Vitamin B_{12} Binders in Rhesus Monkey Serum (36402)

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It has been observed that rhesus monkeys maintained on a protein-deficient diet developed symptoms similar to those of human kwashiorkor (1). To investigate the possibility of using these animals to study changes in vitamin B_{12} binders in protein malnutrition, binders in normal rhesus monkeys were compared to those that occur in normal human serum (2, 3). Beard and Huser (4) using a 2-buffer DEAE-cellulose column chromatographic system did not find any significant differences between rhesus monkey and human binders. Similar results were obtained in our laboratories utilizing the "baby column" method of Retief *et al.* (5). However, it has recently been shown that this technique does not adequately separate all the binders (3). Consequently, normal monkey serum was subjected to DEAE-cellulose column chromatography, utilizing a 4-buffer system (3).

Methods and Materials. Animals were kept under laboratory conditions and fed a standard diet (1). Pooled monkey serum from blood drawn from normal animals was kept at −20°. Three hundred picograms of ^{57}Co B_{12} (sp act 120 μCi/μg) (purchased from Amersham-Searle) were added per milliliter to 30 ml of rhesus monkey serum. After incubation at 37° for 15 min, the serum was dialyzed against 0.0175 *M* sodium phosphate buffer (pH 6.3) and subsequently chromatographed on 3 × 60 cm DEAE-cellulose (Schleicher and Schuell, No. 70) columns, at 4°, and a flow rate of 30 ml/hr. The following buffers were used for stepwise elution: 0.0175 *M* sodium phosphate buffer (pH 6.3), 600 ml; 0.04 *M* sodium phosphate buffer (pH 5.9), 1000 ml; 0.1 *M* sodium phosphate buffer (pH 5.8), 500 ml; and 0.4 *M* sodium phosphate buffer (pH 5.2), 700 ml. Details of the chromatographic procedure have been published (6). Applying this system to human serum transcobalamin II (TCII) is eluted with the 0.04 *M* buffer, the main protein peak binder (MPPB) with the 0.1 *M* buffer, and transcobalamin I (TCI) with the 0.4 *M* buffer. Fractions eluted from the DEAE-cellulose column were chromatographed on Sephadex G-200, 2 × 60 cm columns using 0.005 *M* sodium phosphate (pH 7.4) containing 1.0 *M* NaCl, as eluting buffer. Molecular sizes were estimated by comparison with the elution volumes of proteins of known molecular weight (7).

Results. Since ^{57}Co B_{12} was not eluted from the monkey serum with the 0.04 *M* buffer, a binder similar to human transcobalamin II is not present in the rhesus serum (Fig. 1). Bound ^{57}Co B_{12} was eluted with the 0.1 and 0.4 *M* buffers which separate MPPB and TCI in normal human serum, respectively. Figure 2 shows the radioactive profile obtained by gel filtration of the ^{57}Co B_{12} bound form eluted from the DEAE-cellulose column with 0.1 *M* buffer. It appears that this fraction is composed of molecules of two sizes, approximately 120,000 and 40,000. Bound ^{57}Co B_{12} eluted from normal human serum with 0.1 *M* buffer exhibited a molecular size larger than 120,000 by gel filtration under similar conditions (3). Figure 3 indicates the radioactive profile obtained by gel filtration of the binder in the rhesus serum eluted from the DEAE-cellulose column with 0.4 *M* buffer. The size of this binder is approximately 40,000 (Fig. 3) while the accepted molecular weight for transcobalamin I in human serum is 120,000

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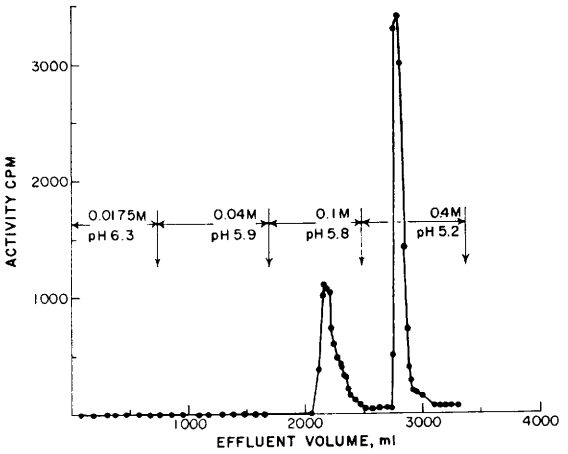

FIG. 1. Radioactive profile of eluent from DEAE-cellulose column chromatography of rhesus monkey serum. Added were 300 pg of ^{57}Co B_{12}/ml of serum. Arrows indicate change in buffer. In normal human serum TCII, MPPB and TCI are eluted with 0.04, 0.1 and 0.4 *M* buffer, respectively.

(3).

Discussion. These data indicate that with 0.04 *M* buffer the eluate from DEAE-cellulose column does not contain a binder (TCII) in normal rhesus monkey which is found in normal human serum. This finding is at variance when the fraction obtained utilizing 2-buffer system of Retief *et al.* was applied (5, 8). The fraction eluted from the monkey serum by DEAE-cellulose column with the 0.1 *M* sodium phosphate was composed of molecules of two sizes, approximately 120,000 and 40,000. Binder eluted from human serum with the same buffer has a molecular weight of approximately 120,000 (3). The binder eluted from monkey serum with the 0.4 *M* buffer was of a molecular size of approximately 40,000. This size is significantly different from 120,000, the accepted size of human TCI (3).

Summary. It appears that vitamin B_{12} binders in normal rhesus monkey serum differ from human TCII in chromatographic behavior on a DEAE-cellulose column and from MPPB and TCI in molecular size, as

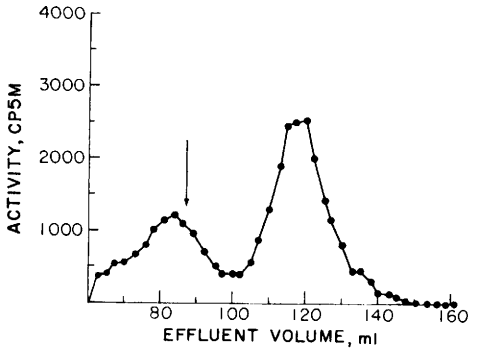

FIG. 2. Radioactive profile obtained by gel filtration on Sephadex G-200 column of fraction eluted from rhesus monkey serum from DEAE-cellulose column with 0.1 *M* sodium phosphate buffer. Arrow indicates elution volume of MPPB from normal human serum.

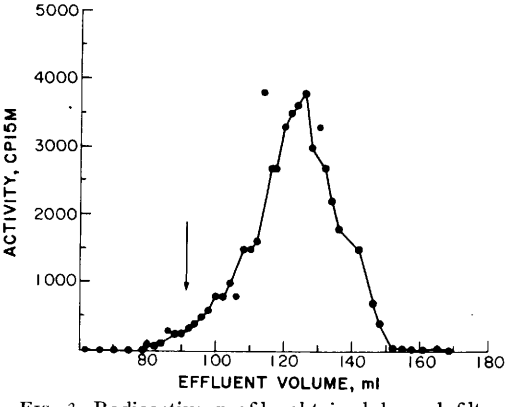

FIG. 3. Radioactive profile obtained by gel filtration on Sephadex G-200 column of fraction eluted from rhesus monkey serum from DEAE-cellulose column with 0.4 *M* sodium phosphate buffer. Arrow indicates elution volume of TCI from normal human serum.

determined by gel filtration on a Sephadex G-200 column.

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