The Blood Picture of Pyridoxine Deficiency in the Monkey

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SINCE ADEQUATE SYNTHETIC DIETS are available for study of isolated deficiencies it was felt desirable to re-study the effects of vitamin deprivation in primates whose metabolic processes are most nearly comparable to those of man. This investigation is concerned with the detailed hematologic changes occurring in rhesus monkeys (macaca mulatta) subjected to prolonged periods of pyridoxine deficiency, and the restoration of the values to normal.

HISTORICAL

Gyorgy1 was the first to recognize the existence of a factor essential for rat nutrition related to the B vitamins other than B1 and B2. Phosphorylated pyridoxal has been demonstrated to be an important enzyme involved in decarboxylation2 of amino acids and also in transamination.3 Recently pyridoxal phosphate has been shown to also participate in desulfuration4 and transsulfuration.5 The essential nature of pyridoxine in protein metabolism is suggestive. Its exact role in tryptophan metabolism is obscure, but it is known that animals deficient in B6 excrete tryptophane as xanthurenic acid.6–10

Nutritional hypochromic microcytic anemias have been produced experimentally in guinea pigs,11 dogs,12 swine,13 chicks14 and monkeys15 on pyridoxine deficient diets. Hemosiderosis and elevation of plasma iron in B6 deficient animals have been reported16,17 and studied extensively by Cartwright and Wintroub18 in swine. Gubler19 found that iron absorption in the rat increased during B6 deficiency in the face of elevated plasma iron, contrary to current theories on regulation of iron absorption.

Both relative and absolute lymphopenia has been observed in B6 deficient monkeys,15 and atrophy of lymphoid tissue with suppression of circulating antibodies occurs in rats.20 Lymphosarcoma implants regressed and new implants failed to “take” in pyridoxine deficient mice.21 Studies in humans afflicted with malignant diseases of lymphoid tissue and placed on B6 deficient diets have been reported.22 No therapeutic effects were noted; however, it appears that the deficient period was too short for adequate evaluation. Pyridoxine deficient swine exhibited epileptiform seizures and alterations in gait associated with degenerative lesions in sensory neurons.23 Our animals did not show these changes. Two of us have reported the production of arteriosclerotic lesions in monkeys on pyridoxine deficient diets.24,25 Cataracts are found in B6 deficient swine26 and revascularization of the cornea in deficient rats.27

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NORMAL VALUES

The few reports on normal blood data of the monkey show considerable latitude in observed values. The RBC count has been reported from 4,941,900/mm$^3$ to 6,330,000/mm$^3$; WBC from 14,151/mm$^3$ to 20,000/mm$^3$ with a preponderance of lymphocytes in the differential. The nuclei of the polymorphonuclear neutrophiles and eosinophiles are more lobate in the monkey, resembling somewhat the hypersegmented neutrophil of pernicious anemia in man. The cytoplasmic granules are similar. Most of the lymphocytes

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Averages of successive readings obtained on each animal prior to period of pyridoxine deprivation, and on 5 control animals during first 6 months of observation are totaled and overall averages for all animals tabulated.

* Control animals.

are of the medium and large variety in contradistinction to the reversed occurrence in man. Difficulty is encountered in differentiating large lymphocytes from monocytes and especially from occasionally occurring "transition forms." A "mast" leukocyte similar to the human basophil is described. Since such variations in total leukocyte counts occur, some authors recommend determining the norm for each animal prior to experimental procedures. Reports on the normal bone marrow in the monkey are scanty but in general agree with our own data. Stasney and Higgins found preponderance of myeloid elements throughout all areas examined except in the tibia. Normal hematologic data was established on each of 16 animals prior to the period of induced deficiency and on 5 control animals (the first 6 months was arbitrarily selected for normal data; see table 1). These values were rounded for the purpose of plotting in graphic form.
EXPERIMENTAL PROCEDURE

Sixteen animals were subjected to varying periods of pyridoxine deficiency lasting from several months in some to over a year in others. All animals were placed on a modified M-3 diet which we have reported in detail. The control animals received the same experimental diet plus the equivalent of one milligram of pyridoxine supplement daily. Routine blood samples were taken weekly on some animals, bimonthly or monthly on others and included RBC, Hgb. (Sahli), PCV, WBC and differential, platelet and reticulocyte counts. The animals were sacrificed after one or more periods of B6 deficiency and complete necropsy studies done.

