The Effect of Pyridoxine Deficiency Induced by Desoxy-pyridoxine on Acute Lymphatic Leukemia of Adults

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In pyridoxine deficiency, there is an absolute decrease in the number of circulating lymphocytes of dogs, monkeys, rats, and mice. In addition, lymphoid atrophy and regression of lymphosarcoma occurs in rats, although atrophy of lymphoid tissue in normal mice and modification of the lymphoid component of the leukemia of Ak mice was not observed. Treatment of lymphosarcoma and acute leukemia in man by inducing pyridoxine deficiency was attempted by Gellhorn and Jones, using the method of Mueller and Vilter. The patients were fed a pyridoxine-deficient diet supplemented by a pyridoxine antagonist, desoxypyridoxine, in doses of 2.5 to 25 mg. per kg. of body weight daily in three divided doses. The treatment period lasted only four to fourteen days. No clinical effect was observed and the trials were discontinued because of the occurrence of convulsions.

One difficulty in inducing pyridoxine deficiency in human beings is the fact that the pyridoxine deficient diet is very unpalatable and patients are very reluctant to stay on it for more than a few weeks at a time. If prolonged maintenance of the deficient state is desired, it would be highly desirable to use another method. In mice, the deficient diet is not necessary, at least for the production of hematologic changes, if adequate amounts of desoxypyridoxine are given. In addition, this method largely obviates the possibility that some of the effects observed may be due to deficiency of some unknown growth factor which is excluded by the use of the basic diet and not included in the usual supplements of known growth factors added to the basic diet.

From the experience with mice, it seemed probable that pyridoxine deficiency could be induced in human beings without the use of a deficient diet, if adequate amounts of desoxypyridoxine were given, and that the patients, eating a normal diet, might be kept in the deficient state for long periods of time. Accordingly, adult patients having acute, malignant lymphoid disease were selected and given varying amounts of desoxypyridoxine in divided doses. They were fed the usual hospital diet.

Case Reports

Case 1

A 29 year old colored man was admitted on June 19, 1951, complaining of malaise of several months' duration and the appearance of lumps in the neck, axillae and inguinal.
regions. Physical examination showed generalized lymphadenopathy, a spleen palpable 3 cm. below the costal margin, and right pleural effusion. On admission he was febrile and X-rays showed slight pneumonitis in addition to pleural effusion. Repeated examinations and cultures of sputum and pleural fluid for tubercle bacilli were negative, as was guinea pig inoculation of pleural fluid. On penicillin therapy, the fever and pneumonitis promptly subsided.

On admission the hemoglobin was 5.0 Gm. and the white blood count 40,000 with a differential count showing 5 per cent polymorphonuclear leukocytes, 45 per cent adult lymphocytes, and 50 per cent immature lymphoid cells and blast forms. Aspirated bone marrow contained no adult granulocytes, 1.6 per cent myelocytes, 27.7 per cent lymphocytes, 56.3 per cent immature cells of the lymphoid series, 11.7 per cent blast forms, and 1.0 per cent nucleated red cells. A diagnosis of acute leukemia, probably lymphatic, was made.

On the twenty-fourth hospital day 400 mg. of desoxypyridoxine daily in divided oral doses was started. This was continued for seven days and then increased to 800 mg. daily which was given for an additional forty-four day period. His hematologic progress is recorded in Fig. 1. After the desoxypyridoxine was discontinued the immature cells practically disappeared from the peripheral blood and polymorphonuclear leukocytes appeared in normal numbers. At this time there were 8 per cent polymorphonuclear leukocytes in the aspirated bone marrow, the differential count being otherwise essentially unchanged. In addition, red cell production improved. He became able to maintain near-normal hemoglobin levels without transfusions, which are indicated by T on figure 1.

Accompanying the improvement in the hematologic state there was marked symptomatic improvement and regression of lymph nodes. By the twenty-sixth day of therapy only a few shotty nodes were palpable. There was little, if any, change in spleen size.

