that in regard to succinate stimulation of TTC reduction and oxygen uptake, tumor tissues behave not in an autonomous, unpredictable fashion, but rather in accord with equally rigid control as obtains in representative normal tissues.

Summary. Studies were made of the endogenous and succinate stimulated *in vitro* reduction of TTC by tissue slices from various human normal and cancer tissues as well as liver, kidney and spleen slices of control and tumor bearing mice. The per cent stimulation by succinate was not found to differ significantly in normal and tumor tissues. These findings were compared with previous studies of oxidative stimulation by succinate. It was also found shown that the presence of a tumor did not change the endogenous or succinate stimulated dehydrogenase activity of the liver, kidney or spleen of mice as measured by the TTC technique.

Received January 23, 1951. P.S.E.B.M., 1951, v76.

Interrelationships of Vitamin B_6, Niacin, and Tryptophan in Pyridine Nucleotide Formation.* (18517)

J. N. WILLIAMS, JR., PHILIP FEIGELSON, S. S. SHAHINIAN, AND C. A. ELVEHJEM.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Since an increased excretion of niacin and some of its derivatives have been shown to result from the administration of tryptophan (1-4), tryptophan probably functions as a metabolic precursor of niacin. These studies have been implemented considerably by the work of Heidelberger *et al.*(5,6). It has also been observed that rats deficient in vitamin B_6 (pyridoxine) do not metabolize tryptophan normally as shown by the excretion of xanthurenic acid in the urine(7-10). Other workers(11,12) have observed that deficiency of combinations of various B vitamins besides pyridoxine also markedly interfere with tryptophan metabolism. Spector(13) reported that pyridoxine apparently had little effect upon the conversion of tryptophan to niacin or N-methylnicotinamide as measured by excretion of the expected metabolites. Recently it has been reported from this laboratory(14, 15) that dietary tryptophan appears to be more important in the maintenance of tissue pyridine nucleotides (DPN and TPN) than dietary niacin although one might expect the reverse to be true because of the presence of the niacin residue in DPN and TPN.

* Published with the approval of the Director of the Wisconsin Agric. Exp. Station. Supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation; and by funds supplied by the Nutrition Foundation, New York, and The Robert Gould Research Foundation, Cincinnati, Ohio.

1. Singal, S. A., Briggs, A. P., Sydenstricker, V. P., and Littlejohn, J. M., *Fed. Proc.*, 1946, v5, 154.

2. Dann, W. J., *Fed. Proc.*, 1946, v5, 231.

3. Rosen, H., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1946, v163, 343.

4. Singal, S. A., Briggs, A. P., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, 1946, v166, 573.

5. Heidelberger, C., Abraham, E. P., and Lepkovsky, S., *J. Biol. Chem.*, 1948, v176, 1461.

6. Heidelberger, C., Abraham, E. P., and Lepkovsky, S., *J. Biol. Chem.*, 1949, v179, 151.

7. Lepkovsky, S., and Nielsen, E., *J. Biol. Chem.*, 1942, v144, 135.

8. Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., *J. Biol. Chem.*, 1943, v149, 195.

9. Miller, E. C., and Baumann, C. A., *J. Biol. Chem.*, 1945, v157, 551.

10. Miller, E. C., and Baumann, C. A., *J. Biol. Chem.*, 1945, v159, 173.

11. Ginoulhiac, E., *Acta Vitaminol.*, 1947, v1, 49.

12. Porter, C. C., Clark, I., and Silber, R. H., *Arch. Biochem.*, 1948, v18, 339.

13. Spector, Harry, *J. Biol. Chem.*, 1948, v173, 659.

14. Williams, Jr., J. N., Feigelson, P., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v137, 597.

15. Williams, Jr., J. N., Feigelson, P., Shahinian, S. S., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

In the present study the effect of a pyridoxine deficiency upon maintenance of liver pyridine nucleotides by dietary tryptophan has been investigated in an attempt to elucidate some of the existing problems concerning the function of pyridoxine in tryptophan metabolism. In addition the role of niacin in pyridine nucleotide maintenance has been further investigated.

