# CHANGES IN FOLIC ACID METABOLISM DURING ADMINISTRATION OF VITAMIN $B_{12}$ ANTIMETABOLITES AND THYROID HORMONES

Z. N. Dzhelieva, G. M. Kyul'yan, and A. V. Trufanov

UDC 615.3:577.164.16.014+615.361.441-092:612.015.641.7

Intramuscular injection of $C^{13}$-2-methyl-2-aminopropanol analog of vitamin $B_{12}$ into guinea pigs caused considerable excretion of formiminoglutamic acid in the urine and a sharp decrease in active forms of folic acid in the blood. At the same time the hemoglobin level and erthythrocyte count fell, while the leukocyte count in the circulating blood rose considerably.

* * *

We showed in a previous paper [1] that the monocarboxylic acid derivative of vitamin $B_{12}$ caused a disturbance of folic acid metabolism in vivo. This disturbance took the form of a lowering of the content of derivatives with folic activity in the blood and an increase in the excretion of formiminoglutamic acid (FIGA) in the urine.

To obtain more profound disturbances, in the present investigation a vitamin $B_{12}$ derivative 33 times more active than the monocarboxylic was used, namely the $C^{13}$-2-methyl-2-aminopropanol analog of vitamin $B_{12}$, synthesized by Friedrich and Heinrich [5]. The index of 50% inhibition of growth of Escherichia coli 113-3 for this antivitamin is 3:1. The action of thyroid hormones was also studied, because of reports [4, 11] that their administration caused vitamin $B_{12}$ deficiency.

## EXPERIMENTAL METHOD

Activity of the folic acid derivatives was determined in whole blood, treated by the method of Toennies and co-workers [12]. Two test organisms were used: Streptococcus faecalis R and Pediococcus cerevisiae. The formula for the single maintenance medium and the principal growth medium for these microorganisms was taken from the paper by Herbert and co-workers [7]. Casein hydrolysate was prepared enzymatically[2].

FIGA was determined in the 24 h urine chromatographically [6] and enzymatically [9]. Before the urine was collected the animals received L-histidine in a dose of 200 mg/kg body weight.

In the experiments of series I the aminopropanol analog was tested on guinea pigs weighing 450-500 g. In the experiments of series II thyroid hormones and methylthiouracil were used on guinea pigs weighing 250-300 g. Each dose of these substances was tested on five animals.

In the experiments of series I the background level of all the indices was determined on all the animals three times in the course of the 1st week. Each guinea pig then received intramuscular injections of the antivitamin (16 $\mu$g on each of the first 2 days, and 8 $\mu$g thereafter) and all the indices were determined again four times. On the 7th day from the start of injection of the antivitamin, the animals were divided into three groups. Groups 1 and 2 included 4 animals, and group 3 (the control) consisted of 2 animals.

Against the background of continuing administration of antivitamin, the guinea pigs of group 1 received daily intramuscular injections of vitamin $B_{12}$ in a dose of 10 $\mu$g for 4 days. The animals of group 2 received pangamic acid by mouth in a dose of 2.5 mg, and the animals of group 3 received antivitamin only. The indices were determined three times. After administration of the antivitamin had ended, for the next 6 days the animals of group 1 received vitamin $B_{12}$ only, and those of group 2 received pangamic acid only. The control animals continued to receive antivitamin.

Laboratory of Biochemistry, Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi (Presented by Active Member of the Academy of Medical Sciences of the USSR V. N. Orekhovich). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 1, pp. 65-68, January, 1968. Original article submitted January 14, 1966.

TABLE 1. Changes in Content of Folic Acid Derivatives in Blood (in mμg/ml) during Tests of the $C^{13}$-2-methyl-aminopropanol Analog of Vitamin $B_{12}$

| Group of animals | Background | | Injection of antivitamin | | Injection of antivitamin with vitamin $B_{12}$ | | Injection of vitamin $B_{12}$ | |
|---|---|---|---|---|---|---|---|---|
| | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae |
| 1 | 13±1,90 | 22,5±2,27 | 5,7±0,67 | 14±1,46 | 8,5±1,7 | 10,6±1,58 | 12,6±0,74 | 18±2,6 |
| | Background | | Injection of antivitamin | | Administration of antivitamin with pangamic acid | | Administration of pangamic acid | |
| 2 | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae | S. faecalis | P. cerevisiae |
| | 12±3,0 | 25,7±4,48 | 4,3±0,64 | 10,3±1,36 | 12,5±5,42 | 13±2,56 | 12,1±1,69 | 17,6±2,2 |
| | Background | | Injection of antivitamin | | | | | |
| 3 | S. faecalis | P. cerevisiae | S. faecalis | | | P. cerevisiae | | |
| | 8,0±1,74 | 31±1,06 | 3,1±0,16 | | | 8,8±0,89 | | |

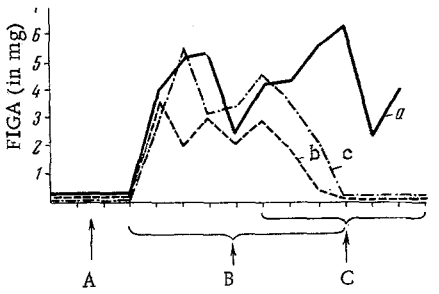


Fig. 1. Excretion of FIGA in the 24 h urine administration of $C^{13}$-2-methyl-2-aminopropanol analog of vitamin $B_{12}$. A) Background; B) administration of $C^{13}$-2-methyl-2-aminopropanol analog of vitamin $B_{12}$; C) administration of vitamin $B_{12}$ and pangamic acid; a) control; b) 10 μg vitamin $B_{12}$ daily; c) 2.5 mg pangamic acid daily.

