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# Serum Vitamin $B_{12}$ Content and UBBC in Gravid and Fetal Rabbits (39045)

HAROLD L. ROSENTHAL AND RONALD P. WILBOIS

*Dept. of Physiological Chemistry and Dept. of Obstetrics and Gynecology, Washington University Medical Center, St. Louis, Missouri 63110*

It is well known that human serum vitamin $B_{12}$ decreases progressively during pregnancy (1–3) with lowest levels present at term. Within 4–6 weeks *post partum*, the maternal serum $B_{12}$ level returns to normal or higher (4–5). In general, fetal cord blood contains more vitamin $B_{12}$ than comparable maternal blood (3). The unsaturated vitamin $B_{12}$ binding capacity (UBBC) of the mother increases during pregnancy and returns to normal within a month of parturition (6, 7). Similar phenomenon have also been found in rats (1). Although pregnant women and gravid experimental rats appear to follow the same pattern with regard to serum vitamin $B_{12}$, no definitive rationale has as yet been offered to explain these effects.

Rabbits are known to contain the highest serum vitamin $B_{12}$ content and UBBC of all laboratory mammals so far tested (8). Because rabbits are often used as models for fetal development, it was of interest to determine the serum $B_{12}$ and UBBC during gravidity and in the fetus.

*Materials. Samples.* Sera of gravid New Zealand rabbits, fetuses near term and sera of both sexes during growth and development were obtained commercially.[1] Sera from New Zealand strain rabbits that were maintained in the laboratory were used for comparative purposes. Sera of normal pregnant women were obtained a few hours prior to parturition and cord blood was obtained from their normal healthy infants. All sera were maintained frozen at −20° until analyzed.

*Vitamin $B_{12}$ assay.* Vitamin $B_{12}$ was determined by a competitive binding assay using porcine intrinsic factor (IF)[2] as the binding protein, high specific activity $^{57}Co$ labelled cyanocobalamin (15 $\mu$Ci/$\mu$g $B_{12}$-60 $\mu$Ci/$\mu$g $B_{12}$)[3] and equilibrium dialysis briefly described as follows:

Dilute 10 $\mu$l of rabbit serum (or use 0.5 ml of undiluted human serum) to 0.5 ml with saline. Add 5.5 ml of 0.1 *M* HAc buffer, pH 4.5 (containing 1 $\mu$g KCN and 50 pg labelled vitamin $B_{12}$ per ml). Cap tubes loosely and place in boiling water bath for 30 min. After cooling tubes in tap water, add 1 ml of IF (in buffer + KCN) sufficient to bind 50–60% of the added radiolabelled vitamin. Pour the tube contents into triple washed visking dialysis tubing and dialyse in capped tubes vs 40 ml of nonradioactive buffer + KCN at 4°C for 64 hr on a slow shaker. Count aliquot portions (5 ml) of the bag contents and external solutions in a well-type scintillation detector. Appropriate serum controls and blanks were included in each run and all procedures were performed in dim light (9, 10). The protein precipitate that forms on heating does not interfere with the assay and need not be removed. All samples were assayed in duplicate. The $B_{12}$ levels were calculated (9, 10) by comparison of the radioactivity retained in the bag, after deduction for dialyzable radioactivity in the outside tube, with a standard curve.

*Unsaturated $B_{12}$ binding capacity (UBBC).* Samples of rabbit serum (10 $\mu$l) or human serum (250 $\mu$l) were diluted to 17.5 ml with 0.1 *M* phosphate buffer, pH 7.3. Duplicate seven (7) ml portions of each dilution were placed in washed visking tubing and dialyzed as above against 15 ml of phosphate buffer containing 100 pg labelled vitamin $B_{12}$/ml (11). Count aliquot portions of inside and outside bags as above. The UBBC was calculated from the radioactivity retained in the

[1] Pel-Freeze Biological, Inc. Rogers, Arkansas.

[2] Abbott Laboratories, North Chicago, Illinois.

[3] Amersham/Searle Corp., Arlington Heights, Illinois.

bag after subtraction of the dialyzable material without corrections for Donnan membrane equilibrium.

*Results and discussion.* The serum vitamin $B_{12}$ content and UBBC of rabbits of both sexes and at different ages are shown in Table I. Gravid rabbits near term contained the highest serum content (138 ng/ml) of any animals tested while fetuses contained the lowest amount—a difference of about six-fold. After birth, the serum $B_{12}$ level increases rapidly to adult levels. The UBBC of fetal serum is higher than that found in either maternal serum or other adult animals. Unlike the data for human pregnancy, the UBBC of gravid rabbits is only slightly elevated from that found in adult animals of either sex. It may also be seen that serum $B_{12}$ levels in male and female virgin rabbits from the same population are essentially the same although serum $B_{12}$ levels of laboratory animals are somewhat lower than sera obtained from commercial sources. Such differences are to be expected in the light of differences of methods of animal husbandry, nutrition and other factors.

In contrast to the data obtained for rabbits, the vitamin $B_{12}$ blood level of pregnant women near term is lower than that found in nonpregnant women (Table II) and cord blood of newly born infants is considerably higher than levels in the adult population. These findings are in agreement with data presented by Luhby *et al.* (12) and reviewed by Kumento (13) but no specific rationale for this phenomenon has been offered (14–16).