Experimental. Two groups of male, weanling rats of the Sprague-Dawley strain weighing 40 to 50 g were used as experimental animals. One group of 30 rats (Group a) was given a pyridoxine-free and niacin-free ration (basal) and the other group of 12 rats (Group b) was given a niacin-free ration, *i.e.*, the basal ration plus adequate pyridoxine. The basal ration plus pyridoxine consisted of vitamin test casein 25, Salts IV(16) 4, corn oil 5, vitamin mix 2, and sucrose 64%. The 2 groups of rats were fed their respective rations and watered *ad libitum* for 9 months during which period 14 of the pyridoxine-deficient animals died with noticeable symptoms of the deficiency. No mortality was observed among the group receiving the niacin-free plus pyridoxine ration. At the end of the 9-month period the average weight of the remaining pyridoxine-deficient rats was approximately one-half that of the control group fed the niacin-free ration. After this period the rats in the pyridoxine-deficient group were separated at random into 3 groups and given the following supplements: Group 1a, none; Group 2a, 1.25 mg pyridoxine hydrochloride per 100 g ration (5 times the normal level); and Group 3a, 150 mg niacin per 100 g ration (100 times the level ordinarily fed to rats in synthetic rations). The group receiving the niacin-free plus pyridoxine ration for 9 months was divided into 2 groups and supplemented as follows: Group 1b, none; Group 2b, 1.35 g DL-tryptophan per 100 g ration (5 times the level fed in an 18% casein ration). The levels of the supplements fed were arbitrarily chosen in order to insure a large excess of each supplement. The supplemented rations were fed to the respective groups for 2 weeks after which the animals were sacrificed for determination of liver pyridine nucleotide concentrations by the method of Feigelson, Williams, and Elvehjem (17). Weight data were recorded throughout the feeding periods to observe if any correlation of weight with liver pyridine nucleotide concentration occurred.

TABLE I. Weight Changes During the Feeding Periods.

Group	Ration	Period (wk)	Avg change in wt per wk, g
a	— Pyridoxine — Niacin	36	+ 4.9
b	+ Pyridoxine — Niacin	36	+ 9.9
1a	— Pyridoxine — Niacin	2	0
2a	+ 1.25 mg % pyridoxine · HCl — Niacin	2	+24.2
3a	— Pyridoxine + 150 mg % niacin	2	— 5.7
1b	+ Pyridoxine — Niacin	2	+ 4.6
2b	+ Pyridoxine — Niacin + 1.35 g % DL-tryptophan	2	— 5.0

Results and discussion. The results of the weight changes during the experiment for the various groups are presented in Table I. The results are expressed as the average change in weight per week in order to compare the smaller supplemented groups with themselves rather than with the 2 large groups as a whole. It can be observed that the animals receiving no pyridoxine (Group a) gained an average of only one-half as much as the group receiving pyridoxine (Group b). Since the growth rate of both groups of rats had reached a plateau before the end of 36 weeks, the average weekly weight gain was lower than would be expected for the young growing rat. Nevertheless, the average weight of Group b was approximately double that of Group a at the end of this period.

After supplementation with the various substances given in the table for 2 weeks,

16. Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, v109, 659.

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

TABLE II. Liver Pyridine Nucleotide Concentrations of the Groups After the 2-week Supplementation Period.

Group	Ration supplement	No. of rats	Liver pyridine nucleotide conc. (γ per g liver)
1a	0	5	807 ± 55*
2a	1.25 mg % pyridoxine • HCl	6	800 ± 54
3a	150 mg % niacin	5	1200 ± 150
1b	0	6	890 ± 60
2b	1.35 g % DL-tryptophan	6	1300 ± 73

* Stand. error of the mean.

definite changes in weight occurred in all the groups except the pyridoxine-deficient group receiving no extra supplement (Group 1a). The inclusion of pyridoxine in the ration of the pyridoxine-deficient rats (Group 2a) caused the expected sharp increase in growth, which was almost as great as the growth of young weanling rats fed a complete ration. When niacin was fed at a 160 mg % level to the pyridoxine-deficient rats (Group 3a) a significant decrease in weight occurred demonstrating a possibly toxic effect of niacin fed at this level. The rats receiving pyridoxine throughout the 9-month period and given no extra supplement for the extra 2-week period (Group 1b) continued to gain weight slowly. When 1.35 g % of DL-tryptophan was added to the control ration (Group 2b), the rats lost weight significantly during the 2-week supplementation period showing a toxic or imbalance effect of the high level of tryptophan.

In Table II are presented the results of the analyses for liver pyridine nucleotides of the various groups of animals after the 2-week supplementation period. The results are expressed as micrograms (γ) DPN plus TPN per gram of fresh liver. From the results of Groups 1a and 2a it appears that a pyridoxine deficiency has no demonstrable effect upon the production of liver pyridine nucleotides from dietary tryptophan. It is possible that enough pyridoxine was retained in the tissues of the rats from the weanling age to satisfy the enzymatic requirements for the conversion. However, some reduction in pyridine nucleotide concentration should have been observed if pyridoxine were highly important in the conversion of tryptophan. In an experiment not reported in this paper in which desoxypyridoxine was fed to both weanling and adult rats for several weeks, the liver pyridine nucleotide concentration was unaffected by the pyridoxine antagonist even when measured in the animals in a moribund state brought on by the antagonist. The animals, however, had not been depleted first of tissue pyridine nucleotides.

