

## Digestion and Absorption of NAD by the Small Intestine of the Rat<sup>1</sup>

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**ABSTRACT** A number of preparations of varying complexity have been used in an effort to elucidate the reactions by which NAD is hydrolyzed to nicotinamide during intestinal digestion. NAD labeled with <sup>14</sup>C in the adenine or pyridine moiety was the substrate used with perfused rat intestine, live rats, perfused live rats, with collection of portal flow, intestinal contents, mucosal tissue, or pancreatic juice. The conclusions reached are that a pyrophosphatase present in the intestinal juice and to a much lesser extent in the pancreatic juice releases 5'-AMP and nicotinamide ribonucleotide. The 5'-AMP was rapidly converted to adenosine then to inosine by bacteria-free intestinal contents. Perfused or intact intestine rapidly hydrolyzed NMN to nicotinamide riboside, which accumulated, but was not absorbed. It was slowly cleaved by an enzyme associated with the mucosal cells to nicotinamide, which was the major if not the only labeled compound absorbed. *J. Nutr.* 113: 412-420, 1983.

**INDEXING KEY WORDS** NAD · intestine · nicotinamide

In animal tissues (1) and probably in foods generally pyridine nucleotides, NAD(H) and NADP(H), account for almost all of the dietary niacin before cooking. It has been assumed that the digestive process gives rise to nicotinamide (NAM) after ingestion of these coenzymes, since NAM appears to be the primary circulating form (2-4) and it is not significantly hydrolyzed to nicotinic acid (4) in the intestine of the rat. However, little information is presently available concerning the sequence of hydrolytic steps, the location of enzymes and the relative rates of reactions that are involved in the hydrolysis of NAD. Several enzymes have been shown to act on NAD (see fig. 1). NAD glycohydrolase or nucleosidase (5), which acts on the N-ribose bond to form NAM and adenosine diphosphate ribose, has been shown to be an abundant membrane-bound enzyme (6-9), but it is inhibited to a great extent *in vivo* (6). Alternatively, NAD can be cleaved at the pyrophosphate linkage to give rise to 5'-AMP (7) and nicotinamide ribonucleotide (NMN), which could then undergo hydrolysis to nicotinamide riboside (NR) (8). NR can be hy-

drolyzed (8) or phosphorylated (9) to give NAM. NAD might also be cleaved by phosphodiesterase to give rise to NR and ADP. The possibility exists that NR may be absorbed without further cleavage.

The purpose of this investigation was to evaluate the role of the small intestine of the rat in the digestion of NAD, and specifically to determine the identity of the products and their rate of formation and absorption utilizing both vascularly perfused rat intestines and live animals and related preparations.

### MATERIALS AND METHODS

[<sup>14</sup>C]carboxamide-labeled NAD, specific activity 53 mCi/mmol, [U-<sup>14</sup>C]adenine-labeled NAD, specific activity 286 mCi/mmol, and [8-<sup>14</sup>C]5'-adenosine monophosphate, specific activity 59 mCi/mmol, (Amersham Corp., Arlington Heights, IL) were radiochemically pure as revealed by paper electrophoresis at pH 3.7 and 7.7 and by paper chromatogra-

© 1983 American Institute of Nutrition. Received for publication 26 July 1982.

<sup>1</sup>Supported in part by National Institutes of Health grant 5R01 AM 19012.

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phy using two solvent systems. All other chemicals were those used previously (4, 10).

Intestinal perfusions were done by a modification of the procedure described by Windmueller et al. (11). Single-pass, vascular perfusions and live animal experiments used in this study have both been described previously (4, 10). Male albino rats of the Sprague-Dawley strain (Simonsen Laboratories, White Bear Lake, MN) and germfree rats (Charles River Breeding Laboratories, Wilmington, MA) weighing between 180 and 280 g were used. The rats were fed a cereal-based, stock diet (Purina laboratory chow, Ralston Purina Co., St. Louis, MO) ad libitum. Animals were fasted 18 hours prior to the experiment, unless otherwise indicated. The intestinal segment studied, both for perfusions and live animals, was 25 to 30 cm in length, tied at each end and cannulated with polyethylene tubing (PE-50, Becton Dickinson & Co., Parsippany, NJ) at the duodenal end, a few centimeters from the stomach. The compounds injected were dissolved in 1.2 ml of 0.9% NaCl. This volume gently distended a majority of the segment insuring contact of substrate with mucosal surface yet not creating excessive pressure. Intestinal perfusions varied from 5 to 20 minutes and live animal experiments varied from 1 to 15 minutes.

