# Histamine Shock and Vitamins. (19793)

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Though there has been much speculation on the antiallergic effect of various vitamins, only recently have experiments been reported which indicate that vitamins possess antianaphylactic and antihistaminic effects. For instance Traina(1) working with guinea pigs found that vit. $B_{12}$ gives protection against anaphylactic shock and against twice the $LD_{50}$ of histamine. Karady and Browne(2) showed that the previous administration of histaminase gives protection against anaphylactic shock, and suggested that vit. $B_{12}$ may activate this enzyme. On the other hand, Guidolin and Ferri(3) in more extensive experiments reached the opposite conclusion, that vit. $B_{12}$ does not protect against anaphylactic shock. Likewise Simon(4), in a clinical investigation, failed to find any antiallergic effect of $B_{12}$. Various authors have tried to relate vit. C to allergy. It has been recommended in high doses for the symptomatic treatment of asthma and other allergic syndromes. On the contrary, Friedell *et al.*(5), Holtz(6), and Wirtschafter and Widmann(7) reported that ascorbic acid contributes to the transformation of histidine into histamine both *in vitro* and *in vivo*. Also, it has been studied in relation to stress, the general adaptation syndrome, and the pharmacology of ACTH and cortisone. Finally, Saylor(8), Black(9), and Clarck and Mackay(10) reported various cases of allergic syndromes, especially urticaria, treated with vit. P and K. These contradictory results have led us to undertake investigations of the possible antihistaminic action of the principal vitamins.

*Procedure and results.* Our experiments were arranged as follows: a) the vitamins were given previous to the histamine shock, in order to measure the possible protective action; b) determinations of the effect of histamine on blood pressure were made before and after administration of the vitamins; c) the antihistaminic effect of the vitamins on the isolated guinea pig's ileum was studied.

a. *Histamine shock.* Histamine was given to guinea pigs as an aerosol in an "asthma chamber." The animals weighed 200 to 250 g. An aqueous solution of histamine (2 or 5 parts in 1,000) was dispersed with an air pressure of 300 mm Hg. The animals were first subjected to the histamine spray without prior treatment and the time from the start of histamine inhalation to loss of upright posture (histamine time, $H_t$) was measured; at $H_t$, the animals were taken out of the chamber. In these pretests, $H_t$ was 2 to 3 minutes with a histamine concentration of 2:1,000 and 1½ to 2 minutes with a 5:1,000 concentration. After the pretests, the guinea pigs were divided into groups of greatest possible homogeneity as to $H_t$. After 5 days, the vitamin was administered subcutaneously 20 minutes before exposure to the aerosol. The results are given in Table I, where n is the number of guinea pigs, $H_t$ the mean histamine time determined in the pretest, $MH_t$ ("modified histamine time") the mean histamine time in the vitamin experiments, and P the probability value indicating the statistical significance of the difference between $H_t$ and $MH_t$.

According to the data in Table I, neither 15 nor 30 $\mu$g/kg of vit. $B_{12}$ gave any protection against histamine shock produced by exposure to histamine aerosol. Results were equally negative with 50 and 100 mg/kg of vit. C and also with vit. $B_1$, B complex and K.

b. *Vasodepressor action of histamine.* The fall of blood pressure produced by intravenous injection of histamine before and one minute after intravenous injection of vit. $B_{12}$ was measured both in cats and in guinea pigs. The details are surveyed in Table II.

In the same arrangement, the effects of still higher intravenous histamine doses, sublethal and lethal, were studied in guinea pigs; blood pressure and respiration were simultaneously registered. The toxic symptoms—hypertensive phase, followed by a hypotensive phase after a lethal dose; bronchospasm; tachypnea followed by bradypnea—were qualitatively

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TABLE I.

| Substance | Dose | n | Histamine spray conc. | $H_t$ | $MH_t$ | P |
|---|---|---|---|---|---|---|
| Vit. $B_1$* | 10 mg/kg | 10 | 5: | 2'10" | 1'55" | .5 |
| $B_{12}$† | 15 μg/kg | 5 | 5: | 1'30" | 1'35" | .8 |
| $B_{12}$ | 30 | 7 | 2: | 3'30" | 3'50" | >.1 |
| C‡ | 50 mg/kg | 10 | 2: 1.000 | 2'00" | 2'25" | .5 |
| C | 100 | 5 | 5: | 1'20" | 1'35" | .7 |
| K§ | 10 | 10 | 5: | 1'25" | 1'35" | .8 |
| B complex‖ | 1 cc/kg | 5 | 2: | 2'05" | 2'15" | .7 |

* Thiamin chloride, 1% sol. † 1.5:100000. ‡ 5% sol. § Menadion, 1% sol.
‖ Thiamin chloride 3 mg, riboflavin 1 mg, pyridoxin 1 mg, niacinamide 10 mg, calcium pantothenate 2 mg/1 cc aqueous sol.

TABLE II.

| Species | Dose: Vit. $B_{12}$, μg/kg | Dose: Histamine, μg/kg | n | $-\Delta p_1$ | $-\Delta p_2$ | P |
|---|---|---|---|---|---|---|
| Cat | 10 | .25 | 10 | 18 | 18 | .9 |
| | 15 | .50 | 9 | 23 | 22 | .8 |
| Guinea pig | 10 | .50 | 10 | 10 | 11 | .9 |
| | 20 | 1 | 10 | 15 | 15 | .9 |

$-\Delta p_1$ = Fall of blood pressure (mm Hg) due to inj. of histamine, before administration of the vit.
$-\Delta p_2$ = Idem, after administration of the vit.

