Hypochromic Anemias of the Tropics Associated with Pyridoxine and Nicotinic Acid Deficiency

By Henry Foy and Athena Kondi

Pyridoxine. Deficiency of pyridoxine (vitamin B₆) has been shown to produce anemia in dogs, rats, pigs, monkeys, chicks and ducks. Poikilocytosis in cattle has been successfully treated with pyridoxine. Ramalingaswami and Sinclair have shown that the hematologic abnormality in pyridoxine-deficient rats is a microcytosis, but it is not necessarily accompanied by an anemia.

Hawkings and his colleagues claim that in rats pyridoxine has no direct anti-anemic action, although when made anemic by hemorrhage the blood regeneration was slower in the B₆-deficient group than in the controls. Further, polycythemia did not develop in normal rats given cobalt if they were also B₆-deficient.

The evidence for a pyridoxine-responding anemia in man is not so precise. Snyderman et al. and Vilter et al. have, however, reported a reticulocytosis following the administration of pyridoxine to pellagrins with macrocytic anemia and in pernicious anemia.

Recently Harris et al. have reported a case of hypochromic anemia in an adult male that failed to respond to iron, folic acid, B₁₂, thiamine, riboflavin, niacin, etc., but did make a response to 200 mg. of intramuscular pyridoxine daily for five days. Following pyridoxine there was a smart reticulocyte response, rapid fall in plasma iron and return of leukocytes, hemoglobin and red blood cells to normal values.

When desoxypyridoxine has been administered to man as a metabolic antagonist of pyridoxine, it produces seborrhoea-like lesions about the eyes, nose and mouth, and many of the patients developed erosions in and around the mouth resembling the cheiloses of riboflavin deficiency; one patient developed severe systemic symptoms. There was no anemia but there was a lymphocytopenia.

In certain types of agranulocytosis in man, B₆ is said to be effective in stimulating the production of white cells.

Pyridoxine deficiency in man and animals leads to excretion of abnormal metabolites most readily when a loading dose of tryptophan is given.

Pyridoxine-deficient anemia is usually hypochromic and microcytic with high plasma iron and moderate leukopenia.

It seems, then, that deficiency of pyridoxine produces a complex of symptoms, of which anemia may in some cases be one.

Below, we report the case of a male African adult with an iron-deficient anemia, erythronormoblastic marrow with rare giant stab-cells, who made no
response whatever to oral ferrous sulphate—30 grains daily—but did respond to 20 mg. of oral pyridoxine daily for 2 weeks.

Methods

Blood samples were taken weekly, without constriction, at approximately 10 A.M., in tubes containing either 4 mg. of potassium oxalate and 6 mg. of ammonium oxalate for each 5 cc. of blood or in tubes containing heparin.

RBC counts were done by the usual methods; packed cell volume by micro hematocrit on a high speed centrifuge.

Hemoglobin was estimated spectrophotometrically by the cyanohemoglobin method of King,24 checked against known hemoglobin standards.

Bone marrow biopsy was done by the usual method using a Salah needle and the dry smears stained with Leishman and Giemsa.

Total proteins were estimated by the Biuret method of Gornall et al.25 Fractionation of the components was carried out by using 26.5 per cent sodium sulphate so as to avoid carrying over any part of the a1 globulin into the albumin fraction, which happens if 22 per cent sodium sulphate is used. Separation of the globulins was done by horizontal paper electrophoresis using a barbiturate buffer with a stabilised pH of 8.6, similarly to avoid carrying over any part of the a1 globulin into the albumin fraction.

The strips were heated for 30 minutes at 110°C. and stained for 3 hours in a mercuric ethanol solution of bromo-phenol-blue and scanned photometrically.

Case 1: A well-developed, well-nourished male African of the Luo tribe, aged 35 years, entered the hospital on 8/23/56 for weakness; he had no significant previous history. Examination revealed pain in the legs with slight edema, breathlessness, palpitation and headache; seborrhoea at nose angles, spleen and liver not enlarged, no temperature and no parasites in stools or blood. Chest, nervous system, heart and tongue were all normal. There was no history of treatment before admission to the hospital. He said that he had been ill for about one week.

