The following procedure has been used successfully on autonomic ganglia of dogs, cats and man, parotid glands of man and pancreas of cats and rats. The tissues were fixed in 10% formalin, Zenker's fixative or 90% alcohol, dehydrated, imbedded and sectioned in the routine manner. Following removal of the paraffin the sections were taken through a degrading series of alcohols and placed in water. One-half of each series of sections was placed in 0.1 N KOH for 45 minutes at room temperature and then thoroughly washed in tap water. The other half of each series of sections was allowed to remain in water during the same time. Both the control and the alkaline treated sections were then stained together in 0.25% or 1% toluidin blue, Harris hematoxylin and eosin or methyline blue-phloxine.

The cytoplasm of the parotid gland cells (Fig. 1) and the acinar cells of the pancreas (Fig. 2) is normally basophilic. Treatment of the sections with ribonuclease prior to staining inhibits the basophilic staining reaction. This indicates that the cytoplasmic basophilia is due to the presence of ribonucleic acid. In all instances, sections of the parotid gland (Fig. 3) and the pancreas (Fig. 4) that were treated with mild alkaline hydrolysis showed no basophilic staining in the cytoplasm. The DNA of the nuclei remained unaltered (Fig. 3 and 4). The basophilic staining reaction of the chromidial substance and the nucleoli of autonomic ganglion cells (Fig. 5), as has been previously reported by Caspersson(1), Gersh and Bodian(8), and others, is due to the presence of RNA. This reaction was inhibited by treatment of the sections with 0.1 N KOH (Fig. 6).

*Conclusions.* The alkaline hydrolysis method described above for the histochemical localization of RNA and its differentiation from DNA is a simple and convenient method for general use. It is apparently also as reliable as any procedure previously reported.

The differential hydrolysis of RNA and DNA probably is due to the position of the phosphoric acid residues connecting the nucleotides. Because of the greater resistance of the DNA to alkaline hydrolysis as compared to the resistance of RNA, Levene and Tipson(9) have advanced the opinion that the hydroxyls of carbons 3 and 5 of neighboring desoxyribose units of DNA are connected. In RNA, the hydroxyls of carbons 2 and 3 of the ribose units probably are involved. The data obtained in the present study afford further evidence of the resistance of DNA to mild alkaline hydrolysis.

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## Comparative Effect of Tryptophan, Niacin, and Nicotinamide in Forming Liver Pyridine Nucleotides.* (18964)

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The ability of tryptophan to replace niacin in the diet of the rat was first demonstrated by Krehl *et al.*(1), who used growth as a criterion of activity. More recent studies in our laboratory indicate that tryptophan and niacin, when incorporated in the ration in equimolar amounts, except at very high levels,

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1. Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A., *Science*, 1945, v101, 489.

TABLE I. Composition of Ration.

| | % |
|---|---|
| Sucrose | 49.2 |
| Gelatin | 40 |
| Sulfasuxidine | 1.5 |
| Salts IV | 4 |
| DL-methionine | .3 |
| Corn oil | 5 |
| Thiamine • HCl | .20 mg |
| Riboflavin | .30 mg |
| Pyridoxine • HCl | .25 mg |
| Calcium pantothenate | 2 mg |
| Biotin | .01 mg |
| Folic acid | .02 mg |
| Inositol | 10 mg |
| Choline • Cl | 200 mg |

Fat soluble vitamins supplied as fortified Haliver Oil, 2 drops per week per rat.

result in about the same production of liver pyridine nucleotides(2). At the levels normally fed in rations tryptophan serves as a physiologically more important precursor of tissue pyridine nucleotides than does niacin(3). The present report is a study on the rate and extent to which single administered doses of niacin, niacinamide and tryptophan are converted to liver pyridine nucleotides in animals depleted of these nucleotides by dietary means.

*Experimental.* Male Sprague-Dawley rats (200-250 g) were maintained on the ration indicated in Table I. When fed at a 40% level gelatin meets the requirements for all the essential amino acids except methionine and tryptophan. With a methionine supplement 40% gelatin serves as an inexpensive source of amino acids that is complete except for tryptophan. The water-soluble vitamins were fed at the usually prescribed levels (Table I) with the exceptions that niacin was omitted and choline fed at twice the normal level. The higher level of choline was included to prevent depletion of methyl groups when large amounts of niacin or its precursors are fed(4). Sulfasuxidine was added to minimize any synthesis of tryptophan or niacin by the intestinal flora. This ration is biologically complete except for the absence of tryptophan and niacin. The rats were fed this incomplete ration *ad libitum* for about 6 weeks to deplete their tissues of pyridine nucleotides. When analyses indicated that the liver pyridine nucleotides had decreased to about one-half the normal value the animals were utilized for the following experimental work.

