Pteroylglutamic Acid, Ascorbic Acid, and Injectable Liver Extract on Dietary Glycine Toxicity in the Rat.* (17400)

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Previous work from this laboratory has demonstrated that the growth retardation resulting from inclusion of sodium benzoate in a purified diet for rats may be in part overcome by the addition of pteroylglutamic acid (PGA) in the diet.[1] Experiments were next designed to study the effect of feeding high glycine diets to rats. In confirmation of the work of Hier *et al.*[2] it was found that rats receiving a purified diet containing 10% glycine grew at a subnormal rate; hence the effects of supplementing this diet with PGA, injectable liver extract, and ascorbic acid were studied. While these experiments were in progress Martel and Gingras[3] reported that the feeding of a 10% glycine diet to rats resulted in growth suppression, mild leucopenia, and an elevated creatinuria, all of which responded to PGA.

Experimental. Six groups of weanling Sprague-Dawley rats, 4 males and 4 females in each group, were fed the diets given in Table I. The rats were fed *ad libitum* and food intake was measured. Complete blood counts were made on the 37th and 46th days of the experiment. The differences in weight gains among the various groups were statistically treated by the analysis of variance method.[4]

Results and Discussion. The growth curves are given in Fig. 1. It may be observed that the inclusion of 10% glycine in the purified diet resulted in a greatly decreased rate of growth. PGA markedly improved the growth of rats receiving the high glycine diet. The addition of liver extract to the high glycine diet resulted in some growth improvement but less than that produced by PGA. The liver extract used was assayed for PGA and found to contain 2.62 γ per cc; this amounted to approximately 0.2 γ of PGA per rat per day. This and the fact that the group receiving PGA plus liver extract grew no faster than those receiving PGA alone would indicate that some of the growth improvement resulting from the liver extract may have been due to its content of PGA.

Johnson and Dana[5] have presented data to indicate that ascorbic acid increased the rate of growth and induced a blood response in rats fed sulfasuxadine. Woodruff and Darby[6] have reported that the inclusion of PGA in the diet of scorbutic guinea pigs reduced the excretion of tyrosine metabolites. Such data indicate a similarity in function of PGA and ascorbic acid and for this reason one group of rats was fed the high glycine diet plus ascorbic acid. It may be seen from the growth curves that ascorbic acid had little effect on the growth of the rats. The difference in rate of gain between the group receiving ascorbic acid and the group receiving high glycine alone was not statistically significant.

Weight gains per gram of food eaten were calculated for the various groups and the average values were: control 0.295, high glycine 0.152, high glycine plus PGA 0.282, high glycine plus liver extract 0.205, high

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1 Totter, John R., Amos, Esther S., and Keith, Cecilia K., *J. Biol. Chem.*, 1949, **178**, 847.

2 Hier, Stanley W., Graham, Claire E., and Klein, David, PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 187.

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

4 Snedecor, George W., Statistical methods applied to experiments in agriculture and biology, The Iowa State College Press, 4th ed., 1946.

5 Johnson, B. Connor, and Dana, Ann S., *Science*, 1948, **108**, 210.

6 Woodruff, Calvin W., and Darby, William J., *J. Biol. Chem.*, 1948, **172**, 851.

TABLE I.
Composition of Experimental Diets.

Dietary component	Groups I	II	III	IV	V	VI
Casein, %	18	18	18	18	18	18
Sucrose, %	75	65	65	65	65	65
Crisco, %	3	3	3	3	3	3
Cod liver oil, %	2	2	2	2	2	2
Salt mix* %	2	2	2	2	2	2
Glycine, %	0	10	10	10	10	10
PGA—mg/100 g	0	0	0.5	0	0.5	0
Liver extr.—cc/kilo	0	0	0	8	8	0
Ascorbic acid—mg/100 g	0	0	0	0	0	100

The liver extract was an anti-pernicious anemia concentrate containing 15 units per cc. The following vitamins were added per 100 g to all diets:

	mg		mg
Thiamine	0.5	Choline chloride	10.0
Riboflavin	0.5	Pyridoxine hydrochloride	0.5
Nicotinic acid	2.0	Biotin	0.005
i-Inositol	10.0	Vit. K	0.025
Calcium pantothenate	1.0		

* Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutr.*, 1937, **14**, 273.

glycine plus PGA plus liver extract 0.291, and high glycine plus ascorbic acid 0.213. With the exception of reversal between the ascorbic acid and liver extract groups, efficiency of gain ranked the same in the various groups as did total weight changes.

