Effects of Penicillin G and Penicillin V on Thiamine Synthesis in the Rat.* (22747)

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A few workers have related the growth promoting ability of antibiotics to elevated levels of certain limiting dietary vitamins in tissues and/or intestinal ingesta(1-6). In this study, an attempt has been made to correlate hepatic and intestinal levels of thiamine with the rate of growth of young rats fed rations containing a sub-optimal concentration of vit. B_1 and supplemented with one of three salts of penicillin G (benzyl penicillin) or with sodium penicillin V (phenoxymethyl penicillin).

Materials and methods. Forty weanling Sprague-Dawley albino female rats of between 40 and 50 g in weight were fed a semi-purified, thiamine-inadequate diet of the following percentage composition: "vitamin-free" casein (Nutritional Biochem. Corp.) 20, dextrinized corn starch 28, sucrose 39, hydrogenated vegetable oil (Crisco) 2.8, corn oil (Mazola) 4, salts (U.S.P. XIV) 4, L-cystine 0.2, and powdered cellulose (Alphacel, Nutritional Biochem. Corp.) 2. The following vitamins were added to the ration (mg/kg): thiamine hydrochloride 0.4, riboflavin 7.0, pyridoxine hydrochloride 5.0, calcium pantothenate 25, nicotinamide 25, i-inositol 100, choline chloride 1,000, folic acid 0.5, biotin 0.1, *para*-aminobenzoic acid 25, 2 methyl-1, 4-naphthoquinone 2.5, and vit. B_{12} 0.025. The corn oil additive contained 0.5 g oleum percomorphum and 125 mg d-alpha tocopherol/100 g. A portion of the carbohydrate component of this ration was in the form of dextrin because Sauberlich(7) reported antibiotics to overcome more readily a thiamine inadequacy if the dietary carbohydrate were dextrin than if it were sucrose. After a 5-day depletion period, groups of 4 animals were subjected to the following regimen:

I	Basal ration
II	*Idem* + potassium penicillin G
III	" + procaine penicillin G
IV	" + benzathine penicillin G
V	" + potassium penicillin V
VI	" + 5.6 mg thiamine hydrochloride/kg
VII	" + injections of potassium penicillin G
VIII	" + " " procaine penicillin G
IX	" + " " benzathine penicillin G
X	" + " " potassium penicillin V

Penicillin was incorporated in the diets at a level of 100 mg/kg ration or injected intraperitoneally (0.5 mg) once every 48 hours. Food and water were provided as needed and the animals were weighed at regular intervals. No record was kept of the amount of food consumed. On the 27th day of treatment, groups I-V were killed by a sharp blow on the head and their intestines and livers excised. The contents of each ileum and cecum were expelled into small screw-cap jars and frozen immediately in a dry ice chest. Each liver was weighed and also frozen. Each of the 3 types of specimens was pooled by ration group. On the 28th day the remaining animals (Groups VI-X) were killed and livers and intestinal contents recovered as described for Groups I-V. The thiamine content of each sample was determined subsequently by means of a microbiologic assay using *Lactobacillus fermenti* 36(8). The livers were thawed and dispersed in water with a Potter-Elvehjem homogenizer prior to assaying them for thiamine. The intestinal samples were thawed as needed, suitably diluted in water, mixed thoroughly and a portion centrifuged at 2,500 rpm in a Serval model SP/X centrifuge. The supernatant liquid was recovered and assayed directly while the portion of the suspension which had not been centrifuged was prepared for assay in the recommended manner by over-night digestion with pepsin and takadiastase. Thus, the intestinal contents were assayed for "free" and "total" thiamine. Just "total" thiamine was estimated from assays of the liver preparations.

* Supported in part by research grant, E-353(C4), from N.I.H., U.S.P.H.S. and Wyeth Laboratories, Philadelphia, Pa.

TABLE I. Effects of Oral and Parenteral Administration of 3 Different Salts of Penicillin G and One of Penicillin V on the Growth of Weanling Rats Fed a Thiamine-Inadequate Diet. Average 27-day net gain in body wt (g) ± stand. dev.

