

## Suppression of Adjuvant Arthritis by Penicillamine in Pyridoxine Deficient Rats<sup>1</sup> (38078)

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About 20 yr ago, a chronic polyarthritis was produced in rats injected with spleen in Freund's adjuvant and was suggested to represent an immunological disorder (1). Early attempts to prevent this experimental disease succeeded with a number of "immunosuppressive agents." They included treatment with lymphocytolytic agents such as cortisol, *N*-mustard, and a vitamin B6 antagonist—desoxypyridoxine (D-B6) (2). The experimental disease, now generally termed "adjuvant arthritis" (AA) was later observed to respond to all "antirheumatic" agents, antiinflammatory and immunosuppressive alike (3), and is widely held to be a meaningful system for testing such substances. Earlier studies with D-B6 had shown that its suppressive effect upon normal and neoplastic lymphoid cells as well as its action suppressing antibody formation and preventing the appearance of arthritic lesions in AA depended on a low intake of vitamin B6 (4).

A number of recent reports deal with the beneficial action of penicillamine (PA) in patients with rheumatoid arthritis (5, 6). While PA was known to antagonize reversibly pyridoxal-PO<sub>4</sub> (7) its antirheumatic activity was not believed to depend on its effect on pyridoxine. Since pyridoxine was thought to reduce PA-toxicity, all previous attempts to evaluate the antirheumatic effect of PA, in man and in experimental

animals, were made in presence of liberal or even very high amounts of dietary vitamin B6.

The experiments summarized in the following deal with the effect of PA on AA in rats fed a diet very low in vitamin B6.

*Materials and Methods.* Adjuvant arthritis was produced in groups of male rats of the Sprague-Dawley strain, weighing from 290 to 310 g. The animals were injected sc in the tail with a suspension of 0.3 mg of dry *M. butyricum* in 0.2 ml mineral oil. In the 4 wk after injection, the animals were weighed weekly and their wrist and ankle joints inspected. In all groups, the incidence of arthritis, the number of arthritic lesions per rat and the severity of the lesions (0-+++) were recorded. Arthritic lesions of fingers or toes, because of their migrant nature, and lesions of knees and elbows, because of the difficulty in judging their presence and extent, were not included in the weekly scoring. All rats were fed a semisynthetic diet very low in pyridoxine. For controls, 10 mg of vitamin B6 was added per kilogram of diet. To the four groups of rats indicated in the table, 50 mg of PA in form of *d,l*- or *d*-PA (Fluka) were administered orally during the first 3 wk of the experiment five times weekly. In order to compare the effects of PA with agents known to modify AA, two groups of sensitized rats were also treated five times weekly for 3 wk, one with 5 mg cortisol acetate (Sanabo) injected sc, the other with 50 mg/kg phenylbutazone (Ciba) by stomach tube. Weekly hematologic studies were performed in all experimental groups

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TABLE I. Adjuvant Arthritis in Rats Treated with Penicillamine in Presence and Absence of Dietary Pyridoxine.

Group	Experiment	No. of rats	Percent			
			Incidence	No. of lesions <sup>a</sup>	Intensity <sup>b</sup> (0 - + + +)	Body weight change
1	Control + B6	21	86	63	38	-9
2	Control - B6	9	89	67	43	-12
3	<i>d</i> -PA + B6	15	87	58	34	-14
4	<i>d</i> -PA - B6	6	50	17*	6*	-9
5	<i>d,l</i> -PA + B6	12	83	63	28**	-7
6	<i>d,l</i> -PA - B6	16	0*	0*	0*	-21
7	Phenylbutazone + B6	6	17*	9*	3*	+10
8	Cortisol acetate + B6	6	0*	0*	0*	-7

<sup>a</sup> 100% = 4 joints per rat.

<sup>b</sup> 100% = 12 pluses per rat.

\* Significantly different from groups 1, 2, 3, and 5 ( $p < 0.01$ ).

\*\* Significantly different from group 1 ( $p < 0.005$ ).

and compared to two unsensitized control groups.

**Results.** Results are summarized in the table. For the sake of presentation they are expressed as percentages, although the original scores were subjected to a  $\chi^2$  test by means of contingency tables. The number of lesions in percent represents the actual number of arthritic lesions counted in relation to the maximal number of lesions that could be obtained in the four joints inspected (4/rat). Similarly, intensity in percent is based on the maximal number of pluses possible (three for each of the four joints) i.e., 12 per rat taken as 100%. Nonsensitized rats (not listed in the table) after 4 wk on the complete ration, gained 32% of their initial body weight, control rats on the B6 deficient diet gained 22%. Weight losses occurred in all sensitized animals except those treated with phenylbutazone (group 7). *d*-PA treated rats with pyridoxine supplement (group 3) developed AA of the same extent as controls (groups 1 and 2) while the same treatment in absence of B6 resulted in partial but significant suppression of arthritic lesions. With the *d,l*-PA, a more potent pyridoxine antagonist, *d,l*-PA in presence of dietary vitamin B6 (group 5), weight loss and the incidence of the arthritic lesions were comparable to untreated controls (groups 1, 2). However, the intensity

