THE EFFECT OF A NICOTINIC ACID DEFICIENCY UPON THE COENZYME I CONTENT OF ANIMAL TISSUES*

By A. E. AXELROD, ROBERT J. MADDEN, and C. A. ELVEHJEM

(*From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison*)

(Received for publication, August 7, 1939)

It has been demonstrated, largely through the work in the Warburg and the von Euler laboratories, that nicotinic acid amide is a constituent of both coenzyme I and coenzyme II. The extreme importance of these pyridine nucleotides in cellular physiology is now well recognized. These facts have stimulated many attempts to establish the vitamin-like nature of nicotinic acid or the amide and the relation of these substances to the synthesis of the pyridine nucleotides in the body.

The importance of nicotinic acid and certain of its derivatives in bacterial nutrition has been established by Knight (1), Mueller (2), Koser *et al.* (3), and Fildes (4). There is no clear cut evidence that nicotinic acid is required preformed in the diet of the rat. Von Euler *et al.* (5) have reported a lowering of both the nicotinic acid and coenzyme I contents in rats fed a vitamin B-free ration. However, it remains difficult to demonstrate an uncomplicated deficiency of the pyridine nucleotides or of any of their possible precursors in the rat. This species, therefore, remains unsuitable for use in studies relating to the function of nicotinic acid. The demonstration of the activity of nicotinic acid in the treatment of canine blacktongue (6) suggested the use of species other than the rat in such studies. In rapid succession it was shown that

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Supported in part by grants from the Wisconsin Alumni Research Foundation.

A preliminary report of this work was given before the Sixth annual meeting of the American Institute of Nutrition at Toronto, Canada, April 26, 1939.

nicotinic acid was also a dietary essential for man (7–10), pig (11), and monkey (12).

In this paper we wish to present data to show that there is a significant decrease in the coenzyme I content of certain tissues in an uncomplicated nicotinic acid deficiency produced in the dog and the pig. This observation is to be taken as evidence for the fact that a part, at least, of the ingested nicotinic acid is utilized for the synthesis of the pyridine nucleotides.

Methods

The dogs were brought to the laboratory shortly after weaning and were given a complete diet for 2 weeks. They were then placed on the modified Goldberger diet previously described (6). The ration was not steamed. This greatly facilitated food consumption studies and no difficulty was encountered in getting the dogs to eat this dry ration. In addition, the dogs were fed daily by pipette 100 micrograms of thiamine[1] and 100 micrograms of riboflavin. The dogs were weighed weekly until their growth rate decreased, when daily weighings were instituted. This permitted a close check on the course of the deficiency. When blacktongue developed, some of the dogs were sacrificed for analysis and the others given nicotinic acid orally for some time before being taken for analysis.

Weanling pigs[2] from different litters were placed on experiment at 10 weeks of age. Their weights ranged from 30 to 38 pounds. The animals were segregated and given the following basal ration: yellow corn 85, purified casein 10, $CaCO_3$ 1, $Ca_3(PO_4)_2$ 1, NaCl 1, Fe (as Fe_2O_3) 0.01, cod liver oil 2. The ration was mixed weekly and fed *ad libitum*. Nicotinic acid was administered, as indicated, either by subcutaneous injection or by incorporating it into the ration. Hemoglobin determinations were made weekly, the blood samples being taken from a superficial ear vein. Weighings were made biweekly.[3]

[1] We are indebted to Dr. D. F. Robertson of Merck and Company, Inc., for generous supplies of thiamine and nicotinic acid.

[2] We are indebted to Professor J. M. Fargo of the Department of Animal Husbandry for the pigs used in our work.

[3] We are indebted to Works Progress Administration Project No. 8649 for assistance in the care of animals.

The method used for the coenzyme I determination is fully described in the preceding publication (13). Two different samples of each tissue were analyzed for coenzyme I and each sample was run in duplicate. Dry weight determinations were made on all tissues.

EXPERIMENTAL

The experiments to be described may be conveniently divided into two classes: Series A, those done before pure coenzyme I became available to us; Series B, those done after we had access to pure coenzyme I.

Series A—Since we had no pure coenzyme I with which to standardize our assays and thus obtain absolute values, it was necessary to control all analyses made on the tissues from deficient animals with simultaneous analyses of the corresponding tissues from a normal animal. All assays were carried out under strictly standardized conditions and the results gave comparative values.

Our first approach was to determine the effect of a nicotinic acid deficiency upon the coenzyme I content of red blood cells.

