However, when the concentration of Mg^{2+} ions was increased, the inhibition of adenosine triphosphatase by 100 mM-

potassium chloride steadily declined from 45 % at 0.25 mM- Mg^{2+} ion to 23 % at 12 mM- Mg^{2+} ion (Fig. 4). Similar results were obtained with potassium nitrate and rubidium chloride. With potassium sulphate, the inhibition of adenosine-triphosphatase activity decreased precipitously

Fig. 1. Release of inorganic phosphate from ATP after incubation of a mitochondrial-membrane suspension (preparation no. 1). The incubation medium contained ATP (tris salt) (3 mM, initial concentration), $MgCl_2$ (3 mM), tris-acetate buffer, pH 7.4 (50 mM), and the membrane suspension. The final volume was 2.5 ml. The experiment at 23° was performed on a different day from the one at 37°. Conditions: ○, 0.32 mg. of protein/ml. at 37°; ●, 0.32 mg. of protein/ml. at 23°; □, 0.08 mg. of protein/ml. at 23°; ■, 0.08 mg. of protein/ml. at 37°; △, 0.02 mg. of protein/ml. at 37°.

Fig. 2. Effects of different concentrations of KCl (●) and NaCl (○) on the release of inorganic phosphate from ATP after incubation of a mitochondrial-membrane suspension (preparation no. 2) for 15 min. at 23°. The incubation medium contained 0.12 mg. of protein/ml. Other conditions were the same as those given in Fig. 1.

when the concentration of Mg^{2+} ions was increased from 0.25 to 1 mM. With higher concentrations of Mg^{2+} ions, the inhibition tended to remain relatively constant. When Ca^{2+} ions were substituted for Mg^{2+} ions, increasing concentrations of Ca^{2+} ions resulted in increased adenosine-triphosphatase activity (Fig. 5). Again, 150 mM-potassium chloride inhibited the enzyme at all concentrations of Ca^{2+} ions, the inhibition remaining between 30 and 40 % when the concentration of Ca^{2+} ions was increased from 0.5 to 12.0 mM.

To determine whether the inhibition of adenosine triphosphatase by alkali-metal ions was reversible, the membrane suspension was incubated as usual and at the end of the 15 min. incubation period the tubes were plunged into a mixture of ice and water. After dialysis of the suspension for 23 hr. at 0° against 3 l. of 50 mM-tris-acetate

buffer, pH 7.4, the incubation was repeated, starting the reaction by the addition of ATP. Analysis of the dialysed medium for K^+ ions showed that it contained less than 0.1 mM of the cation. Although 155 mM-potassium chloride inhibited the enzyme by about 40 %, this was reversed almost completely by dialysis (Table 2). The undiluted enzyme lost 37 % of its activity when left at 0° for 23 hr. but the potassium chloride inhibited the aged enzyme to the same extent as the fresh preparation. The markedly lower activity of the aged dialysed preparation compared with the aged undialysed enzyme may be due to differences in protein concentration during the 23 hr. of aging: 3.0 mg./ml. of the undiluted preparation and 0.12 mg./ml. of the dialysed preparation. When the membrane suspension was preincubated at 23° for 15 min. or 1 hr. with

Table 1. *Inhibition of mitochondrial adenosine triphosphatase by salts and sugars*

Mitochondrial-membrane suspensions (preparation no. 2 in Expts. 1 and 2 and preparation no. 3 in Expt. 3) were incubated for 15 min. at 23°. The incubation medium contained $MgCl_2$ (3 mM), tris-acetate buffer, pH 7.4 (50 mM), protein (0.12 mg./ml.), ATP (tris salt) (3 mM, initial concn.), and other additions as indicated. The total volume was 2.5 ml.

Expt. no.	Addition	Final concn. of addition (mM)	Adenosine-triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1	None	—	7.20	—
	KCl	150	4.37	-39
	NaCl	150	4.44	-38
	RbCl	150	4.44	-38
	CsCl	150	4.24	-41
	Choline chloride	150	5.00	-31
	NH_4Cl	150	3.90	-46
	LiCl	150	4.10	-43
	KNO_3	150	3.06	-57
	$NaNO_3$	150	2.73	-62
	Sucrose	300	6.77	-6
2	None	—	6.84	—
	KCl	150	4.00	-42
	NaCl	150	4.20	-39
	K_2SO_4	100	5.74	-16
	Na_2SO_4	100	5.60	-18
	NH_4Cl	150	3.67	-46
	$(NH_4)_2SO_4$	100	3.74	-45
	LiCl	150	3.80	-44
	$LiNO_3$	150	2.00	-71
	Sucrose	300	5.80	-15
	Glucose	300	5.87	-14
3	None	—	5.10	—
	KCl	50	4.20	-18
	KNO_3	50	2.60	-49
	$KCl + KNO_3$	50 + 50	2.46	-52
	Choline chloride	50	4.27	-16
	NaCl	50	4.00	-22
	Choline chloride + NaCl	50 + 50	3.74	-27
	Na_2SO_4	50	4.60	-10
	$NaNO_3$	75	1.73	-66
	$Na_2SO_4 + NaNO_3$	50 + 75	1.40	-72
	$KCl + NaCl$	50 + 50	3.40	-33

150 mM-potassium chloride and either 3 mM-Mg²⁺ ion or 12 mM-Ca²⁺ ion, the inhibition of adenosine triphosphatase was the same for the non-preincubated and the preincubated enzyme. Both of the preincubated preparations showed a small, but significantly greater, adenosine-triphosphatase activity (20 % with Mg²⁺ ions) than the non-preincubated control.

