PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 675–677 (1974)

Vitamin B_{12} Binding Proteins in the Human Embryo (37873)

DAVID W. SONNEBORN AND M. J. IWANIK
(Introduced by H. J. Hansen)
Department of Anatomy, Medical College of Virginia, Richmond, Virginia 23298

No data has been published on the unsaturated [that is, without vitamin B_{12} (B_{12})] binding proteins in embryos of any species. This is in contrast to the many studies which have elucidated these proteins in adults (1–5). In humans, three proteins are present which bind B_{12}. These are intrinsic factor (I.F.) with a molecular weight of 55,000 daltons produced in the gastric mucosa and functioning in transporting B_{12} across the intestinal mucosa; transcobalamin II (TC-II) with a molecular weight of 35,000 transports B_{12} into cells of peripheral tissues, and transcobalamin I (TC-I) which has a molecular weight of 110,000 and whose function is not understood (1). The sites of synthesis of TC-I and TC-II are not known. TC-I and TC-II are found in serum. In addition, TC-I is found in many body fluids including gastric juice, saliva, and milk (1). Evidence has been presented that a third serum binder transcobalamin III with distinctive charge properties exists (6–8). It is not certain, however, that this is not one of the above glycoproteins with different sugars producing the different charge characteristics.

The extensive work of Kumento has shown that cord serum from term human infants contains TC-III and a protein with a molecular weight of 110,000 which differs in charge from TC-I, but which, as discussed above, may be a very similar molecule (9). The present work was undertaken to establish the sequence of appearance of the above proteins during human development. As such, it presents the first information of embryonic vitamin B_{12} binding proteins of any species. We establish that in the earliest samples studied embryonic stomachs contain proteins similar if not identical to I.F. and TC-I. Furthermore, unsaturated serum binding was present only in the oldest sample and in this case resembled TC-I.

Materials and Methods. Specimens were obtained from abortuses surgically removed for reasons unrelated to these experiments.[1] Fetuses ranged in crown-heel length from 15 to 26 cm and were from 16 to 20 weeks gestation. Blood obtained from the umbilical cord was centrifuged at 3000*g* for 10 min at 4° and the resulting serum removed for assay. Stomachs were removed, weighed, and homogenized with 20 vol (w/v) cold isotonic saline in a Virtis homogenizer at half full speed. A supernate of this was prepared by centrifugation as described above for the preparation of the serum.

B_{12} ^{57}Co (sp act 243.5 mCi/mg) was obtained from Philips-Duphar, Holland. Radioactivity was measured in a Nuclear Chicago Well Scintillation Detector. Levels of proteins which bind B_{12} were measured with the charcoal technique (10).

Molecular weights of B_{12} binding proteins were estimated using Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, NJ) in 2.5 × 100 cm columns eluted by gravity with pH 7 0.1 *M* Tris buffer in 0.9% NaCl with 0.02% azide added as preservative as previously described (4). Levels of endogenous B_{12} was measured using a microbiological assay (11).

Results. Table I presents the results of charcoal assay for B_{12} binding proteins in preparations from fetuses of various ages. The various proteins were identified by their molecular weight as determined by Sephadex

[1] This work was approved by the Medical College of Virginia Committee for the Conduct of Clinical Research.

TABLE I. Levels of Unsaturated Vitamin B_{12} Binding Proteins in the Human Fetus.[a]

	Crown-heel length (cm)						
	15	17	20	21	24	25	26
No. of fetuses	1	2	1	1	1	1	1
Gastric I.F.	+	+	+	+	+	+	+
Gastric TC-I	+	+	+	+	+	+	+
Serum TC-I	No data	—	—	—	—	—	0.13 ng/ml
Serum TC-II	No data	—	—	—	—	—	—

[a] + denotes presence of binder; — denotes absence of binder.

gel filtration. All stomachs contained intrinsic factor and TC-I. Segments of gut distal and proximal to the bowel did not contain any B_{12} binding protein (results not shown). Serum binders were only found in the oldest specimen and in this all binding was associated with a protein of molecular weight of 110,000. A protein of this size and one with a molecular weight of 35,000 were present in cord serum collected from term infants. This last is in agreement with the findings as Kumento (9), and we have found two binders present in cord serum from ten infants, one with a molecular weight of 35,000 and the other with a molecular weight of 110,000 (unpublished results).

Figure 1 shows an example of Sephadex G-100 gel filtration of a representative stomach sample. A binder with a molecular weight intermediate between IgG and albumin (TC-I) and a binder smaller than albumin (I.F.) are present.

Figure 2 shows gel filtration of a serum sample from the 26 cm crown-heel length. The only binder present is intermediate in size between IgG and albumin and represents TC-I [TC-F of Kumento (9)].

Table II shows levels of B_{12} in the fetal serum samples as determined by microbiological assay (11). All samples contain B_{12} at a level approximately equal to those found in adults.

