Liver as the Site of Synthesis of Nicotinic Acid from Tryptophane in Rats.* (21094)

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The dietary tryptophane is converted into nicotinic acid in the body of the rat(1). While some workers claim that this transformation takes place in the intestine by the activity of the intestinal micro-organisms(2,3) others have shown that it takes place in the tissues of the animal(4,5). On incubation of liver homogenates with tryptophane small amounts of nicotinic acid and quinolinic acid have been obtained, which indicate that possibly liver is the organ concerned in this transformation(6-9). Henderson and Ramasarma (10), however, did not confirm the above observation. In order to determine whether liver is the organ concerned in the synthesis of nicotinic acid from tryptophane in rats urinary excretions of some of the metabolites of nicotinic acid after administration of tryptophane were estimated in rats after their livers were poisoned by injection of carbon tetrachloride. The effect of incubation of livers from normal and carbon tetrachloride-treated rats with tryptophane on the production of nicotinic acid and quinolinic acid was also studied.

Experimental. Twenty-four hour urinary excretion of nicotinic acid, quinolinic acid, and N-methyl nicotinamide in normal and carbon tetrachloride injected rats. Rats, weighing 125-175 g, were housed in individual metabolism cages and urine collected in flasks containing one cc concentrated hydrochloric acid. They were fed a nicotinic acid-deficient diet (11) with which dl-tryptophane was mixed at the level of 1%. The daily urinary excretions of nicotinic acid, quinolinic acid, and N-methyl nicotinamide (N-MN) were estimated for one week. After this period all the animals were given a daily intraperitoneal injection of carbon tetrachloride (0.03 cc/kg), and the same metabolites were estimated in the urine. All the animals were weighed on alternate days and their food consumptions were noted. The results are given in Table I.

TABLE I. 24 Hr Urinary Excretion of Nicotinic Acid, Quinolinic Acid, and N-MN by 12 Rats before and after Injection of Carbon Tetrachloride. Avg of 6 days' excretion.

Carbon tetrachloride	Nicotinic acid (γ)	Quinolinic acid (γ)	N-MN (γ)
Before inj.	50 ± .09*	474 ± 67	603 ± 94
After 7 days inj.	29 ± .07	117 ± 29	187 ± 18

* Stand. error.

Incubation of liver homogenates with tryptophane. Two groups of rats (60-150 g in weight) were used. The first group received 1% dl-tryptophane-supplemented nicotinic acid-deficient diet only, while the second group received the same diet with a daily intraperitoneal injection of carbon tetrachloride for 7 days (0.03 cc/kg). Injection of carbon tetrachloride was given to the animals of the second group 2 days before they were placed on experimental diet. The animals were then killed and their livers quickly removed and homogenized in a chilled mortar with ice cold Krebs Ringer phosphate buffer solution of pH 7.4. Aliquots of the liver homogenates (400-500 mg) were taken in 2 weighed flasks. One of the flasks contained 2 cc Krebs Ringer phosphate buffer and 5 cc distilled water, while the second flask contained in addition 10 mg of dl-tryptophane in 5 cc solution. The flasks were quickly weighed after the addition of liver homogenate. The first flask was immediately boiled for half an hour and the second flask was incubated for half an hour at 37°C. After incubation the flask was boiled for half an hour; 2 cc of 50% trichloroacetic acid was then added to the flask and the volume made up to 25 cc and filtered. Ten cc filtrate was then taken for the estimation of nicotinic acid according to the method previously described (12). Another 10 cc portion was autoclaved for one hour with N sulfuric acid according to the method of Sarett(13), nicotinic acid was then estimated by the chemical method

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TABLE II. Incubation of Liver Homogenate from Normal and Carbon Tetrachloride Treated Rats with Tryptophane. (Incubation period 30 min.)

	Nicotinic acid (mg %)		Quinolinic acid (mg %)	
	Without substrate	With substrate	Without substrate	With substrate
Normal (7)	14.33 ± 2.1	14.58 ± 1.8	50.94 ± 13.8	86.28 ± 12.8
Injected (7)	12.22 ± .9	12.10 ± 1.1	35.10 ± 10.7	52.90 ± 7.2
t	.07	.03	1.2	2.26

Figures in parentheses indicate the number of animals.

(12), and quinolinic acid values have been calculated by multiplying the increase of nicotinic acid by 5.8 as described by Sarett(13). The results are shown in Table II.

Discussion. It will be seen from Table I that the urinary excretion of nicotinic acid, quinolinic acid and N-MN was greatly decreased in rats injected with carbon tetrachloride, which damaged the liver. Incubation studies showed that livers of both groups of animals could not convert tryptophane to nicotinic acid. This observation agrees with that of Henderson and Ramasarma(10). It was observed that homogenized liver from normal rats could convert tryptophane to quinolinic acid more efficiently than the livers of animals receiving injection of carbon tetrachloride. This finding supports the observation of Wang *et al.*(7), but was in disagreement with works of Henderson(10). These studies indicate that liver plays a role in the transformation of tryptophane to nicotinic acid. It may be either the site for the synthesis of nicotinic acid or it might supply the essentials necessary for the conversion of tryptophane to nicotinic acid.

Summary. 1. Intraperitoneal injections of carbon tetrachloride in rats fed a dl-tryptophane supplemented nicotinic acid-deficient diet lowered urinary excretion of nicotinic acid, quinolinic acid and N-MN. 2. Liver homogenates of normal rats converted tryptophane to quinolinic acid more efficiently than that of carbon tetrachloride-treated rats. 3. It is suggested that liver is the principal site of synthesis of nicotinic acid and quinolinic acid from tryptophane.

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