From the results of Group 3a it appears that niacin at the high level fed is able to spare liver pyridine nucleotides. Whether the niacin acts to spare the nucleotides by utilization in their synthesis or by interfering with the enzymatic breakdown of those already present is still open to question at this time. It is known that niacinamide is an inhibitor of the enzymes that destroy the pyridine nucleotides(18) as well as those that utilize them(19).

The results from Groups 1b and 2b indicate that tryptophan in excess of the amounts required for building protein can be converted to pyridine nucleotides rather than being completely removed by other metabolic pathways.

When the results in Tables I and II are compared there appears to be no positive correlation between weight changes brought about by the supplements in question and liver pyridine nucleotide concentration. In fact the 2 groups with the highest pyridine nucleotide concentrations (Groups 3a and 2b) actually lost weight during the supplementation period. The group gaining weight at the fastest rate (Group 2a) moreover has the same liver pyridine nucleotide concentration as the group neither gaining nor losing weight (Group 1a). Although the pyridine nucleotides are undoubtedly necessary for the normal functioning of an animal and therefore for growth, it is probable that the range of concentrations observed in these experiments

18. Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, 1941, v35, 502.

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

is actually in excess of the amounts necessary for adult rats. Pyridine nucleotide concentrations which could be correlated with growth changes would probably be obtained when the concentrations are lower than those required by the animal for normal function.

Summary. 1. It has been observed that a pyridoxine deficiency in the rat does not disturb the normal conversion of tryptophan to liver pyridine nucleotides to any demonstrable extent. 2. Niacin fed to pyridoxine-deficient rats spares liver pyridine nucleotide concentrations. 3. Tryptophan fed in excess along with normal dietary protein in a complete ration increases liver pyridine nucleotides concentrations above normal demonstrating that the excess metabolite can be utilized rather than simply degraded and excreted.

†One hundred g of vitamin mixture contained the following vitamins in a sucrose base: thiamine hydrochloride 10 mg, riboflavin 15 mg, pyridoxine hydrochloride 12.5 mg, calcium pantothenate 100 mg, biotin 0.5 mg, folic acid 1 mg, choline chloride 5 g, and i-inositol 0.5 g.

Received January 24, 1951. P.S.E.B.M., 1951, v76.

Toxicity of Rubber Stoppers for Tissue Cultures.*† (18518)

RAYMOND C. PARKER, JOSEPH F. MORGAN, AND HELEN J. MORTON.
From the Connaught Medical Research Laboratories, University of Toronto.

During studies on the development of a synthetic medium for animal cells in tissue culture(1-3), it was observed that chance contact of the synthetic feeding mixtures with the rubber stoppers used to close the culture tubes often killed the cells within 48 hours. The following experiments were undertaken in an attempt to reduce the toxicity of the rubber stoppers by various methods of chemical treatment and to test the relative toxicity of various types of rubber.

Methods. Chick embryo mesenchyme tissues, derived from skeletal muscle, were cultivated on the inner surface of standard Pyrex test tubes and supplied with a fluid synthetic mixture (No. 199), which was withdrawn and replaced at regular intervals. Full details of the culture methods and of the synthetic mixture have already been reported(1). Unless otherwise specified, the rubber stoppers that were tested were of the standard black laboratory variety, size No. 1, obtained from commercial supply houses. After chemical treatment (Tables I and II), 9 stoppers

TABLE I. Effect of Solvent Extraction on the Toxicity of Black Rubber Stoppers Tested in Tissue Culture.

Exp. No.	Treatment*	Survival (days)†
A	Control. Culture fluid not exposed to rubber	33
B	Control. Culture fluid exposed to untreated black stoppers	2
1	Hot ether (6 hr)‡	6
2	Hot acetone (6 hr)‡	3
3	Hot alcohol (6 hr)‡	3.2
4	Hot ether (2 hr), hot acetone (2 hr), hot alcohol (2 hr)‡	9.2
5	Cold ether (1 wk)§	8.6
6	Cold acetone (1 wk)§	2.5
7	Cold alcohol (1 wk)§	7
8	Cold ether (2 days), cold acetone (2 days), cold alcohol (2 days)§	7.3

* In each treatment, 9 new black stoppers were extracted with 250 ml of solvent, under the conditions specified, and were then boiled in 3 changes of distilled water.

† Each figure represents the average survival time of 6 cultures in each of the fluids tested.

‡ Extracted in a Soxhlet apparatus.

§ Solvent changed daily.

* This work was supported, in part, by grants from the National Cancer Institute of Canada and the Ontario Cancer Treatment and Research Foundation.

† With the technical assistance of Miss B. J. Barrett, Miss E. A. Burr and Miss O. E. Rockett.

1. Morgan, J. F., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

2. Morton, H. J., Morgan, J. F., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 22.

3. Parker, R. C., Morgan, J. F., and Morton, H. J., *J. Cell. Comp. Physiol.*, 1950, v36, 411.