Effect of Sulfasuxidine on the Interrelation of Folic Acid, Vitamin B_{12} and Vitamin C.* (18123)

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In a previous communication(1), we reported that the addition of vitamin C and vitamin B_{12} to semi-purified rations stimulated chick growth and the *in vivo* synthesis of folic acid (PGA) as measured by liver storage. We also reported that PGA produced an increase in the amount of vitamin B_{12} stored in the liver. We suggested that the effects noted were due, particularly in the case of vitamin C, to a stimulation of intestinal synthesis. It is the purpose of this paper to report further studies on this problem.

Experimental. Straight run (New Hampshire ♂♂ X Single Comb White Leghorns ♀♀) crossbred chicks, which were the progeny of hens fed diet B-1 described previously(2), were used in all studies. The chicks were housed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum.* The chicks were wing banded and weighed at one day of age. Weights were recorded at weekly intervals.

All chicks were placed on a PGA-deficient ration containing sucrose 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts V(3) 6 g, soybean oil 5 g, L-cystine 0.3 g, thiamine hydrochloride 0.3 mg, riboflavin 0.6 mg, nicotinic acid 5.0 mg, pyridoxine hydrochloride 0.4 mg, calcium pantothenate 2.0 mg, choline chloride 150 mg, biotin 0.03 mg, inositol 100 mg, 2-methyl-1, 4-naphthoquinone 0.05 mg, and α-tocopherol 0.3 mg. Fortified haliver oil (60,000 U.S.P. units of vitamin A, 6,000 U.S.P. units of vitamin D_3 per g) was given by dropper (2 drops per bird per week).

In all cases, groups of 12 chicks were used throughout except in Exp. 3 where groups of 10 birds were used. Crystalline vitamin B_{12} was injected into the pectoral muscle with a 1 ml calibrated syringe. At the termination of the depletion period, which in all cases was 2 weeks, the chicks were sorted and divided into groups according to weight and percent gain. A test period of 2 weeks followed, at the termination of which the birds were sacrificed, and the livers removed, frozen immediately, and stored at freezing temperatures until assayed. PGA and vitamin B_{12} were extracted as described previously(1) except that one-half the amount of trypsin originally called for was used. PGA was measured microbiologically with *Streptococcus faecalis* R as a test organism using the medium of Luckey *et al.*(4). Vitamin B_{12} was measured similarly with *Lactobacillus leichmannii* ATCC 4797 using the medium of Skeggs *et al.*(5), modified by Thompson *et al.*(6).

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TABLE I. Effect of Sulfasuxidine on Chick Growth, Liver Storage, and Caecal Content of PGA and Vitamin B_{12}.* Exp. 1.

Supplement	Wt during gain test, g	Liver storage: PGA γ/g	Liver storage: PGA Storage equivalent†	Liver storage: Vit. B_{12} γ/g	Liver storage: Vit. B_{12} Storage equivalent†	Caecal contents: PGA γ/g dried wt	Caecal contents: Vit. B_{12} γ/g dried wt
50 γ PGA/100 g of ration	40	1.17	3.90 ± 0.39	0.04	0.05 ± 0.00	2.65 ± 0.25	4.50 ± 0.37
50 γ PGA/100 g of ration + vit. B_{12} (0.1 γ/day)‡	65	1.04	3.90 ± 0.59	0.07	0.17 ± 0.01	9.57 ± 0.45	6.25 ± 0.34
50 γ PGA/100 g of ration + vit. C (100 mg %)	42	2.42	6.01 ± 0.69	0.02	0.06 ± 0.00	11.77 ± 2.11	5.20 ± 0.68
50 γ PGA/100 g of ration + sulfasuxidine (1%)	57	1.38	5.42 ± 0.87	0.09	0.12 ± 0.05	0.00 ± 0.00	4.30 ± 0.41
50 γ PGA/100 g of ration + sulfasuxidine (1%) + vit. B_{12} (0.1 γ/day)‡	59	1.69	4.44 ± 0.56	0.12	0.24 ± 0.05	0.00 ± 0.00	5.10 ± 0.62
50 γ PGA/100 g of ration + sulfasuxidine (1%) + vit. C (100 mg %)	56	1.34	5.18 ± 0.62	0.01	0.06 ± 0.02	0.88 ± 0.27	3.42 ± 0.24

* Eight observations/group including standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† Storage equivalent $= \frac{\gamma/\text{liver}}{\text{body wt}} \times 100.$ ‡ Injected.