Effects of bivalent cations. In the absence of Mg²⁺ ions, the mitochondrial-membrane preparation showed very little adenosine-triphosphatase activity (Table 3: Expt. 1). Both Ca²⁺ and Cu²⁺ ions increased the enzyme activity but not as much as did Mg²⁺, Mn²⁺, Co²⁺, Zn²⁺, Cd²⁺ and Fe²⁺ ions. Magnesium sulphate was as effective as magnesium chloride in stimulating adenosine triphosphatase. Similar results have been obtained with intact mitochondria (Myers & Slater, 1957; Kielley & Kielley, 1953; Potter, Siekevitz & Simonson, 1953; Lardy & Wellman, 1953; Novikoff, Hecht, Podber & Ryan, 1952), with fragments from mechanically or ultrasonically disrupted mitochondria (Bronk & Kielley, 1957; Gamble & Lehninger, 1956), with digitonin extracts of mitochondria (Cooper, 1958; Cooper & Lehninger, 1957), with a membrane fraction from 0.3% deoxycholate-disrupted mitochondria (Siekevitz *et al.* 1958), and with purified soluble adenosine triphosphatase (Pullman *et al.* 1960). With each of these bivalent cations, 150 mM-K⁺ ion inhibited adenosine triphosphatase by 22-49 % with all preparations. There did not seem

to be any correlation between the inhibition of adenosine-triphosphatase activity by K⁺ ions and the relative enzyme activity with various bivalent cations. For example, although the absolute

Fig. 4. Effects of different concentrations of MgCl₂ on the inhibition of mitochondrial adenosine triphosphatase by various salts. Conditions were the same as those given in Fig. 3. Conditions: ▲, 150 mM-KNO₃ with preparation no. 4; ●, 100 mM-KCl with preparation no. 2; ■, 100 mM-RbCl with preparation no. 4; ▼, 100 mM-K₂SO₄ with preparation no. 3.

Fig. 3. Effects of different concentrations of MgCl₂ in the presence (●) and absence (○) of 100 mM-KCl on the release of inorganic phosphate from ATP after incubation of a mitochondrial-membrane suspension (preparation no. 2) for 15 min. at 23°. The incubation medium contained ATP (tris salt) (3 mM, initial concentration), tris-acetate buffer, pH 7.4 (50 mM), 0.12 mg. of protein/ml., and different concentrations of MgCl₂ in a total volume of 2.5 ml.

Fig. 5. Effects of different concentrations of CaCl₂ in the presence (●) and absence (○) of 150 mM-KCl on the release of inorganic phosphate from ATP after incubation of a mitochondrial-membrane suspension (preparation no. 3). Other conditions were the same as those given in Fig. 3.

Table 2. *Reversibility of the inhibition of mitochondrial adenosine triphosphatase*

A sample (0.3 mg.) of mitochondrial-membrane protein (preparation no. 6) was added to a medium containing ATP (tris salt) (3 mM, initial concn.), $MgCl_2$ (3 mM), tris-acetate buffer, pH 7.4 (50 mM), and, when present, KCl (155 mM), in a final volume of 2.5 ml. (a). A 0.9 mg. sample of protein was added to the same medium in a final volume of 7.5 ml. (c). Both (a) and (c) were incubated for 15 min. at 23° and (a) was assayed for inorganic phosphate in the usual manner. (c) was cooled rapidly to 0° and then dialysed at 0° for 23 hr. against 3 l. of 50 mM-tris-acetate buffer, pH 7.4. At the end of the dialysis, 0.1 ml. of 75 mM- $MgCl_2$ was added to 2.3 ml. of the dialysed medium during the usual 10 min. equilibration period at 23°. The assay was started by the addition of 0.1 ml. of 75 mM-ATP. (b) was assayed in the same manner as (a) except that 0.3 mg. of membrane protein, which had been kept at 0° for 23 hr., was added to the incubation medium.

Conditions	KCl	Adenosine-triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
(a) Fresh	—	5.10	—
	+	3.03	-41
(b) Aged for 23 hr. at 0°	—	3.23	—
	+	2.03	-37
(c) Dialysed for 23 hr. at 0°	—	1.99	—
	+*	1.92	-3

* No KCl was added to the incubation medium after the dialysis. The salt had been added to the medium before the first incubation.

Table 3. *Activation of mitochondrial adenosine triphosphatase by bivalent cations and its inhibition by potassium chloride*

Conditions were the same as those given in Table 1. Mitochondrial-membrane preparation no. 3 was used. The concentration of the bivalent cation was 3 mM.