In about forty-five days after the discontinuance of therapy, hematologic and clinical relapse began. A second course of desoxypyridoxine therapy, 1000 mg. daily for forty-five days, was given without evident effect on the clinical course. At no time during or after either course of therapy were toxic effects observed which could be attributed to the desoxy-

![Fig. 1.—Case 1. Hematologic remission. On the sixty-third day the immature cells had practically disappeared from the peripheral blood and granulocytes had reappeared in normal numbers. Note subsequent maintenance of hemoglobin.](image-url)
pyridoxine. Seborrheic dermatitis did not appear. Further treatment included ACTH, cortisone, x-ray therapy, transfusions, and antibiotics. He died one year after admission. Permission for autopsy was not obtained.

Comment on Case 1

Desoxypyridoxine therapy appears to have effected a satisfactory clinical and hematologic remission of brief duration. A second course of therapy failed to be effective. As in mice the hematologic effect may have been obtained without complete pyridoxine deficiency.

Case 2

Six months before admission this forty-eight year old white man developed inguinal adenopathy. In spite of x-ray therapy and cortisone the adenopathy increased and became generalized. He developed weakness, dyspnea, abdominal distension, and edema and was admitted on Dec. 5, 1951. Examination showed generalized adenopathy, enlargement of liver and spleen, bilateral pleural effusion, ascites, and edema. Morphologically the cells of the blood and bone marrow were entirely similar to those of case 1.

On the fifth hospital day 600 mg. of desoxypyridoxine daily in divided doses was started. This was increased to 800 mg. on the seventh day, to 1000 mg. on the eleventh day, and to 1400 mg. on the forty-ninth day. This dose was continued until death on the eightieth day. His hematologic progress is recorded in figure 2. The total white count declined to the normal level and abnormal cells practically disappeared from the peripheral blood. There was no significant, sustained change in the total granulocyte count. A gradual decline in hemoglobin value occurred, but only two transfusions were necessary during the eighty day period. It is possible that without the therapy more transfusions would have been necessary.

During therapy some regression of lymph nodes occurred. The size of the liver and spleen were unchanged. On the sixty-fifth day seborrheic dermatitis of the central area of the face was evident and persisted until death. At the same time he felt symptomatically improved.

![Fig. 2.—Case 2. Reduction of immature cells in peripheral blood.](image-url)
INDUCED PYRIDOXINE DEFICIENCY AND LEUKEMIA

He was able to get out of bed, and he shaved himself and wanted to go home on a trial visit. On the morning of the eightieth day he was found dead in bed.

Postmortem examination revealed massive diffuse enlargement of superficial and deep lymph nodes; the largest measured 3 cm. in diameter. The spleen weighed 775 Gm. and on section, revealed a bulging, brick red, moderately firm surface. There was marked subcutaneous edema of the lower extremities, pleural and peritoneal effusions, and atelectasis of the lungs. No significant hemorrhage was present.

Histologic examination showed patchy and focal leukemic infiltration of the epicardium, lungs, gastric mucosa, liver, kidneys, testes, and periaireolar tissues. Sections of spleen revealed large infiltrating follicles without germinal centers which extended extensively into the surrounding pulp. These follicles consisted of large and small round cells with scanty cytoplasm and large and small round, intensely basophilic nuclei. In some of the larger nuclei, there were nucleoli. In the lymph nodes (fig. 3) there was focal retention of the architecture but for the most part there was a uniform replacement by the small and large cells noted in the spleen. There was marked capsular infiltration. Section of marrow from the lumbar vertebra showed a marked leukemic infiltration with a few small foci of the erythromyeloid cells and a normal number of megakaryocytes.

There was focal destruction of the inner half of the zona fasciculata of the adrenals with evidence of regeneration of cells in this area. In the convoluted tubules of the kidneys there were crystals which stained light gray to green in the hematoxylin and eosin preparation, some of these crystals were surrounded by a giant cell reaction. These crystals were not identified. Other histologic changes which might have been due to pyridoxine deficiency were not noted.

