Study of Folic Acid and Vit. B_{12} in Blood and Urine During Normal Pregnancy.* (22995)

HERMAN BAKER, RUTH ERDBERG, INEZ PASHER, AND HARRY SOBOTKA
Departments of Chemistry, Obstetrics and Gynecology, Mt. Sinai Hospital, New York City

This report deals with blood and urine levels of cyanocobalamin (vit. B_{12}) and folic acid (PGA) simultaneously determined in the same subjects during pregnancy, not complicated by hemorrhage and abortion. Recent investigations have shown vit. B_{12} level in serum to be low during pregnancy(1,2). In the latter communication, mean levels of vit. B_{12} in serum before delivery were as low as those reported in cases of pernicious anemia (3). Since the functions of vit. B_{12} and PGA are interrelated, the present study was undertaken in an attempt to correlate their involvement during pregnancy.

Methods. Methods for determination of vit. B_{12} in serum and urine have been described(4,5). All assays for vit. B_{12} were made in parallel using *Euglena gracilis* (Z strain) and *Ochromonas malhamensis;* a comparison which permits elimination of responses to congeners of the vitamin(4). PGA in urine was estimated with a thermophilic bacillus(6).† A modification of this method for assay of blood was devised, as suggested by earlier work.‡ Blood is diluted 1:5 with citrate buffer at pH 6.1, to which 0.05% ascorbic acid has been added. After incubating the blood-buffer mixture for 2 hours, the proteins can be completely removed after a 10 minute autoclaving period by centrifugation; the supernatant is then diluted again with medium 1:2-1:100 (final dilutions 1:10-1:500). Recovery experiments using this technic yielded satisfactory results for a range from 0.3-100 mμg/ml. Patients were selected from the pre-natal clinic of the hospital. The cases that developed complications during pregnancy were excluded. The patients received vitamin preparations free of PGA and vit. B_{12} to avoid interference with the assay. Ordinary diets do not perceptibly disturb PGA levels(7), however vit. B_{12} levels might be altered; blood and urine were obtained in the morning; patients were not placed on controlled diet. Several blood and urine determinations were carried out on the same patients whenever possible during duration of pregnancy. A total of 47 patients were studied.

TABLE I. B_{12} and PGA Content of Blood and Urine during Normal Pregnancy.

Quartile	B_{12} (μμg/ml) Blood	B_{12} (μμg/ml) Urine	PGA (mμg/ml) Blood	PGA (mμg/ml) Urine
1st	80–108	0–19	0–10	0–10
2nd	108–193	19–55	10–19	10–18
3rd	193–360	55–95	19–57	18–72
4th	>360	>95	>57	>72
Median	193	55	19	18
No. of patients	51	54	54	54

Results. The occasional high values in these determinations tend to distort the picture, if it were represented by average values. We have therefore given the medians, rather than the means, in the following presentation.

B_{12} in urine and blood. The vit. B_{12} content in urine and blood is given in Table I. Fifty-four specimens were included in the urine study, and 51 in blood studies. The median of urinary B_{12} levels was 55 μμg/ml and of blood levels 193 μμg/ml. Moreover, 35 instances of B_{12} in urine were crowded in the group below 60 μμg/ml; of these, 14 in the group below 25 μμg/ml. In blood values, 17, or one-third of the total, were in the group below 125 μμg/ml.

The PGA content of urine and blood was determined in 54 specimens; results are rendered in Table I. The median of the PGA level in urine and blood was 19 mμg/ml. In both cases the distribution curve shows a

* Aided by grants from U. S. Public Health Service and Am. Cyanamid Co., Lederle Laboratories.

† The basal medium for this assay is under revision and will be reported separately. The medium as used now, contains 2% glycerol, inst. of 2% sucrose, because some sucrose samples contain a PGA-like substance which contaminates the assay medium and results in high blanks.

‡ Reported in *Fed. Proc.*, 1954, p. 311.

much slower decrease towards higher values than in normal non-pregnant subjects.

Discussion. The study indicates that PGA increases during pregnancy in blood and urine. Judging from previous reports on PGA levels in blood and urine of normal cases(3, 8,9), the median value of 19 mμg/ml is from 2-8 times as high as normal. Since the renal threshold for PGA is low, it is not surprising that the median urinary PGA values are also increased above the normal values reported.

Fetal development poses a problem of cellular and tissue synthesis for the pregnant woman. The need for facilitation of all mechanisms involving such synthesis is pressing; PGA and B_{12} are known to be involved (10). Any inhibition of PGA can lead to fetal death or developmental abnormalities. This effect has been shown in chick embryos (11). Because pregnancy places abnormal demands on maternal metabolism, the available stores of these vitamins are brought into circulation to continue this state and to further fetal development.

PGA is also a factor in hormonal response; estrogen activity in birds and mammals has been inhibited by PGA deficiency(12). Aminopterin, a PGA antimetabolite, can terminate pregnancy by inhibiting progesterone activity. Since estrogen and progesterone are vital hormones in pregnancy, it is likely that PGA is involved in pregnancy by enhancing hormonal activity or overcoming any inhibition. Ovarian hormones fail to maintain pregnancy in PGA deficient animals(13); it is thus reasonable to assume that in this deficient state hormonal inhibitors are operative. The finding of high levels of PGA during pregnancy, previously reported(14), and also observed by us, supports the assumption that PGA is needed to insure proper hormonal activity by antagonizing inhibitors.

Vit. B_{12} levels in this study were low. Median urine values were 2-5 times lower than normal(3,15), and blood values up to 4 times lower. The low serum B_{12} observed, as pregnancy progressed, suggests that the fetus may attract maternal B_{12} across the placenta, thereby causing a depletion of this vitamin in the maternal circulation. Recent results bear out this premise(2,16). A study of placental transfer of B_{12}, as compared with PGA, is now under way in our laboratories.