Expt. no.	Salt	150 mM-KCl	Adenosine-triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1	None	—	0.33	—
	None	+	0.20	-39
2	$MgCl_2$	—	4.77	—
	$MgCl_2$	+	3.37	-29
	$CaCl_2$	—	0.93	—
	$CaCl_2$	+	0.63	-32
	$MnCl_2$	—	5.30	—
	$MnCl_2$	+	2.97	-44
	$CoCl_2$	—	4.74	—
	$CoCl_2$	+	2.76	-42
	$ZnCl_2$	—	2.93	—
	$ZnCl_2$	+	1.50	-49
	$CdCl_2$	—	3.20	—
	$CdCl_2$	+	1.70	-47
3	$MgCl_2$	—	5.34	—
	$MgCl_2$	+	3.16	-41
	$MgSO_4$	—	5.50	—
	$MgSO_4$	+	3.16	-42
	$CaCl_2$	—	0.90	—
	$CaCl_2$	+	0.63	-30
	$FeCl_2$	—	4.07	—
	$FeCl_2$	+	3.16	-22
	$CuCl_2$	—	0.50*	—
	$CuCl_2$	+	0.27	-46

* Duplicate samples: 0.53 and 0.47.

enzyme activity with Cu^{2+} ions was only one-tenth of that with Mg^{2+} ions (Table 3; Expt. 3), the relative inhibition by 150 mM- K^+ ion was similar.

Effects of 2,4-dinitrophenol. It is now well established that 2,4-dinitrophenol causes activation of Mg^{2+} ion-stimulated mitochondrial adenosine triphosphatase (Lardy & Wellman, 1953; Myers & Slater, 1957; Pullman *et al.* 1960). With a mitochondrial-membrane preparation, 0.1 mM-2,4-dinitrophenol caused a small activation that is not apparent in the presence of a concentration of 150 mM of various salts (Table 4). The decreased inhibitory effect of the various alkali-metal ions in the presence of 2,4-dinitrophenol is a result of the sum of the inhibitory effect of the metal ion and the activating effect of the 2,4-dinitrophenol. There is no synergistic or antagonistic action between the two compounds.

Effects of different substrates. To determine whether the inhibition of adenosine triphosphatase by alkali-metal ions was specific for ATP, the sodium salts of a number of other nucleotides were used as substrates (Table 5). No adenosine-triphosphatase activity was detected with ADP as substrate, and very little with AMP (Table 5; Expt. 1). The inhibition of adenosine triphosphatase by 150 mM- K^+ ion was about the same when GTP, ITP or UTP was substituted for ATP despite large differences in enzyme activity in the absence of K^+ ions. The relative adenosine-triphosphatase activities with various triphosphates as substrates were similar to those reported by Pullman *et al.* (1960) for their purified adenosine-triphosphatase preparation and agree with the observations of

Gamble & Lehninger (1956) and Kielley & Kielley (1953).

Effects of calcium ions. When Ca^{2+} ions were substituted for Mg^{2+} ions as the activating bivalent cation, alkali-metal ions still caused a significant

inhibition of the enzyme, despite the decreased adenosine-triphosphatase activity (cf. Pullman *et al.* 1960; Cooper, 1958; Potter *et al.* 1953; Lardy & Wellman, 1953). However, qualitatively the inhibitory effects of alkali-metal ions were different

Table 4. *Effects of salts and of 2,4-dinitrophenol on mitochondrial adenosine triphosphatase*

Conditions were the same as those given in Table 1. Mitochondrial-membrane preparation no. 2 was used. The concentration of 2,4-dinitrophenol was 0.1 mM.

Expt. no.	Addition	Final concn. of addition (mM)	2,4-Dinitrophenol	Adenosine-triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1	None	—	—	6.94	—
	None	—	+	8.00	+15
	KCl	150	—	4.34	-37
	KCl	150	+	6.10	-12
	NaCl	150	—	4.44	-36
	NaCl	150	+	5.90	-15
2	None	—	—	6.54	—
	None	—	+	7.60	+16
	RbCl	150	—	4.34	-34
	RbCl	150	+	5.17	-21
	LiNO_3	150	—	1.83	-72
	LiNO_3	150	+	2.76	-58
	K_2SO_4	100	—	5.50	-16
	K_2SO_4	100	+	6.37	0
	Choline chloride	150	—	4.77	-27
	Choline chloride	150	+	5.57	-15
	Sucrose	300	—	6.10	-7
	Sucrose	300	+	7.14	+9

Table 5. *Inhibitory effect of salts on mitochondrial adenosine triphosphatase with different substrates*

Conditions were the same as those given in Table 1. Mitochondrial-membrane preparation no. 3 was used. In both experiments the sodium salts of the nucleotides were used in an initial concentration of 3 mM.

Expt. no.	Substrate	Salt	Final concn. of salt (mM)	Adenosine-triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1	ATP	—	—	4.03	—
	ATP	KNO_3	150	1.37	-66
	ATP	NaCl	100	3.23	-20
	ADP	—	—	0	—
	AMP	—	—	0.20	—
	AMP	KNO_3	150	0.20	0
	AMP	NaCl	100	0.13	-35
2	ATP	—	—	4.07	—
	ATP	KCl	150	3.13	-23
	GTP	—	—	2.53	—
	GTP	KCl	150	1.70	-33
	ITP	—	—	4.73	—
	ITP	KCl	150	2.87	-39
	UTP	—	—	0.87	—
	UTP	KCl	150	0.50	-42
	CTP	—	—	0.27*	—
	CTP	KCl	150	0.17†	-37

* Duplicate samples: 0.33 and 0.20.

