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Effect of Feeding Pyridine Derivatives to Young Rats on a High Protein Diet.

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The inhibition of the growth of young rats on a low protein diet by nicotinamide but not by nicotinic acid at a dietary level of 1% led Handler and Dann[1] to postulate that the more rapid methylation of nicotinamide resulted in a greater decrease in the available "methyl donors" than that which occurred following ingestion of nicotinic acid. They were able to demonstrate that the administration of methionine to rats fed large amounts of nicotinamide resulted in an increase in the "trigonelline"* fraction of the urine and an increase in the growth rate. However, the need for methylation in the process of detoxication does not fully explain the reason for the high toxicity of coramine (nikethamide) which is much more slowly methylated than even the relatively nontoxic nicotinic acid.[2,3] It is possible that much

[1] Handler, P., and Dann, W. J., *J. Biol. Chem.*, 1942, **146**, 337.

* This "trigonelline" fraction has since been shown to consist chiefly of nicotinamide methochloride (N1 methyl nicotinamide).

[2] Ellinger, P., and Coulson, R. A., *Biochem. J.*, 1944, **38**, 265.

of the toxicity of pyridine, nicotinamide and coramine is inherent in the molecular structure and is not due solely to any secondary effect such as the depletion of the store of methionine or choline since the subcutaneous administration of these compounds in relatively small doses leads to immediate manifestations of toxicity.[4]

This communication reports the influence of the oral administration of 17 pyridine derivatives on rat growth and on the weight and on some aspects of the composition of the rat liver.

Experimental. Preparation of Basal Rat Diets. For the entire series of experiments the rats were maintained on a comparatively high protein diet which consisted of cottonseed oil, 15, casein, 25, salts,[5] 5 and starch, 55 parts. The high protein diet was adopted as a protective measure inasmuch as many of the compounds are quite toxic. Experimental diets were prepared by inclusion of the appropriate supplement in the basal diet. Each rat also received 1.0 ml of Brewers' yeast extract a day (equivalent to 1 g of dry yeast) and 1 drop of Percomorph oil a week. The experimental animals were an inbred mixture of the Sprague-Dawley and Illinois strains. To avoid any difficulties due to sexual differences equal numbers of males and females initially weighing from 45-50 g were utilized for each group. In no instance were 2 rats from the same litter used in the same experimental group.

Effect of the Methylated Pyridine Nucleus on the Growth Rate. His[6] reported the isolation of methyl pyridinium hydroxide from the urine of a dog which was fed repeated doses of pyridine. Since this publication in 1887 it has been generally supposed that pyridine is methylated in the process of "detoxication" and eliminated in the urine as the methyl derivative.

Experiments were devised to test the effect of feeding several methylated and nonmethylated pyridine derivatives to young rats. Groups of rats were fed nicotinic acid, nicotinamide, coramine, alpha, beta and gamma picoline, isonicotinic acid and alpha picolinic acid at levels of 1% of the diet for 28 days. The respective N-methyl derivatives of these compounds were synthesized by methylating the parent substance with methyl iodide or dimethyl sulphate followed by the subsequent conversion to the chloride salt through treatment with silver chloride. These methylated compounds were recrystallized several times and added to the basal diet at concentrations which were equivalent to 1% of their immediate precursors on the basis of their respective molecular weights.

Whereas methylation of some of the pyridine derivatives resulted in a decrease in the degree of growth inhibition (Table I) other compounds were unaffected or actually rendered more toxic by this procedure. In general, the rats grew at a rate which was roughly proportional to their food intake regardless of the compound administered. This would indicate that although the appetite decreased these substances had no effect on the intestinal absorption or the assimilation of the diet. There was little correlation between the acute toxicity as measured by subcutaneous injection[4] and the toxicity as measured by the degree of growth inhibition following prolonged administration by dietary means. The addition of choline or methionine to diets containing beta picoline or coramine failed to increase the growth rate significantly over that of rats which did not receive any lipotropic compound. It would appear that any growth inhibition caused by beta picoline or coramine is not reduced by the addition of substances that are necessary for transmethylation, or, that the process of methylation has little connection with the decreased appetite and the resultant growth decrease if an otherwise adequate high protein diet is fed.

Detoxication Products of Pyridine and Coramine. In an effort to determine whether the rat actually does methylate pyridine a group of rats were fed for 10 days a diet

[3] Coulson, R. A., and Stewart, C. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 364.

[4] Brazda, F. G., and Coulson, R. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 19.

[5] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

[6] His, W., *Arch. expt. Path. u. Pharm.*, 1887, **22**, 253.

TABLE I.
Effect of Oral Administration of Pyridine Derivatives to Young Rats on a High Protein Diet for 28 Days.