Results and discussion. The addition of vitamin C or vitamin B_{12} produced a small growth response. The addition of sulfasuxidine at the level of 1% also produced a growth response which was not increased when vitamin C or vitamin B_{12} was added to the ration.

In studying the interrelation of vitamins of the B-complex through liver storage, one is limited by the inefficient manner in which the water soluble vitamins are stored in this as well as other tissues. Evaluation is further complicated by the difference in the total weights of the chicks receiving the different rations. This difficulty can be compensated for to some extent by reducing all values to a common base, namely, γ per liver per 100 g of body weight (storage equivalent). Our results (see tables) are presented as both γ per g liver and liver storage equivalent.

The results obtained upon the analysis of the livers of these chicks for PGA and vitamin B_{12} were in accordance with previous work, namely, the addition of vitamin C resulted in a definite increase in the PGA stored in the liver. Such a stimulation was not observed when sulfasuxidine was added to the ration. Caecal analysis of these groups (Table I) proved far more striking. The administration of vitamin B_{12}, 0.1 γ per day by injection, produced a definite increase in the caecal level of PGA. The addition of vitamin C also increased the caecal PGA. The increase in caecal level of PGA upon administration of vitamin B_{12} was as great as that observed upon the addition of vitamin C. There may be several explanations for the observed results. First, the flora in the caecum may differ from that in the areas of the tract where the major part of absorption occurs. However, work by Shapiro *et al.*(7) appears to rule this out since evaluation of their data shows that the normal chick, at the age of 4 weeks, has the same ratio of different types of flora regardless of the section of the tract analyzed. Secondly, vitamin B_{12} may interfere with the absorption of PGA. If this were the case, one would expect an increase in the caecal PGA upon

7. Shapiro, S. K., and Sarles, W. B., *J. Bact.*, 1949, v58, 531.

TABLE II. Effect of Sulfasuxidine on Chick Growth and Liver Storage of PGA and Vitamin B_{12} in Presence of Excess Vitamin B.*

Exp.	Supplement	Wt gain during test, g	PGA γ/g	PGA Storage equivalent†	Vit. B_{12} γ/g	Vit. B_{12} Storage equivalent†
2	None	10	1.09	4.51 ± 0.92	0.02	0.08 ± 0.00
	Vitamin B_{12} (0.1 γ/day)‡	27	0.79	3.12 ± 0.53	0.07	0.24 ± 0.00
	Vitamin C (100 mg %)	17	1.48	7.32 ± 0.53	0.03	0.08 ± 0.02
	Sulfasuxidine (1%)	11	1.01	3.28 ± 0.46	0.02	0.08 ± 0.00
	Sulfasuxidine (1%) + vit. B_{12} (0.1 γ/day)‡	21	0.74	2.48 ± 0.38	0.15	0.41 ± 0.13
	Sulfasuxidine (1%) + vit. C (100 mg %)	11	1.64	6.14 ± 1.58	0.01	0.05 ± 0.00
	50 γ PGA/100 g of ration	74	1.17	4.00 ± 0.68	0.02	0.07 ± 0.00
	50 γ PGA/100 g of ration + vit. B_{12} (0.1 γ/day)‡	111	1.18	5.60 ± 0.65	0.08	0.24 ± 0.04
	50 γ PGA/100 g of ration + vit. C (100 mg %)	106	1.95	8.85 ± 1.37	0.05	0.14 ± 0.03
	50 γ PGA/100 g of ration + sulfasuxidine (1%)	73	1.56	6.32 ± 0.85	0.03	0.07 ± 0.04
	50 γ PGA/100 g of ration + sulfasuxidine (1%) + vit. B_{12} (0.1 γ/day)‡	113	2.13	6.83 ± 0.65	0.06	0.17 ± 0.04
	50 γ PGA/100 g of ration + sulfasuxidine (1%) + vit. C (100 mg%)	73	1.20	4.18 ± 0.76	0.01	0.06 ± 0.00

* Eight observations/group including standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† Storage equivalent $= \frac{\gamma/\text{liver}}{\text{body wt}} \times 100.$

‡ Injected.

the administration of vitamin B_{12} and sulfasuxidine. This was not observed. Another possibility is that vitamin B_{12} either inhibits the storage of PGA, aids in, or is the agent in the conversion and/or storage of a PGA like substance that lacks microbiological activity for *S. faecalis*.