† Duplicate samples: 0.20 and 0.13.

with Ca^{2+} ions. Caesium sulphate, ammonium sulphate and potassium sulphate caused significantly greater inhibitions than did the corresponding chlorides (Table 6). With Mg^{2+} ions, however, iso-osmotic amounts of the chlorides had significantly greater inhibitory actions than did the corresponding sulphates (cf. also Table 1). However, with either Ca^{2+} ions or Mg^{2+} ions as the activating bivalent cation, the inhibition by nitrates was similar and was greater than with either the corresponding chlorides or the sulphates (cf. Table 1).

Effects of anions and tris buffers. Since anions as well as univalent cations appeared to inhibit adenosine triphosphatase, tris buffers were used that had been titrated to pH 7.4 with hydrochloric acid, sulphuric acid, nitric acid, or acetic acid. The effects of 150 mM-potassium chloride were studied with no activating bivalent cation, with 3 mM- Ca^{2+} ion, or with 3 mM- Mg^{2+} ion. In these experiments, 1.7 ml. of the 2.5 ml. of incubation medium consisted of the different tris buffers. The added potassium chloride, ATP and membrane suspension were prepared in the standard 50 mM-tris-acetate buffer, pH 7.4. Adenosine-triphosphatase activity was least with the tris-nitrate buffer, and, in the presence of Mg^{2+} ions, it was greatest in the tris-acetate buffer. In general, 150 mM- K^+ ion in-

hibited the enzyme to about the same extent in all four buffers.

The added alkali-metal ions were dissolved in various tris buffers so that only 0.3 ml. of the incubation medium represented tris-acetate (Table 7). The effects of sodium acetate, sodium chloride, sodium nitrate and sodium sulphate were then studied (Table 7: Expt. 1). In the absence of Na^+ ions, adenosine-triphosphatase activity was highest in the tris-sulphate buffer followed, in order of decreasing activity, by tris-acetate, tris-chloride and tris-nitrate. With the exception of sodium acetate, the other three sodium salts inhibited the enzyme by 21–31%. Since sodium acetate only had a very small inhibitory action in this experiment, the effects of potassium acetate, lithium acetate and ammonium acetate also were studied (Table 7: Expt. 2a). In a tris-acetate buffer, potassium acetate inhibited the enzyme only slightly, whereas lithium acetate and ammonium acetate inhibited it by 27%. However, when Ca^{2+} ions were substituted for Mg^{2+} ions (Table 7: Expt. 2b), the inhibition by potassium acetate increased from 5 to 46%. In tris-chloride, tris-sulphate, or tris-nitrate buffers, potassium acetate again had only a small inhibitory action (Table 7: Expt. 2c). With Ca^{2+} ions instead of Mg^{2+} ions, the inhibitory action of potassium acetate was in-

Table 6. *Effects of calcium ions and magnesium ions on the inhibition of mitochondrial adenosine triphosphatase by salts*

Conditions were the same as those given in Table 1. In Expt. 1, mitochondrial-membrane preparation no. 3 was used, and in Expt. 2 preparation no. 4 was used.

Expt. no.	Salt	Final concn. of salt (mM)	Adenosine- triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1 (a): 3 mM- Mg^{2+} ion	None	—	5.94	—
	Li_2SO_4	100	4.16	-30
	LiCl	150	2.80	-53
	LiNO_3	150	1.43	-76
	Cs_2SO_4	100	5.34	-10
	CsCl	150	3.16	-47
	CsNO_3	150	1.73	-71
1 (b): 3 mM- Ca^{2+} ion	None	—	1.33	—
	Cs_2SO_4	100	0.77	-42
	CsCl	150	1.17	-12
	CsNO_3	150	0.40*	-70
2: 3 mM- Ca^{2+} ion	None	—	1.83	—
	NH_4Cl	150	0.40	-78
	$(\text{NH}_4)_2\text{SO}_4$	100	0.20	-89
	LiCl	150	0.67	-63
	Li_2SO_4	100	0.73	-60
	LiNO_3	150	0.43†	-76
	KCl	150	1.30	-29
	K_2SO_4	100	0.77	-58
	KNO_3	150	0.53	-71

* Duplicate samples: 0.43 and 0.37.

† Duplicate samples: 0.40 and 0.47.

creased markedly (Table 7: Expt. 3*a*). Further, in tris-acetate buffer, 150 mM-lithium acetate inhibited adenosine triphosphatase much more than did potassium acetate. This is similar to what was observed when Mg^{2+} ions were used (Table 7: Expt. 2*a*). In the absence of either Ca^{2+} or Mg^{2+} ions (Table 7: Expt. 3*b*), the inhibitory action of 150 mM-potassium acetate in tris-acetate buffer was similar to that when Ca^{2+} ions were present.

Since sodium acetate and potassium acetate behaved differently from the corresponding chlorides, the concentration of Mg^{2+} or Ca^{2+} ions was varied from 0.1 to 12.0 mM (Fig. 6). The inhibitory action of 150 mM-potassium acetate decreased

rapidly as the concentration of bivalent cation changed from 0.1 to 1.0 mM and then tended to remain constant. The shape of both inhibition curves is similar to that for potassium sulphate (Fig. 4). With both potassium acetate and potassium sulphate the inhibition of adenosine triphosphatase at an ATP: Mg^{2+} ion molar ratio of 1:1 was much less than that with the other salts.