Compound %	No. rats	Avg final wt g	Avg liver wt wet g	Avg % solid	Avg liver wt wet as % body wt	Avg % fat wet wt	Avg % fat dry wt	Avg food intake g/day	Avg wt gains g/day
Pyridine methochloride 1.64%	6	113.5	5.05	35.05	4.45	4.30	12.39	5.55	2.26
Alpha picoline 1%	5*	110.8	4.96	38.03	4.29	3.77	10.91	4.84	2.34
Alpha picoline methochloride 1.54%	5†	50.6	2.09	30.62	4.17	1.76	5.78	1.91	0.50
Beta picoline methochloride 1.54%	6	97.3	3.66	31.53	3.70	3.42	11.38	4.00	1.91
Gamma picoline 1%	6	100.9	4.47	35.30	4.56	6.23	18.54	4.08	1.96
Gamma picoline methochloride 1.54%	6	92.0	3.42	28.32	3.77	2.25	7.62	3.69	1.69
Alpha picolinic acid 0.70%	5*	141.5	6.06	32.15	4.28	2.78	8.65	7.76	3.40
Alpha picolinic acid methyl betaine 1.12%	6	77.4	3.59	31.75	4.64	2.42	7.19	3.76	0.73
Nicotinic acid 1%	6	147.8	7.50	36.00	5.08	6.68	18.56	8.30	3.54
Trigonelline 1.12%	6	169.2	7.08	33.47	4.18	4.14	12.45	9.95	4.33
Isonicotinic acid 1%	8	131.8	5.20	34.04	3.92	4.19	12.43	6.79	3.07
Isonicotinic acid methyl betaine 1.12%	6	135.1	5.21	33.78	3.86	3.38	9.89	6.20	3.15
Nicotinamide 1%	8	105.6	5.12	34.57	4.85	6.89	19.96	5.98	2.18
Nicotinamide methochloride 1.41%	6	159.1	7.60	33.95	4.78	4.62	13.59	9.11	3.95
Coramine methochloride 1.28%	6	167.5	7.05	32.62	4.21	3.97	11.75	10.06	4.16
Control	12	155.5	6.59	33.82	4.25	4.16	12.36	8.18	3.91

The content of methylated pyridine compounds in the diet is equivalent, in each case, to a 1% content of the immediate unmethylated precursor.

* The 5 rats in these groups represent the survivors of the starting groups of 6.

† Alpha picoline methochloride proved to be too toxic to permit the experiment to continue for 28 days. This group represents the survivors of a 14-day experiment.

which contained 1% pyridine. The urine was collected and attempts were made to isolate pyridine methochloride by essentially following the procedure described by His.[6] No free pyridine was detected in the urine nor was it possible to isolate any of the methyl derivative. On the other hand, it was possible to isolate pyridine methochloride if the rats were fed this compound. Nicotinamide methochloride was isolated from the urine of rats which received nicotinic acid, nicotinamide, or coramine. We could find no evidence for the direct methylation of coramine to coramine methochloride. Similar studies involving the metabolism of the other compounds in this series are in progress and the results will appear in a subsequent publication.

It would be premature to conclude that coramine and pyridine are not directly methylated since the "detoxified" methyl derivative might be converted to some other substance before being eliminated by the kidney. However, it does seem unlikely that any considerable fraction of these compounds is methylated by the rat and it is probable that the rat uses some means other than methylation to reduce their concentration in the body. No information is available on the degree of absorption of these compounds from the intestine of the rat. However, coramine, nicotinic acid and nicotinamide are known to be quickly absorbed from the human intestine.[2]

Effect of Pyridine Derivatives on Liver Fat. The rats were killed at the end of a 28-day feeding period, the livers were removed and the water and total lipids were determined. A sample of each liver was saved and sectioned for histological examination. It is evident that the use of the high protein diet prevented the development of livers which were as fatty as those usually developed on a low protein diet. (Tables I and

TABLE II.
Effect of Addition of Choline or Methionine to Diet of Young Rats Receiving β-picoline or Coramine for 28 Days.

Compound %	No. rats	Avg final wt g	Avg liver wt wet g	Avg % solid	Liver wt wet as % of body wt		Liver fat as % wet liver wt		Avg % fat dry wt	Avg food intake g/day	Avg wt gains g/day
					Range of values	Mean	Range of values	Mean			
Beta picoline 1%	6	97.5	4.61	39.02	4.05 5.29	4.51±0.13*	5.33 21.10	11.33±1.44	29.95	4.00	1.87
Beta picoline 1%+ 0.5% choline	6	114.3	4.99	33.87	4.00 5.05	4.36±0.10	2.98 5.70	3.91±0.24	11.24	4.72	2.37
Beta picoline 1%+ 1.2% methionine	6	102.0	4.69	33.26	4.07 4.91	4.61±0.08	2.58 4.49	3.40±0.20	10.32	3.96	1.94
Coramine 1%	12	121.5	10.73	36.96	6.82 9.71	8.19±0.27	3.62 7.34	5.78±0.33	17.47	6.71	2.68
Coramine 1%+ choline 0.50%	6	108.8	7.88	32.23	6.01 7.80	7.06±0.16	1.87 3.47	2.38±0.14	7.40	5.69	2.32
Coramine 1%+ methionine 1.2%	6	117.1	9.17	34.13	6.68 9.56	7.83±0.27	2.89 5.35	3.62±0.22	11.76	5.69	2.58
Control	12	155.5	6.59	33.82	3.82 4.83	4.39±0.09	2.76 5.01	3.57±0.18	12.36	8.18	3.91