Comment on Case 2

A partial hematologic remission consisting of marked reduction of the immature lymphocytes circulating in the peripheral blood occurred with some
symptomatic improvement. Seborrheic dermatitis appeared after sixty days of treatment. Postmortem examination failed to reveal any significant modification of the leukemic process in the tissues, although therapy was continued until death.

Case 3

This 36 year old white man was admitted to the hospital in October 1951 for study and treatment of a chronic neurologic disease. The admission blood studies were normal. One month after admission he was given intravenous ACTH daily for ten days. Three months after admission the routine blood studies showed the white blood cell count to be 16,400 with 20 per cent immature lymphoid cells and blast forms. At that time the hemoglobin level was 15.1 Gm. In the bone marrow 84 per cent of the cells were immature lymphoid cells and blast forms. Treatment with desoxypyridoxine was started two weeks later and continued for thirty-three days. For two days he received 800 mg. daily in divided oral doses, then 1000 mg. for two days, then 1400 mg. for twenty-four days, then 600 mg. for five days. Thirteen days after the start of treatment seborrheic dermatitis of the face appeared and persisted (fig. 4). The clinical course was progressively downhill with enlarging spleen and increasing anemia, and thrombocytopenia. Terminally, the white blood cell
Induced Pyridoxine Deficiency and Leukemia

count fell to 1000 indicating that the number of immature cells in the peripheral blood had been greatly reduced. There was no increase in the circulating neutrophils. He died suddenly fifty days after the malignant process was discovered and three days after desoxyxypyrnodoxine therapy was stopped.

Postmortem examination revealed two primary processes, syringomyelia involving the cervical, thoracic, and upper lumbar spinal cord and an acute lymphocytic leukemia. The spleen weighed 455 Gm. There were two small accessory spleens and a moderate enlargement of both superficial and deep lymph nodes.

Histologic examination of the liver, kidneys, renal pelves and ureters, and adrenal glands showed focal leukemia infiltration and recent hemorrhages. There were still a few widely separated lymphoid follicles within the spleen with a marked diffuse lymphocytic and lymphoblastic infiltration of the pulp. Section taken from a lumbar vertebra showed complete replacement of the marrow by leukemia cells. There was only an occasional megakaryocyte. The sections of lymph nodes were very interesting (fig. 5) in that there was retention of the normal sinusooidal pattern, marked reticuloendothelial hyperplasia, and large primary and secondary follicles, which consisted of closely packed lymphocytes and lymphoblasts without any discernible germinal centers. There was a marked invasion of the nodal capsule. No phagocytosis was noted.

The adrenals showed some cortical degeneration. Aside from this finding and the focal hemorrhages in the liver, kidneys, and adrenals, no other histologic changes which might have been due to pyridoxine deficiency were discovered.

Comment on Case 3

Desoxyxypyrnodoxine therapy failed to produce any clinical improvement in this patient. Pyridoxine deficiency, as evidenced by seborrhoeic dermatitis, was established. It is possible that the deficiency had something to do with his
death, although clinical evidence for this in the form of convulsions or massive hemorrhage did not occur. At the end of the course of desoxypyridoxine therapy there was marked reduction in the number of immature cells circulating in the peripheral blood. Postmortem examination showed retention of the architecture of the lymph nodes with marked reticuloendothelial proliferation. This may represent a modification of the leukemic process by the pyridoxine deficiency.

Case 4

This 24 year old white man was admitted complaining of malaise, weakness, weight loss, and increasing pallor. On examination he appeared chronically ill. There was enlargement of the spleen and lymph nodes in the cervical region. The hemoglobin level was 10.6 Gm. The white blood cell count was 20,650 with 45 per cent immature lymphoid cells and blast forms in the peripheral blood. In the bone marrow 75 per cent of the cells were immature lymphoid cells and blast forms. Four days after admission treatment with desoxypyridoxine was started. He received 500 mg. daily in divided oral doses for thirty-six days. During this period the white blood cell count fell to 4,100 but there was no increase in circulating neutrophils. Symptomatically he was somewhat improved but this could be attributed to transfusions, of which he received fourteen during this period.