Dog I—This dog rapidly developed the deficiency and just before death exhibited very severe symptoms of blacktongue; *i.e.*, an excessive and very viscous salivary excretion, diarrhea, fiery red appearance of the periphery of the tongue and of the buccal mucosa, and extreme weight loss. Both the hematocrit and hemoglobin values remained normal. Blood samples were taken before any symptoms could be detected and during the period of extreme deficiency. Arrows in the growth curve (Fig. 1) mark the times at which analyses were made. A number of normal dogs were used for controls and their blood was always assayed simultaneously with that of the experimental dog. At no time could we detect any variation from the normal in the coenzyme I content of the red blood cells of the deficient dog.

Dog II—In this case, nicotinic acid was given orally at the times indicated in the growth curve (Fig. 1). As with Dog I, blood samples were taken during varying stages of the deficiency and the coenzyme I content of the red blood cells compared with that of normal dogs. The times of analyses are indicated by arrows in the growth curve. Again, we could detect no change in the coenzyme I content of the red blood cells even under conditions

of extreme deficiency. These results were fully confirmed by experiments to be described later and we may conclude that a severe nicotinic acid deficiency had no effect upon the coenzyme I content of the blood of dogs.

Our attention was then directed to a study of the coenzyme I content of other tissues from these deficient animals.

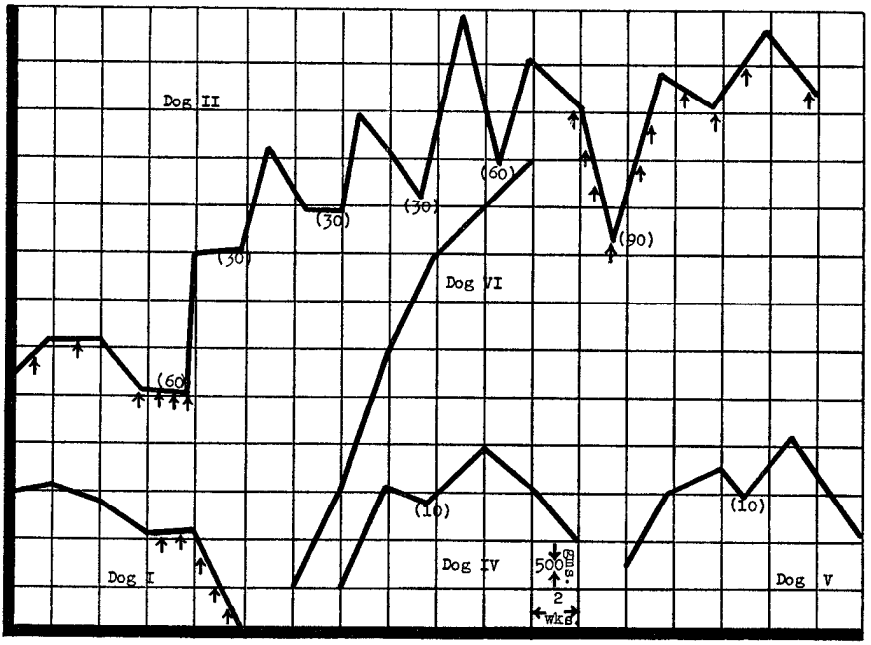

FIG. 1. Growth curves of Dogs I, II, IV, V, VI. The arrows indicate the times at which the blood was analyzed for its coenzyme I content. The numbers in parentheses indicate the mg. of nicotinic acid fed.

Dog II was sacrificed while in the last stages of the deficiency and the brain (gray matter), liver, kidney cortex, muscle (gastrocnemius), and blood were analyzed for their coenzyme I content. The corresponding tissues from Dog III were used for normal controls. Dog III, an adult animal weighing 15 kilos, had been kept on the modified Goldberger diet for 3 months without showing any signs of a nicotinic acid deficiency. 240 mg.

of nicotinic acid were administered orally during the 10 day period previous to the analysis. The following results were obtained: (*a*) the brain, kidney cortex, and blood of Dog II showed no significant change, and (*b*) the liver of Dog II showed a decrease of 70 per cent below the value for the liver from Dog III and the muscle showed a decrease of 22 per cent below the value for the muscle taken from Dog III.

Pig 423—After a depletion period of 5 weeks on the basal ration, growth had practically ceased and the diet was then supplemented with 100 mg. of nicotinic acid per kilo. The growth response was pronounced and normal growth was maintained during the entire course of the experiment. At no time did the animal evidence any deficiency symptoms.

Pig 378—The ration of this pig was not supplemented with nicotinic acid. Growth was very slow and the deficiency symptoms noted at the end of the experiment were (1) severe diarrhea, (2) unhealthy, scurfy skin, and (3) general moribund condition.

