February 24, 1950

Dr. Waldo E. Cohn
Biology Division
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Oak Ridge, Tennessee

Dear Waldo:

I am sorry about the poor results of those two preparations, since I feel partly to blame. The nicotinamide-ribose linkage is very labile to alkali. Schlenk reports a 50 per cent destruction in 0.1 N NaOH at 20 degrees after 17 minutes. My own experience enforces this observation. I would guess that the NMN at pH 11 was partially disintegrated. I will send you a preparation that has the following history:

> TPN of about 75 per cent purity was split by nucleotide pyrophosphatase in glycylglycine buffer. The adenine moieties were precipitated with lead acetate. The supernatant was decomposed with $H_2S$ and is the NMN preparation I am sending you. There are approximately 20 cc., containing in micromoles per cc. 0.09 ortho P, 4.68 organic P, 4.55 pentose and 4.87 nicotinamide nucleoside on the basis of U-V absorption (using Schlenk's coefficient of $5.0 \times 10^6$ cms.$^2$ x mole$^{-1}$).

It would be a reasonable caution not to exceed pH 7. I would guess there are about 400 micromoles of glycylglycine in the preparation.

Concerning the riboflavin phosphate preparation, I can give you very little information since it is a commercial product prepared by a synthetic method. We tested for its

activity as a coenzyme for cytochrome reductase (Horecker's liver preparation) and found it almost as active as material obtained from enzymatic cleavage of FAD. There was a suggestion of an inhibitory effect, and I would not be surprised if it were not a homogeneous product.

The TPN adenosine diphosphate split is nil. I set about to prepare some for you and discovered that the TPN preparation which had been purified to the point where the phosphate content was theoretical (although it was only 75 per cent pure in terms of dry weight) had now deteriorated to a purity of only 58 per cent. This means, of course, that cleavage of such a preparation would very likely yield a mixture of adenine nucleotides, although I have no clear notion of the basis for the deterioration of the TPN. Since our data for establishing the identity of the TPN nucleotide with your adenylic a seems fairly complete, there is no immediate need to go to the painful lengths of preparing more TPN of high purity (It took us over two months to get a few hundred milligrams). If you are willing to work with the preparation which might contain only 50-75 per cent of its adenine in the form of "adenosine diphosphate," I will be very happy to work some up for you. This will be very little trouble.

I have had several opportunities to run mixtures of adenosine monophosphates on Dowex 1 columns according to your description, and it is uncanny how precise the duplication is. It is really an elegant technique.

Please give my best regards to Nick; and I will write him soon about some of our recent results.

With kindest regards,

Sincerely,

Arthur Kornberg