Similarity of Hematologic Effect of Pyridoxine Deficiency, Cortisone, and Myeloid Metaplasia Factor of Human Urine.* (18295)

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Previous work done in this laboratory and studies reported by others have shown that similar hematologic effects have been produced in human beings or animals by pyridoxine deficiency induced by desoxypyridoxine, by cortisone and adrenocorticotropic hormone, and by a myeloid metaplasia factor recoverable from human urine in certain disease states. More detailed experiments have been performed in mice to study further the similarity of effect produced by these apparently unrelated compounds and to discover whether there is any evidence that the effects are mediated through a common mechanism.

Pyridoxine deficiency. A previous study (1) in mice showed that pyridoxine deficiency was associated with granulocytosis and lymphopenia. The regimen used produced only a partial deficiency, however, as evidenced by the absence of weight loss, anemia, lymphoid atrophy and convulsions. A more complete deficiency was produced in the present experiment by administering the desoxypyridoxine† orally rather than parenterally. It was added to the Purina dog chow diet in percentages of 0.05%, 0.1% and 0.15% and given *ad libitum* to 3 groups of 5 young adult Swiss mice. These animals ate 3 to 6 g daily so that the 3 groups received 1.5-3 mg, 3-6 mg, and 4.5-9 mg daily of desoxypyridoxine, respectively. Hematologic changes occurred in all 3 groups within 5 days. Granulocytosis and lymphopenia were equally great in all groups indicating that the smallest amount of desoxypyridoxine administered produced maximal results. Three mice died on the 18th, 23rd and 31st days. By the 32nd day the other 12 had significant weight loss. Six of these which had moderately severe dermatitis were killed and autopsied. The remaining 6 animals were continued on the diet containing 0.05% desoxypyridoxine to which was added 0.15% of pyridoxine HCl, providing each animal with 4.5 to 6 mg of pyridoxine daily. The blood picture was restored to normal promptly and weight gain occurred. Definite tissue abnormalities were present in the animals having dermatitis, in contrast to negative findings in the previous experiment(1) (Fig. 1b). There was slight enlargement of the spleens with extensive infiltration of the pulp by neutrophils and large, immature, mononuclear cells, with occasional mitoses. Megakaryocytes were slightly increased in number and erythropoietic elements were abundant. Atrophy of the splenic follicles did not occur. Changes in the liver were not striking, only a few small areas of infiltration with neutrophils and mononuclear cells being present.

Atrophy of lymphoid tissue might have been expected(2), but none occurred. In addition to the intact splenic follicles, the thymus and lymph nodes were of normal size and histologic structure. Mild adrenal cortical hyperplasia was evidenced by a measurable increase in the width of the cortex as compared with normal animals.

Cortisone effect. The occurrence of lymphopenia, granulocytosis and regression of lymphoid tissue associated with administration of ACTH and cortisone has been described(3-8). To compare this effect with

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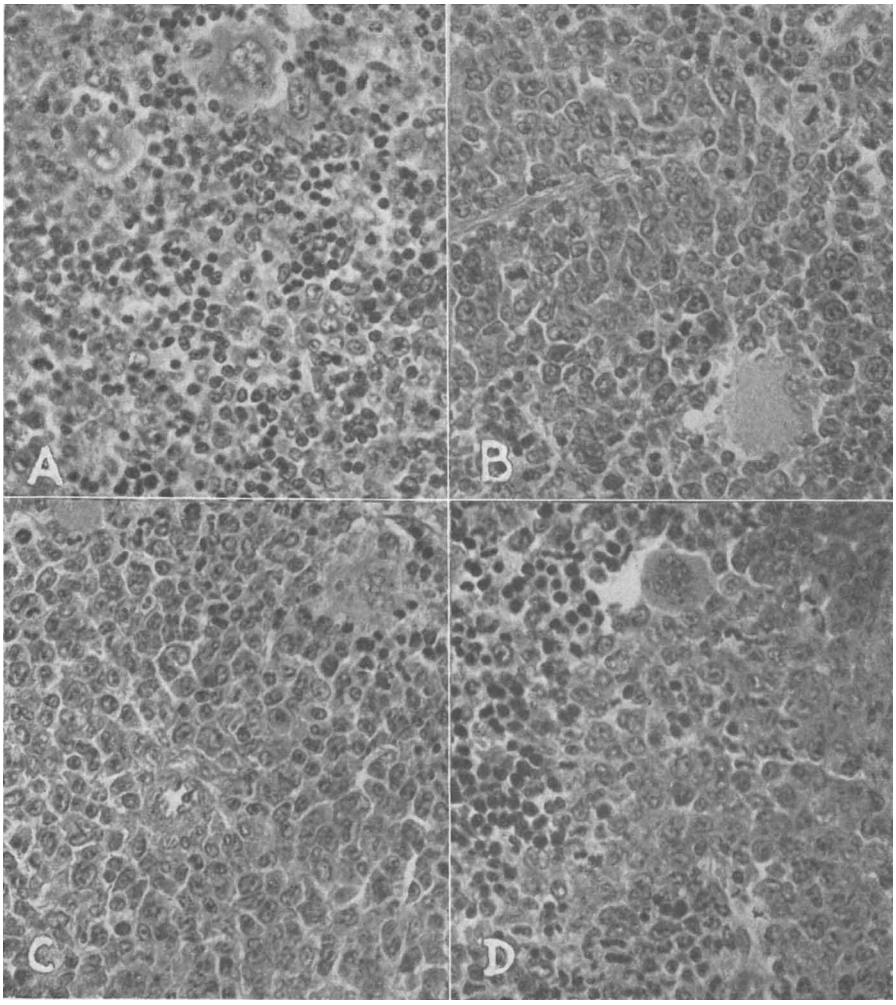