Pig 877—After 4 weeks on the basal ration this pig exhibited very severe symptoms and therapy was begun immediately. 6 gm. of nicotinic acid were injected over a period of 6 weeks. The ration was then supplemented with 100 mg. of nicotinic acid per kilo. A slow response was noted after 3 weeks of therapy and, at the completion of the experiment, all symptoms had disappeared and the animal was growing at a normal rate.

Pig 392—This pig was given only the basal ration. After 10 weeks, growth had practically ceased and mild deficiency symptoms appeared. At this time, a decided growth response was noted. Suspecting coprophagy, we removed the pig to a wire-bottomed cage which was flushed with water twice daily. Growth was halted for 3 weeks, when another growth response occurred. The pig was slaughtered at this time. It is difficult to explain the anomalous behavior of this animal.

None of the pigs evidenced any signs of anemia. Relative values were obtained by comparing the tissues of Pigs 423 and 877 with those of Pigs 378 and 392 respectively. The brain, kidney cortex, liver, muscle, and red blood cells were analyzed.

In Pigs 423 and 378, the coenzyme I contents of the kidney cortex and brain were approximately the same, while the liver of

Pig 378 showed a decrease of 50 per cent and its muscle a decrease of 87 per cent below the values of the corresponding tissues from Pig 423.

In Pigs 877 and 392, only the muscle of Pig 392 showed a decrease in its coenzyme I content. This was a decrease of 95 per cent. It is to be noted that Pig 392 behaved in the anomalous manner described above and its failure to show a decrease in the coenzyme I content of the liver may be due to the ingestion of small amounts of nicotinic acid which this pig was obviously able to procure. This small amount of nicotinic acid was unable to maintain the coenzyme I content of the muscle.

TABLE I

Effect of Nicotinic Acid Deficiency upon Coenzyme I Content of Dog Tissues

Tissue	Coenzyme I per gm. fresh tissue		
	Dog IV	Dog V	Dog VI
	micrograms	*micrograms*	*micrograms*
Kidney cortex	1130	1070	1000
Blood*	60	66	61
Liver	714	650	1185
Gastrocnemius muscle	295	427	490

* No significant changes were found in the hematocrit values.

No change was noted in the coenzyme I content of red blood cells from the deficient pigs. However, the small coenzyme I content of pig blood makes these determinations rather unreliable.

Series B—Owing to the availability of pure coenzyme I, these assays could be standardized and the absolute values for the coenzyme I content determined.

Dogs IV and V were kept on the modified Goldberger ration except for a single oral supplement of 10 mg. of nicotinic acid after the first indication of decreased growth. At the time of sacrifice Dog V exhibited mild symptoms and Dog IV very severe symptoms of blacktongue (Fig. 1).

Dog VI was given the basal diet plus 5 mg. of nicotinic acid daily by pipette. Normal growth was maintained throughout the course of the experiment and no deficiency symptoms were noted.

Table I lists the tissues analyzed; the values are given in micrograms of coenzyme I per gm. of fresh tissue. Significant decreases

in coenzyme I content are to be noted in the livers of Dogs IV and V and in the muscle of Dog IV.

The moisture content of the tissues from the deficient animals remained normal.

It became of interest to determine whether the coenzyme I content of tissues could be increased beyond their normal value by the administration of nicotinic acid. The rat was chosen as the experimental animal. An amount equivalent to 1 mg. of nicotinic acid per kilo of live weight was administered daily to three rats by intraperitoneal injection. The rats were kept on the stock diet. The tissues of three normal rats of approximately the same age and weight were also analyzed. The results are

TABLE II

Effect of Addition of Excessive Amounts of Nicotinic Acid upon Coenzyme I Content of Rat Tissues

Tissue	Coenzyme I per gm. fresh tissue					
	(13)*	(14)*	(22)*	Normal	Normal	Normal
	micro-grams	*micro-grams*	*micro-grams*	*micro-grams*	*micro-grams*	*micro-grams*
Liver	1260	1170	1040	1070	935	1210
Brain gray matter	340	378	348	300	300	320
Kidney cortex	1100	1160	1080	965	1050	1130
Gastrocnemius muscle	805	850	770	710	705	850

* The values in parentheses indicate the number of injections given.

given in Table II as micrograms of coenzyme I per gm. of fresh tissue.

It is evident that the coenzyme I content of the tissues of the rat was not increased by the administration of extra amounts of nicotinic acid. These findings confirm those obtained by von Euler (5).