Laboratory Findings (8/27/56)

<table>
<thead>
<tr>
<th>R.B.C's</th>
<th>4,133,000 cu. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>8.3 Gm./100 ml.</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>34%</td>
</tr>
<tr>
<td>M.C.V.</td>
<td>83 µ</td>
</tr>
<tr>
<td>M.C.H.</td>
<td>20γγ</td>
</tr>
<tr>
<td>M.C.H.C.</td>
<td>24%</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>3.5%</td>
</tr>
<tr>
<td>W.B.C's</td>
<td>4,830 cu. mm.</td>
</tr>
<tr>
<td>Indirect Van</td>
<td>0.4 mg./100 ml.</td>
</tr>
<tr>
<td>den Bergh</td>
<td></td>
</tr>
<tr>
<td>Sternal marrow</td>
<td>Active erythronormoblastic with rare giant stab-cells. (G.S.C.)</td>
</tr>
</tbody>
</table>

Treatment and Progress

Oral ferrous sulphate, 30 grains daily, was started on 8/28 and continued until 9/27. On 9/6 the R.B.C. hemoglobin and P.C.V. had fallen to 4,000,000/cu. mm., 8 Gm./100 ml. and 32%, respectively; on 9/13 all these values had again fallen slightly (see fig. 1). The reticulocytes during this period fluctuated between 1.0% and 3.5%. Since all the blood values had fallen during the period of iron treatment and the patient was clinically worse, 20 mg. of oral pyridoxine daily was started on 9/13 and continued until 9/27. Following this, on 9/16 the reticulocytes rose to 7.5%; on 9/20 the R.B.C. hemoglobin and P.C.V. increased to 4,790,000/cu. mm., 10.1 Gm./100 ml. and 37%; on 9/27 the values were 4,900,000/cu. mm., 11.0 Gm./100 ml. and 39%, respectively, and the clinical condition of the patient was so very much improved that he was discharged on 9/27. Had he remained longer, no doubt his blood values would have reached normal (fig 1).

During the course of iron treatment the total proteins fell from 6.0 Gm./100 ml. (albumin 2.6, globulin 3.4) to 5.5 Gm./100 ml. (albumin 2.0, globulin 3.5). On 9/20 the total protein
was 6.2 Gm./100 ml. (albumin 2.5, globulin 3.7). On 9/27 the total protein fell to 5.7 Gm./100 ml. (albumin 2.5, globulin 3.2). The protein picture for the whole course of the illness is shown in table 1.

**Nicotinic Acid.** Anemia is not constantly associated with nicotinic acid deficiency, but its interrelation with amino acid metabolism may occasionally associate it with anemia.

It is known that tryptophan can be converted into nicotinic acid in man, the rat and other animals. Benesch has suggested that nicotinic acid is synthesized by the flora of the intestinal tract in man, and competing organ-

<table>
<thead>
<tr>
<th>Date</th>
<th>Total Proteins</th>
<th>Albumin</th>
<th>α₁</th>
<th>α₂</th>
<th>β</th>
<th>γ</th>
<th>Total Globulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/27/56</td>
<td>6.0</td>
<td>2.6</td>
<td>.24</td>
<td>.71</td>
<td>.95</td>
<td>1.5</td>
<td>3.4</td>
</tr>
<tr>
<td>9/6/56</td>
<td>5.5</td>
<td>2.0</td>
<td>.46</td>
<td>.72</td>
<td>1.1</td>
<td>1.2</td>
<td>3.5</td>
</tr>
<tr>
<td>9/13/56</td>
<td>5.5</td>
<td>2.0</td>
<td>.22</td>
<td>.68</td>
<td>1.1</td>
<td>1.5</td>
<td>3.5</td>
</tr>
<tr>
<td>9/20/56</td>
<td>6.2</td>
<td>2.5</td>
<td>.42</td>
<td>.78</td>
<td>1.1</td>
<td>1.4</td>
<td>3.7</td>
</tr>
<tr>
<td>9/27/56</td>
<td>5.7</td>
<td>2.5</td>
<td>.26</td>
<td>.62</td>
<td>1.0</td>
<td>1.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Fig. 1.—Blood values of Case 1.
isms may be present in the gut which reduce the quantity of nicotinic acid available for man; this may be especially so on marginal tropical diets. The nicotinic acid-responding case of anemia was similarly erythronormoblastic with rare giant stab-cells and typical pellagrous symptoms accompanied by mental confusion. The patient did not respond to oral iron alone, but did so when nicotinic acid was added.