FIG. 1. Spleens. ×370.
(A) Normal. (B) Pyridoxine deficiency. (C) Cortisone. (D) Urine factor.

that produced by pyridoxine deficiency, 5 normal mice were given 1 mg of cortisone acetate daily in a single subcutaneous injection. One died on the 4th day. Within 5 days the mean lymphocyte count of the remaining 4 decreased from 15,250 to 4,750 per cmm and remained below this level during the remaining 25 days of the experiment. During the same time, the mean granulocyte count rose from 4,250 to 10,500 per cmm, the highest recorded count being 38,400 per cmm. Unlike the lymphocyte counts the granulocyte counts showed considerable day to day variation. Three animals lost 1.9, 2.2, and 0.9 g in weight, respectively; one gained 0.1 g. After 30 days of cortisone administration the animals were killed and autopsied. The spleens were uniformly about half normal size and there was extensive cellular infiltration of the pulp identical with that occurring in the pyridoxine deficient mice (Fig. 1c). In 2 mice, the splenic follicles were smaller and less ac-

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tive than normal while in the other 2 the follicles were normal. There were no significant changes in the livers or marrows. Marked narrowing of the adrenal cortex with reduction in size and granularity of the cells was evident, with advanced atrophy of the cortex in one animal.

Myeloid metaplasia factor from human urine. The production of myeloid metaplasia in guinea pigs by the injection of fractions of urine from patients with chronic myeloid leukemia has been described(9). Subsequent reports(10-14) confirmed this finding and showed, in addition, that the active material was also present in the urine of certain individuals not having chronic myeloid leukemia. Preliminary experiments(14) indicate that mice developed similar but more pronounced lesions than guinea pigs. Urine fractions known to have produced changes in guinea pigs have now been administered to 263 mice. The test solutions were prepared from urine by a benzoic acid adsorption technic(15) and further fractionated as previously described(13). The mice received a total of 5 ml of solution derived from 500 ml of urine in 10 daily subcutaneous doses of 0.5 ml. Leukocyte and differential counts were done at the beginning and end of the 10-day period. The animals were killed and autopsied on the 10th day.

Polymorphonuclear leukocytosis of greater degree than that previously produced in guinea pigs, occurred regularly in the mice. The degree of leukocytosis correlated well with histologic changes in the hematopoietic organs. In one group of 39 mice the mean granulocyte count rose from 3,200 to 30,000 per cmm. Unlike the pyridoxine deficient and cortisone treated animals, there was no change in the mean lymphocyte count, the mean control count being 10,600 per cmm as compared with 10,800 per cmm at the conclusion of the experiment.

Changes occurred in the spleen, liver and bone marrow. The spleen size increased 3 to 4 times. The number and size of the follicles and the activity of the germinal centers was diminished. The pulp (Fig. 1d) was infiltrated with neutrophils, and large, immature mononuclears showing mitoses. The latter cell often predominated. Megakaryocytes and nucleated red cells were greatly increased in number. The changes were qualitatively identical with those produced by cortisone and pyridoxine deficiency, differing only in the degree of infiltration and regression of the follicles.