At the termination of each experiment the intestine was quickly removed and the contents collected by flushing with water. The intestinal tissue was boiled for 1 minute in water and homogenized. Protein in the contents and intestinal tissue was precipitated with 2 volumes of absolute ethanol. For perfusion experiments the perfusate was also treated with ethanol. The samples were then filtered, and the volume of the filtrates was reduced in vacuo. The samples were subjected to high-voltage paper electrophoresis (pH 3.7), to paper chromatography in two solvent systems as described previously (4), and to separation by low-voltage (320 v for 90 minutes) electrophoresis at pH 7.7 (0.2 M triethanolamine, 0.002 M EDTA) in preparation for counting each labeled compound.

A series of 15-minute, live-animal experiments was done to determine the sequence of appearance of products from carboxamide-labeled NAD in the lumen of the intes-

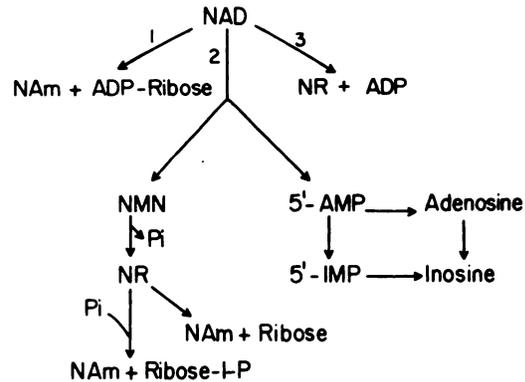


Fig. 1 Possible pathways for the degradation of NAD by the small intestine of the rat.

tinal segment. Samples of contents (0.1 ml) were withdrawn at times between 0.5 and 15 minutes by inserting a 24-gauge needle through the intestinal wall. Samples were either immediately spotted and dried on paper for chromatography and electrophoresis or were added to 0.2 ml of absolute ethanol to prevent further hydrolytic activity. Ethanol-treated samples were also analyzed immediately after each experiment by paper electrophoresis and paper chromatography to avoid variability in results found with samples subjected to long work-up times. With these techniques the distribution of isotope could not be accurately determined at the end of the experiment, since samples had been removed during the experiment, hence a second series of live-animal experiments was done without removal of serial samples. For these experiments the percent distribution of isotope and the distribution of isotope in hydrolysis products after 15 minutes of digestion was determined for the intestinal contents and intestinal tissue as previously described.

Intestinal contents of unfasted rats, killed by cervical dislocation, were removed from the proximal 30 cm of the small intestine with a small volume (2 to 3 ml) of water. The contents, which contained both pancreatic juice and intestinal secretions were sterilized by membrane filtration through a 0.20- $\mu$ m filter to remove bacteria and particulate matter and were incubated at 24° with [<sup>14</sup>C]carboxamide-labeled NAD or [<sup>14</sup>C]adenine-labeled NAD to determine the extent of hydrolysis without the presence of intes-

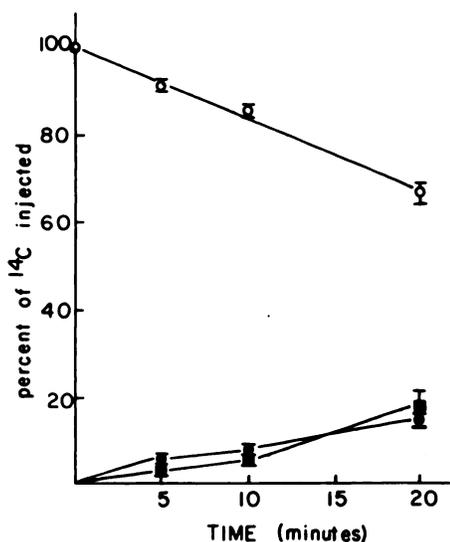


Fig. 2. Transport of  $^{14}\text{C}$  from the lumen (O) of the perfused intestine into intestinal tissue (●) and perfusate (■), when carboxamide-labeled NAD is the substrate.

tinal cells. Labeled NAD ( $9 \times 10^{-5} \text{ M}$ ) was incubated with intestinal contents, and samples were removed during 15-minute experiments. These samples were analyzed for labeled products as described above. To determine the contribution of pancreatic juice to the hydrolysis of NAD, pancreatic juice was collected from a live rat after cannulation of the pancreatic duct with PE-50 polyethylene tubing. Incubation with NAD and analysis of products was done as described for intestinal contents. Intestinal contents were also incubated with  $^{14}\text{C}$ 5'-AMP to determine the sequence of degradation steps involved in the hydrolysis of the 5'-AMP moiety of NAD.  $^{14}\text{C}$ 5'-AMP ( $2.4 \times 10^{-4} \text{ M}$ ) was incubated with contents only or with the addition of one of the following hydrolysis products: 600-fold excess of inosine, 50-fold excess adenosine or 50-fold excess 5'-IMP.

