Effect of Riboflavin and Pteroylglutamic Acid on Growth and White Cell Production of Rats.* (18638)

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(Introduced by P. L. Day)

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Previous work from this laboratory has indicated that the growth depression brought about by feeding a high (10%) level of glycine to rats can be partially overcome by the addition of pteroylglutamic acid (PGA)(1). It has also been reported by Martel and Gingras(2) that the feeding of a 10% level of glycine to rats resulted in a mild leucopenia which responded to PGA. The present experiments were undertaken in an effort to determine if riboflavin or choline could substitute for PGA in these functions. Since glycine oxidase is a flavin-containing enzyme riboflavin could possibly increase the rate of destruction of the glycine. It has been shown that one or more of the methyl groups of choline may be converted to a "one-carbon fragment" and condensed with glycine to form serine(3).

Experimental. Nine groups of male weanling Sprague-Dawley rats were fed diets of the composition given in Table I. Each animal was given 6 g of food per day for the first 16 days; 7 g per day from the 16th-32nd experimental day; and 8 g per day from the 32nd-56th day. The supplements of riboflavin and PGA were added daily to the food. On the restricted food intake employed in these experiments the animals of all groups consumed most of the food and supplements given them. Complete blood counts were made on the 32nd day of the experiment. The differences in weight gain and in white blood cells were statistically treated by the analysis of variance method(4).

Results and discussion. The average weight gains and the weight gains per g of food eaten for the various groups are given in Table II. It is evident from these data that neither a high level of riboflavin (Group V) nor a high level of choline (Group VII) had any effect in preventing the growth suppression brought about by the toxic level of glycine. The combination of choline and riboflavin (Group VIII) was also ineffective. PGA was found to partially but not completely overcome this growth suppression when given along with the riboflavin. The response with the combination of riboflavin and PGA, however, was no better than that reported by Dinning *et al.* (1) using PGA alone on high glycine diets. It would thus seem that riboflavin cannot replace PGA nor does it act synergistically with it in preventing the growth suppression brought about by the inclusion of 10% glycine in the diet.

The hematological data are presented in Table II. A mild leucopenia developed on the 10% glycine diet which is in accord with the results of other investigators(1,2). The addition of riboflavin to the high glycine and high glycine plus choline diets brought about a reduction in white cells as compared to the unsupplemented controls. This reduction was not significant in the animals receiving a high level of both glycine and choline. In all cases where additional riboflavin and PGA were supplied in the diets (Groups III, VI, and IX) there was a statistically significant increase in the white cell count over that of the basal group and also over that of their

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2. Martel, F., Page, E., and Gingras, R., *Rev. Canadienne de Biologie,* 1947, v6, 802.

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TABLE I. Composition of Diets.

Components	I	II	III	IV	V	VI	VII	VIII	IX
	%								
Casein	18	18	18	18	18	18	18	18	18
Sucrose	68	68	68	58	58	58	56	56	56
Crisco	10	10	10	10	10	10	10	10	10
Cod liver oil	2	2	2	2	2	2	2	2	2
Salt mix	2	2	2	2	2	2	2	2	2
Glycine	0	0	0	10	10	10	10	10	10
Choline	0	0	0	0	0	0	2	2	2
	Supplements								
Riboflavin, γ/day	0	100	100	0	100	100	0	100	100
Folic acid, γ/day	0	0	100	0	0	100	0	0	100

The following vitamins were added per 100 g to all diets:

	mg		mg
Thiamine	.5	Choline chloride	10
Riboflavin	.5	Pyridoxine hydrochloride	.5
Nicotinic acid	2.	Biotin	.005
i-Inositol	10.	Menadione	.025
Calcium pantothenate	1		

TABLE II. Weight Gain and Hematological Data of Rats Receiving Riboflavin and Folic Acid on Various Purified Diets.

