# Pteroylglutamic Acid Nutrition in Vitamin C-Deficient Guinea Pigs (34428)

SACHCHIDANANDA BANERJEE AND MONOMOHAN NANDY

*Research Division, Dey's Medical Stores (Mfg.) Private Limited, Calcutta—19, India*

Ascorbic acid helps in the conversion of pteroylglutamic acid (PGA) to *N*-formyltetrahydro PGA (CF) in rats (1). Aminopterin-induced PGA deficiency in rats diminished urinary excretion and tissue concentrations of ascorbic acid (2, 3). Both scorbutic guinea pigs (4) and monkeys (5) show the presence of macrocytes in the peripheral blood although the anemia is of the normocytic normochromic type. The utilization of PGA may be defective in the scorbutic condition. Megaloblastic anemia responding to ascorbic acid has also been reported (6, 7). In PGA deficiency, administered histidine is eliminated in the urine as formiminoglutamic acid (Figlu). The present report deals with studies on PGA nutrition in scorbutic guinea pigs. Serum levels of PGA and cyanocobalamin and urinary excretions of PGA, CF, and Figlu were determined in normal and scorbutic guinea pigs. Hematological studies were also undertaken.

*Methods.* Guinea pigs were selected and fed a scorbutogenic diet with or without a supplement of ascorbic acid by the paired feeding technique as described previously (8), with the difference that additional vitamins (thiamine hydrochloride, 0.9 mg; riboflavin, 1.2 mg; pyridoxine hydrochloride, 0.3 mg; niacinamide, 7.5 mg; PGA, 0.5 mg; and cyanocobalamin, 0.3 μg) were mixed with each 100 g of the scorbutogenic diet. Animals which did not receive the ascorbic acid supplement developed scurvy at the end of 3 weeks of the scorbutic regimen. They and the normal controls were fed 100 mg of histidine monohydrate and urine was collected for 16 hr under toluene. Blood samples were drawn by cardiac puncture. Hematological studies were undertaken on whole blood. Plasma and urinary PGA was estimated microbiologically using *Lactobacillus casei*, ATCC 7469. Plasma cyanocobalamin was estimated using *Lactobacillus leichmannii*, ATCC 7830. Urinary CF was estimated with *Leuconostoc citrovorum*, ATCC 8061 using leucovorin as the reference standard (3). Urinary Figlu was estimated by paper electrophoresis and staining with Ninhydrin (9).

*Results.* There were no significant changes in the plasma levels of PGA and cyanocobalamin or the urinary excretion of PGA and CF in the scorbutic guinea pigs as compared to normal controls. Figlu could not be detected in the urine of either the normal or scorbutic guinea pigs after they were fed histidine monohydrate. Scorbutic guinea pigs showed normocytic and normochromic anemia. The results are given in Tables I and II.

*Discussion.* The normal excretion of CF

TABLE I. PGA Status of Normal and Scorbutic Guinea Pigs.[a]

| | | Normal | Scorbutic | *t* |
|---|---|---|---|---|
| Plasma PGA (mμg/100 ml) | (12) | 77 ± 5 | 74 ± 8 | 0.31 |
| cyanocobalamine (mμg/100 ml) | ( 5) | 46 ± 11 | 42 ± 16 | 0.20 |
| Urinary PGA (mμg/16 hr) | (10) | 609 ± 38 | 832 ±148 | 0.72 |
| CF (mμg/16 hr) | (13) | 98 ± 17 | 95 ± 11 | 0.15 |
| Figlu after administration of 100 mg of histidine | (13) | Could not be detected | Could not be detected | |

[a] PGA = pteroylglutamic acid; CF = *N*-formyl-tetrahydro PGA; Figlu — formimino-glutamic acid; values in parentheses indicate the number of animals; all the values of *t* are insignificant.

TABLE II. Hematological Picture of Normal and Scorbutic Guinea Pigs.

| | Normal (12)[a] | Scorbutic (12) | $t$ |
|---|---|---|---|
| RBC (millions /mm$^3$) | 5.11 ± 0.08 | 3.57 ± 0.27 | 5.3[b] |
| PVC (%) | 45 ± 0.3 | 32 ± 2.7 | 4.7[b] |
| Hb (g/100 ml) | 14.43 ± 0.16 | 10.5 ± 0.82 | 4.7[b] |
| MVC (m$\mu$) | 89 ± 1.9 | 90 ± 2.3 | 0.33 |
| MCH ($\mu\mu$g) | 28.2 ± 0.4 | 29.4 ± 1.12 | 1.01 |
| MCHC (%) | 31.6 ± 0.56 | 32.5 ± 0.74 | 0.96 |

[a] Values in parentheses indicate the number of animals.

[b] These values of $t$ are highly significant.

and absence of Figlu in the urine after administration of histidine, the normal blood levels of PGA in the scorbutic guinea pigs indicated that the metabolism of PGA was not disturbed when the animals developed vitamin C deficiency. The hematological picture of the scorbutic animals, which received more PGA and cyanocobalamin than the scorbutic animals reported by Chakrabarty and Banerjee (4), was again normocytic and normochromic. Occasional macrocytes were present in the peripheral blood (4). The above results indicate a limited role of ascorbic acid in the intermediary metabolism of PGA in guinea pigs.

*Summary.* Blood levels of PGA and cyanocobalamin and the urinary excretion of PGA and CF were estimated in scorbutic and paired-fed normal guinea pigs. The urinary excretion of Figlu after administration of histidine also was determined. No significant changes in the blood levels of PGA and cyanocobalamin or in the urinary excretion of PGA, CF, and Figlu were observed in the vitamin C-deficient guinea pigs. The intermediary metabolism of PGA did not seem to be affected as a result of vitamin C deficiency. It seems that in guinea pigs, unlike rats, ascorbic acid has a limited role in the conversion of PGA to CF.

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