$^{14}\text{C}$ NR was prepared by hydrolyzing  $^{14}\text{C}$ NAD to  $^{14}\text{C}$ NMN with intestinal contents, then incubating the  $^{14}\text{C}$ NMN with snake venom phosphatase according to the methods of Rowen and Kornberg (12). The incubation mixture was treated with two volumes of absolute ethanol and centrifuged to remove protein. The supernatant was taken to dryness in vacuo and resuspended in water. By this method 89% of the radioactivity was in NR, 9% in Nam and 2% in NMN.

Intestinal contents was then incubated with  $^{14}\text{C}$ NR to determine whether its hydrolysis to  $^{14}\text{C}$ NAM would occur in the absence of intestinal cells. The techniques were the same as described earlier. To determine the effect of inorganic phosphate on the conversion of NR to NAM, a crude homogenate of intestinal mucosa was incubated with  $^{14}\text{C}$ NR with and without phosphate. Mucosal cells were scraped from an intestine and suspended in 8 ml of 0.9% NaCl, homogenized in the cold and centrifuged at  $10,000 \times g$  for 15 minutes. The supernatant was dialyzed overnight against a large volume of water. The dialyzed enzyme (140  $\mu\text{l}$ ) was then incubated with  $^{14}\text{C}$ NR in 0.01 M Tris buffer (pH 8.0) with and without 0.01 M phosphate (total volume 0.2 ml). Aliquots were removed for determination of  $^{14}\text{C}$ NAM by electrophoresis.

## RESULTS

When  $^{14}\text{C}$ carboxamide-labeled NAD (188 nmol) was injected into the duodenum of the perfused small intestine of the rat, the loss of radioactivity from the intestinal contents was linear for at least 20 minutes. After 20 minutes, 14.7% of the dose was found in the intestinal tissue and 18.1% had reached the perfusate (see fig. 2). Table 1 compares intestinal perfusions to live-animal experiments described in the methods section and to a "perfused live-animal" technique which was designed to relate better the perfused intestine results with those of the live animal. Rats were anesthetized with sodium pentobarbital and a midline incision was made. The intestine was placed in a thermostated bath con-

TABLE 1

Percent distribution of isotope in NAD hydrolysis products 5 minutes after luminal administration of  $^{14}\text{C}$ NAD<sup>1</sup>

	Intestinal contents	Intestinal tissue	Perfusate or blood
Perfused isolated intestine	91.3 $\pm$ 1.3	5.8 $\pm$ 1.0	2.5 $\pm$ 1.1
Perfused live animal	90.4 $\pm$ 1.2	7.0 $\pm$ 1.1	2.4 $\pm$ 0.3
Live animal	88.1 $\pm$ 2.0	4.5 $\pm$ 1.3	ND <sup>2</sup>

<sup>1</sup> Mean values for 3 experiments  $\pm$  SEM. <sup>2</sup> ND, not determined.