Group*	Diet	Avg wt gain 56 days, g	Wt gain per g food eaten, g	R.B.C., $10^6/mm^3$	W.B.C.,† $10^3/mm^3$	Lymphocytes, $10^3/mm^3$
I	Basal	156	.354	8.30	16.58	13.81
II	Basal + riboflavin	153	.358	8.18	17.28	14.06
III	Basal + riboflavin + PGA	152	.356	7.97	20.82	17.89
IV	High glycine	85	.235	7.92	14.05	11.08
V	" " + riboflavin	78	.211	7.92	13.60	9.50
VI	" " + " + PGA	132	.322	8.77	19.55	15.43
VII	High glycine + choline	85	.252	7.85	19.37	15.89
VIII	High glycine + choline + riboflavin	94	.258	8.79	14.60	11.42
IX	High glycine + choline + riboflavin + PGA	127	.319	9.30	25.44	20.94

* Six or 7 animals in each group.
† A difference of 2.37 × 10^3 white blood cells per mm^3 is statistically significant ($P = 0.01$).

unsupplemented controls. In the present experiments the increase in white cell production brought about by riboflavin and PGA was much greater when there was a high level of dietary choline (Group II). This would indicate that choline or some product of choline metabolism influences such cell production. That PGA alone will not bring about this increase with high levels of glycine, choline or a combination of these substances is indicated by the results shown in Table III. Recent work has indicated that "methyl" groups are necessary for the formation of white blood cells and that these methyl groups can be supplied by betaine when there is sufficient vit. B_{12} present(5). In the present experiments casein was used as the protein source which would supply sufficient vit. B_{12} to meet the requirements of the rat. A relationship of PGA to flavin-containing enzymes (6) and to choline oxidase(7) has been postulated. Results such as those obtained in the present experiments might be explained on

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TABLE III. Hematological Data on Rats Receiving High Levels of Glycine and Choline With and Without Added PGA.

Diet	No. of animals	R.B.C. 20th day $10^6/mm^3$	W.B.C. 20th day $10^3/mm^3$
Experiment I			
Basal	4	7.63	17.8
Basal + 500 γ PGA/100 g	7	7.30	18.7
Basal + 10% glycine	5	7.43	18.0
Basal + 10% glycine + 500 γ PGA/100 g	5	7.10	16.6
Basal + 10% glycine + 2% choline	5	9.23	17.1
Basal + 10% glycine + 2% choline + 500 γ PGA/100 g	5	9.78	17.9
Experiment II		32nd day	
Basal	8	8.31	18.8
Basal + 6% choline	8	8.49	18.9
Basal + 6% choline + 500 γ PGA/100 g	8	8.84	17.8

the basis of an altered rate of conversion of choline to betaine.

The red cell counts, hemoglobin and hematocrits of the various groups showed no significant differences.

Summary. Weanling albino rats were fed a standard purified diet, a similar diet with 10% glycine replacing an equal amount of sucrose, and a diet with 10% glycine and 2% choline replacing an equal amount of sucrose. Other groups received these diets supplemented with 100 γ of riboflavin per day or 100 γ of riboflavin and 100 γ of PGA per day. The growth of the rats receiving the high glycine and high glycine plus high choline diets was greatly reduced as compared to the basal group. Supplementation of these diets with riboflavin brought about no effect on the growth; the combination of riboflavin and PGA overcame the growth depression to a considerable extent and also brought about a marked increase in gain per unit of food consumed. Supplementing the various diets with both riboflavin and PGA brought about a leucocytosis which was very marked when the diet contained a high level of both glycine and choline. The data would suggest that there is an interrelationship between riboflavin and PGA in white cell production.

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Further Observations on the Microbiological Assay for Vitamin B_{12} Using *Lactobacillus leichmannii.* (18639)

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Conflicting reports have appeared regarding the comparative potencies of vit. B_{12} and B_{12b} in microbiological assays with *Lactobacillus leichmannii* and *L. lactis* Dorner. The two compounds were found to have approximately equal activity for *L. leichmannii* by Pierce *et al.*(1,2). However, in subsequent studies in our laboratories, the potency of vit. B_{12b} often appeared to be only 0.7 to 0.8 that of vit. B_{12} in the *L. leichmannii* assay. In contrast, Jackson and co-workers(3) found that vit. B_{12b} was 1.4 to 1.5 times as potent as vit. B_{12} when the vitamins were added

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