Effects of different enzyme preparations. The adenosine-triphosphatase preparation used in the present study represents primarily mitochondrial membranes with little, if any, adhering mitochondrial 'matrix' (Watson & Siekevitz, 1956). Other preparations obtained from chemically or

Table 7. *Effects of salts and tris buffers on mitochondrial adenosine triphosphatase*

The tris buffers were prepared by titrating 0.25 M-tris to pH 7.4 with 0.25 N-HCl, 0.25 N- HNO_3 , 0.25 N- H_2SO_4 , or 0.25 N-acetic acid, and then diluting the buffer to a final concentration of 50 mM. In Expt. 1, the sodium salts were dissolved in the tris buffers indicated before addition to the incubation medium. In the other experiments, the salts were dissolved in 50 mM-tris-acetate buffer, pH 7.4. In Expts. 2 (*b*) and 3 (*a*), 3 mM- $CaCl_2$ was used instead of 3 mM- $MgCl_2$. In Expt. 3 (*b*), no Ca^{2+} or Mg^{2+} ions were added. Other conditions were the same as those given in Table 1. Mitochondrial-membrane preparation no. 4 was used. Ac represents $CH_3CO\cdot O$.

Expt. no.	Tris buffer	Salt	Final concn. of salt (mM)	Adenosine- triphosphatase activity (μ moles of inorganic phosphate/mg. of protein)	Change (%)
1	Acetate	—	—	7.47	—
	Acetate	NaAc	150	7.10	- 5
	Chloride	—	—	6.70	—
	Chloride	NaCl	150	4.97	- 26
	Nitrate	—	—	4.16	—
	Nitrate	$NaNO_3$	150	2.87	- 31
	Sulphate	—	—	8.47	—
	Sulphate	Na_2SO_4	100	6.66	- 21
2 (<i>a</i>)	Acetate	—	—	7.90	—
	Acetate	KAc	150	7.50	- 5
	Acetate	LiAc	150	5.80	- 27
	Acetate	NH_4Ac	150	5.77	- 27
2 (<i>b</i>)	Acetate	—	—	1.43	—
	Acetate	KAc	150	0.77	- 46
2 (<i>c</i>)	Chloride	—	—	7.27	—
	Chloride	KAc	150	6.17	- 15
	Sulphate	—	—	7.97	—
	Sulphate	KAc	150	7.27	- 9
	Nitrate	—	—	3.97	—
	Nitrate	KAc	150	3.80	- 4
3 (<i>a</i>)	Chloride	—	—	2.16	—
	Chloride	KAc	150	1.23	- 43
	Sulphate	—	—	2.16	—
	Sulphate	KAc	150	1.23	- 43
	Nitrate	—	—	1.50	—
	Nitrate	KAc	150	0.97	- 35
	Acetate	—	—	1.80	—
	Acetate	KAc	150	1.17	- 35
	Acetate	LiAc	150	0.43*	- 76
3 (<i>b</i>)	Acetate	—	—	0.43†	—
	Acetate	KAc	150	0.20‡	- 53
	Acetate	LiAc	150	0.17§	- 60

* Duplicate samples: 0.53 and 0.33.

† Duplicate samples: 0.27 and 0.13.

‡ Duplicate samples: 0.40 and 0.47.

§ Duplicate samples: 0.20 and 0.13.

physically disrupted mitochondria were studied to determine whether the adenosine triphosphatase in these preparations also was inhibited by alkali-metal ions. The adenosine triphosphatase in a preparation of particles isolated from mitochondria by treatment with digitonin by the method of Cooper & Lehninger (1956) is inhibited by alkali-metal ions in a manner similar to that of membranes from deoxycholate-disrupted mitochondria (Table 8). With either Mg^{2+} ion or Ca^{2+} ion as the activating bivalent cation, the inhibition by potassium nitrate was significantly greater than that by potassium chloride (cf. Table 1). The lack of an effect of sucrose and the inhibition by potassium chloride of the Mg^{2+} ion-stimulated

enzyme in these particles is similar to the observations of Cooper & Lehninger (1957).

Effects of pH. Although there is as yet no agreement on the existence of more than one mito-

Fig. 6. Effects of different concentrations of $MgCl_2$ (Δ) and $CaCl_2$ (●) on the inhibition of mitochondrial adenosine triphosphatase (preparation no. 4) by 150 mM-potassium acetate. Other conditions were the same as those given in Fig. 3.

Fig. 7. Effects of pH in the presence (●, ■) and absence (○, □) of 150 mM-NaCl on the release of inorganic phosphate from ATP by a mitochondrial-membrane suspension (preparation no. 5). 50 mM-Tris-acetate buffers were prepared as described by Myers & Slater (1957). The incubation medium contained ATP (tris salt) (3 mM, initial concentration), $MgCl_2$ (○, ●) or $CaCl_2$ (□, ■) (3 mM), and 0.12 mg. of protein/ml. in a total volume of 2.5 ml. The incubation time was 15 min. and the temperature 23°.

Table 8. *Effects of salts and sucrose on the adenosine triphosphatase of an enzyme complex from digitonin-disrupted rat-liver mitochondria*

Conditions were the same as those given in Table 1. In Expt. 1 (a), 3 mM- $MgCl_2$ was used; in Expt. 1 (b), 3 mM- $CaCl_2$ was used. The protein concentration in the incubation medium was 0.12 mg./ml.