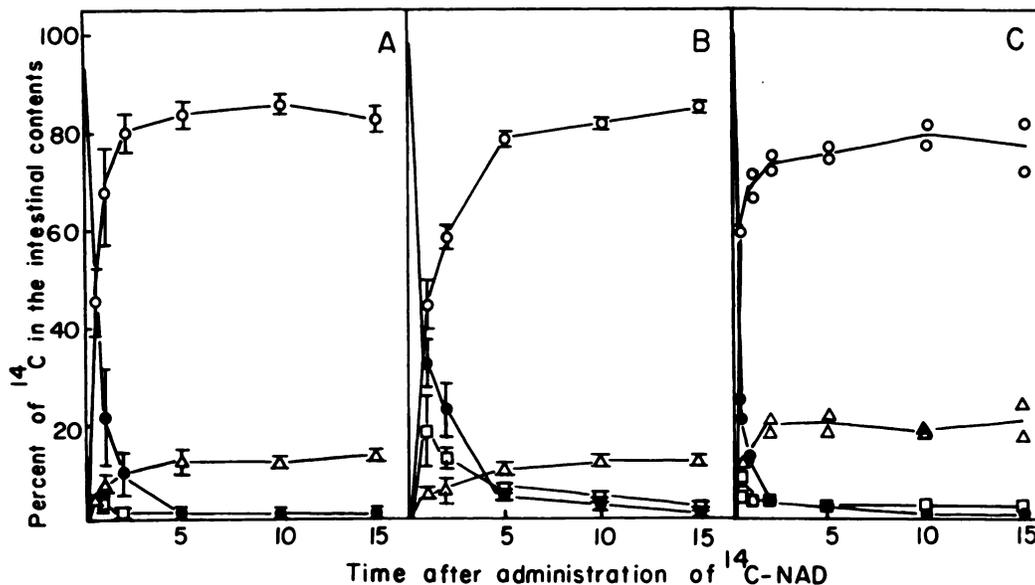


Fig. 3 Spectrum of labeled products [NAD (●); NR (○); NMN (□); NAM (Δ)] in the contents of the intestine of the live animal when 94.4 nmol of [<sup>14</sup>C]carboxamide-labeled NAD was injected into the duodenum of: 3A Twenty-four-hour fasted rats. 3B Unfasted rats. 3C Unfasted germfree rats. Vertical bars represent the SEM for three animals.

taining Earle's buffer and a PE-50 polyethylene cannula was placed in the lumen about 2 cm from the stomach. The portal vein was cannulated with a 16-gauge curved needle and, the vena cava was cannulated with an 18-gauge straight needle. Freshly drawn rat blood was diluted to 5% hematocrit with the perfusate medium used for intestinal perfusions. This mixture was infused into the vena cava at 10 ml/minute. After injection of carboxamide-labeled NAD into the lumen, flow from the portal vein was collected and analyzed for radioactivity. The advantage of this procedure is that it simulates the live animal without returning radioactivity to the animal for recirculation. With each of the above experimental techniques, the contents at 5 minutes still contained most of the radioactivity (91% for intestinal perfusions, 90% for perfused live animals and 88% for live animals). These data indicate that the loss of <sup>14</sup>C to the circulation and therefore to other tissues was slow enough to justify the use of the live animal for further studies.

There was no significant difference between the contents of fasted and unfasted rats on the rate of hydrolysis when a dose of 94 nmol was used (see fig. 3A, 3B). In both cases NAD decreased rapidly within the first min-

ute, resulting in an initial rise in NMN followed by a large increase in NR, which became the primary product (80–85%) within 1 minute. The other significant product was NAM, which represented about 10–20% of the label found in the contents in the first minute. Since intestinal perfusions and live animals showed that 70–80% of the label was still in the contents after 15 minutes, NR represented an accumulating intermediate in the breakdown of NAD and suggests that the nucleosidase or phosphorylase are rate limiting. Analysis of the intestinal tissue and perfusate indicated that 60–80% of the radioactivity associated with these fractions was in NAM. The same results were obtained when [<sup>14</sup>C]NAM was the substrate presented to the perfused intestine (4). This suggests that hydrolysis of NR to NAM occurs before absorption.

The contribution of intestinal bacteria to the degradation of NAD was also considered. Unfasted, germfree rats were used in a parallel experiment to those mentioned above. No difference in the rate of hydrolysis or the distribution of isotope were found (see fig. 3C) except for a slightly higher level of isotope in the NAM.

The effect of increasing the dose of NAD

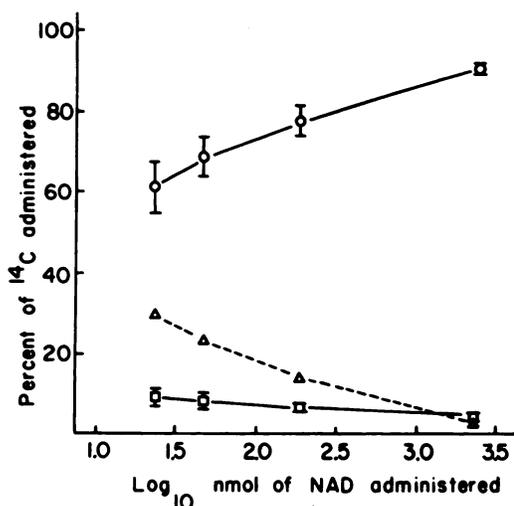


Fig. 4 Effect of dosage on the distribution of  $^{14}\text{C}$  from [ $^{14}\text{C}$ ]carboxamide-labeled NAD in intestinal contents (○); intestinal tissue (□); and  $^{14}\text{C}$  removed, obtained by difference (△). Vertical bars represent the SEM for three animals 15 minutes after injection into the duodenum of the fasted rat.