Case 2: A female Sudanese about 40 years old, who lived in Nairobi for about 10 years, entered the hospital on 9/4/56 in a state of mental confusion, with pellagrous skin manifestations, perleche + + weakness, breathlessness and palpitation. Her temperature was normal; liver and spleen not enlarged, and tongue, chest and heart all normal. No ova or parasites were in her blood or stool.

Laboratory Findings (9/5/56)

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>8.6 Gm./100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.B.C's</td>
<td>5,300,000/cu. mm.</td>
</tr>
<tr>
<td>P.C.V.</td>
<td>34%</td>
</tr>
<tr>
<td>M.C.V.</td>
<td>83μ g</td>
</tr>
<tr>
<td>M.C.H.</td>
<td>20γγ</td>
</tr>
<tr>
<td>M.C.H.C.</td>
<td>25%</td>
</tr>
<tr>
<td>W.B.C's</td>
<td>5,630/cu. mm.</td>
</tr>
<tr>
<td>Indirect Van den Bergh</td>
<td>0.4 mg./100 ml.</td>
</tr>
<tr>
<td>Sickling</td>
<td>Negative</td>
</tr>
<tr>
<td>Marrow</td>
<td>Erythronormoblastic with rare giant stab-cells and 5% plasma cells.</td>
</tr>
</tbody>
</table>

Treatment and Progress

Oral ferrous sulphate, 30 grains daily, was started on 9/5 and continued until 10/6. On 9/12 the hemoglobin had fallen to 8.0 Gm./100 ml. and the R.B.C. and P.C.V. to 5,000,000 and 32%, respectively. Giant stab-cells were still present in the marrow. The clinical condition of the patient had deteriorated and she had become seriously mentally confused; 100 mg. of intravenous nicotinic acid was therefore given on 9/16 and continued until 9/23 daily. Four days later (9/20) her mental condition was normal, the perleche and pellagrous skin had cleared, and the hemoglobin, R.B.C. and P.C.V. had risen to 9.0 Gm./100 ml., 5,200,000/cu.mm. and 33%, respectively. Oral nicotinic acid, 100 mg. daily was started on 9/24, and continued until 9/29. On 9/27 her hemoglobin was 10.8 Gm./100 ml., R.B.C. 5,700,000, P.C.V. 40%. On 10/6 her hemoglobin was 12.4 Gm./100 ml., R.B.C. 6,200,000 and the patient was discharged. As can be seen from figure 2 the reticulocytes made no response when the patient was on iron, but did so four days after the commencement of nicotinic acid. In table 2 the serum protein values are given.

Discussion and Summary

Two cases of hypochromic anemia with erythronormoblastic marrows and giant stab-cells are reported. Both cases deteriorated when only on iron treatment; the first responded to pyridoxine therapy and the second to nicotinic acid.

Neither of the patients had diarrhea or steatorrhea and all were on the normal tropical hospital diet, similar to that given to the other patients who responded to iron therapy alone. The patients were under our constant control and were in the hospital from three to four weeks and had no other treatment. The diet of these two patients before they entered hospital did not appear to differ from that of other Africans with whom we have been dealing.

The presence of G.S.C. in the marrow is in our opinion associated with a deficiency of folic acid, due either to malabsorption following the anemia or
low intake associated with poor appetite. In many cases G.S.C. disappear during treatment with iron alone, perhaps due to improvement in absorption or appetite. In others supplementary folic acid is necessary.31,32,34

Bone marrow biopsy done in eight other cases of pellagra showed no giant stab-cells and the marrow was normal except for an increase in plasma cells and eosinophils.

We do not think that pyridoxine or nicotinic acid were responsible for their presence in these cases, since giant stab-cells are found in all megaloblastic anemias and in some cases of hypochromic anemia, as well as in other conditions where there is no evidence of either pyridoxine or nicotinic acid deficiency.