Expt. no.	Addition	Final concn. of addition (mM)	Adenosine-triphosphatase activity (μmoles of inorganic phosphate/mg. of protein)	Change (%)
1 (a)	None	—	8.27	—
	KCl	150	6.23	-25
	KNO ₃	150	3.67	-56
	Sucrose	300	8.23	0
1 (b)	None	—	1.73	—
	KCl	150	1.27	-27
	KNO ₃	150	0.87	-50
	Sucrose	300	1.40	-19

chondrial adenosine triphosphatase (Lehninger & Wadkins, 1962; Racker, 1961), evidence has been presented by Myers & Slater (1957) that there are four mitochondrial adenosine triphosphatases with pH optima at 6.3, 7.4, 8.5 and 9.4. The effect of pH on the inhibition of adenosine triphosphatase by alkali-metal ions at different pH values was investigated by using 50 mM-tris-acetate buffers, prepared as described by Myers & Slater (1957). The sodium chloride solutions were prepared by dissolving the salt in 250 mM-tris, adjusting the pH with 250 mM-acetic acid, and diluting with water to give a final tris concentration of 50 mM. With either Mg^{2+} or Ca^{2+} ion as the activating cation, the pH optimum was between 8 and 9 (Fig. 7). This is similar to the pH optimum for a purified soluble adenosine triphosphatase from ox-heart mitochondria (Pullman *et al.* 1960). The presence of 150 mM-sodium chloride inhibited the adenosine triphosphatase over the entire pH range. In the presence of Mg^{2+} ions the inhibition was similar at all pH values tested, ranging from 32 % to 37 %. With Ca^{2+} ions, however, the inhibition increased from 18 % at pH 5 to 56 % at pH 7, and then decreased to 30 % at pH 10.

DISCUSSION

The inhibition of mitochondrial adenosine triphosphatase by alkali-metal ions appears to be non-specific. A salting-out effect probably can be ruled out since the inhibition occurred with concentrations of potassium chloride or sodium chloride as low as 10 mM (Fig. 2). Others have observed a similar inhibitory action of mitochondrial adenosine triphosphatase by alkali-metal ions in a variety of intact and disrupted mitochondrial preparations. Lardy & Wellman (1953) found that, in the presence or absence of Mg^{2+} ions, the adenosine-triphosphatase activity of intact rat-liver mitochondria usually was greater with 150 mM-sucrose than with 77 mM- or 92 mM-potassium chloride or -sodium chloride. Similar observations were made by Myers & Slater (1957). Both groups of investigators observed an enhanced effect of 2,4-dinitrophenol on adenosine-triphosphatase activity when sucrose was replaced by potassium chloride or sodium chloride as the suspending medium. In the present study, alkali-metal ions diminished the activating effect of 2,4-dinitrophenol when mitochondrial membranes were used.

Bronk & Kielley (1957) found that, with Mg^{2+} ion as the activating bivalent cation, Rb^{+} ions at a concentration of 2 mM inhibited the enzyme by 13 % in mitochondrial fragments. Further, in their initial studies with digitonin extracts of mitochondria, Cooper & Lehninger (1957) reported that,

whereas sucrose caused relatively insignificant inhibition of Mg^{2+} ion-stimulated adenosine triphosphatase, potassium chloride caused a substantial inhibition of the enzyme.

Permeability barriers that have presumably been abolished in the disrupted mitochondrial preparations and extracts can be ruled out as an explanation for the inhibitory action of alkali-metal ions. The adenosine triphosphatase in intact mitochondria also is affected in the same manner, but it is less clear whether this effect would be observed in truly undamaged mitochondria where the particles are reported to possess little or no adenosine-triphosphatase activity (Hunter, 1951; Kielley & Kielley, 1951; Potter *et al.* 1953; Chappell & Perry, 1954); that potassium chloride or sodium chloride had no effect on the adenosine-triphosphatase activity of intact liver mitochondria when these cations replaced sucrose is relevant (Potter *et al.* 1953). When observed for short periods, freshly prepared mitochondria possess little adenosine-triphosphatase activity whether or not Mg^{2+} ions are present (Potter *et al.* 1953). However, in intact liver mitochondria potassium chloride inhibits at low levels of adenosine-triphosphatase activity (Table 3 of Myers & Slater, 1957). In the present study, inhibition also was observed at low levels of adenosine-triphosphatase activity (Table 3).

Myosin adenosine triphosphatase also has been reported to be inhibited by alkali-metal ions (Watanabe, Tonomura & Shiokawa, 1953; Bowen & Kerwin, 1954; Mommaerts & Green, 1954; Bowen & Martin, 1962). Perry & Grey, (1956) found that potassium chloride inhibited myofibrillar adenosine triphosphatase, and Perry & Chappell (1957) showed that the inhibition of Ca^{2+} ion-activated L-myosin by potassium chloride was not specific since sodium chloride was equally effective. However, other studies have shown an activating effect of univalent cations on myosin adenosine triphosphatase (Mommaerts & Seraidarian, 1947; Bowen & Gershfeld, 1957).