of the arthritic lesions was significantly less than that of the controls (group 1). *d,l*-PA treatment on the basic diet without added B6 resulted in complete suppression of the AA in all animals but with an average weight loss of 21%. Thus, in confirmation of a previous report (8) *d*-PA did not modify the extent of AA in rats fed a complete diet. Others have reported (9) that *d,l*-PA diminished the intensity of the arthritic lesions. This latter finding appears confirmed in the present experiments (group 5).

The hematologic findings will be published elsewhere in greater detail. Briefly they were the following: One week after the injection of *M. butyricum* and unrelated to the appearance of arthritic lesions, a moderate but significant normochromic, normocytic anemia with a reduction of hemoglobin amounting to about 15% was noted. A very striking granulocytosis and a moderate but significant thrombocytosis were also observed. The peak of the hematological alterations appeared 2 wk after the injections of the mycobacteria. From then on the granulocytosis and thrombocytosis subsided while the anemia persisted. Compared to the values in sensitized control rats, the granulocytosis was reduced with all of the treatments which suppressed the development of the AA. *M. butyricum* injected rats treated

with cortisol acetate or with PA in the absence of dietary pyridoxine developed striking lymphopenia. The thrombocytosis was corrected by cortisol acetate and phenylbutazone but not by PA. The anemia was significantly reversed by cortisol acetate but not by any of the other treatments.

*Discussion.* In view of earlier observations with D-B6, the effect of another, perhaps more potent pyridoxine antagonist upon AA was not surprising. The possibility that a deficiency in vitamin B6 may have a beneficial action in diseases of hypersensitivity was considered many years ago (10). This was based on findings in a study undertaken to scrutinize more closely the phenomenon of lymphoid atrophy (10, 11) caused by starvation. In these experiments, the state of thymic and other lymphoid tissue was compared among groups of rats fed many variations of semisynthetic diets each deficient in a single essential nutritional factor. Duration of the experiment and total body weight changes served as basis of comparison. While all forms of prolonged malnutrition, lead to weight loss and cause lymphoid atrophy, a deficiency of vitamin B6 for short duration which gave little effect on body growth resulted in extreme lymphoid atrophy. In addition low serum antibody concentrations were noted. Subsequently, especially after the first competitive inhibitor of pyridoxine became available, the immunosuppressive effect of "acute pyridoxine deficiency" (4) was studied in greater detail. It was established that the effect of pyridoxine deprivation upon lymphoid cells and antibody levels was not mediated by the adrenal cortex and that the low antibody titers were a consequence of inhibited antibody synthesis rather than accelerated degradation. The immunosuppressive effect of "acute pyridoxine deficiency" was even more strikingly evident with regard to delayed immunity (homografts) (12) a situation widely assumed to be related to AA (13). PA was introduced as an antirheumatic agent for two theoretical reasons. One held that the chelation of  $\text{Cu}^{2+}$  was desirable (5) while the other initially emphasized the depolymerizing action of PA upon macroglobulins (6). Neither of these

theories has received adequate experimental support as present. We would therefore raise the question as to whether partial suppression of pyridoxal- $\text{PO}_4$  by PA may represent an alternative explanation. The inability of PA to appreciably modify AA in rats on a complete ration may possibly depend on the habit of almost continuous feeding in this species which might prevent an even transient, interprandial action of the antivitamin.

Attempts at immunosuppression with l-PA therapy in conjunction with a low pyridoxine intake may offer certain advantages over other agents. In contradistinction to the bone marrow suppressive effect which attends all radiomimetic substances (*N*-mustard, antifolics, etc.) vitamin B6 deprivation, like excess cortisol, affects only lymphocytes and does not destroy cells of either the erythrocytic or granulocytic series. While vitamin B6 deficiency causes readily reversible skin manifestations and microcytic anemia, it is not likely to cause any of the numerous undesirable side effects of cortisol. Also the prompt reversibility of even advanced deficiency states by restitution of the vitamin may be an advantage over the slow recovery from excess cortisol.

*Summary.* The administration of *dl*-penicillamine—a potent pyridoxine antagonist—to rats fed a diet low in vitamin B6 completely prevented the development of adjuvant arthritis. On the same regimen, *d*-penicillamine—a comparatively weak antivitamin—proved less effective. Neither the feeding of a pyridoxine deficient diet alone nor treatment with *d*-penicillamine on a complete ration modified the experimental disease. However, *dl*-penicillamine, even on a complete diet, partially reduced the intensity of the arthritic lesions without lowering their incidence.

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