It is clear from our results that pregnancy exacts overwhelming demands on PGA and B_{12} stores. High maternal stores of these vitamins during pregnancy should alleviate some of the stress, which fetal development places upon maternal metabolism, and could prevent intrauterine injury(17).

Summary. A comparison of PGA and vit. B_{12} levels in blood and urine during pregnancy shows that levels of PGA are high, and those of B_{12} are low. Possible explanations for these findings are discussed.

We are indebted to Dr. Alan F. Guttmacher, Director of Department of Obstetrics and Gynecology of Mt. Sinai Hospital, and to Dr. Lazar Margulies for generous cooperation.

1. Boger, W. P., Wright, L. D., Beck, G. D., and Bayne, G. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 140.

2. Okuda, K., Helliger, A. E., and Chow, B. F., *Am. J. Clin. Nutr.*, 1956, v4, 440.

3. Nieweg, H. O., Faber, J. G., de Vries, J. A., and Kroese, W. F. S., *J. Lab. Clin. Med.*, 1954, v44, 118.

4. Baker, H., Sobotka, H., Pasher, I., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 636.

5. Hutner, S. H., Bach, M. K., and Ross, G. I. M., *J. Protozool.*, 1956, v3, 101.

6. Baker, H., Hutner, S. H., and Sobotka, H., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 210.

7. Jukes, T. H., Franklin, A. L., Stokstad, E. L. R., and Boehne, J. W., *J. Lab. Clin. Med.*, 1947, v32, 1350.

8. Spray, G. H., and Witts, L. J., *Clin. Sci.*, 1952, v11, 273.

9. Toennies, G., Frank, H. G., and Gallant, D. L., *J. Biol. Chem.*, 1953, v200, 23.

10. Jukes, T. H., Broquist, H. P., and Stokstad, E. L. R., *Arch. Biochem. and Biophys.*, 1950, v26, 157.

11. Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

12. Hertz, R., and Tullner, W. W., *Endocrinology*, 1949, v44, 278.

13. Nelson, M. N., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 614.

14. Knott, E. M., and Suarez, R. M., *Fed. Proc.*, 1949, v8, 387.

15. Mollin, D. L., and Ross, G. I. M., *J. Clin. Path.*, 1952, v5, 129.

16. Chow, B. F., Barrow, L., and Ling, C. T., *Arch. Biochem, and Biophys.*, 1951, v34, 151.

17. Lepkovsky, S., Barson, H. J., Bouthilet, R., Pencharz, R., Singman, D., Dimick, M. K., and Robbins, R., *Am. J. Physiol.*, 1951, v165, 79.

Received January 7, 1957. P.S.E.B.M., 1957, v94.

Comparison of Some Suggested Anticariogenic Compounds in the Rat.* (22996)

JOSEPH C. MUHLER
Indiana University, Bloomington, Ind.

This report concerns an evaluation of 4 substances which have been suggested for reducing human dental caries by means of topical procedures. These include stannous fluoride(1,2), sodium fluoride(3), sodium oxalate (4), and sodium N-palmitoyl sarcosinate(5).

Materials and methods. A total of 280 weanling Sprague-Dawley strain rats were divided according to initial body weight into 7 groups. Each group was further subdivided equally according to sex. Groups 1, 2, and 3 received sodium fluoride, stannous fluoride,† or sodium fluorostannite‡ respectively, at a fluoride concentration of 30 parts/million. Group 4 received sodium oxalate at an oxalate concentration of 30 parts/million. Group 5 received 1% solution of sodium N-palmitoyl sarcosinate. Group 6 received a solution of sodium N-palmitoyl sarcosinate and sodium fluoride, the concentrations of which were identical with those which were used in Groups 1 and 5 above. Group 7 received distilled water and served as control. In all instances the compounds were put in drinking water, which the animals received *ad libitum*. All solutions were made fresh each morning, and any solutions remaining in the drinking tubes from previous day were discarded. No attempt was made to regulate amount of solution the animals received. However, frequent estimates were made concerning amount of water which an entire group drank during a 24-hour period. These data initially indicated that the 2 sarcosinate groups and the stannous fluoride groups consumed somewhat less solution, but after about 30 days these differences were not pronounced. The animals all received the same stock corn cariogenic diet§ and were housed in pairs in raised-screen cages in air-conditioned room. The duration of the study was 140 days, after which the animals were sacrificed by ether and the heads removed for dental caries evaluation as previously described(6).

Results. A summary of the pertinent data (Table I) indicates that the 3 fluorides were the only substances tested which numerically decreased the mean number of dental cavities, when compared to the control. However, of the 3 fluorides only stannous fluoride and sodium fluorostannite were significantly effective (probability of 0.05). When sodium fluoride was added to the sarcosinate solution at the same concentration as in Group 1, no effect on dental caries was observed. The reason for the apparent difference between the 2 sodium fluoride groups, with and without sarcosinate, is not known. Sodium oxalate was without effect, while 2 groups receiving sodium N-palmitoyl sarcosinate had an apparent but not significant increase in incidence of dental caries.

In both groups receiving the sarcosinate solution there was less weight gain during the

* This study was supported in part by grant from Procter and Gamble, Ivorydale, O.

† Obtained from Metal and Thermit Co., Rahway, N. J.

‡ Synthesized in Department of Chemistry at Indiana University.

§ Composition of diet was as follows: yellow corn grits, 52.7%; ground yellow whole corn, 11.3%; powdered whole milk, 30%; alfalfa, 4.8%; iodized sodium chloride, 1%; and irradiated yeast, .2%.