

questionable whether one may, at this time, associate the calcification process in inorganic phosphate and glycerophosphate solutions with two completely discrete mechanisms. 4. Strontium, barium, and manganous ions share with magnesium the property of mediating the cyanide inhibition of calcification.

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Effects of a Combined Deficiency of Vitamins E and B₆ on Blood Picture of Rat.* (20854)

JAMES S. DINNING, J. M. YOUNG, JR., MARY R. SIMMONS, AND PAUL L. DAY.

From the Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock.

One sign of acute vit. E deficiency in monkeys and rabbits is leucocytosis(1,2). There is a marked increase in the numbers of circulating granulocytes with little change in the numbers of blood lymphocytes(1,2). Vitamin B₆ is also concerned in hemopoiesis. It has been shown that B₆-deficient dogs(3) and pigs(4) develop a microcytic anemia. Weir *et al.*(5) reported that the injection of a vit. B₆ antagonist (desoxypyridoxine) into mice resulted in a decrease in circulating lymphocytes and an increase in granulocytes. These changes could be reversed by the administration of pyridoxine. Quite recently Vilter *et al.*(6) reported the effects of administering desoxypyridoxine to man. They observed an absolute lymphopenia with a tendency toward polymorphonuclear leucocytosis. In contrast

Wintrobe *et al.*(4) observed no significant change in leucocytes as a result of vit. B₆ deficiency in pigs.

In general, the leucocyte changes which have been observed in vit. B₆ deficiency resemble those of vit. E deficiency. There is some biochemical data which suggest a relationship between vit. E and vit. B₆ in metabolism(7,8). For these reasons a study was made of the effect of a combined deficiency of vit. E and vit. B₆ on the hemogram of the rat.

Methods. Weanling littermate Sprague-Dawley rats of both sexes were fed a basal

TABLE I. Weight Changes and Food Consumption of Rats Fed the Experimental Diets.

Diet	No. of rats	Mean initial wt, g	Mean final wt, g	Mean daily food intake, g
Basal	9	50	102	4.6
" + E	7	49	106	5.4
" + B ₆	7	47	194	8.6
" + B ₆ + E	7	48	223	8.3

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TABLE II. Effects of Vit. E and B₆ on Peripheral Leucocytes of Rats (Leucocytes Reported as Thousands/ μ l).

Diet	Total leucocytes	Lymphocytes	Monocytes	Neutrophils	Eosinophils
Basal	18.4	6.9	.6	10.5	.4
" + E	8.5	5.9	.1	2.4	.1
" + B ₆	10.0	7.2	.4	2.2	.2
" + B ₆ + E	10.8	8.8	.4	1.5	.1

diet consisting of casein, 18.6 g; sucrose, 67.4 g; lard, 8.0 g; cod liver oil, 2.0 g; salt mix (*J. Nutrition*, v14, 273), 4.0 g; choline chloride .1 g; inositol, 10 mg; riboflavin, .8 mg; thiamin HCl, .5 mg; Ca pantothenate, 2 mg; nicotinic acid, 2 mg; and menadione, .44 mg. This diet is deficient in vit. E and B₆. One group of rats was given this diet without supplement. Three other groups of rats were given the basal diet supplemented with pyridoxine, α -tocopheryl acetate, and the combination of the two, respectively. Pyridoxine was mixed with the diet at a level of 10 mg per kilo. Tocopherol was given orally 2 times weekly. The animals were given 2 mg of α -tocopherol acetate per dose until they reached a body weight of 100 g at which time the dose was increased to 4 mg. Food and water were given *ad libitum* and food intake records were kept. The rats were fed these diets for 105 days. Complete blood counts on tail blood were made at frequent intervals, using standard hematological technics.

Results. The effects of the supplements on weight gain and food intake are given in Table I. Vit. B₆ markedly increased growth and food consumption. Supplementation of the basal diet or the vit. B₆-containing diet with vit. E had no significant effect on growth or food intake.

Erythrocyte counts and hemoglobin, and hematocrit determinations were made on tail blood from all rats at frequent intervals. The results were all within the normal limits for the rat and the data are not given.