from 23 to 2300 nmol was to decrease the percentage of the label being removed from the contents in 15 minutes from 38.6% to 8.5% (see fig. 4, upper curve). Analysis of the products in the contents at the end of 15 minutes showed that with a dose of 23 nmol, 70% of the  $^{14}\text{C}$  was in the form of NR, 21.0% in the form of NAM and 5.5% was in the form of NMN, whereas with 2300 nmol 88.0% of the  $^{14}\text{C}$  was in the form of NR, 2.9% was in the form of NAM and 9.1% in the form of NMN. Luminal NR therefore represents 42% of total  $^{14}\text{C}$  in the 23 nmol dose and 80% of the 2300 nmol dose at 15 minutes (fig. 5). The following evidence indicates that the radioactivity removed from the contents, which was determined by difference between radioactivity administered and radioactivity recovered, was probably NAM. Analysis of products in the intestinal tissue gave results similar to those obtained when [ $^{14}\text{C}$ ]NAM was injected into the duodenum (4). NAM was the major product in the intestinal tissue, representing 75 to 95% of the radioactivity. Analysis of the perfusate from intestinal perfusions also showed NAM to be the major product. No NR was found in the intestinal tissue of the live animals or in the

intestinal tissue or perfusate of perfused intestines.

To evaluate further the relationship of the reactions involved, 94 nmol of [ $^{14}\text{C}$ ]NAD and 94  $\mu\text{mol}$  unlabeled NMN was injected into the live animal. Analysis of contents removed from the intestine at varying times (fig. 6 in contrast to fig. 3) indicated that NAD was hydrolyzed more slowly and that when a large amount of the unlabeled intermediate, NMN was present, [ $^{14}\text{C}$ ]NMN was trapped. Sixty-six percent of the label in the contents at 10 minutes was present in NMN. [ $^{14}\text{C}$ ]NR was formed more slowly, reaching only 34% in 15 minutes; and [ $^{14}\text{C}$ ]NAM formation was insignificant, perhaps due to inhibition of NR phosphorylase.

The hydrolysis of NAD, by intestinal contents isolated from unfasted rats, was evaluated during 20-minute experiments. The rate of hydrolysis varied with each preparation of intestinal contents and with the concentration of NAD studied, but the products were always the same. NAD was hydrolyzed almost exclusively to NMN by the enzymes present in the intestinal contents, and only small amounts of NAM were occasionally found (see fig. 7A). NAM shown in this representative figure was a contaminant of the initial substrate. No difference was found between nonsterile intestinal contents, and contents sterilized by membrane filtration.

Pancreatic juice was examined for hydrolytic enzymes which could act on NAD. Only about 9% of the NAD was converted to NMN in 15 minutes at 24° (see fig. 7C). No other

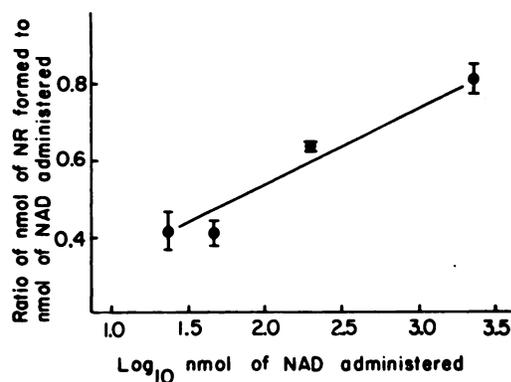


Fig. 5 Effect of dosage of NAD on the nature of its hydrolysis products formed in the intestinal segments of the live animals used for the data in fig. 4.

products were found. It therefore appears that pancreatic enzymes have a minor effect on the intestinal hydrolysis of NAD.

Isolated intestinal mucosal tissue was also incubated with carboxamide-labeled NAD to determine the extent of hydrolysis in the presence of cells and membrane fragments. A suspension of mucosal cells was prepared by scraping the mucosa with a microscope slide and suspending 1.0 ml of packed cells in 9.0 ml of 0.9% NaCl. Fifty-microliter aliquots of mucosal cell suspension were incubated with 4.7 nmol of [ $^{14}\text{C}$ ]NAD. At times varying from 1 to 15 minutes 150  $\mu\text{l}$  of absolute ethanol was added. Each sample was homogenized with a hand-operated glass homogenizer and centrifuged. The supernatants were analyzed for labeled products (table 2). Less than 10% of the radioactivity remained in the pellet. Almost complete hydrolysis to NAM occurred within 5 minutes.

Isolated intestinal sacs were also examined, where 20-cm segments were tied at both ends and bathed in Earle's buffer at 37°. Carboxamide-labeled NAD was injected into the sac and allowed to incubate. At 15 minutes the luminal contents were examined. Most of the radioactivity in the lumen was in the form of NAM with only a small amount in NR (table 3). A slower breakdown in NMN to NR was also observed as compared to live animals. Results from these intestinal sac experiments were not as consistent with increasing dosage (table 3), as were live animals, nor was the percent distribution very reproducible, except that NAM was always the major component.