TABLE I. VITAMIN $B_{12}$ CONTENT AND UBBC OF RABBIT SERUM.[a]

| Age | No. | Serum $B_{12}$ (ng/ml ± SEM) | UBBC |
|---|---|---|---|
| Fetus | | | |
| Female | 8 | 19 ± 2 | 59 ± 6 |
| 8–12 weeks | 10 | 54 ± 5 | 33 ± 2 |
| 1–3 years | 7 | 65 ± 5 | 24 ± 2 |
| 1–3 years (L) | 8 | 26 ± 2 | 20 ± 3 |
| Gravid | 8 | 138 ± 23 | 38 ± 5 |
| Male | | | |
| 8–12 weeks | 10 | 55 ± 5 | 33 ± 2 |
| 1–3 years | 10 | 71 ± 9 | 27 ± 2 |
| 1–3 years (L) | 12 | 47 ± 7 | 41 ± 9 |

[a] The L stands for laboratory animals; all other sera from commercial source.

TABLE II. VITAMIN $B_{12}$ CONTENT AND UBBC OF HUMAN SERUM.

| | No. samples | Serum $B_{12}$ | UBBC |
|---|---|---|---|
| | | (ng/ml ± SEM) | |
| Cord blood | 10 | 1.07 ± 0.23 | 1.94 ± 0.14 |
| Mother | 12 | 0.33 ± 0.05 | 2.83 ± 0.24 |
| Nonpregnant | 10 | 0.41 ± 0.05 | 1.23 ± 0.44 |

The wide variation of vitamin $B_{12}$ serum levels between rabbits and other common mammals is difficult to explain at this time. It is well-known that native vitamin $B_{12}$ is tightly bound to at least three-protein components in rabbit serum (17) and these carrier proteins are present at concentrations much higher than in other mammals (18). The present data in rabbits suggests that the synthesis of vitamin $B_{12}$ binding proteins in fetal serum is limited while the production of maternal serum binding proteins is increased during gravidity. The fact that UBBC in both fetal rabbit and human cord serum is elevated may not be relevant since native serum $B_{12}$ is probably methylcobalamin (19) rather than cyanocobalamin and the latter may represent nonspecific binding. Graber *et al.* (20) have suggested either an active transport process across the placenta or alternatively, the presence of fetal and placental $B_{12}$ binding protein(s) with a much higher affinity for vitamin $B_{12}$ than present in maternal plasma. Although it appears unlikely that the mechanism for placental transfer of vitamin $B_{12}$ for rabbits and other mammals is basically different, it is conceivable that the rate, quantity, and structure of carrier protein synthesis may be markedly different.

1. Young, J. E., Barrows, C. Jr., Okuda, K., and Chow, B. F., Obstet. Gynecol. **14,** 149 (1959).
2. Okuda, K., Helliger, A. E., and Chow, B. F., Amer. J. Clin. Nutr. **4,** 440 (1956).
3. Lowenstein, L., Lalonde, M., Deschenes, E. B., and Shapiro, L., Amer. J. Clin. Nutr. **8,** 265 (1960).
4. Metz, J., Festenstein, H., and Wilch, P., Amer. J. Clin. Nutr. **16,** 472 (1965).

5. Ball, E. W., and Giles, C., J. Clin. Pathol. **17,** 165 (1964).
6. Gottlieb, C. W., Retief, P., Pratt, P. W., and Herbert, V., J. Clin. Invest. **45,** 1121 (1967).
7. Sullivan, L. W., Greene, R. J., and Liu, Y. K., J. Clin. Invest. **46,** 1121 (1967).
8. Rosenthal, H. L., and Brown, C. L., Jr., Proc. Soc. Exp. Biol. Med. **86,** 117 (1954).
9. Lau, K. S., Gottlieb, C., Wasserman, L. R., and Herbert, V., Blood **26,** 202 (1965).
10. Liu, Y. K., and Sullivan, L. W., Blood **39,** 426 (1972).
11. Rosenthal, H. L., O'Brien, G., and Austen, S., Arch. Biochem. Biophys. **99,** 319 (1962).
12. Luhby, A. L., Feldman, R., Marley, J., Odang, O., and Cooperman, J., Fed. Proc. Abstract, Fed. Amer. Soc. Exp. Biol. **20,** 451 (1961).
13. Kumento, A., Acta Paediatr. Scan. Suppl. 194, (1969).
14. Wertalik, L. F., Metz, E. N., LoBuglio, A. F., and Balcerzak, S. P., J. Amer. Med. Asso. **221,** 1371 (1972).
15. Bianchine, J. R., Bonnlander, B., Macaraeg, P. V. J., Hersey, R., Bianchine, J. W., and McIntyre, P. A., J. Clin. Endocrinol. **29,** 1425 (1969).
16. Lawrence, C., and Klipstein, F., Ann. Int. Med. **66,** 25 (1967).
17. Rosenthal, H. L., Haessler, I. E., and Hill, R. C., Biochem. Biophys. Acta **104,** 46 (1965).
18. Rosenthal, H. L., and Austen, S., Proc. Soc. Exp. Biol. Med. **109,** 179 (1962).
19. Stahlberg, K. G., Scand. J. Haematol. Suppl. **1,** (1967).
20. Graber, S. E., Scheffel, U., Hodkinson, B., and McIntyre, P. A., J. Clin. Invest. **50,** 1000 (1971).

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