For chromatography, a 0.1-ml aliquot of sample was mixed with 2 ng/ml of the high-specific-activity B_{12} ^{57}Co, thus labeling the proteins with radioactive vitamin. To remove nonspecific binding, a great excess (100 ng) of nonradioactive B_{12} was added. Then 0.5 ml of normal human serum was added to serve as a protein marker and the result placed on a G-100 column as described above.

Discussion. Intrinsic factor and TC-I were found in all stomach samples. Although for technical reasons we are unable to quantitate the exact amount of binder present, in no case was there binder present to bind with

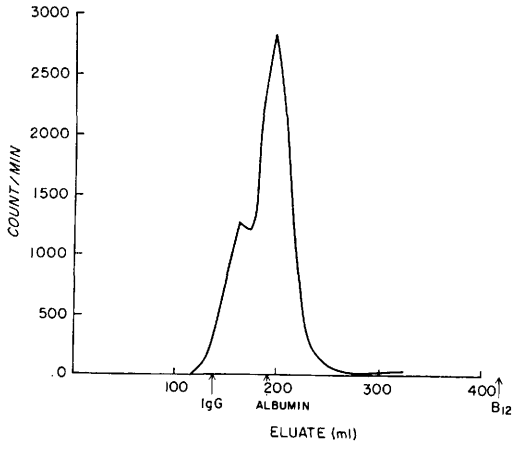

FIG. 1. Gel filtration of the gastric B_{12} binding proteins on Sephadex G-100 showing the presence of two binders—intrinsic factor and TC-I.

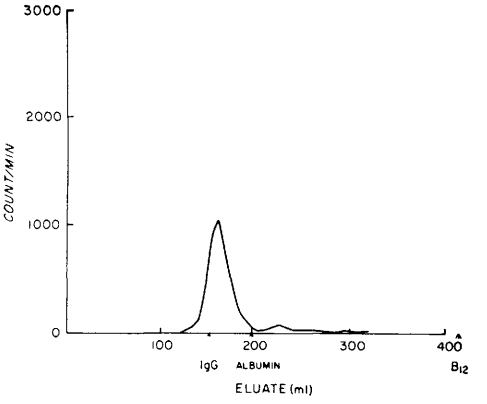

FIG. 2. Sephadex gel filtration of the only serum sample (26-cm crown-heel length) with any unsaturated B_{12} binding protein showing that this protein is intermediate in size between IgG and albumin.

TABLE II. Levels of Vitamin B_{12} in Samples of Fetal Serum (Microbiological Assay).

Crown-heel length	Endogenous vitamin B_{12} (pg/ml)
16	575
17	500
20	1833
24	1650
26	595

more than 5 ng of B_{12}. This is a very small amount of binder when compared to that found in adult stomachs (1). Apparently, the genes responsible for producing I.F. and R-binder are activated at a very low level in these embryonic periods as compared to their level of activation in the adult. This low level of protein for transporting the vitamin may be related to the observation of Deren *et al.* (7) who showed that no protein was necessary for transporting vitamin B in embryonic rat yolk sac. This suggests that embryonic tissues may not require the binding proteins for transport of B_{12} across cell membranes. If this should be true in humans, it could account for the small amount of binders that are present.

Kumento (9) has reported that 20% of endogenous B_{12} is bound to a protein with charge properties of TC-II. Perhaps there are levels of TC-II in fetal serum which we cannot measure because they contain endogenous B_{12}. Another possibility is that the TC-II contained in cord serum is of maternal origin. It seems, however, plausible that the genes for TC-II synthesis are not activated until after 20 weeks.

It is apparent from the work above that there are similarities in embryonic B_{12} binding proteins and those found in adults. Intrinsic factor and TC-I are present in the stomachs of both—albeit at different levels. However, serum differs markedly between adults, in which two different unsaturated binders are present, and embryos, in which no unsaturated binders are present in the early stages.

1. Grasbeck, R., Prog. Hematol. **6**, 233 (1969).
2. Simons, K., Prog. Gastroenterol. **1**, 195 (1969).
3. Hall, C. A., and Finkler, A. G., Biochim. Biophys. Acta. **78**, 234 (1963).
4. Sonneborn, D. W., and Hansen, H. J., Science **168**, 591 (1970).
5. Sonneborn, D. W., and Hansen, H. J., Comp. Biochem. Physiol. **38B**, 467 (1971).
6. Gizis, E. J., and Meyer, L. M., Proc. Soc. Exp. Biol. Med. **140**, 326 (1972).
7. Bloomfield, F. J., and Scott, J. M., Brit. J. Haematol. **22**, 33 (1972).
8. Lawrence, C., Blood **33**, 899 (1969).
9. Kumento, A., Acta Paediactrica Scand. Suppl. **194**, 1 (1969).
10. Miller, O. N., Arch. Biochem. Biophys. **68**, 255 (1957).
11. Hunter, S. H., Bach, M. K., and Ross, G. I. M., J. Protozool. **3**, 101 (1956).
12. Derer, J. J., Padykula, H. A., and Wilson, T. H., Devel. Biol. **13**, 349 (1966).

Received May 21, 1973. P.S.E.B.M., 1974, Vol. 145.