The presence of 0.01 M phosphate greatly increased the conversion of [ $^{14}\text{C}$ ]NR to [ $^{14}\text{C}$ ]NAM by a crude enzyme preparation from intestinal mucosa (fig. 8). In the presence of phosphate 90% of the initial [ $^{14}\text{C}$ ]NR was converted to NAM, whereas in the absence of phosphate only 25% was converted.

Intestinal contents were incubated with [ $^{14}\text{C}$ ]NR to determine whether or not the presence of intestinal cells was necessary for the conversion of NR to NAM. Less than 5% conversion to [ $^{14}\text{C}$ ]NAM occurred in 30 minutes when other membrane filter sterilized contents or nonsterile contents was incubated with [ $^{14}\text{C}$ ]NR (53 mCi/mmol) at a concentration of  $3.8 \times 10^{-5}$  M.

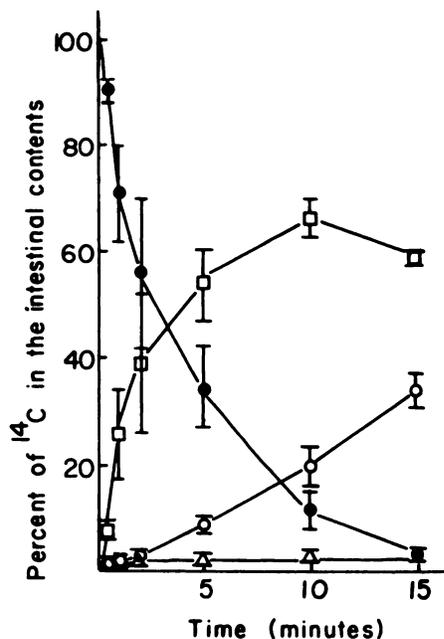


Fig. 6 Spectrum of labeled products in the contents of the intestine of the live animal when 94 nmol of [ $^{14}\text{C}$ ]carboxamide-labeled NAD was injected into the duodenum of a fasted rat together with 94  $\mu\text{mol}$  unlabeled NMN. Symbols are those used in fig. 2.

To determine the extent to which NAD is hydrolyzed by the stomach, a cannula was placed at the esophageal entrance to the stomach of a 3-hour fasted rat. A tie was placed at the pyloric sphincter to prevent radioactivity from passing to the duodenum. Carboxamide-labeled NAD (189 nmol) was injected into the stomach. At 30 minutes, the stomach was flushed with water and the contents analyzed for labeled products. Greater than 90% of the radioactivity was recovered in NAD.

The fate of the 5'-AMP portion of NAD was examined using NAD labeled with  $^{14}\text{C}$  in the adenine moiety. Hydrolysis by intestinal contents was very rapid and compared well with the rate of NMN formation when [ $^{14}\text{C}$ ]carboxamide-labeled NAD was incubated with the same preparation of intestinal contents (fig. 7B). When the labeled hydrolysis product was examined it was found to be inosine. The formation of inosine from 5'-AMP requires at least two enzyme reactions. Either 5'-AMP is dephosphorylated to adenosine and then deaminated to form inosine

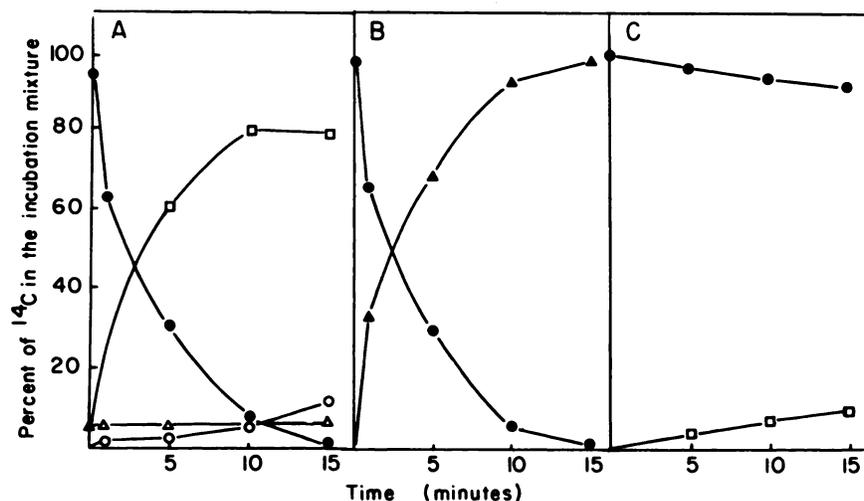


Fig. 7 A representative example from one experiment of 7A Pyrophosphatase action on [ $^{14}\text{C}$ ]carboxamide-labeled NAD of sterile, isolated intestinal contents, 7B Formation of [ $^{14}\text{C}$ ]inosine from [ $^{14}\text{C}$ ]adenine-labeled NAD by the same preparation of sterile isolated intestinal contents as used in 7A, 7C Incubation of [ $^{14}\text{C}$ ]carboxamide-labeled NAD with isolated pancreatic juice, when  $9 \times 10^{-5}$  M NAD was used as substrate. Symbols: ●, NAD; □, NMN; ○, NR; △, NAm; ▲, inosine.