In the liver, 2 types of infiltration occurred, the first characterized by portal and perivascular infiltration by neutrophils and medium-sized and large mononuclear cells having occasional mitotic figures. In the second type, the same cells were irregularly distributed throughout the parenchyma, in a morphologic pattern identical with that occurring in human chronic myeloid leukemia. There was an increase in the neutrophil and immature mononuclear cells in the marrow, with a decrease in nucleated red cells. Cortisone and pyridoxine deficiency produced only minimal changes in the liver and none in the marrow.

Mild hyperplasia of the adrenal cortex, similar to that induced by pyridoxine deficiency, occurred. Regression of lymphoid tissue did not occur. The thymus and lymph nodes were normal in size and histology.

While urines from normal individuals consistently contain little or none of the myeloid metaplasia factor, it is impossible to correlate the amount present in pathologic urines with the disease state of the patient. The substance cannot be isolated from the urine of all patients with chronic myeloid leukemia and it may be present in large amounts in the urine of patients with such

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conditions as carcinoma, fever, chronic lymphoid leukemia and monocytic leukemia. These comments refer to results obtained with the isolation procedure described above, which are not necessarily comparable to the results obtained with the fat soluble fractions of Miller and Hause(12).

Discussion. Similar hematologic and histologic changes occur in mice as the result of pyridoxine deficiency, cortisone administration, and administration of a myeloid metaplasia factor from human urine. Granulocytosis and myeloid metaplasia were constant features of the 3 conditions. The splenic infiltration was not reported in other experiments(16,17) in which mice received 1.25-2.5 mg of cortisone daily for periods of 6-9 days. Lymphopenia occurred with cortisone administration and pyridoxine deficiency, but was not produced by the urine fractions. Unlike animals treated with adrenal cortical extract(4) or humans given ACTH(8), the lymphopenia which occurred in 2 of the 3 groups persisted for the 30-32 days of these experiments instead of disappearing during the course of the experiment. In adrenalectomized animals(4) and in humans with adrenal insufficiency(8), ACTH produced granulocytosis without lymphopenia, a change similar to that produced by the urine fractions.

In our experiments with cortisone the lymph nodes and thymus were normal in size and histology, without regression of lymphoid tissue. It has been suggested(18) that prolonged administration of cortisone might produce an initial regression followed by compensatory regeneration of lymphoid structures. If this occurred, it was not reflected in the peripheral blood, since the lymphopenia, once established, persisted unchanged.

The fact that similar changes occurred with 3, apparently unrelated substances, suggests the possibility that some common mechanism is operating in the 3 conditions. In previous work(14) with the urine fractions it was considered that the active material might be a regulatory hormone, in which event a high degree of structural specificity and some correlation between the disease state of the patient and the amount of material in the urine might be expected. Alternatively, the factor might be one of a group of substances which, although differing widely in chemical structure, could alter the production of a regulatory hormone, which in turn would produce the hematopoietic changes. The fact that similar changes occurred in all 3 groups in the present experiment lends support to this hypothesis. It should be noted that significant differences did occur, however. The urine fractions did not produce lymphopenia. With pyridoxine deficiency the hematologic changes were maintained for 30 days, a result not characteristic of the alarm reaction, in which there is a reversal toward normal within a brief period of time. The fact that the effects of pyridoxine deficiency could be completely abolished by the addition of pyridoxine to the diet while continuing desoxypyridoxine indicates that the effect of this chemical is exclusively that of a displacer of the active B_6 vitamin and that, in itself, it does not activate the alarm reaction.

While it is possible that the effects of pyridoxine deficiency might be mediated by the alarm reaction, it seems just as valid to consider the possibility that the hematopoietic results of cortisone administration might be the result of the establishment of an effective deficiency of the active vitamin B_6 coenzyme, occurring at some stage in the metabolic cycle with which the vitamin is concerned.

Summary. Similar hematologic and histologic changes occurred in mice made pyridoxine deficient, in mice treated with cortisone, and in mice treated with a myeloid metaplasia factor from human urine. It is concluded that the changes produced by pyridoxine deficiency and by the urine fractions are not necessarily mediated through the mechanism of the alarm reaction. Other mechanisms of action are considered.

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