There was nothing significant about the serum proteins which were within normal range; the albumin globulin ratio was, however, reversed in both patients (tables 1 and 2) and remained so on discharge from hospital. This

---

**TABLE 2.—Serum Proteins**

<table>
<thead>
<tr>
<th></th>
<th>Total Proteins</th>
<th>Albumin</th>
<th>a1</th>
<th>a2</th>
<th>β</th>
<th>γ</th>
<th>Total Globulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/5/56</td>
<td>8.1</td>
<td>4.1</td>
<td>.19</td>
<td>.21</td>
<td>.88</td>
<td>2.7</td>
<td>4.0</td>
</tr>
<tr>
<td>9/12/56</td>
<td>7.1</td>
<td>3.4</td>
<td>.19</td>
<td>.49</td>
<td>1.0</td>
<td>2.2</td>
<td>3.9</td>
</tr>
<tr>
<td>9/20/56</td>
<td>7.7</td>
<td>3.1</td>
<td>.43</td>
<td>.70</td>
<td>1.2</td>
<td>2.3</td>
<td>4.6</td>
</tr>
<tr>
<td>9/27/56</td>
<td>8.0</td>
<td>3.7</td>
<td>.32</td>
<td>.61</td>
<td>1.0</td>
<td>2.3</td>
<td>4.2</td>
</tr>
</tbody>
</table>
reversed A/G ratio is common in many Africans and Indians, is not related to anemia and may be associated with diet or be genetically determined.\textsuperscript{31-33,36}

Until the exhibition of pyridoxine and nicotinic acid the patients were making no progress, but afterwards they made an uninterrupted cure and were discharged from the hospital feeling well.

The reticulocytosis was the expected one for the blood count and hemoglobin level, and as will be seen from figures 1 and 2 did not occur until after either pyridoxine or nicotinic acid had been given.

As in Dinning’s and Day’s pyridoxine deficient rats we also found in our pyridoxine-treated patients that there were increased R.B.C. counts and low hemoglobins with the mean corpuscular hemoglobin of 20\,\gamma\,g. and 17 \,\gamma\,g.

In eight other cases of typical pellagra there was, however, no sign of anemia at all, the hemoglobin range being between 15 to 16 Gm. per 100 ml.

At this altitude (6,000 feet) “pure” iron deficient anemia is rather rare\textsuperscript{32} and it was no surprise to us that these two cases failed to respond to iron therapy alone and that they needed other factors.

Pyridoxine appears in some way to be associated with hemopoiesis. Its absence does not in all cases give rise to anemia but sometimes produces abnormalities in red and white cell maturation and hemoglobin production in man and animals.

The role of nicotinic acid in hemopoiesis is obscure.

We realize that two swallows do not make a summer, and we have no idea how important a part pyridoxine and nicotinic acid play in hemopoiesis. Since, however, such pyridoxine and nicotinic acid-responding cases are rather infrequent we thought it worthwhile to record these two cases.

**Summario in Interlingua**

Es reportate duo casos de anemia hypochromic con medulla erythronormoblastic e gigante leucocytes bandiforme. Ambe casos se deteriorava durante un tractamento con solmente ferro. Le prime respondeva a un therapia a pyridoxina, le secunde a un therapia a acido nicotinic.

Ni le un ni le altre del duo patientes habeva diarrhea o steatorrhea. Ambes recipeva le normal dicta hospitalari tropic, simile a illo recipite per le altre patientes le quales respondeva al tractamento con solmente ferro. Le patientes esseva constantemente sub nostre survelantia. Illes esseva al hospital durante inter tres e quatro septimanas e recipeva nulle altre tractamento. Le dieta del duo patientes ante lor admission al hospital non pareva differer ab le dieta de altre africanos con qui nos ha habite a facer.

Le presentia de gigante leucocytes bandiforme in le medulla es associate, in nostre opinion, con carentia de acido folic in consequentia de malabsorption post le anemia o in consequentia de inadequatia del ingestion a causa del pauco bon appetito. In multe casos le gigante cellulas bandiforme dispare durante le tractamento con solmente ferro. Il es possibile que isto es le consequentia de un melliorate absorption o de un melliorate appetito. In altre casos le administration supplementari de acido folic es necessari.

Biopsias de medulla ossee in octo altre casos de pellagra monstrava nulle
gigante leucocytos bandiforme, e le medulla esseva normal con le exception
de un augmento de plasmocytos e de eosinophilos.

Nos non opina que pyridoxina o acido nicotinic esseva responsabile pro
lor presentia in iste casos, proque gigante leucocytos bandiforme se trova
in omne anemias megaloblastic e in certe casos de anemia hypochromic, si
ben como in altere conditiones in que il existe nulle evidentia de un manco
de pyridoxina o de acido nicotinic.

