OCCURRENCE OF FREE NICOTINIC ACID IN THE LIVER OF NICOTINAMIDE-INJECTED RATS

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The administration of NAm to the rat is followed by an increase of about 10-fold in the NAD content of the liver (Bonsignore and Ricci,1949). The same phenomenon has subsequently been described in the mouse (Kaplan et al.,1956). Equimolecular doses of NAc are far less effective than NAm in promoting the NAD synthesis in the liver (Kaplan et al.,1956). This observation suggested a scheme for the NAD biosynthetic pathway which involved NMN as an intermediate. More recently, the isolation of Des-NAD from the liver of NAm injected mice has been reported, and it is now widely accepted that the NAm conversion to hepatic NAD proceeds through a pathway similar that present in human erythrocytes, which involves NAc-nucleotides as intermediates (Langan et al.,1959). NAm deamidation at the mononucleotide level by rat liver preparations has been described (Sarma et al.,1961); however it has not yet been possible to detect the NAm conversion to NMN by rat liver, in vitro or in vivo. The NAm deamidase ac=

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Abbreviations: NAm,Nicotinamide; NAc, Nicotinic acid; NAD,Nicotinamide-Adenine-Dinucleotide; NMN,Nicotinamide Mononucleotide; Des-NAD, Nicotinic acid-Adenine-Dinucleotide; Des-NMN, Nicotinic acid Mononucleotide.

tivity, previously reported to be absent in rat liver (Rajagopalan et al.,1960), has now been found to be present in an inhibited form (Petrack et al.,1963). The inhibition is released after hypophysectomy (Greengard et al.,1963).

Traces of NAc have been detected in the liver of NAm-treated mice (Langan et al.,1959), but quantitative data were still lacking. Using a more sensitive exper= imental procedure, we have been able to find that in the rat the level of free NAc in liver is greatly in= creased after NAm injection, and follows a curve which reaches a maximum at the 8^{th} hr.

Experiments were conducted on adult female rats of the Wistar strain, with a weight ranging between 130 and 150 g, and fed a standard laboratory diet. The rats were injected intraperitoneally with 100 mg of NAm (as 10% solution) per 100 g of body wt. NAm was pur= chased from the Merck A.G., and determined to be free from contaminating pyridine compounds by paper chromato= graphy in two different solvent systems. After the injection, food was withdrawn and only water supplied freely. Injections were made on a time schedule so that all the rats were decapitated within a short period of time. Two rats were sacrificed at each time interval.

Percloric acid extracts from 8 g of liver was prepared according to the procedure of Hurlbert et al., (1954). An aliquot of the neutralized extract was kept for an enzymatic assay of NAD (Holzer et al.,1958). A second aliquot corresponding to 6 g of liver was chromatographed on a 1 x 12 cm column of Dowex 1 ($HCOO^-$) 200-400 mesh, following the procedure of Hurlbert et al.(1954), using a 250 ml mixing chamber and 4N HCOOH in the reservoir.

The NAm was washed away from the column with water,

and was determined in this fraction by the method of Hughes and Williamson (1953). As checked by chromatography on Whatman 1 in the solvent C of Preiss and Handler (1958), at each time interval after injection of NAm, NAm is the tertiary pyridine compound present in the water eluate. In addition, there are slight traces of quaternary pyridine derivatives which do not interfere appreciably in the method of Hughes and Williamson.

NAc was eluted from the Dowex 1 column together with NAD. In order to separate NAc from NAD and its hydrolytic products, the fractions in the peak were pooled, lyophylized and taken up in 1 ml of water. Aliquots corresponding to 1.5 g of liver, were chromatographed on Whatman 31 paper using n-butanol:water as the descending solvent with 1% ammonia on the bottom of the jar. Under these conditions NAc migrated with a Rf of about 0.33. The region of the paper corresponding to NAc, as revealed on a adjacent lane by the method of Kodicecek and Reddi (1951), was cut out and eluted with 4 ml of 0.1 N HCl with the descending technique. The eluates were brought to pH 6-7 and NAc was determined by the method of Hughes and Williamson. Under these conditions the material eluted from the paper gave the A_{350}/A_{370} ratio of 0.6 which is characteristic of NAc. Known amounts of NAc were run through the separation procedure and NAc was recovered with a yield of 95-100%. Des-NAD was eluted from the column with a higher HCOOH concentration, and was determined by its reaction with KCN (Lamborg et al.,1958).