DISCUSSION

In the light of our present knowledge, the basal rations employed produced a definite nicotinic acid deficiency in the dog and the pig. The ability to produce such a deficiency has greatly facilitated all efforts directed toward a study of the function of nicotinic acid. Our experiments demonstrate that a nicotinic acid de-

ficiency results in a lowered coenzyme I content of the liver and muscle. The extent to which the coenzyme I content was affected varied considerably and can be considered to be a function of the degree of the deficiency. At the present, however, no positive correlations can be made.

It is significant that the brain, kidney cortex, and blood always maintained their normal coenzyme I content, decreases being noted only in the case of liver and muscle. We may assume that the normal level of coenzyme I in the former tissues is absolutely essential, a decrease being incompatible with life. This assumption does not hold true for the liver and muscle which apparently can maintain their vital functions, although presumably to a greatly lessened degree, in the absence of their normal content of coenzyme I. The possibility still exists, however, that in the absence of coenzyme I the transfer of hydrogen from metabolite to oxygen is effected through the medium of other carrier systems. In this manner the normal metabolism could be maintained.

Knight (1) and Fildes (4) have demonstrated the requirement of *Staphylococcus aureus* and *Proteus* respectively for nicotinic acid or certain of its closely related derivatives. It is known that these organisms can synthesize the V factor, which is thought to be either coenzyme I or coenzyme II, and the conclusion was drawn that nicotinic acid serves as a precursor for the synthesis of these coenzymes. Evidence supporting the existence of such a relationship in the case of the human has been given by Kohn (14) and Vilter, Vilter, and Spies (15). Kohn has demonstrated an increase in the blood factor V of both normal subjects and pellagra patients following the ingestion of large amounts of nicotinic acid and Vilter, Vilter, and Spies have observed a decrease in the factor V content of the blood of pellagrins which could be remedied through nicotinic acid therapy. In confirmation of our findings, Kohn and Dann (16) found no significant change in the blood factor V level of the dog during a nicotinic acid deficiency. Intravenous administration of nicotinamide had no effect upon the blood factor V level.

It is to be noted that our experiments yielded no information concerning the coenzyme II content of the tissues studied. Assuming the existence of a state of equilibrium between coenzyme I and coenzyme II, we may expect to find changes in the coenzyme II content which correspond to those found for coenzyme I.

Whether the decrease in the coenzyme I content of the liver and muscle observed in the animals used in this study is sufficient to account for the gross symptoms observed cannot be answered at this time.

The ability of the vitamins thiamine and riboflavin to serve as precursors of essential coenzymes is now well established. Evidence is now being rapidly accumulated to show that a third vitamin, nicotinic acid, may also function in such capacity.

SUMMARY

1. A nicotinic acid deficiency in the dog and pig results in a lowered coenzyme I content of the liver and muscle.

2. No effect is noted upon the coenzyme I content of the brain, kidney cortex, and blood.

3. The coenzyme I content of rat tissues cannot be increased by the administration of excessive amounts of nicotinic acid.

BIBLIOGRAPHY

1. Knight, B. C. J. G., *Biochem. J.*, **31,** 731 (1937).
2. Mueller, J. H., *J. Biol. Chem.*, **120,** 219 (1937).
3. Koser, S. A., Dorfman, A., and Saunders, F., *Proc. Soc. Exp. Biol. and Med.*, **38,** 311 (1938).
4. Fildes, P., *Brit. J. Exp. Path.*, **19,** 239 (1938).
5. von Euler, H., Schlenk, F., Melzer, L., and Högberg, B., *Z. physiol. Chem.*, **258,** 219 (1939).
6. Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Biol. Chem.*, **123,** 137 (1938).
7. Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. Am. Med. Assn.*, **109,** 2054 (1937).
8. Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *Proc. Soc. Exp. Biol. and Med.*, **37,** 405 (1937).
9. Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. Am. Med. Assn.*, **110,** 622 (1938).
10. Harris, L., and Hassan, A., *Lancet*, **2,** 1467 (1937).
11. Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., *Biochem. J.*, **32,** 10 (1938).
12. Harris, L. J., *Biochem. J.*, **32,** 1479 (1938).
13. Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, **131,** 77 (1939).
14. Kohn, H. I., *Biochem. J.*, **32,** 2075 (1938).
15. Vilter, R. W., Vilter, S. P., and Spies, T. D., *J. Am. Med. Assn.*, **112,** 420 (1939).
16. Kohn, H. I., and Dann, W. J., *Proc. Am. Physiol. Soc., Am. J. Physiol.*, **126,** 557 (1939).