For the next three months his symptomatic improvement was maintained. During this time he was given seventeen transfusions. The white blood cell count varied between 10,000 and 40,000. During the next month there was gradual weight loss and increasing malaise, and thrombocytopenia developed. A second course of desoxypyridoxine was started four months after the completion of the first course, five months after admission. For three days he received 600 mg. in divided oral doses, then 1,000 mg. for eleven days, then 800 mg. for eight days, the total duration of treatment being twenty-two days. During this time the white

Fig. 6.—Case 4. Lymph node, X190.
blood cell count fell from 14,500 to 150. There was no rise in neutrophils. The fall could be accounted for on the basis of disappearance of the immature cells from the peripheral blood. Seborrheic dermatitis did not appear. He died three days after desoxypyridoxine therapy was discontinued.

Postmortem examination was interesting because grossly it was consistent with chronic lymphoid leukemia: the spleen weighed 600 Gm., the liver 4000 Gm., and the deep lymph nodes were moderately to markedly enlarged. Histologically, however, only the liver and kidneys showed diffuse and marked leukemic infiltrations. The sections taken from the spleen and lymph nodes (fig. 6) showed marked reticuloendothelial hyperplasia, marked phagocytosis, and retention of the normal architecture. The lymphoid follicles of both the spleen and lymph nodes were composed of large and small lymphocytes and no germinal centers. There was no leukemic infiltration of the capsules of the lymph nodes and there were foci of extramedullary hematopoiesis in the spleen. Section of a lumbar vertebra revealed a moderate diminution of the erythromyeloid cells and serous atrophy of the adipose tissue. There was only a slight lymphocytic infiltration of the marrow.

There was moderate degeneration of the adrenal cortex with lipoid depletion of the zona fasciculata. As in case 2 dark green crystals were present in the convoluted tubules of the kidneys; some were surrounded by a giant cell reaction.

Comment on Case 4

Desoxypyridoxine given in doses of 500 mg. daily failed to produce evident effect. A second course with a maximum dose of 1000 mg. daily was featured by marked reduction of the circulating immature cells. Death occurred within a few days after the second course was completed.

Postmortem examination showed retention of normal architecture, marked phagocytosis, and marked reticuloendothelial hyperplasia in the spleen and lymph nodes. In addition, the bone marrow showed only minimal lymphocytic infiltration with persistence of hypoplastic but otherwise normal hematopoiesis. These findings in the spleen, lymph nodes, and marrow were considered probably to be modification of the leukemic process by the therapy.

Discussion

Of these four cases of malignant lymphoid disease maintained on a normal diet and treated with oral desoxypyridoxine, two developed seborrheic dermatitis. We consider this to be reasonably conclusive evidence that pyridoxine deficiency was induced in these cases. It is therefore evident that pyridoxine deficiency can be produced in human beings on a normal diet, if sufficient quantities of desoxypyridoxine are given. The dose required to do this appears to be in excess of 500 mg. daily. When the dose was increased to 800 mg. daily in one case and continued for forty-four days, a satisfactory hematologic remission was obtained and no toxic effects or seborrheic dermatitis were observed. A state of partial pyridoxine deficiency may have been induced. When the dose was increased to 1400 mg. daily, two patients developed seborrheic dermatitis after they had been on this dose for sixteen or eighteen days. One had been given 1000 mg. for the previous thirty-eight days and the other 800 or 1000 mg. for the previous four days. It might seem that doses in excess of 1000 mg. daily were necessary for the production of seborrheic dermatitis, but, obviously, conclusions about dosage cannot be drawn from these two cases, particularly as the intake of B6 vitamins in the diet was not controlled.