The content of NAc of the liver at various times after NAm injection is reported in the Table I.

The value shown at zero time was obtained from normal rats and is near the lower limit of the assay

TABLE I

NAc and other pyridine compounds in the liver of NAm treated rats.

(Values expressed as μmoles/g of fresh tissue)

Hours after injection	NAm	NAc	Des-NAD	NAD
0	0.22	0.001	0.010	0.49
2	5.14	0.034	0.234	1.58
5	5.00	0.040	0.265	2.92
8	4.34	0.068	0.241	2.56
12	1.90	0.062	0.232	5.60
16	1.03	0.005	0.047	3.08
24	0.10	0.001	0.022	0.62

method. The administration of NAm is very soon followed by a rise in the NAc level and this amounts to a 70-fold increase after 8 hr. The values and the time curves for the other compounds are in close agreement with the data from other authors (Langan et al.,1959; Greengard et al.,1961).

The time curves for NAc and NAD follow a similar pattern. A sharp increase is noted immediately after NAm administration, with a peak from the 8^{th} to the 12^{th} hr, followed by a sharp decrease to normal values. On the other hand, the curve of NAm shows an earlier maximum. It can be noted that the maximum level of free NAm around the 2^{nd} hr almost equals the maximum level reached by NAD at the 12^{th} hr.

The isolation of NAc from the liver of NAm-injected rats further substantiates the conversion of NAm to NAD through desamidated compounds. The involvement of NAc in NAD biosynthesis is clearly indicated by the fact that its level in the liver is relatively high during the time when NAD is being synthesized. After the 12^{th} hr it drops to lower values. The same can be noted for Des-NAD.

The eventual accumulation of free NAc can be explained by assuming that the deamidation of NAm occurs

at a higher rate than the reaction of NAc with 5-phosphoribosyl-1-pyrophosphate. In fact, it has been recently reported by Imsande (1964) that the specific activity of rat liver Des-NMN pyrophosphorylase is relatively low as compared to that of other biological systems.

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REFERENCES

Bonsignore A.and Ricci C.,Boll.Soc.It.Biol.Sper. 25, 710,(1949)

Greengard P.,Kalinsky H.J.and Petrack B., Biochim. Biophys.Acta 52,408,(1961)

Greengard P., Petrack B., Craston A. and Kalinsky H.J., Biochem.Biophys.Res.Comm.13,478,(1963)

Holzer H.,Busch D. and Kroeger H., Z.Physiol.Chem. 313, 184,(1958)

Hughes D.E. and Williamson D.H., Biochem.J. 55,851, (1953)

Hurlbert R.B., Schmitz H., Brumm A.F. and Potter V.R., J.Biol.Chem. 209,23,(1954)

Imsande J., Biochim.Biophys.Acta 82,445,(1964)

Kaplan N.O., Goldin A., Humphreys S.R., Ciotti M.M., and Stolzenbach F.E., J.Biol.Chem. 219, 287,(1956)

Kodiceck E. and Reddi K.K., Nature 168,475,(1951)

Lamborg M., Stolzenbach F.E. and Kaplan N.O., J.Biol. Chem. 231,685,(1958)

Langan T.A., Kaplan N.O. and Shuster L., J.Biol.Chem. 234,2161,(1959)

Petrack B., Greengard P., Craston A. and Kalinsky H.J., Biochem.Biophys.Res.Comm. 13,472,(1963)

Preiss J. and Handler P., J.Biol.Chem. 233,493,(1958)

Rajagopalan K.V., Sarma D.S.R., Sundaram T.K. and Sarma P.S., Enzymologia 21,277,(1960)

Sarma D.S.R., Rajalakshmi S. and Sarma P.S., Biochem. Biophys.Res.Comm. 6,389,(1961)