Separate 0.0203 molar solutions of niacin, niacinamide, and DL-tryptophan were prepared. The tryptophan was dissolved with aid of dilute sodium hydroxide. Two ml per 100 g body weight of the appropriate solution was fed by tube directly into the stomach of the rat. This volume supplied the equivalent of 5 mg niacin per 100 g body weight. At the times after the stomach tubing indicated in Fig. 1, the animals were sacrificed, livers removed, frozen in a mixture of dry ice and acetone and analyzed for pyridine nucleotides by the method of Feigelson, Williams, and Elvehjem(5). This method measures the sum of diphospho- and triphosphopyridine nucleotides and this will be referred to simply as liver PN. The results are shown in Fig. 1, where each point represents the mean value of from 2-4 animals. The average deviation from the mean is ± 66.

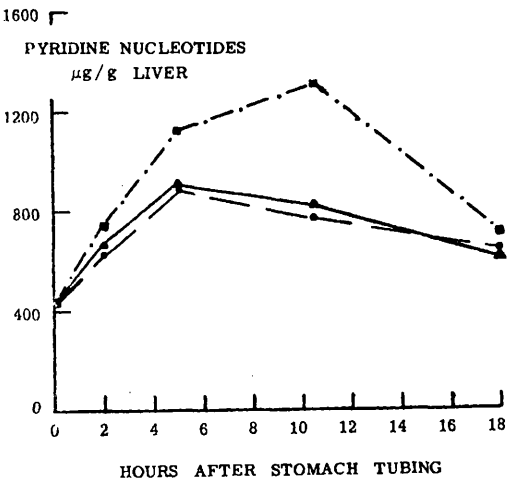


FIG. 1. The effect of a single dose of niacin, niacinamide, or tryptophan on the pyridine nucleotide level of rat liver. —▲— niacin, —■— • — niacinamide, —●— — tryptophan.

2. Feigelson, Philip, Williams, J. N., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

3. Williams, J. N., Jr., Feigelson, P., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v187, 597.

4. Handler, P., and Dann, W. J., *J. Biol. Chem.*, 1942, v146, 357.

5. Feigelson, Philip, Williams, J. N., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v185, 741.

TABLE II. Effect of Equimolar Supplements of Niacin, Niacinamide and Tryptophan Upon Liver Pyridine Nucleotide Formation.

| No. of animals | Supplement | Avg μg PN/g liver | | Range |
|---|---|---|---|---|
| 7 | 0 | 444 | 213 | 424-464 |
| 6 | 70 mg % niacin | 111 657 | | 553-780 |
| 6 | 70.5 mg % niacinamide | 565 | | 486-612 |
| 6 | 116 mg % DL-tryptophan | 635 | | 526-762 |

It is apparent that liver PN rapidly increased following the administration of all of the supplements. After 2 hours a definite increase was noted, and the PN level continued to rise to a maximum until 5-10 hours. This was followed by a decrease in PN until at 18 hours the liver PN was almost back to its starting level. Almost identical changes in PN followed the equimolar doses of tryptophan or niacin. The PN level rose to a maximum of 900 μg/g liver 5 hours after supplementation, then decreased slowly until at 18 hours it reached a level of 650 μg/g liver. The response to an equimolar amount of niacinamide, also administered by stomach tube as a single dose, was quite different from that of niacin and tryptophan as shown in the figure. Following niacinamide administration the PN level reached a maximum level of 1310 μg/g liver in 10 hours, which declined sharply to 720 μg/g liver at 18 hours.

To study this phenomenon further more animals were depleted of tissue PN as described above. They were divided into 4 groups and fed the basal ration plus the supplements, indicated in Table II. The supplements of tryptophan, niacin or niacinamide were equimolar and were incorporated directly into the ration. The level of the supplements was chosen so that the rats would consume daily approximately the same amounts of the supplements force-fed in the preceding experiments. The 4 groups of animals were fed the respective supplemented rations *ad lib.* for 2 weeks, and their livers analyzed for PN. The results are shown in Table II. In all supplemented groups there is a significant increase in PN level above the group receiving no supplement. There is no indication that when niacinamide is incorporated into the ration it is superior to either niacin or tryptophan as a precursor of PN.

*Discussion.* The present study was conducted under conditions of severe tryptophan deficiency and, even under such conditions, tryptophan administration resulted in the same PN formation as equimolar amounts of niacin. The identity of the curves after force-feeding equimolar amounts of niacin and tryptophan (Fig. 1) implies that, at least in the rat, the conversion of tryptophan to PN is not a minor but rather a major metabolic route of tryptophan metabolism. The apparently greater efficiency of a single dose of niacinamide as a precursor of PN as compared to niacin or tryptophan might be explained by the fact that niacinamide does not have to be amidated before forming PN, whereas niacin does.

The return of the liver PN level to almost the original value after 18 hours indicates a relatively short lifetime for the PN molecule and an inefficient conservation of the niacinamide residue from the catabolized PN.

*Summary.* Changes in the level of liver pyridine nucleotides (PN) of rats were studied at various times following the administration by stomach tube of equimolar amounts of tryptophan, niacin, or niacinamide. Tryptophan was observed to be equally effective as a PN precursor as equimolar amounts of niacin, whereas niacinamide produced significantly higher levels of liver PN than either tryptophan or niacin. When incorporated directly into the ration, however, niacin and tryptophan were again equivalent as PN precursors whereas, under these conditions, niacianamide was a slightly less efficient PN precursor than niacin or tryptophan.

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