* Probable error of the mean result.

II). None of the methylated compounds produced any significant increase in the fat content of the liver as determined by gravimetric analysis or microscopic examination. In spite of the small number of rats in each group it is statistically improbable that any of these methylated compounds are capable of increasing liver lipids since none of the 53 rats that received a methylated compound showed any increase in liver fat. Gamma picoline, nicotinic acid, and nicotinamide produced some increase in the fat content (Table I) in spite of the high protein diet. The significance of this change is not clearly established. However, it has been demonstrated that nicotinic acid and nicotinamide definitely produce fatty livers in rats maintained on low protein diets.[1] Beta picoline and coramine produced a definitely significant increase in the liver fat content (Table II). There was good correlation between the histological appearance and the chemical analysis in all groups. Alpha picoline, alpha picolinic acid and isonicotinic acid did not produce any significant change in the fat content (Table I). The effect of pyridine, under these experimental conditions, will be published later.

Effect of Coramine on Liver Size. In addition to the increase in liver fat caused by coramine this compound invariably caused a great increase in the absolute nonfat liver weight. The average liver weight of the 24 rats that received coramine, either alone or supplemented with choline or methionine, was very nearly twice that of the controls. This increase is not due to hydration since the per cent solid content remains normal. None of the other substances reported in this study had any effect in this regard. The effects produced by beta picoline and coramine are compared in Table II. Although the addition of 0.5% choline or 1.2% methionine prevented the increase in liver fat† caused by either beta picoline or coramine the great increase in the nonfat weight

† Preliminary experiments with coramine indicated that neither 0.15% choline nor 0.6% methionine was able to prevent the increase in liver lipids.

due to coramine was not prevented by the addition of choline or methionine. The fact that a combination of lipotropic factors and high protein diet was not capable of preventing the abnormal liver growth would suggest that this action of coramine is not due to any decrease in factors necessary for transmethylation but to a specific action of the coramine molecule.

Summary. 1. The effect of the ingestion of a series of pyridine derivatives by immature rats on a high protein diet is described. 2. None of the N-methyl derivatives of this series had any appreciable influence on either the fat content or the absolute liver weight. 3. Gamma picoline, nicotinic acid, and nicotinamide produced slight increase in liver fat; beta picoline and coramine produced significant increases in liver fat. In the case of beta picoline and coramine this increase in fat could be prevented by the inclusion of 1.2% methionine or 0.5% choline. The effect of methionine or choline on the metabolism of the other compounds reported was not determined. 4. Coramine produced a great increase in the fat-free liver weight which could not be prevented by the addition of choline or methionine to the diet. 5. The growth inhibition following the administration of beta picoline or coramine was not appreciably affected by the addition of choline or methionine to the high protein diet.

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Growth and Reproduction in Rats on Synthetic Rations.*

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Introduction. It is now generally recognized that rats grow at a rapid rate when given synthetic diets containing only 6 B vitamins. When sulfonamides are added, the growth is greatly retarded but the effect is counteracted by the addition of folic acid and biotin. Ransone and Elvehjem[1] reported that certain folic acid concentrates from liver produced a growth response beyond that which could be attributed to the known vitamins present. Recently evidence has been presented for the presence in fresh liver and other materials, of a factor necessary for continued growth and a normal blood picture in the monkey.[2] Further work has shown the existence of an unknown factor(s) in liver which stimulates the growth of *S. faecalis* when this organism is cultured on a "complete" synthetic medium.[3,4] The present work was undertaken to determine the effect upon growth and lactation of rats receiving a purified ration containing 10 crystalline B vitamins.

Experimental. Male weanling rats (Sprague-Dawley) weighing between 38 and 45 g were used in all growth experiments. They were housed in individual cages and were fed the

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1 Ransone, B., and Elvehjem, C. A., *J. Biol. Chem.*, 1943, **151**, 109.

2 Cooperman, J. M., Ruegamer, W. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 101.

3 Cooperman, J. M., Ruegamer, W. R., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, **163**, 769.

4 Ruegamer, W. R., Cooperman, J. M., Sporn, E. M., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **167**, 861.