CONTROLS

The control animals warrant special comment. All did exceedingly well, gained weight and maintained excellent appetites for 40 weeks in one to almost three years in others. The Hgb. and RBC values remained constant during these months. It must be emphasized that the anemia of pyridoxine deficiency developed well within this time period, invariably manifest by the second month following withdrawal of B6. However, 4 control monkeys developed a macrocytic anemia after 140 weeks, 100 weeks, 64 weeks and 40 weeks respectively. This anemia was reflected by a rise of the mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) to over 100 cu. μ. The anemia was not corrected by small doses of vitamin B12, folic acid or iron. The effect of larger doses of vitamin B12 is at present under study.

RESULTS AND INTERPRETATION

During the initial control period all animals gained weight and seemed to thrive in the absence of complicating infections. When dietary pyridoxine was removed their food consumption dropped by the second or third week, accompanied by weight loss. The clinical appearance has been described. Briefly, this included diminished vigor, unkempt appearance, apathy or hyper-irritability, partial loss of hair with greying, fissuring of the skin of the palms and soles, loosening of the teeth, abnormalities in dentition and orbital edema. The pathologic findings of the vascular and hepatic changes have been described elsewhere.

The anemia is invariably manifest by the second month and is unremitting until either the animal expires or receives pyridoxine supplement. Late in the deficient period the RBC and hemoglobin values rise temporarily without concomitant clinical improvement. This phenomenon probably reflects dehydration with hemoconcentration as it does not materially alter the course of the anemia (fig. 1). The corollary to this is seen following the administration of B6 supplement. There is a temporary drop in the RBC, hemoglobin and PCV (fig. 2), undoubtedly the manifestation of corrected fluid balance mirroring the transitory rise occurring late in the deficient period. The relationship between dehydration and the phenomenon of "target" cells will be discussed.

There are two prominent morphologic changes occurring in the red cells during B6 deficiency (fig. 3). The production of red cells depleted of hemoglobin results in severe hypochromia. The mean corpuscular hemoglobin drops 20 per cent from a normal of 24.0 μg to less than 19.0. This would probably be of greater magnitude were it not for the relative constancy of the red blood cell volume. The second change is the increase in cell diameter from an average of
7.0 μ to slightly over 8.0 μ (approximately 15 per cent).* This has not been reported in pyridoxine deficient animals to date. The reason for the paradox of "hypochromic macrocytes" is not evident but may be related to structural

changes in the liver (i.e., fatty infiltration with cirrhosis in some) seen in most of the deficient animals at necropsy.

* Readings obtained by use of the halometer, C. A. Hauser & Son. Spot checks on this method by direct measurement consistently gave readings approximately 0.5 μ less than those obtained with the halometer.
Since the red cell volume drops less than 10 per cent (from 82.0 cu.μ to 76 cu.μ) and the mean diameter rises, it follows that the mean corpuscular thickness (MCT) must fall. By calculation, an average decrease from 2.12 μ to 1.50 μ was observed, or a 34 per cent drop (fig. 3). These two changes together with a manifest dehydration resulted in the appearance of numerous leptocytes, abnormally thin “target-cells,” in the peripheral circulation (fig. 4). At the height of the anemia there was low-grade reticulocytosis, marked anisocytosis, slight poikilocytosis, polychromatophilia and severe hypochromia. Microcytes were numerous but were outnumbered by large thin forms. Normoblasts occurred in the peripheral blood with increasing frequency as the anemia progressed, in one animal (3338) rising to as high as 26 per 100 WBC. Many red cells not classified as normoblasts contained scattered granular nuclear fragments and nuclear dust. Typical megaloblasts of the pernicious anemia type were not observed, either in the peripheral blood or in marrow aspirates.

