

species differences can be offered on the basis of the results of these experiments.

Summary. Two series of 36 and 24 guinea pigs were studied. Cortisone was given to a third of the animals, ACTH to a third and the remaining third served as controls. The administration of the hormones, in moderate or large doses, was started before giving the sensitizing injections of egg white, and was continued while they were being given and

for varying periods after their completion. No treatment had any effect on the severity of anaphylactic reactions induced by the subsequent intravenous injection of the antigen. Used in adequate amounts, both hormones produced pronounced and sustained reductions in the number of circulating eosinophils in the blood of male and nonpregnant female guinea pigs.

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Negative Effects of Vitamin B₁₂ on Blood of Healthy Individuals, with Special Reference to Eosinophils.* (18705)

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While examining blood smears from patients ill with pernicious anemia, who responded to therapy with vit. B₁₂, we were surprised to find that there had been no specific increase in eosinophilic polymorphonuclear leucocytes. We had expected to find a rise in eosinophilic cells since it is well known that oral or intramuscular therapy with whole liver or with liver extracts commonly produces an eosinophilia in patients ill with pernicious anemia or in healthy individuals(1-9), which may reach as high as 75% of the total white

blood cell count. Because of this, observations were made on the effects of therapy with relatively large amounts of vit. B₁₂ given, either by mouth or by injection, to 3 healthy young adults and to 5 healthy children.

Methods. Three adult males and 5 school children served as subjects. They were healthy and none of them gave a history of previous allergic disease, anemia, or blood dyscrasia. In the adults, studies of their blood were made 3 times a week; during a control period of 2 weeks, during a 28 day vit. B₁₂ therapy period, and during the 2 weeks of the post-therapy period. Blood from the children was observed twice during the pre-therapy period and at weekly intervals during the therapy and post-therapy periods. Fasting blood was collected in heparinized tubes and from these determinations of hematocrit, hemoglobin, and red blood cell counts were made by standard technics. Absolute eosinophil counts and white blood cell counts were made by a modification of Randolph's technic using 3 parts of phloxine to one part of methylene blue(11). Differential counts of stained blood were made by standard technics using fresh blood cover-glass smears and counting 200 white blood cells.

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1. Minot, G. R. and Murphy, W. P., *J.A.M.A.*, 1925, v89, 759.

2. Watkins, C. H. and Gerglund, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1927, v25, 206.

3. Whitley, L. E. H., *Lancet*, 1928, v1, 285.

4. Smith, K. S. and Whitley, L. E. H., *Lancet*, 1928, v2, 277.

5. Ordway, T. and Gorham, L. W., *J.A.M.A.*, 1928, v91, 925.

6. Meulengracht, E., *Am. J. M. Sci.*, 1930, v179, 199.

7. Adler, A. and Schiff, A., *Deutsch Arch. Clin. Med.*, 1938, v161, 282.

8. Allen, R. N. and Meyer, O. O., *J. Lab. and Clin. Med.*, 1940, v26, 457.

9. Kark, R. M. and Muehroke, R. C., unpublished data.

11. Randolph, T. G., *Allergy*, 1944, v15, 89.

TABLE I. Absolute and Differential Counts of Eosinophiles in 8 Healthy Persons before and after Treatment with vit. B₁₂.

Subject	Sex	Age	B ₁₂ μg per day	Absolute Eosinophile Counts per mm ³				Differential Count of Eosino- philes: % Total White Blood Cells			
				Pre Vit. B ₁₂		Post Vit. B ₁₂		Pre Vit. B ₁₂		Post Vit. B ₁₂	
P.	M	11	10*	136	176	239	220	2	2	3	2
W.	M	7	10†	116	132	117	83	2	1	1	1
H.	M	10	10†	156	242	261	225	4	6	4	3
D.	M	8	10†	117	99	75	77	1	1	1	2
H.W.	F	12	10†	50	77	89	84	2	1	2	0
C.	M	20	300†	176	149	193	187	1	2	2	4
L.	M	27	300†	321	308	385	264	1	3	3	2
M.	M	28	75*	303	396	297	401	1	3	1	2

* Intramuscular therapy.

† By mouth.

The dosage of vit. B₁₂ was regulated to provide approximately similar amounts of crystalline B₁₂ to that present naturally in 1½ lb of raw calves liver(12). Accordingly, each of 2 adults received 300 μg vit. B₁₂ a day by mouth for 28 days, and the third subject was given 75 μg of vit. B₁₂ a day by intramuscular injection for the same period of time. The children received 10 μg of the vitamin each day for 28 days; 4 took this by mouth and the fifth received it by intramuscular injection.

Results and discussion. It is apparent that at least two biochemical mechanisms associated with liver therapy stimulate an increase in circulating eosinophils. The first type of liver eosinophilia is the result of sensitization to liver protein or to the protein of the animal from which the liver or liver extract was obtained. This commonly produces clinical manifestations of sensitization in the patient receiving the liver(6). The second type of liver eosinophilia is a specific effect of liver therapy and occurs uniformly, even in healthy individuals, following the ingestion of 1½ lb of raw fresh calves liver for 28 days(9). This latter type of eosinophilia was commonly seen in patients ill with pernicious anemia, who lived on raw liver, in the years immediately following Minot and Murphy's(1), discovery of the value of a diet high in liver for treatment of the disease. This eosinophilia is symptom free and is not associated with clinical manifestations of allergy.

Vit. B₁₂ did not produce an eosinophilia in our subjects (Table I). Clinically there was no evidence of development of toxic or allergic disturbances in either adults or children, who remained healthy throughout the period of study. Hematologic data obtained in the control period were not altered significantly in any subject following therapy. The data, as far as the children are concerned, are somewhat at variance with those obtained by Wetzel *et al.*, who found an 8% eosinophilia in children after treatment with vit. B₁₂(10). The small rise in the eosinophils reported by Wetzel may be a normal variation when making differential counts of cells from slides(13), which is not as statistically valid as counts made by direct counting technics(14).

Summary. 1. Oral or intramuscular administration of large amounts of vit. B₁₂ was without effect on the blood of healthy adults and children. In particular, an eosinophilia was not observed. 2. Neither allergic nor toxic symptoms were noted during or after therapy. 3. It is concluded that vit. B₁₂ is not the eosinophilic factor present in raw calves liver.

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10. Wetzel, N. C., Fargo, W. C., Smith, I. H., and Helikson, L., *Science*, 1949, v110, 651.

13. Goldner, F. M. and Mann, W. N., *Guys Hosp. Rep.*, 1938, v88, 54.

14. Best, W. R. and Samter, M., *Blood*, 1951, v6, 67.