Relationship of Pyridoxine Deficiency to Adrenal Function in Production of Leucocytes in Mice.* (18762)

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Granulocytosis, lymphopenia, and myeloid metaplasia in the spleen have been produced in mice either by treatment with cortisone or by the production of pyridoxine deficiency induced with desoxypyridoxine(1,2). This similarity of effect suggested a common mechanism of action. The possibility that pyridoxine deficiency induced by desoxypyridoxine merely activated an alarm reaction, mediated through the adrenal, was considered, although significant differences in the hematologic pictures produced by the 2 methods were pointed out. The results of 3 other experiments reported here support the view that the mechanism responsible for the production of the hematologic abnormalities in pyridoxine deficiency in the mouse is independent of the adrenal gland.

Exp. 1. 15 weanling Swiss mice weighing within 7 g of one another were placed in individual cages and divided into 3 groups of 5 each. Group I was fed ground Friskies dog chow to which was added 0.1% desoxypyridoxine HCl. Group II was fed dog chow and given 1 mg of cortisone subcutaneously daily. Group III received both cortisone and desoxypyridoxine. At the beginning of the experiment and twice weekly thereafter total and differential leucocyte counts were done and the animals weighed. Sections of spleen, liver, bone marrow, adrenal glands, and lymph nodes were obtained at autopsy. The simultaneous induction of pyridoxine deficiency and administration of cortisone apparently produced greater changes in the circulating neutrophiles and lymphocytes than either alone (Fig. 1). The leucocyte shift was more complete on the 4th day and the lymphopenia was more severe with earlier mortality and greater weight loss. Histologic changes in the organs were the same as described before(2) and were not significantly different in the 3 groups. The greater effect produced by pyridoxine deficiency plus cortisone as compared to the effect of either alone indicated that the mechanism of action of the one may differ from the other.

Exp. 2. Bilateral adrenalectomy through a single dorsal incision was done on 22 weanling Swiss mice. The entire group was given 0.85% salt solution for drinking water and a diet of Friskies dog chow. They were divided into 2 groups of 11 each. The first group received 0.1% desoxypyridoxine added to the diet. The other group served as controls. On the 15th day, after hematologic changes had occurred, 0.3% pyridoxine HCl was added to the diet of all the survivors. The leucocyte changes in the adrenalectomized mice given desoxypyridoxine (Fig. 2) were identical with those occurring in intact mice given desoxypyridoxine, as previously reported(1). Absolute neutrophilia and lymphopenia occurred which was reversed by the addition of pyridoxine to the diet. Although 7 of the 11 mice receiving desoxypyridoxine died in the first week, hematologic changes had already occurred. Only 2 of this group survived the entire experimental period. There was no significant change in the leucocyte counts of the control group of 11 adrenalectomized mice not given desoxypyridoxine (Fig. 3). Although the lymphocyte counts increased moderately, they remained within normal limits. Only 2 mice had died at the end of the first week and 4 survived the experimental period. Histologic sections from animals which died before pyridoxine was added to the diet were unsatisfactory because of post-mortem degeneration. In the spleens of animals killed at the end of the experiment there was a slight, but

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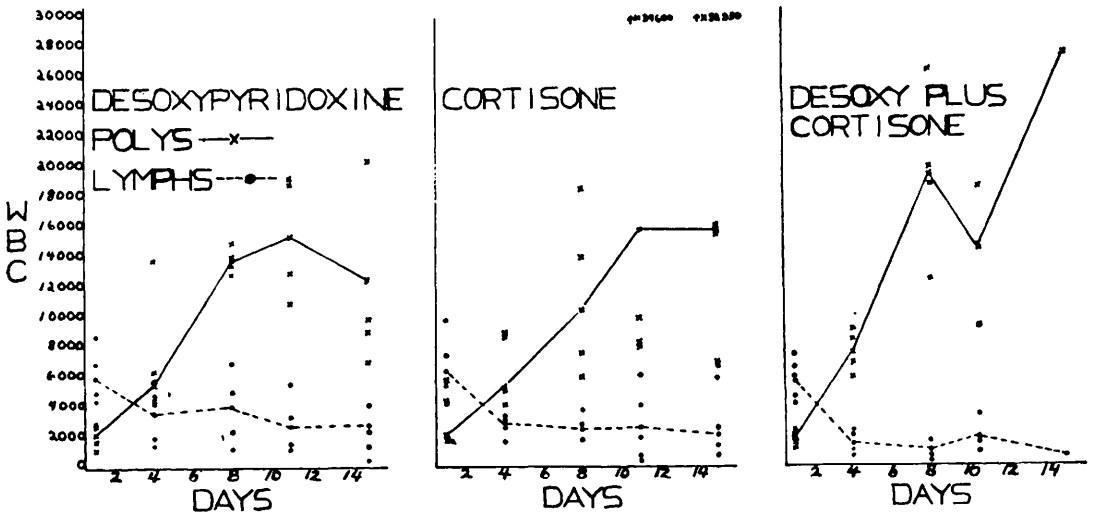

FIG. 1. Effect on circulating leucocytes of cortisone, of pyridoxine deficiency, and of the two combined.