The bone marrow (sternal and tibial) was cellular, with normoblastic hyperplasia resulting in reversal of the myeloid-erythroid ratio in 2 animals. Cytoplasmic vacuoles were observed, particularly in the erythroid series.

Peculiar basophilia was observed within the cytoplasm of polymorphonuclear neutrophiles. This was not ingested nuclei or “hematoxylin bodies” of the “L. E.
cell" type. Similar focal areas of basophilia could be found in the metamyelocytes and late myelocytes, suggesting irregular maturation of the cytoplasm.

The total leukocyte count dropped in 50 per cent of the animals who had been deficient for an average of 13 months (range: 9 to 21 months). The fall affected

![Composite Graph of Blood Indices](image)

**Fig. 3.—** Blood indexes plotted as percentage of normal. Note relative constancy of MCV. Points plotted represent vertical average of values obtained on each animal during comparable elapsed time interval of deficiency period.

the granulocytes and the mononuclears proportionately without selective lymphopenia as is reported by others. In no case was there a persistent leukopenia although occasional counts of 3,000 cu. mm. were observed. The remaining 8 animals showed no alteration in the total or differential counts; these had been deficient a shorter period of time, an average of 9 months (range: 5 to 16 months).
A tendency for low values in the WBC was also seen in some of the control animals.

The blood platelets fluctuated widely, but there was no consistent deviation either during the control or deficient period. There was no increased coagulability of the blood as has been reported in the chick associated with decreased clotting time and hyperprothrombinemia.\textsuperscript{14}

Normal red cell fragility as measured in hypotonic saline (control animals) began at 0.42 per cent and was complete at 0.33 per cent. Cells from deficient monkeys showed increased resistance, complete hemolysis being delayed to 0.24 per cent. This is to be expected and was predictable since hypochromic cells are more resistant to hemolysis by virtue of their greater capacity to imbibe fluid prior to bursting.\textsuperscript{34} Furthermore, "target" cells can be produced experimentally in vivo by rendering the plasma hypertonic through induced dehydration.\textsuperscript{35} These same cells, then, have a greater margin of safety prior to becoming spherocytic and bursting.

Hemosiderosis was conspicuously absent at necropsy; serum iron determinations made during life showed no consistent elevation. This is contrary to the experience of others.\textsuperscript{18} The effect of excessive dietary iron intake was not studied.

Deficient animals receiving supplementary $B_6$ showed immediate reticulocytosis (fig. 2), reaching a peak within a few days. The hemoglobin, RBC and PCV values showed initial temporary fall, reflecting correction of the pre-existing...
dehydration and hemoconcentration. Subsequently all erythroid values including the Win trobe indexes returned to the pre-deficient levels. In those animals showing low white counts during the deficient period a definite rise was observed, although not always returning to the pre-deficient levels. “Target” cells persisted in the peripheral blood three to four weeks after supplementary B6 had resulted in correction of the dehydration, indicating a factor (s) in addition to dehydration was responsible for their formation. Since most of the deficient animals showed structural liver damage (i.e., fatty alteration with cirrhosis in some) at necropsy, some even with manifest functional damage (i.e., jaundice, hypoproteinemia, edema), there is a distinct possibility that the peculiar morphology of the “target” cells may be related to the former. The period of dehydration possibly initiates the “target” change in previously malformed red cells which then persists for the life of the cell.

SUMMARY AND CONCLUSIONS

1. Pyridoxine deficiency in the monkey results in a severe anemia characterized by hypochromia, increase in mean diameter, decrease in mean cell thickness, dehydration, the appearance of “target” cells and increased resistance to hemolysis.

2. The administration of pyridoxine results in restoration of hematologic values to normal, correction of dehydration and disappearance of “target” cells.

3. Further evidence is offered relating “target” cells to dehydration and, in addition, possibly to hepatic damage.

4. A delayed macrocytic anemia which was not corrected by low doses of vitamin B12, folic acid or iron is described in control animals. This anemia occurred chronologically one to two years later than the induced anemia of pyridoxine deficiency and is not necessarily related. The cause of this anemia is unknown.

5. Normal hematologic data on twenty-one M. rhesus monkeys is presented.

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