Treatment	Mode of administration of penicillin	
	Oral*	Parenteral†
K penicillin G (II & VII)	98.38 ± 7.57	88.25 ± 22.10
Procaine penicillin G (III & VIII)	91.13 ± 10.66	75.25 ± 15.99
Benzathine penicillin G (IV & IX)	99.00 ± 7.60	61.50 ± 14.72
K penicillin V (V & X)	88.25 ± 11.21	73.25 ± 11.62
Thiamine-insufficient control (I)	50.75 ± 21.20	
Thiamine-sufficient " (VI)	105.63 ± 7.14	

* By means of analysis of variance of these data (Group VI excluded from analysis) a highly significant difference exists. $LSD_{.01} = 26.4$.

† No statistically significant difference was found in these groups. Calculated as indicated in footnote *.

Results. The growth data assembled in Table I reveal that: a) the level of thiamine employed in the basal ration was not adequate for normal growth; b) supplementation of the deficient basal ration with excess thiamine resulted in normal growth rates; c) ingestion of penicillin stimulated growth significantly (indicative of an anticipated thiamine-sparing action); and d) intraperitoneal administration of the penicillins was not accompanied by a statistically-significant growth response. It is of interest that the three salts of penicillin G and the one of penicillin V were essentially equal in their ability to spare the dietary requirement for thiamine when given *per os*. It would appear that potassium penicillin G was superior to the other penicillins when administered parenterally, although the growth response was not significant when subjected to an analysis of variance.

The results of the thiamine assays of the livers are seen in Table II. Note the consistently higher concentrations of hepatic thiamine in rats given penicillin than in the deficient control rats (Group I). It is apparent also that the oral route of administration of penicillin was more effective than the parenteral one in enhancing storage of thiamine in the liver. When expressed as the amount of thiamine per liver, the stimulatory action of penicillin on the hepatic stores of vit. B_1 is even more striking than an expression of concentration. Hence, penicillin had an effect upon the size of the liver in these rats. Also, it would seem that potassium penicillin G was more effective in encouraging liver stores of thiamine than the other penicillins tested.

The data in Table III are not particularly illuminating in suggesting reasons for effects of penicillin on liver stores of thiamine. Note that in the ileum both "free" and "total" thia-

TABLE II. Effect of Penicillin on Weights and Thiamine Levels of Livers from Rats Fed a Thiamine-Inadequate Ration. Expressed as fresh wt of liver.

Treatment		Avg thiamine content		Avg liver wt (g)
		Conc. (μg/g)	Total (μg/liver)	
Thiamine-insufficient control		.84	4.27	5.11 ± 1.12*
Thiamine-sufficient "		2.82	21.09	7.48 ± 1.53
K penicillin G	Oral	1.54	10.76	7.01 ± .29
	Parenteral	1.64	9.93	6.04 ± 1.47
Procaine penicillin G	Oral	1.29	8.98	6.96 ± 1.21
	Parenteral	1.07	5.56	5.18 ± .96
Benzathine penicillin G	Oral	1.21	8.99	7.45 ± 1.11
	Parenteral	1.06	5.33	5.03 ± 1.17
K penicillin V	Oral	1.22	8.44	6.93 ± .91
	Parenteral	1.06	5.77	5.45 ± 1.08

* ± stand. dev.

TABLE III. Thiamine Levels in Ileal and Cecal Contents of Rats Given Penicillin and a Thiamine-Inadequate Diet. Results expressed as μg/g wet wt.

Treatment		Ileum		Cecum	
		"Free"	"Total"	"Free"	"Total"
Thiamine-insufficient control		.34	.70	.38	1.00
Thiamine-sufficient "		.90	1.69	.63	2.68
K penicillin G	Oral	.17	1.57	.18	.76
	Parenteral	.005	.51	.31	1.37
Procaine penicillin G	Oral	.45	1.53	.03	.53
	Parenteral	.02	.52	.91	
Benzathine penicillin G	Oral	.19	.64	.30	.51
	Parenteral	.005	.47	.69	1.49
K penicillin V	Oral	.17	.60	.45	.85
	Parenteral	.005	.005	.83	1.47

mine existed at greater concentrations in animals given the antibiotic orally than in those which had received penicillin intraperitoneally. However, in no case was the concentration of "free" thiamine in ileal contents increased by the administration of penicillin; in only two cases (orally-administered potassium and procaine penicillin G) did penicillin raise the level of "total" thiamine in ileal ingesta. The only consistent pattern in cecal contents is that parenteral administration of penicillin, with but one exception, elevated the levels of "free" and "total" thiamine while oral administration of the drug had an opposite effect.

