AMP-monosulphates: Adenosine-5′-phosphosulphate (APS). Inorganic sulphate was rapidly liberated from this compound by acid hydrolysis. It was somewhat more resistant to alkaline and CaO hydrolysis. It was degraded to adenosine, phosphate and sulphate by snake venom and prostatic phosphatases. It was an intermediate in the enzyme system for sulphate activation [4]. Adenosine-2′-sulphate-5′-phosphate and adenosine-3′-sulphate-5′-phosphate decomposed at a slower rate than APS during acid hydrolysis and were very slowly degraded by alkaline and CaO hydrolysis. Digestion with snake venom phosphatase produced adenosine, phosphate and sulphate. Prostatic phosphatase produced adenosine-sulphates + phosphate.

AMP-disulphates: The disulphates were probably a mixture of three different compounds, which were not further separated. Two of the compounds conceivably contained one phosphosulphate and one ribose-sulphate linkage. The third compound contained both sulphates attached to ribose.

AMP-trisulphate: Adenosine-2′,3′-disulphate-5′-phosphosulphate was degraded to adenosine-2′,3′ - disulphate - 5′-phosphate and sulphate during acid, alkaline and CaO hydrolysis. Further decomposition occurred at a slower rate. Snake venom phosphatase produced adenosine-2′,3′-disulphate-5′-phosphate + sulphate. Prostatic phosphatase produced adenosine-2,3′-disulphate, phosphate and sulphate.

The factors influencing the yield of the different AMP-sulphates were studied. APS appeared as the primary product. By increasing the amount of sulphuric acid in the reaction mixture and prolonging the reaction time a total yield of AMP-sulphates amounting to 90—100 % (calc. on AMP) could be obtained. It was possible to obtain a 40—50 % conversion of AMP to APS without more than small amounts of other AMP-sulphates.

The different AMP-sulphates could be separated from each other by column electrophoresis on a gram scale.

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4. We wish to thank Dr. P. W. Robbins for testing the enzymic activity of our APS.

Complex Formation of Nucleoside Triphosphates with Metal Ions *

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Transphosphorylation reactions with nucleoside triphosphates as the phosphate donor require metals as cofactors, especially Mg^{2+}, Mn^{2+} or Ca^{2+}. It has therefore been suggested that metal nucleotide complexes are the actual coenzymes. In the present work the dissociation constants of several nucleoside triphosphates of Ca^{2+}, Mg^{2+}, Co^{2+} and Mn^{2+} have been determined. An ion exchange method according to the principle reported by Schubert has been adopted. An anion exchange resin (Dowex-1) in the chloride form has been used. The dissociation constants of mononucleotides with different metals have been determined under the following conditions. A total volume of 20 ml contained (concentrations): 0.5×10^{-4} M nucleotide, 0.1 M NaCl, 0.005 M tris(hydroxymethyl)aminomethane buffer, pH 8.2, and usually 50 mg resin. Equilibration was obtained without and with addition of metal salts to the mixture by shaking for 3 h at 23 °C. The concentration of nucleotide not bound to the resin was determined by reading the extinction at appropriate wavelenghts in ultraviolet region on a Beckman spectrophotometer. From these data the dissociation constants k_c for ATP with metals have been calculated.

$$k_c\ \mathrm{Ca} > k_c\ \mathrm{Mg} > k_c\ \mathrm{Co} > k_c\ \mathrm{Mn}$$
$$1.7 \times 10^{-4}\quad 0.9 \times 10^{-4}\quad 0.23 \times 10^{-4}\quad 0.17 \times 10^{-4}$$

Dissociation constants for different metal complexes with ADP and AMP show the same sequence as reported for ATP. However, as an approximation complexes with ADP are 10 times more dissociated and complexes with AMP are 100 times more dissociated than the corresponding ATP metal complexes. This can be illustrated in the case of complexes with Mg

$$\mathrm{ATP} < \mathrm{ADP} < \mathrm{AMP}$$
$$0.9 \times 10^{-4}\quad 6.4 \times 10^{-4}\quad 1.1 \times 10^{-3}$$

The dissociation constants for CTP, GTP, UTP and ITP with the different metals show, with a few exceptions, the same sequence as reported for ATP. The values approximately correspond to those given for ATP metal complexes.

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