The hematological data are presented in Table II. Data for males and females are given separately only in Groups IV and V, since there were no sex differences in the other groups. The feeding of the high glycine diet did not produce a significant leucopenia in these rats when compared to the controls; however, addition of PGA to this diet did elevate the white cell count. Addition of liver extract and liver extract plus PGA to the high glycine diet resulted in an increased white count in male rats but was without effect in the females.

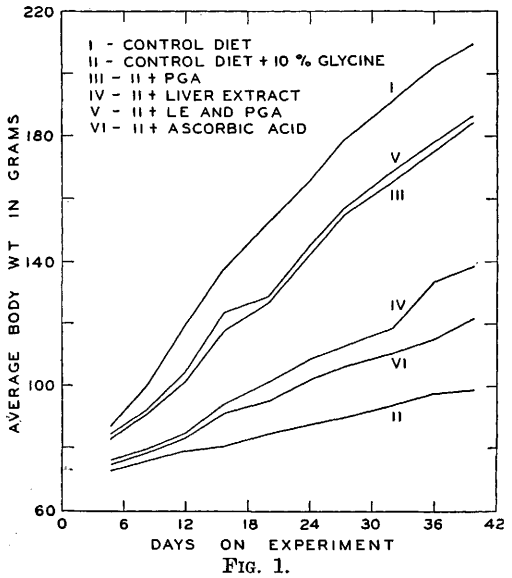

FIG. 1.
Growth curves for rats receiving the various diets.

Although the red cell counts of rats receiving the high glycine diet were lower than the control group, none of the supplements tested were effective in restoring the counts to the level of the controls. Hemoglobin values were also reduced by the high glycine diet and were improved only by the addition of the combination of PGA plus liver extract or by ascorbic acid. There were no consistent differences in hematocrit or differential white cell count among the various groups. The rats receiving ascorbic acid were found to have a higher mean corpuscular volume than any of the other groups. It may also be noted that rats receiving ascorbic acid had the lowest red count of any of the groups, but had a higher hemoglobin level than any of the others except the control group and the

TABLE II.
Average Hematological Data for Rats Receiving the Various Diets.

Group	Wbc., 10^3/mm³	Reticulocytes, % of rbc.	Granulocytes, % of wbc.	Lymphocytes, % of wbc.	Rbc., 10^6/mm³	Hb., g/100 cc	Vol. packed Rbc. cc/100 cc	Mean corpuscular vol., microns³
I. Control	17.9	2.6	11	89	7.29	14.9	51	70
II. High glycine	16.4	2.2	17	83	6.75	13.9	49	74
III. High glycine + PGA	19.1	2.2	10	90	6.70	13.2	49	74
IV. High glycine + L.E.								
♂	20.7	2.3	20	80	6.29	13.1	46	73
♀	14.5	2.5	12	88	6.09	13.9	49	81
V. High glycine + PGA + L.E.								
♂	26.5	3.5	17	83	6.80	14.9	51	75
♀	17.5	3.6	9	91	6.27	14.6	50	79
VI. High glycine + ascorbic acid	16.4	3.1	13	87	6.20	14.1	50	82

group receiving liver extract plus PGA.

There is not sufficient evidence available at present to permit an explanation of the growth-promoting effect of PGA when added to a high glycine diet for rats. Holland and Meinke[7] have presented evidence which indicates that PGA may function in the synthesis of serine by *Streptococcus faecalis.* It has been shown by Sakami[8] that glycine is converted to serine and then to glycogen by the rat. It is possible that this conversion may be a factor in explanation of our results. Experiments are in progress to study the effects of PGA on the conversion of glycine to serine by liver homogenates.

Summary. Weanling albino rats were fed a standard purified diet, and a similar diet containing 10% glycine replacing an equal amount of sucrose. Groups of rats were given the glycine-containing diet supplemented respectively with PGA, injectable liver extract, PGA plus liver extract, and ascorbic acid. The growth of rats receiving the high glycine diet was greatly reduced as compared to the control group. Supplementation of the high glycine diet with PGA and with PGA plus liver extract resulted in a marked improvement in growth rate; liver extract alone was less effective; and ascorbic acid did not significantly improve rate of growth of rats receiving the 10% glycine diet. Rats receiving the high glycine diet did not develop a marked leucopenia when compared with control rats; however, supplementation of this diet with PGA resulted in an increased white count while supplementation with liver extract and with liver extract plus PGA resulted in an increased white count in male rats but was without effect in females. The mean corpuscular volume was found to be highest in rats receiving the high glycine diet plus ascorbic acid.

7 Holland, Bryant R., and Meinke, W. W., *J. Biol. Chem.*, 1949, **178**, 7.

8 Sakami, W., *J. Biol. Chem.*, 1948, **176**, 995.

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