The mitochondrial adenosine triphosphatase studied in the present investigation and the Na^{+} ion-plus- K^{+} ion-dependent adenosine triphosphatase found in a variety of cell membranes have certain features in common. Both enzymes are located in the membrane (Post *et al.* 1960; Whittam & Wheeler, 1961). Both are Mg^{2+} ion-dependent, the optimum ATP: Mg^{2+} ion molar ratio for maximum adenosine-triphosphatase activity being about 1:1 (Skou, 1960; Dunham & Glynn, 1961). When the concentration of Mg^{2+} ions exceeds that of ATP, or when the ATP concentration is greater than that of the Mg^{2+} ions, the enzymic activity is inhibited (Skou, 1960). The pH optimum of both enzymes is between 7 and 8 (Dunham & Glynn,

1961; Skou, 1957; Whittam & Wheeler, 1961; Järnefelt, 1961), although with Ca^{2+} ion as the activating bivalent cation the pH optimum for the mitochondrial adenosine triphosphatase shifts to 9. Both enzymes catalyse the hydrolysis of ITP (Skou, 1960), the hydrolysis of ITP being greater than that of ATP. Although Post *et al.* (1960) reported that the adenosine triphosphatase in their erythrocyte preparations did not split ITP, this discrepancy may be due to the use of peripheral-nerve microsomes by Skou (1960).

Although some of the properties of the so-called 'glycoside insensitive' or ' Na^+ ion-plus- K^+ ion-independent' component of membrane adenosine triphosphatase are similar to those of the mitochondrial enzyme, e.g. the activation by both Mg^{2+} and Ca^{2+} ions (Dunham & Glynn, 1961), several properties of the Na^+ ion-plus- K^+ ion-dependent adenosine triphosphatase are strikingly different from the mitochondrial adenosine triphosphatase. First, although either Na^+ or K^+ ions alone have little or no effect on the Na^+ ion-plus- K^+ ion-dependent enzyme (Post *et al.* 1960; Dunham & Glynn, 1961; Whittam & Wheeler, 1961), either alkali-metal ion alone inhibits mitochondrial adenosine triphosphatase. Maximal activation of the Na^+ ion-plus- K^+ ion-dependent adenosine triphosphatase usually is achieved only if both ions are present, whereas in mitochondria the inhibition caused by the presence of both Na^+ and K^+ ions is merely additive. However, both Skou (1957) and Järnefelt (1961) have reported activation by Na^+ ions alone of microsomal adenosine triphosphatase from brain and peripheral nerve. Secondly, the inhibition of mitochondrial adenosine triphosphatase is non-specific when different alkali-metal ions are used (with the same anion). With the Na^+ ion-plus- K^+ ion-dependent enzyme, however, not only do Na^+ and K^+ ions have a specific effect, e.g. depending on whether they are on the inside or the outside of the erythrocyte membrane (Glynn, 1962; Whittam, 1962*a, b*), but the amount of activation by Li^+ , NH_4^+ , K^+ , Rb^+ and Cs^+ ions is different (Skou, 1960). Thirdly, the mitochondrial adenosine triphosphatase is activated by Ca^{2+} ions; the Na^+ ion-plus- K^+ ion-dependent enzyme is inhibited by Ca^{2+} ions (Skou, 1957; Dunham & Glynn, 1961). Fourthly, Skou (1960) found that the activation of his Na^+ ion-plus- K^+ ion-dependent enzyme preparation from peripheral-nerve microsomes shifted the $\text{ATP}:\text{Mg}^{2+}$ ion molar ratio for optimum activity from 1:1 to 1:2. With mitochondrial adenosine triphosphatase, no shift in this ratio was observed when the enzyme was inhibited by potassium chloride.

With intact brain mitochondria, Deul & McIlwain (1961) reported small and relatively inconsistent effects of Na^+ and K^+ ions on adenosine

triphosphatase. Recent studies of liver adenosine triphosphatase suggest that, if there is a Na^+ ion-plus- K^+ ion-dependent enzyme in this organ, it may be present only in the liver membranes. Emmelot & Bos (1962) found that the adenosine-triphosphatase activity in liver membranes was increased by 0.1M-sodium chloride if K^+ ions were present in the medium, but the microsomal adenosine triphosphatase was not stimulated by Na^+ ions (cf. Skou, 1957; Järnefelt, 1961). Bonting, Caravaggio & Hawkins (1962) found that in liver almost all of the Na^+ ion-plus- K^+ ion-dependent adenosine triphosphatase was present in the nuclear fraction, which presumably would contain the cell membranes. In a study of the microsomal adenosine triphosphatase of brain, muscle, testis, liver and kidney, Järnefelt (1962) found a significant activation of adenosine triphosphatase by Na^+ and K^+ ions only in brain and muscle.

If it is assumed that a Na^+ ion-plus- K^+ ion-dependent adenosine triphosphatase is involved directly in cation transport across the membranes of, for example, erythrocytes and peripheral nerves, it is not surprising that unequivocal evidence for such an enzyme has not been found in mitochondria. Whereas there is good evidence for coupled active transport of Na^+ and K^+ ions by erythrocytes (Glynn, 1957) and peripheral nerves (Hodgkin & Keynes, 1955), no unequivocal evidence has been presented so far for the active transport of Na^+ ions by mitochondria either into or out of these particles. Further, there is no evidence that the accumulation of K^+ ions by mitochondria against a concentration gradient is linked to the movement of Na^+ ions (see references at the beginning of the present paper).