Le proteinas del sero monstrava nihil significative. Lor valores esseva intra
le limites del norma. Tamen, in ambe patientes le proportion de albumina a
globulina esseva revertite (vide tabulas 1 e 2). Iillos remaneva revertite
quando le patientes esseva dimititite ab le hospital. Iste reversion del pro-
portion de albumina a globulina es commun inter africanos e indianos. Illo
non es relationale a anemia e es forsan associate con le dieta o es determinate
geneticamente.31-32,35,36

Usque al introduction de pyridoxina e acido nicotinic in le regime, le duo
patientes non progredeva del toto. Postea lor recuperation esseva continue,
e illes se sentiva ben quando illes esseva dimititite.

Le reticulocytosis non deviava ab lo que esseva expectate super le base del
numeration de cellulas sanguinec e del nivello de hemoglobina. In plus, como
figuras 1 e 2 lo monstra, illo non occurreva usque post le initiation del curso
de pyridoxina o de acido nicotinic.

Como Dinning e Day lo constatava in lor rattos a carentia de pyridoxina,
nos trovava in nostre patientes a tractamento pyridoxic que le numeration
erthrocytic esseva elevate e que le nivello de hemoglobina esseva basse.
Le valores medie pro hemoglobina corpuscular esseva 20 g/g e 17 g/g.

Tamen, octo altere casos de pellagra exhibiva nulle signo de anemia del
toto, e le valores de hemoglobina remaneva inter 15 e 16 g per 100 ml.

A iste altitude (6.000 pedes), “pur” anemia a carentia de ferro es satis
rar,52 e nos non trovava surprendente que iste duo casos non respondeva al
therapia a solmente ferro e que altere factores therapeutic esseva requirite.
Nos recognosce que “duo hirundines non face Ic primavera,” e nos non es
preste a fixar le grado de importantia que pyridoxina e acido nicotinic possede
pro le mechanismo hemopoietic. Tamen, viste que casos que responde a
pyridoxina e acido nicotinic es satis rar, nos credeva que il valerea le pena
publicar le presente reporto.

Il pare un facto que pyridoxina es associate in un manera o un altere con
le processos del hematopoiese. Su absentia non resulta necessarmente e in
omne cases in anemia. On pote asserer solmente que su absentia produce a
VICES anormalitates del maturation erythro- e leucocytic e del generation de
hemoglobina in humanos e in animales.

Le rolo de acido nicotinic in le hematopoiese es obscur.

REFERENCES

1. Fouts, P. J., Helen; O. M. and Leovsky, S.: Nutritional microcytic hypochromic anemia
2. ———, ———, ——— and Jukes, T. H.: Production of microcytic hypochromic anemia in
due to pyridoxine deficiency, together with data on phenylhydrazine anemia. J. Biol.
Chem. 153:171, 1944.
5. McCall, K. B., Waisman, H.A., Elvehjem, C. A. and Jones, E. S.: A study of pyri-
doxine and pantothenic acid deficiencies in the monkey (Macaca Mulatta). J. Nutri-
tion 31:885, 1946.
1945.
10. Reid, J. T., Huffman, C. F. and Duncan, C. W.: The therapeutic effect of yeast and
doxine and pantothenic acid deficiencies in the monkey (Macaca Mulatta). J. Nutri-
tion 31:885, 1946.
1945.
Churchill, 1953.
19. Cantor, M. M. and Scott. J. W.: Agranulocytic angina effectively treated with intra-
22. Heidelberger, C., Abraham, E. P. and Lepovsky, S.: Tryptophan metabolism. II. Con-
cerning the mechanism of the mammalian conversion of tryptophan into nicotinic
23. Heidelberger, C., Abraham, E. P. and Lepovsky, S.: Tryptophan metabolism. II. Con-
cerning the mechanism of the mammalian conversion of tryptophan into nicotinic
24. Heidelberger, C., Abraham, E. P. and Lepovsky, S.: Tryptophan metabolism. II. Con-
cerning the mechanism of the mammalian conversion of tryptophan into nicotinic
Hypochromic Anemias of the Tropics Associated with Pyridoxine and Nicotinic Acid Deficiency

HENRY FOY and ATHENA KONDI