TABLE III.

| Vitamin | Dose per 50 cc bath | n | $h_{c1}$ (mm) | $h_{c2}$ (mm) | P |
|---|---|---|---|---|---|
| $B_{12}$ | 1 mg | 9 | 22 | 23 | .7 |
| | 15 | 10 | 21 | 22 | .8 |
| | 30 | 9 | 21 | 20 | .7 |
| C | 1 μg | 8 | 23 | 22 | .9 |
| | 10 | 10 | 20.5 | 23 | .7 |
| | 100 | 9 | 22 | 21 | .8 |

$h_{c1}$ = Ht of contraction due to histamine before addition of vitamin.
$h_{c2}$ = Idem, after addition of vitamin.

and quantitatively no different in untreated animals and in those pretreated 1 to 15 minutes beforehand with 15 or 30 μg/kg of vit. $B_{12}$.

c. *Histamine action on excised guinea pig gut.* The preventive action of varied doses of vit. $B_{12}$ and C against histamine spasm of the isolated gut was studied with ileum strips suspended in 50 cc Ringer solution. Before and one minute after addition of the vitamin dose, 1 μg of histamine was added to the bath fluid. After each histamine test, the intestine was washed with 100 cc Ringer solution. The mean value of the height of the histamine contraction (in mm) was determined in approximately 10 experiments in each group. Mean values were practically the same in the presence and in the absence of the vitamins, as shown in Table III. Thus, on this test object vit. $B_{12}$ and C proved also to be devoid of antihistaminic action. Large doses of both vitamins (100 mg of C and 15 μg of $B_{12}$) produced marked relaxation, but not even in the relaxed state was the sensitivity of the intestine to histamine decreased.

*Discussion.* In the work here reported, vit. $B_{12}$ failed to give any protection against the respiratory, the vasodepressor, the general toxic and the lethal actions of histamine or against the histamine-induced spasms of the isolated intestine. Against some of these histamine actions, vit. $B_1$, C, K, and B complex were also tried with the same negative result. The present experiments differ from those of Traina(1), who came to the opposite conclusions, in that we employed a greater number of test objects, conducted a larger number of experiments and extended the experiments into a higher dosage range of the vitamins; we also avoided the intracardial administration of vitamin and histamine, exclusively employed in Traina's experiments.

*Summary.* 1. Even in high doses, vit. $B_1$, $B_{12}$, C, K, and B complex did not protect guinea pigs against histamine shock produced by inhalatory administration of histamine aerosol. 2. Vit. $B_{12}$ and C did not exhibit any antihistaminic action in assays of blood pressure in cats and guinea pigs. 3. Vit. $B_{12}$ and C did not show any antihistaminic action in assays on the isolated guinea pig ileum; in high doses both vitamins caused the intestinal

strip to relax, but without decrease of its histamine sensitivity.

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## Activity of Citrovorum Factor for the Chick.* (19794)

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Sauberlich and Baumann(1) reported the existence of a substance which was closely related to folic acid and which was necessary for the growth of *Leuconostoc citrovorum.* The isolation of citrovorum factor (CF) in crystalline form from horse liver was reported by Keresztesy and Silverman(2). This substance was found to be approximately twice as active for *L. citrovorum* as the synthetic compound, 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid (leucovorin(3), folinic acid-SF (4)). The biological activity of the natural crystalline product in folic acid deficient animals has not been reported thus far. Various reports have indicated that synthetic compounds with CF activity prepared from folic acid are at least partially active in folic acid deficient chicks. Hill and Briggs(5) reported that a synthetic form of folinic acid which differed from folinic acid-SF† was as effective as pteroylglutamic acid (PGA) when given in the diet. Broquist *et al.*(6) recently found that leucovorin was 50% as active as PGA when injected. Orally administered leucovorin, however, had considerably lower activity. The authors suggested that leucovorin might be a mixture of diastereoisomers in order to account for its having only one-half as much activity as PGA when injected. Recently, Sauberlich(7) compared the activity for the chick of PGA with a concentrate of CF and concluded that they had the same activity when incorporated in the diet.

Because of the differences in the reported activities of the various products and because of the recent availability of crystalline natural CF, it became of interest to determine the comparative efficacy of crystalline CF, folinic acid-SF, leucovorin, and PGA for the chick.

*Experimental.* New Hampshire female chicks from a commercial hatchery were equilibrated according to weight in groups of 6 and placed on experiment when one day old. They were raised in electrically heated brooders with wire screen floors. Feed and water were supplied *ad libitum* and the experiments were terminated at the end of 4 weeks. The basal diet, which contained no added folic acid, consisted of the following in g/kg: Cerelose (glucose) 615, vitamin-free casein 200, gelatin 80, DL-methionine 3, corn oil 40, Chick Salts A 60,‡ and choline chloride 2.

* The sample of leucovorin was supplied by Lederle Laboratories, Pearl River, N. Y., and the sample of folinic acid-SF by Dr. William Shive, University of Texas, and Eli Lilly and Co., Indianapolis.

† A personal communication from Dr. William Shive informs us that the folinic acid used by Hill and Briggs(5) was a purified amorphous synthetic sample which differed from folinic acid-SF, and did not contain any free or conjugated PGA.

‡ Sixty g of Chick salts A contributed the following in g/kg of diet: $CaCO_3$ 15, $K_2HPO_4$ 9, $Na_2HPO_4$ 7.3, $Ca_3(PO_4)_2$ 14, NaCl 8.8, $MgSO_4 \cdot 7H_2O$ 5, ferric citrate (16.7% Fe) 0.4, $MnSO_4 \cdot 4H_2O$ 0.42, KI 0.04, $ZnCO_3$ 0.02 and $CuSO_4 \cdot 5H_2O$ 0.02. All salts were U.S.P. grade or purer.