Dunham & Glynn (1961), in their study of the activation by Na^+ and K^+ ions of erythrocyte adenosine triphosphatase, were not able to decide from their results whether the glycoside-insensitive and the glycoside-sensitive components of the adenosine triphosphatase were two separate enzymes. The recent report by Schatzmann (1962) on the inhibition by lecithinase of both the Na^+ ion-plus- K^+ ion-dependent and the Na^+ ion-plus- K^+ ion-independent adenosine triphosphatases of erythrocytes indicates that both components may be lipoprotein in nature. The isolation of a purified soluble adenosine triphosphatase from heart mitochondria by Pullman *et al.* (1960) might allow the use of solubilized and purified non-mitochondrial membrane adenosine triphosphatases that are activated by Na^+ ions plus K^+ ions. Such studies might show whether differences in the response of adenosine triphosphatase to alkali-metal ions in different membranes are due to separate enzymes, or to differences in orientation of the enzyme within or on the membrane.

The present results give little indication of the mechanisms involved in the inhibition of mitochondrial adenosine triphosphatase by alkali-metal ions. The similarity of the inhibition with various nucleotide triphosphates as substrates suggests that the alkali-metal ions do not react with the purine moiety of the substrate. Although activation of the enzyme by different bivalent cations had little effect on the inhibition by alkali-metal ions, the decrease in inhibition with increasing concentrations of Mg^{2+} ions suggests a possible competition between the Mg^{2+} ion and the alkali-metal ion for a reactive group on the enzyme. Such an interpretation is complicated by the observation that the inhibition decreases in spite of a decrease in adenosine-triphosphatase activity when the ATP: Mg^{2+} ion molar ratio becomes greater than 1:1. Further, alkali-metal ions inhibit the enzyme even in the absence of an activating bivalent cation.

The relatively non-specific inhibition suggests that quantitative changes in the ionic milieu of the mitochondria would have a marked effect on the coupling of oxidation to phosphorylation, since many believe that mitochondrial adenosine-triphosphatase activity represents a partial reversal of oxidative phosphorylation (for reviews see Racker, 1961; Lehninger & Wadkins, 1962). One of the functions of the alkali-metal ions within the mitochondria may be to keep adenosine-triphosphatase activity at a low level for maximum efficiency of oxidative phosphorylation. The observations that 'aged' mitochondria lose K^+ ions (Ulrich, 1959) and that 'aging' uncouples oxidative phosphorylation (Lehninger, 1949) tend to support such an idea. Pressman & Lardy (1955) have reported that K^+ ions are necessary for maximum rates of respiration and phosphorylation by rat-liver mitochondria with a phosphate-accepting system present.

SUMMARY

1. The effects of alkali-metal ions on the adenosine-triphosphatase activity of a membrane suspension from deoxycholate-disrupted rat-liver mitochondria were studied.

2. The alkali-metal ions Na^+ , K^+ , Li^+ , Rb^+ , Cs^+ and NH_4^+ inhibited the adenosine triphosphatase to about the same extent. With Na^+ or K^+ ions, significant inhibition was observed at a concentration of 10 mM, and the inhibition increased progressively with increasing concentrations.

3. Although various bivalent cations, Mg^{2+} , Mn^{2+} , Ca^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} and Cd^{2+} , activated the adenosine triphosphatase to a variable extent, the inhibition by alkali-metal ions was similar. The enzyme hydrolysed ATP, ITP, CTP,

UTP and GTP at different rates, but again there were only small differences in the inhibition by alkali-metal ions.

4. The activation of adenosine triphosphatase by 0.1 mM-2,4-dinitrophenol was diminished in the presence of an alkali-metal ion.

5. With the same alkali-metal ion and in a tris-acetate buffer, pH 7.4, the anions inhibited the adenosine triphosphatase in the decreasing order: NO_3^- ; Cl^- ; SO_4^{2-} . These differences were decreased when the cation was incubated in a tris buffer containing the same anion as the alkali-metal salt.

6. The inhibition was reversible and preincubation of the adenosine triphosphatase with the alkali-metal ion did not affect the inhibition.

7. With Mg^{2+} ion as the activating cation, the pH optimum of the adenosine triphosphatase was about 8. This was shifted to about 9 with Ca^{2+} ion. Inhibition of the adenosine triphosphatase by an alkali-metal ion was similar over the pH range 5-10.

8. The results are discussed in relation to the similarities and differences in the properties of myosin adenosine triphosphatase, mitochondrial adenosine triphosphatase and the ' Na^+ ion-plus- K^+ ion-activated' or 'glycoside-sensitive' adenosine triphosphatase of several membrane preparations.

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Basic and Other Proteins in Microsomes of Rat Liver

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Several physical properties of microsomal ribonucleoprotein particles and the molecular weight of the RNA component of samples from different species have been determined (McQuillen, 1962); the results of these studies suggest that by these criteria ribosomes from all the cells examined appear to be similar in many respects. Some chemical properties of the total protein have been described, e.g. for ribosomes from yeast (Yin & Bock, 1960), pea seedlings (Setterfield, Neelin, Neelin & Bayley, 1960) and *Escherichia coli* (Waller & Harris, 1961; Spahr, 1962), but little is

known about the nature of different protein species present. Butler, Cohn & Simson (1960) and Cohn, Simson & Butler (1960) reported that basic proteins were present in ribonucleoprotein preparations obtained from microsomes of rat liver. Extraction of such ribonucleoprotein particles with dilute hydrochloric acid was found to produce a basic fraction in which the proportions of some of the principal *N*-terminal groups were different from those in the total protein. The results of the experiments described below confirm the presence of substantial amounts of basic proteins in pre-