In all four cases there was a marked reduction in the numbers of immature
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lymphoid cells circulating in the peripheral blood. In case 1 this modification of the leukemic process was accompanied by decrease in the severity of the anemia and by the appearance of normal numbers of polymorphonuclear leukocytes circulating in the peripheral blood. In fact the peripheral blood picture became practically normal for a brief period. In the bone marrow, adult granulocyte elements increased from none to 8.7 per cent. This improvement in granulocyte production did not occur in the other three cases. There could be several reasons for its occurrence in the one case: it could depend upon the reciprocal relationship between lymphocytes and granulocytes seen under a wide variety of conditions including pyridoxine deficiency; it could be due to possible stimulation of granulocyte production in pyridoxine deficiency as discussed elsewhere; or it could be due to suppression of the abnormal immature elements in the bone marrow allowing better granulocyte formation. Of course, it must be remembered that the brief remission which occurred in case 1 could have been due to unknown factors, possibly the transfusions. It seems clear that tuberculosis was not the cause for the remission, although without autopsy this cannot be entirely ruled out.

In two of the four cases, histologic examination of the tissues showed possible modification of the leukemic process. In one there was only minimal leukemic infiltration in the spleen, lymph nodes, and marrow; in the other, this finding was observed in the lymph nodes only.

Other changes which might be attributed to pyridoxine deficiency consisted of adrenal cortical degeneration in all three autopsied cases and, in two, crystalline deposits in the convoluted tubules of the kidneys. Significant hemorrhage did not occur.

In none of the cases were any toxic clinical manifestations observed which could be directly attributed to the desoxycoenzyme therapy and which could not be explained on the basis of the disease process itself.

From the observations there seems to be some reason to be optimistic that the induction of pyridoxine deficiency may play a role in the control of malignant lymphoid disease. However, in only one of four cases was a satisfactory remission obtained. This was of brief duration and, as often occurs in the induction of folic acid deficiency in the treatment of childhood leukemia, could not be reproduced by a second course of therapy. Although we are somewhat pessimistic about the possibility that the induction of any type of vitamin deficiency will have a lasting effect on leukemia or any other type of malignant disease, still we do believe that further trials of such therapy should be carried out. Malignant cells have been shown to change their metabolic requirements for a vitamin with the result that control of the malignant process is lost or it becomes necessary to establish such a complete vitamin deficiency that the patient dies of the deficiency itself. However, little is known about the adaptive metabolic potentialities of malignant cells, so that a wholly pessimistic attitude is not justified.

Summary

1. Pyridoxine deficiency can be induced in human beings by giving large doses of desoxycoenzyme while maintaining the patients on a normal diet.
2. In four cases of acute lymphoid disease of adults some modification of the
disease appears to have been accomplished by the induction of pyridoxine deficiency. In one case a brief clinical remission occurred which seemed almost complete.

3. Possible other effects of the deficiency were adrenal cortical degeneration and deposition of crystals in the convoluted tubules of the kidneys. The adrenal changes could well be due to postmortem degeneration.

4. Other toxic effects of the desoxypyridoxine therapy were not observed.

5. Further trials of this type of therapy appear to be justified.

SUMMARIO IN INTERLINGUA
1. Carentia de pyridoxina pote esser provocat.e in humanos per le administratio de grande doses de desoxypyridoxina durante que le patiehtne es mantenite a un dieta normal.

2. In quatro casos de acute morbo lymphoide de adultos alicun modification del morbo pare haber resultate del provocatiots de carentia pyridoxinic. In un caso le breve remissioti clitsic que occurreva pareva esser quasi complete.

3. Altere effectos possibile del carentia esseva degeneration del cortice adrenal e deposition de crystallos in le tubulos convolute del renes. Le cambiamentos adrenal pote ben esser attribuite al degeneration postmortal.

4. Altere effectos toxic del therapia a desoxypyridoxina non esseva observate.

5. Il pare que cetere essayos con iste typo de therapia es justificate.

REFERENCES
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