Discussion. This report adds weight to the accumulating evidence that antibiotics aid in the vitamin economy of certain animals. However, the manner by which this is achieved is unknown. True, the intestinal flora is affected(3,5,6), a fact which we felt unnecessary to establish in this report. Moreover, absorption from the intestinal lumen should be considered in view of reports that antibiotics cause a thinning of the intestinal wall(9,10). Unfortunately, our data on the influence of penicillin on the thiamine content of the intestinal ingesta are inconclusive. Nevertheless, the positive effect of penicillin on liver stores of thiamine may be a reflection of intestinal synthesis and absorption, a supposition advocated by others(2,4,9).

Apparently the different physical properties of the penicillin salts tested had little to do with the thiamine-sparing activity of penicillin, since the 3 salts of penicillin G and the one salt of penicillin V differed but slightly in their effects on the rate of growth. It is obvious further that incorporation of the antibiotic in the ration gave a growth response superior to that obtained with widely-spaced parenteral injections of penicillin. It must be remembered, however, that all of the penicillin given parenterally is excreted within 6 to 8 hours; thus the tissues of rats which received penicillin parenterally were exposed to penicillin approximately 8 of the 48 hours that elapsed between injections. On the other hand, bacitracin, an antibiotic that is poorly absorbed from the intestinal tract, has been reported to stimulate the growth of rats fed a vit. B_{12} deficient diet only when administered intraperitoneally(11). Presumably, if penicillin had been given to the rats parenterally every 8 hours instead of every 48 hours a growth response may have occurred. Despite the long interval between injections of penicillin, the total amount and concentration of thiamine in the livers of these animals was appreciably greater than in the thiamine-deficient control animals. Moreover, of the parenterally administered penicillins, potassium penicillin G enhanced the rate of growth as well as the liver's stores of thiamine to the greatest degree. Correlation of the growth rates with the content of thiamine in the livers and the liver weights is excellent (compare Tables I and II). Nevertheless, the thiamine-sufficient control rats had approximately twice the amount of thiamine in their livers than did the deficient rats showing the best response to penicillin.

Summary. Three salts of penicillin G and one of penicillin V were found to be essentially equal when administered orally in sparing the rat's dietary requirement for thiamine, as determined by rates of growth and thiamine levels in the liver. Growth was not stimulated to a statistically significant degree upon administering these antibiotics intraperitoneally every other day. although thiamine levels in the livers were elevated by such treatment. The effects of penicillin on thiamine levels in ileal and cecal contents were inconclusive.

The author expresses appreciation for the assistance of Mrs. Ruth H. Christenson. Dr. Elliott R. Weyer, Chas. Pfizer & Co., Brooklyn kindly supplied potassium and procaine penicillin G; Dr. Edward F. Roberts, Wyeth Laboratories, Philadelphia, donated benzathine penicillin G and sodium penicillin V. In addition we are indebted to Dr. Stanton M. Hardy, Lederle Laboratories, Pearl River, N. Y. for furnishing many of the dietary ingredients and to Dr. Karl Folkers, Merck and Co., Rahway, N. J. for the gift of vit. B_{12}.

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Received August 27, 1956. P.S.E.B.M., 1956, v93.

Effect of Stimulation of Adrenal Cortex on Blood Pepsinogen in Animals and Man. (22748)

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The level of pepsinogen in blood and urine constitutes an index of gastric activity(1-4). Uropepsinogen excretion reportedly increases following administration of adrenocorticotrophic hormone or adrenal steroids, and after acute emotional and physical stress; therefore it has been suggested as a measurement of adrenal activity. These data and their implications concerning the relationship of gastric activity to stress and the General Adaptation Syndrome have been reviewed recently by Gray *et al.*(5). However, Hirschowitz and his associates were not able to demonstrate increases in blood pepsinogen following ACTH administration(6), and Necheles and his co-workers recently reported failure of cortisone to affect uropepsinogen excretion (7). Similarly, in animal studies, we were unable to demonstrate elevation in blood pepsinogen following either acute or chronic noxious stimulation in cats and rats even when adrenal hypertrophy was observed(8,9). A study was undertaken, therefore, to determine if direct stimulation of the adrenals by exogenous ACTH influenced blood pepsinogen in animals. The effect of a single injection of ACTH on blood pepsinogen in humans was also evaluated and titres in several patients on chronic steroid therapy were measured.

* Supported by P.H.S. Grants M-739 and H-1954.