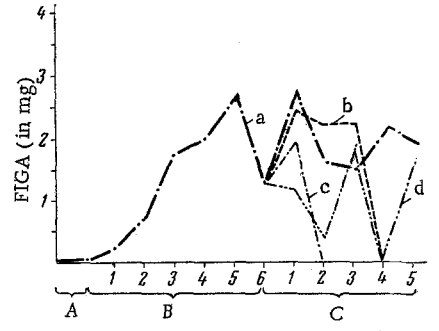


Fig. 2. Excretion of FIGA in the 24 h urine during administration of thyroid hormones and methylthiouracil. A) background; B) administration of monocarboxylic acid derivative of vitamin $B_{12}$; C) administration of thyroid hormones and methylthiouracil together with antivitamin $B_{12}$; a) control; b) 0.0025 mg tri-iodothyronine (daily; c) 0.03 mg thyroxine (daily); d) 250 mg methylthiouracil (daily).

In the experiments of series II, against the background of administration of the monocarboxylic acid derivative of vitamin $B_{12}$ thyroxine was tested in doses of 0.01, 0.03, and 0.06 mg, tri-iodothyronine in doses of 0.0025 and 0.01 mg, and methylthiouracil in doses of 250 and 500 mg per animal.

## EXPERIMENTAL RESULTS

It will be seen in Table 1 that the aminopropanol analog of vitamin $B_{12}$ considerably lowered the concentraion of derivatives with folic activity in the blood.

Their level remained low even after administration of vitamin $B_{12}$ or pangamic acid together with the antivitamin. Only after administration of the antivitamin had ended, but administration of vitamin $B_{12}$ or pangamic acid continued, did the concentration of compounds with folic activity in the blood come close to the background level.

The dynamics of the FIGA content in the urine is illustrated in Fig. 1. Before administration of the antivitamin no FIGA was found in the urine, whereas after its administration the content of this acid increased considerably. FIGA was found in the urine even when vitamin $B_{12}$ or pangamic acid was administered against the background of continuing injection of the antivitamin. Excretion of FIGA ceased completely only after administration of antivitamin and vitamin $B_{12}$ or pangamic acid had ended.

Administration of the aminopropanol analog of vitamin $B_{12}$ lowered the hemoglobin concentration by 2.5-4.2% and the erythrocyte count by 1-2.5 million cells /$mm^3$ blood. After injection of antivitamin and treated with vitamin $B_{12}$ or pangamic acid had ended the blood picture returned to normal. The guinea pigs receiving the aminopropanol analog of vitamin $B_{12}$ became apathetic, developed liquid stools, and lost 15-30% of their body weight. A similar clinical picture was also observed when the monocarboxylic derivative of $B_{12}$ was used, although it was less severe.

Thyroxine and tri-iodothyronine, given against the background of administration of the monocarboxylic acid derivative of vitamin $B_{12}$ led to a decrease in the hemoglobin concentration and erythrocyte count, and also lowered the FIGA content in the urine. The minimal dose of thyroxine preventing the appearance of FIGA in the urine was 0.03 mg, and of tri-iodothyronine 0.0025 mg, per guinea pig (Fig. 2). Methylthiouracil in a dose of 250 mg had no appreciable effect on the lowering of the hemoglobin level and the erythrocyte count or on the FIGA content in the urine. A dose of 500 mg was toxic and the animals died after the third dose.

On the basis of Herbert and Zalusky's hypothesis [8] it may be postulated that in vitamin $B_{12}$ deficiency caused by the aminopropanol analog or the less active monocarboxylic acid derivative of $B_{12}$, folic acid metabolism is blocked at the stage of 5-methyltetrahydrofolic acid, which is inactive against S. faecalis and P. cerevisiae. However, the problem why vitamin $B_{12}$ and pangamic acid have the same effect on restoration of derivatives with folic activity in the blood and on disappearance of FIGA from the urine remains unsolved.

The lowering of the hemoglobin concentration and the erythrocyte count in the blood and the simultaneous removal of FIGA from the urine brought about by thyroid hormones indicate that these hormones and antivitamin $B_{12}$ have an antagonistic action on folic acid metabolism.

## LITERATURE CITED


1. Z. N. Dzhelieva, G. M. Kyul'yan, and A. V. Trufanov, Vopr. Med. Khimii, No. 4, 386 (1964).
2. O. I. Pushkinskii and A. S. Kutseva, In the book: Vitamin Resources and Their Use [in Russian], Moscow (1955), p. 166.
3. Biochemical Preparations, New York, 5, 100 (1957).
4. P. Fatterpaker, U. Marfatia and A. Sreenivasan, Nature, 176, 165 (1955).
5. W. Friedrich, H. Heinrich, E. Königk, et al., Ann. New York Acad. Sci., 112, 601 (1964).
6. M. R. S. Fox, W. J. Ludwig, H. A. Barker, et al., Proc. Soc. Exp. Biol., New York, 105, 145 (1960).
7. V. Herbert, R. Fisher, and B. J. Koontz, J. Clin. Invest., 40, 81 (1961).
8. V. Herbert and R. Zalusky, Ibid., 41, 1263 (1962).
9. M. Meiss, D. P. Peyser and A. Miller, J. Lab. Clin. Med., 64, 512 (1964).
10. S. M. Milcou, L. Vaisler, and E. Costiner, Therapie, 16, 868 (1961).
11. C. A. Nichol, L. S. Dietrich, N. V. Cravens, et al., Proc. Soc. Exp. Biol., New York, 70, 40 (1949).
12. G. Toennies, E. Usdin and P. Phillips, J. Biol. Chem., 221, 855 (1956).