or deaminated to form 5'-IMP and then dephosphorylated to inosine. The hydrolysis pathway for 5'-AMP was examined by incubating [ $^{14}\text{C}$ ]5'-AMP with sterile intestinal contents. Use of labeled NAD or 5'-AMP as the substrate produced the same results. Inosine was formed almost exclusively and rapidly in both cases. Figure 1 shows two possible pathways for the formation of inosine. To determine the sequence of reactions, intestinal contents were then incubated with [ $^{14}\text{C}$ ]5'-AMP and unlabeled inosine (600-fold excess), adenosine (50-fold excess), or 5'-IMP (50-fold excess). Adenosine was the only intermediate containing label when 600-fold excess of inosine or 50-fold excess of aden-

osine was added. Fifty-fold excess of 5'-IMP did not produce any labeled intermediates, but it did slow the conversion to inosine presumably by influencing the phosphatase (see fig. 9). Conversion to inosine was inhibited by 600-fold excess of either adenosine or 5'-IMP.

Two intestinal perfusions were done where [ $^{14}\text{C}$ ]NAD labeled in the adenine position was used as the substrate. With a dose of 80 nmol, 60% of the label was transported to the perfusate in 15 minutes, 8% was found in the intestine, and 32% remained in the contents.

TABLE 2

<i>Hydrolysis of NAD by rat mucosal scrapings<sup>1</sup></i>		
Compound	Percentage of $^{14}\text{C}$ by time	
	1 min	5 min
NAD	34.6	4.0
NMN	16.1	8.6
NAm	49.3	87.5

<sup>1</sup> Representative data from one experiment.

TABLE 3

*Percentage distribution of  $^{14}\text{C}$  in contents of intestinal sacs 15 minutes after injection*

Compound	Percent distribution, dose, <sup>1</sup> nmol		
	23.7	94.4	188.9
%			
NAD	0.4	1.5	0.6
NMN	21.3	35.4	14.0
NR	13.8	11.3	10.6
NAm	64.5	51.7	74.7

<sup>1</sup> Each dose represents one experiment.

When the dose was increased to 713 nmol, 44% was transported to the perfusate, 8% was found in the intestine and 49% remained in the contents. Analysis of products was not carried out for these experiments. The rate of hydrolysis and transport for the 5'-AMP portion of NAD was considerably faster than the hydrolysis and transport of the NMN portion.

DISCUSSION

Dietary NAD was hydrolyzed primarily in the small intestine of the rat. The initial step was shown to be cleavage to NMN and 5'-AMP, which is catalyzed by a pyrophosphatase found in intestinal secretions or released from desquamated cells. Pancreatic juice did not contribute significantly in the hydrolysis of NAD. NMN was rapidly hydrolyzed to NR, and NR more slowly to NAm. Both reactions required the presence of intestinal cells indicating that the enzymes are membrane bound or intracellular.

A recent abstract by Baum et al. (13) concerning the hydrolysis of NAD by intestinal brush border membranes suggests that the brush border contains two enzymes for the hydrolysis of NAD, one forming NAM and another forming NMN, which after a lag period is then converted to NR. They looked at different fractions from homogenates of the small intestine of the rat for NAD hy-

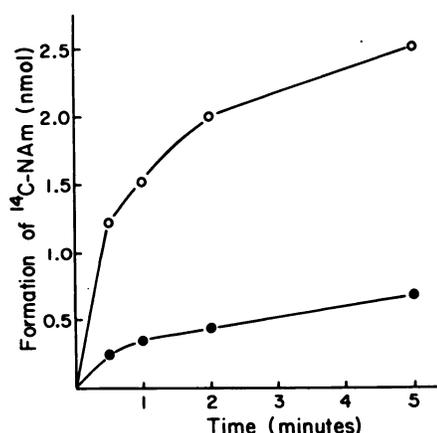


Fig. 8 A representative assay for the conversion of [<sup>14</sup>C]NR (2.8 nmol, 1.4 × 10<sup>-5</sup> M) to [<sup>14</sup>C]NAm by a crude dialysate of homogenized intestinal mucosa in the presence (○) and absence (●) of 0.1 M phosphate.