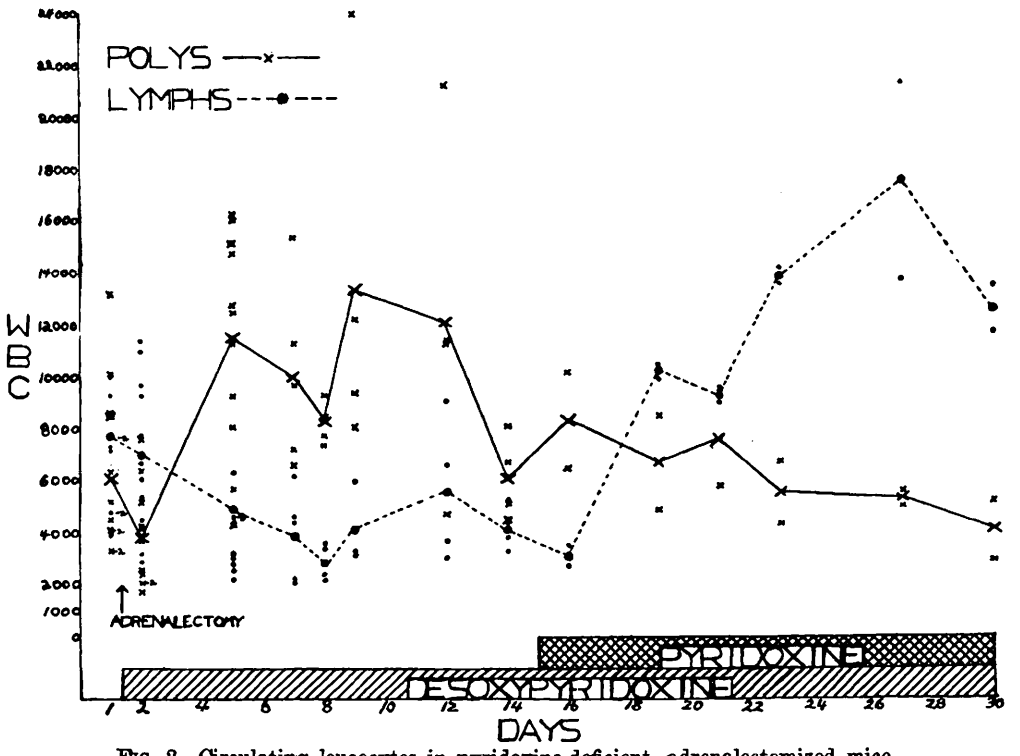

FIG. 2. Circulating leucocytes in pyridoxine-deficient, adrenalectomized mice.

definite, increase in the size, number, and activity of the follicles, with increased numbers of nucleated red cells in the pulp. The fact that adrenalectomy failed to prevent the leucocyte changes caused by pyridoxine deficiency is clear evidence that the mechanism of production of these changes is not mediated through the adrenal.

Exp. 3. It was suggested previously(2) that one of the effects of cortisone might be

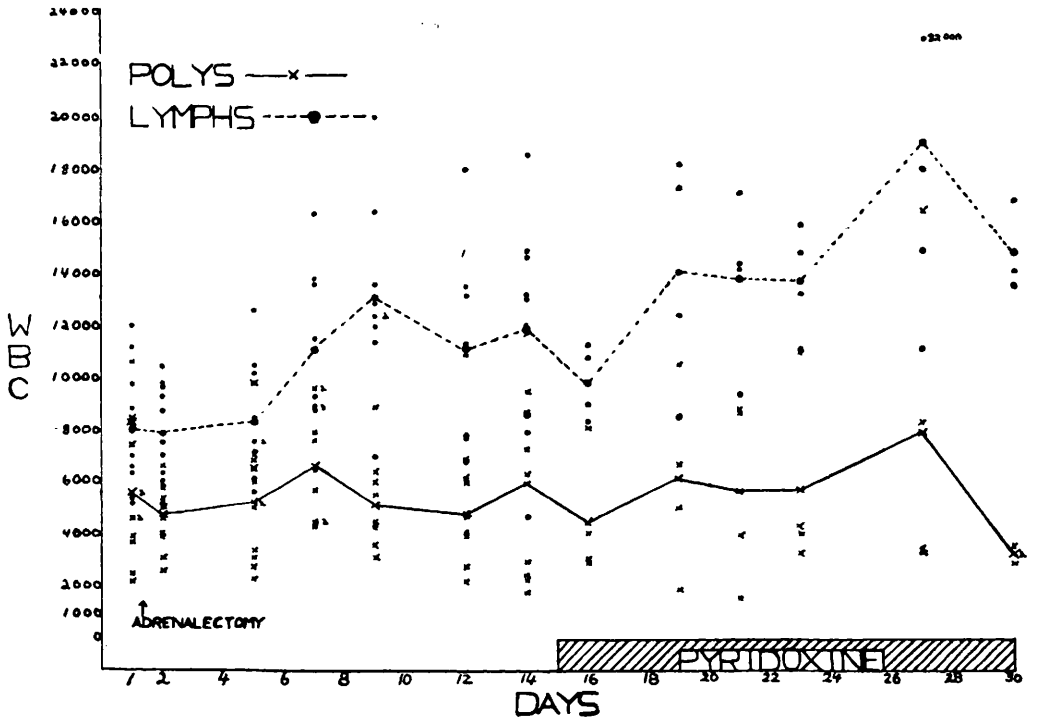

FIG. 3. Circulating leucocytes in untreated, adrenalectomized mice.