The leucocyte data given in Table II were obtained between the 95th and 105th experimental days. The values are the mean of 2 to 3 bleedings for each rat in the various groups. Monocytes and eosinophils were present in insignificant numbers in all groups. Animals receiving the diets without

vit. B₆ exhibited slightly lower lymphocyte counts than did the vit. B₆-supplemented groups. This difference in number of lymphocytes was found to be statistically significant when the data were analyzed by the analysis of variance method ($P < 0.05$). It must be realized that animals in the vit. B₆-deficient groups consumed less food than did the B₆-supplemented animals and inanition has been shown to result in a reduction in the numbers of peripheral lymphocytes of rats (9).

The most striking effect observed was the great increase in circulating neutrophils in rats deficient in both vit. E and B₆. The numbers of peripheral neutrophils of animals in this group were elevated 7-fold when compared with control animals receiving both vitamins. Supplementation of the basal diet with either vit. E or B₆ markedly reduced the numbers of neutrophils. The combination of the 2 vitamins reduced neutrophils only slightly more than either of them separately.

In Fig. 1 neutrophil counts are plotted as a function of time on the various diets. The increase in number of neutrophils of rats fed the basal diet began about the 50th day of feeding and continued rapidly thereafter.

Discussion. The most striking finding in these experiments was the observation that rats deficient in both vit. E and B₆ exhibit a marked granulocytosis and that this condition is prevented by supplementing the diet with either vit. E or B₆. This indicates that these 2 vitamins are closely related in some metabolic mechanisms. Extensive biochemical studies were made on these animals and will be reported in detail elsewhere but it is appropriate to mention here that creatine excretion followed a pattern quite similar to that of the peripheral neutrophils. Animals receiving the basal diet exhibited an increased creatinuria beginning about the 50th experi-

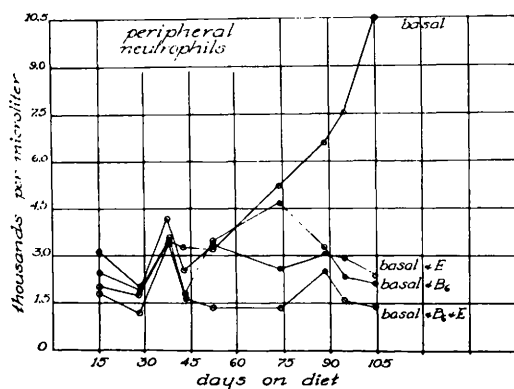


FIG. 1. Effects of vit. E and B_6 on peripheral neutrophils of rats.

mental day. This was approximately the same time the increased neutrophil levels became apparent. Creatinuria serves as a good indication of the beginning of nutritional muscular dystrophy in rabbits(10). Thus, rats deficient in both vit. E and B_6 behaved like rabbits deficient in vit. E, and granulocytosis may be a result of the metabolic alterations which instigate nutritional muscular dystrophy.

Summary. Sprague-Dawley rats deficient in both vit. E and B_6 exhibited a greatly increased peripheral neutrophil count. Sup-

plementation of the diet with either of these vitamins prevented this increase. The B_6 -deficient rats exhibited a slight lymphopenia which was not affected by vit. E. None of the animals developed anemia.

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Identification and Quantitative Determination of Serotonin (5-hydroxytryptamine) in Blood Platelets.*† (20855)

MARJORIE B. ZUCKER, BERNARD K. FRIEDMAN, AND MAURICE M. RAPPORT.†

From the Department of Physiology, New York University College of Dentistry, New York City.

The vasoconstrictor substance in blood serum was identified by Rapport and his co-workers as 5-hydroxytryptamine (serotonin) (1). Serotonin is not present in plasma(2), but appears in serum when clotting occurs in the presence of platelets(3,4). The fact that not only coagulation but also agitation, aging, freezing or exposure to ultrasonic frequencies

cause the appearance of vasoconstrictor activity in platelet-rich plasma indicates that the clotting process *per se* is of no importance in the evolution of serotonin and suggests that the material is simply released from the platelets by these procedures. The occurrence of smooth muscle-stimulating material in crude platelet extracts supports this suggestion. In preliminary experiments, paper chromatograms of dog and rabbit platelet extracts were found to show spots which corresponded to 5-hydroxytryptamine(5). In the absence of information on the reliability of paper chro-

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‡ Present address: Sloan-Kettering Institute, New York City.