material remaining after dialysis was lyophilized and then hydrogenated with 10% platinum on Darco. After hydrolysis and analysis for amino acids, the yields of alanine and of α-aminobutyric acid were found to be at least 20 and 35% of the theoretical values, respectively. Keratosulfate from shark cartilage was allowed to react with alkali in the presence of Adams catalyst (5) and hydrogen. At least 50% of the theoretical yields of alanine and α-aminobutyric acid were obtained.

The formation of alanine and of α-aminobutyric acid confirms the postulated mode of linkage and the mechanism of its cleavage in alkali. The similarity of the action of alkali on cartilage keratosulfate and some glycoproteins further underlines the previously indicated similarity of this type of keratosulfate with the blood group substances (6).

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Regulation of Nicotinamide Adenine Dinucleotide Biosynthesis by Adenosine Triphosphate

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In 1958, Preiss and Handler (1, 2) reported the formation of niacin ribonucleotide from niacin and 5-phosphoribosyl 1-pyrophosphate by extracts of human erythrocytes (Scheme I, Reaction I). The enzyme was subsequently purified from beef liver and referred to as niacin ribonucleotide pyrophosphorylase by Imsande and Handler (3). Recent reports from this laboratory indicated that niacin ribonucleotide is also produced from quinolinic acid by a 5-phosphoribosyl 1-pyrophosphate-dependent reaction catalyzed by quinolinate transphosphoribosylase (Reaction II) (4-6). In the presence of ATP, niacin ribonucleotide thus formed is converted to deamido-NAD (Reaction III), which is formed is converted to deamido-NAD (Reaction III), which is

![Scheme 1. Biosynthesis of NAD from tryptophan and niacin, and the regulation of these pathways by ATP. PP-ribose-P, 5-phosphoribosyl-1-pyrophosphate.](http://www.jbc.org/)

![Fig. 1. Effects of ATP on the activity of niacin ribonucleotidase (A) and niacin ribonucleotide pyrophosphorylase (B). A, the reaction mixture (0.5 ml) contained 7 mmoles of niacin ribonucleotide-14C (8 × 10^4 c.p.m. per µmole), 3 µmoles of MgCl₂, 50 µmoles of phosphate buffer, pH 7.3, 20 µg of the purified enzyme preparation, and ATP as indicated. B, the reaction mixture (0.5 ml) contained the same ingredients as A, except that 7 mmoles of niacin-14C (8 × 10^4 c.p.m. per µmole) and 0.4 µmole of 5-phosphoribosyl 1-pyrophosphate were added in the place of niacin ribonucleotide-14C. Incubation was carried out for 30 minutes at 37°.

Niacin ribonucleotide-14C was prepared from niacin-7-14C, ATP, and ribose 5-phosphate with crude extracts of human erythrocytes by the method of Preiss and Handler (2). The niacin ribonucleotide-14C was obtained by hydrolysis of niacin ribonucleotide-14C by prostatic phosphomonoesterase (5). These compounds were isolated with a Dowex 1 column and purified by paper chromatography as previously described (5).
protein per hour, respectively. At 1 X 10⁻⁶ M ATP, these were 1.0 X 10⁻⁶ M and 0.9 μmole per mg of protein per hour, respectively.

When the enzyme preparation was subjected to sedimentation analyses under the same conditions. The nicotinamide was probably produced from NAD by the action of NADase present in the extract. Neither deamido-NAD nor NAD were produced. When 2 μmole of ATP were added to the incubation mixture, neither niacin nor niacin ribonucleoside was converted to niacin ribonucleotide. In a similar set of experiments with 1.0 μmole of niacin ribonucleotide-¹⁴C (8 X 10⁶ c.p.m. per μmole), 5 μmole of MgCl₂, 1 μmole of L-glutamine, 50 μmole of phosphate buffer, pH 7.5, and 4.4 mg of the liver preparation treated with charcoal, was incubated for 90 minutes at 37°, niacin (542 μmole), niacin ribonucleoside (74 μmole), and niacin ribonucleotide (383 μmole) were recovered. Neither deamido-NAD nor NAD were produced. When 2 μmole of ATP were added to the incubation mixture, neither niacin nor niacin ribonucleoside was formed but deamido-NAD (617 μmole), NAD (157 μmole), nicotinamide (81 μmole), and niacin ribonucleotide (155 μmole) were recovered under the same conditions. The nicotinamide was probably produced from NAD by the action of NADase present in the extract. These reaction products were isolated by paper chromatography, and the radioactivity was determined as previously described (5).

In a similar set of experiments with 1.0 μmole of niacin ribonucleotide-¹⁴C (8 X 10⁶ c.p.m. per μmole) as substrate, niacin (932 μmole) was produced in the absence of ATP. However, on the addition of ATP, the substrate was converted to niacin ribonucleotide (127 μmole), deamido-NAD (292 μmole), NAD (197 μmole), and nicotinamide (98 μmole).

It may be concluded, therefore, that when a sufficient amount of ATP is available, niacin ribonucleotide activity disappeared and Reactions I, III, IV, and VI operate, thus quinolinole (tryptophan), niacin, and niacin ribonucleoside are all utilized for the synthesis of NAD and no niacin accumulates. On the contrary, when the concentration of ATP is low, the activity of niacin ribonucleotidease predominates and Reactions I, III, IV, and VI do not operate; thus the precursors mentioned above are almost totally converted to niacin, and no NAD is produced. Further studies are currently in progress in order to investigate the reversibility of the ATP-dependent conversion of the two

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2 At 2 X 10⁻⁸ M ATP, K₅₇₆ and V₇₆₆₆ values were 1.4 X 10⁻⁶ M and 5.2 μmole per mg of protein per hour, respectively. At 1 X 10⁻⁸ M ATP, these were 1.0 X 10⁻⁶ M and 0.9 μmole per mg of protein per hour, respectively.

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3 Albino rats were decapitated, and the livers were removed and homogenized for 2 minutes with 2 volumes of 0.14 M KCl in a Waring Blender. The homogenate was centrifuged for 10 minutes at 12,000 X g, and the supernatant was treated with activated charcoal (15% w/v).
enzyme activities and also to see if the enzyme is phosphorylated in the presence of ATP or if ATP acts as a modifier of protein conformation.

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