Administration of sulfasuxidine, in all cases, greatly lowered the caecal level of PGA. This is in confirmation of previous work(8,9). This effect was observed regardless of whether vitamin C or vitamin B_{12} were present. On the other hand, the administration of sulfasuxidine had no effect on the caecal level of vitamin B_{12}.

Exp. 2 (Table II) was similar to Exp. 1 except that in this case the level of B vitamins in the ration was doubled in order to compensate for any other B vitamin, besides PGA, that might be made limiting by the administration of sulfasuxidine. The results are essentially the same as in Exp. 1 except that a growth stimulation was observed upon the addition of vitamin B_{12} and sulfasuxidine showing that sulfasuxidine does not inhibit the growth response attributed to vitamin B_{12} if the level of the other B vitamins is adequate.

Another interesting phenomena is one that has been noted many times in this laboratory, namely the inclusion of sulfasuxidine in the ration, produces variable results. The effect of sulfasuxidine on the intestinal flora and the, in turn, effect on vitamin synthesis is well known. Consequently, if a new born chick receives an inoculum high in organisms that synthesize necessary vitamins, the inclusion of sulfasuxidine in the diet will result in a growth depression. On the other hand, if the inoculum received is high in organisms that have a high requirement for the necessary vitamins,

8. Luckey, T. D., Moore, P. R., Elvehjem, C. A., and Hart, E. B., *Science*, 1946, v103, 682.

9. Wright, L. D., Skeggs, H. R., and Sprague, K. L., *J. Nutrition*, 1945, v29, 431.

TABLE III. Effect of Injected PGA and Vitamin C on Chick Growth, Liver Storage, and Caecal Content of PGA and Vitamin B_{12}.*

			Liver storage				Caecal contents	
			PGA		Vit. B_{12}			
Exp.	Supplement	Wt gain during test, g	γ/g	Storage equivalent†	γ/g	Storage equivalent†	PGA γ/g dried wt	Vit. B_{12} γ/g dried wt
3	None	20	0.98	3.22 ± 0.43	0.01	0.01 ± 0.00	4.71 ± 0.82	2.50 ± 0.22
	PGA (20 γ/day)	134	2.31	5.95 ± 0.77	0.07	0.16 ± 0.00	11.10 ± 0.67	4.70 ± 0.62
	Vit. C (25 mg/day)	20	1.27	3.40 ± 0.73	0.04	0.09 ± 0.10	4.25 ± 0.52	3.60 ± 0.54

* Six observations/group including standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† Storage equivalent $= \frac{\gamma/\text{liver}}{\text{body wt}} \times 100.$

the inclusion of sulfasuxidine in the diet will result in a growth stimulation since more of the vitamin injested by the animal would be available for absorption. Shapiro(7), has shown that in the case of the normal chick, on a natural ration, the predominant flora in the tract are lactobacilli. The high vitamin requirements of the lactobacilli are well known. If this is also the case with a sucrose semi-purified ration, the generally observed growth stimulation resulting upon the inclusion of sulfasuxidine in the diet is readily explained. Such a concept is further borne out by the observation that in Exp. 2 no change in growth occurred when sulfasuxidine was included in the ration.

In Exp. 3 (Table III) the injection of PGA at the level of 20 γ per day resulted in a definite growth stimulation and increase in the PGA stored in the liver. A marked increase in the liver storage of vitamin B_{12} was also observed. The injection of vitamin C at the level of 25 mg per day had no effect on growth, or liver storage of PGA and vitamin B_{12}. Analysis of the caecal contents were in correlation with the liver analysis. The injection of PGA resulted in a higher caecal level of this vitamin, showing that PGA may be excreted into the tract under the proper conditions.

Summary. The administration of sulfasuxidine eliminates the growth response produced by vitamin C in the chick, on a semi-purified ration. No effect is observed on the growth response produced by vitamin B_{12}, if the levels of the other B vitamins are adequate.

The administration of sulfasuxidine to a semi-purified ration eliminates the increase of the PGA liver storage produced by vitamin C. Both vitamin C and vitamin B_{12} raise the caecal concentration of PGA in the chick. The administration of sulfasuxidine lowers the caecal levels of PGA to a minimum in all cases. Sulfasuxidine has no effect on the caecal level of vitamin B_{12} in the chick.

Vitamin C, when injected, has no effect on growth, liver storage or caecal concentration of PGA in the chick. The injection of PGA markedly increased the liver storage and caecal levels of both PGA and vitamin B_{12}.

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