the induction of an effective deficiency of the active vit. B_6 coenzyme, occurring at some stage in the metabolic cycle in which the vitamin is concerned. In order to obtain evidence for or against this hypothesis an attempt was made to block the hematologic effect of cortisone by feeding large quantities of the B_6 vitamins to cortisone-treated mice. A diet was prepared containing 0.3% each of pyridoxine HCl, pyridoxal HCl, and pyridoxamine dihydrochloride added to ground Friskies dog chow. Since a mouse eats 3 to 6 g of diet daily, this formula provided 9 to 18 mg of each metabolite to each mouse daily. Twenty Swiss mice were divided into 4 groups of 5 each. Group 1: B_6 diet and 1 mg cortisone. Group 2: B_6 diet and 0.5 mg cortisone. Group 3: normal diet and 1 mg cortisone. Group 4: normal diet and 0.5 mg cortisone. Cortisone was given subcutaneously daily. Groups 1 and 2 were maintained on the supplemented diet for 8 days before cortisone was started.

The animals eating the normal diet developed the expected neutrophilia and lymphopenia. These changes were more marked in the animals receiving 1 mg of cortisone than in those receiving 0.5 mg. There was no evidence that the hematologic response to cortisone was in any way modified by the added B_6 vitamins in Groups 1 and 2. In spite of the enormous doses of the vitamins the findings were entirely comparable with those of the control groups. This may indicate that the mechanism for the production of hematologic changes induced by cortisone differs from that of pyridoxine deficiency but the possibility still exists that cortisone may have a role in the B_6 metabolic cycle, perhaps by blocking the production or utilization of pyridoxal phosphate or pyridoxamine phosphate.

Discussion. It has been shown(2) that in pyridoxine deficiency induced by desoxypyridoxine the hematologic changes can be maintained for at least 30 days, a result not characteristic of the alarm reaction, in which there is a reversal towards normal in a brief period of time. Also, the fact that the hematologic picture can be restored to normal by the addition of pyridoxine indicates that the effect of

desoxypyridoxine is probably that of a metabolic competitor to the active B_6 vitamin and, in itself, does not activate an alarm reaction. The demonstration that the hematologic abnormalities occur in pyridoxine-deficient, adrenalectomized mice proves conclusively that the mechanism is not that of an alarm reaction mediated through the adrenal. Thus, the mechanism for the production of these abnormalities remains to be elucidated. It may conceivably involve interference with some enzyme activity in nucleoprotein metabolism not understood at this time.

Since simultaneous pyridoxine deficiency and cortisone administration produced greater changes than either alone, the possibility should be considered that cortisone actually accelerated the production of pyridoxine deficiency. Stoerk(3) considered this when he observed that the combination was more deadly in rats than either one alone. He offered the hypothesis that cortisone, by increasing the utilization of amino acids, increased the body's demand for vitamin B_6 and thereby further aggravated the pyridoxine deficiency. Against this hypothesis is our finding that enormous amounts of pyridoxine, pyridoxal, and pyridoxamine in no way modified the hematologic effect of cortisone. Since the hematologic changes are so similar in these two states, the possibility exists, however, that the ultimate mechanism may be the same. Thus, the effect might be on the "end-organ" whether this be nucleoprotein synthesis or some other unknown metabolic reaction. This would adequately explain all the results of these experiments.

Summary. 1. The production of pyridoxine deficiency in adrenalectomized mice calls forth the same hematologic response, neutrophilia and lymphopenia, as in intact animals. This is positive evidence that these blood changes occurring in pyridoxine deficiency are not the result of an alarm reaction mediated through the adrenal gland. (2) Pyridoxine deficiency and cortisone administration produce similar hematologic responses. When combined, a synergistic effect results. This may be evidence that the mechanism of the production of the hematologic changes is different in the 2 conditions. (3) Further evidence that the mechanisms may differ is the fact that the cortisone effect cannot be blocked by giving large amounts of pyridoxine, pyridoxal, and pyridoxamine, although the possibility exists that cortisone has a role at another site in the B_6 cycle.

Pyridoxine, pyridoxal, pyridoxamine, and desoxypyridoxine were supplied by Merck and Co.

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Interrelationships of Folic Acid and Ascorbic Acid in Rat Liver.* (18763)

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Nichol and Welch(1,2) reported that one probable role of ascorbic acid in animal tissues is to aid in the conversion of folic acid to the *Leuconostoc citrovorum* factor (LCF). Recently we have reported that the choline oxidase system of rat tissues is intimately related to folic acid, ascorbic acid, LCF, and vit. B_{12}(3,4) as demonstrated by *in vitro*

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