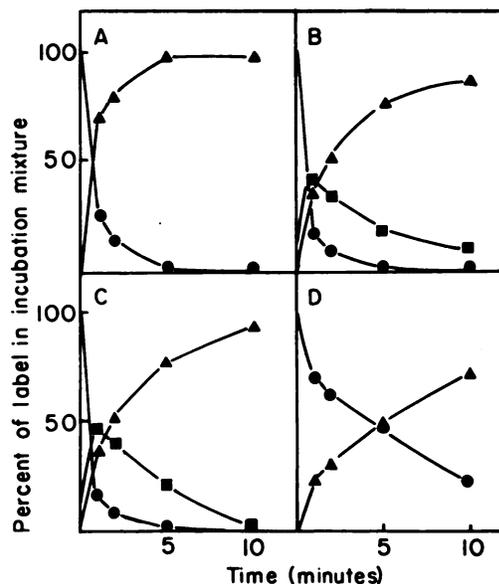


Fig. 9 Effect of overloading with unlabeled potential intermediates on the labeling of hydrolysis products of [<sup>14</sup>C]-5'-AMP (●); inosine (▲); adenosine (■) during incubation with sterile intestinal contents. 9A Unlabeled 0.24 mM 5'-AMP. 9B A plus 144 mM inosine. 9C A plus 12 mM adenosine. 9D A plus 12 mM 5'-IMP mM.

drolytic activity and found it largely in the particulate fractions, with the highest activity in the brush-border membrane fraction. They also found that at pH 5-6 the principal product was NAm, whereas at pH 7.0 approximately equal amounts of NMN and NAm were formed.

Increasing the dosage of NAD caused a decrease in the percentage of labeled products removed from the intestine and a decrease in the percentage of NR that was converted to NAm. The uptake of [<sup>14</sup>C]-carboxamide-labeled NAD was relatively slow (24% in 15 minutes) compared to the uptake of [<sup>14</sup>C]NAm (80% in 15 minutes), shown previously to be a nonsaturable process (10). This evidence indicates that NR is converted to NAm before absorption occurs and that this reaction is the rate-limiting step. It also indicates that the glycohydrolase or nucleoside phosphorylase involved here is saturable. Work by Grossman et al. showed that NRase from erythrocytes could be inhibited by NAm (14). Further evidence that cleavage to NAm occurs before absorption

is the observation that NR was not found in intestinal tissue or the perfusate fractions.

The conversion of NR to NAm probably occurs by phosphorylation. Purine nucleoside phosphorylases are common and have been shown to act on NR to varying degrees depending on the species and tissues being studied (15). It is also possible, however, that the phosphate effect seen in this study resulted from a stabilizing effect on the enzyme (15). The initial reaction in NAD digestion is not the result of intestinal bacterial action, since sterile intestinal contents formed NMN at the same rate as did nonsterile contents and the results obtained with germfree rats were the same as those found with normal animals. Fasting for up to 18 hours did not significantly alter the rate of hydrolysis or pattern of products formed.

The hydrolytic reaction sequence for the 5'-AMP portion of NAD was dephosphorylation to form adenosine, then deamination to form inosine. The reactions occurred very rapidly in the presence of isolated bacteria-free intestinal contents. Wilson et al. (16) showed this sequence of hydrolytic steps with 5'-AMP in everted sacs of hamster small intestine, but they did not distinguish between the action of intestinal secretions and that of epithelial cells. This study shows that mucosal membranes are not required to catalyze the hydrolysis of NAD to inosine.

Another difference in the intestinal handling of the two portions of NAD was the rate of absorption of radioactivity. Absorption of isotope was more rapid when adenine-labeled NAD was placed in the lumen of a perfused intestine than when carboxamide-labeled NAD was given (60% vs. 24%, respectively, in the perfusate at 15 minutes). These results clearly indicate that after the initial fission of NAD to NMN and 5'-AMP, the latter is rapidly hydrolyzed by intestinal secretions while the NMN is acted on by phosphatase and phosphorylase associated with, but not secreted by, the mucosal cells. Earlier work by Reis (8) indicated that in many animal and human tissues, the activity of specific 5'-nucleotidase is much greater than that of nonspecific phosphatases with the exception of a few tissues including intestinal mucosa. Also, alkaline phosphatase is known to be located in the brush border,

so the hydrolysis of NMN to NR by a non-specific phosphatase would be expected only in the presence of intestinal cell membranes.

#### ACKNOWLEDGMENTS

We would like to thank Larry Karnitz for his technical assistance with some of the experiments.

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