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Delivery of Vitamin B_{12} to Human Lymphocytes by Transcobalamins I, II and III[1] (38185)

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It has been established that three binders of vitamin B_{12} can be separated by DEAE-cellulose column chromatography following addition of labelled vitamin to normal serum (1–4). All 3 binders are present in trace amounts. They are known as transcobalamin I (TC I), transcobalamin II (TC II), and transcobalamin III (TC III) or "main protein peak binder" (MPPB). TC II and TC III have an electrophoretic mobility of α-globulin, while TC I has the mobility of β-globulin (1, 2).

Labelled vitamin B_{12} is cleared rapidly from intravenously administered TC II ^{57}Co B_{12} but much more slowly from TC I ^{57}Co B_{12} (2, 5, 6). The differences in clearance are less marked, however, when the transcobalamins are labelled with orally administered labelled vitamin B_{12}. The rapid clearance of ^{57}Co B_{12} from intravenously administered ^{57}Co B_{12}—TC II has been attributed to partial denaturation of the protein during its purification *in vitro* (7). Finkler and Hall (8) reported that HeLa cells take up ^{57}Co B_{12} from TC II *in vitro* but not from TC I while Retief *et al.* (9) observed that TC II delivers vitamin B_{12} to reticulocytes at a faster rate than TC I. Uptake by perfused rat liver of labelled vitamin B_{12} from ^{57}Co B_{12} labelled TC II and TC III from serum of normal donors and pernicious anemia patients was substantially greater than from ^{57}Co B_{12} labelled TC I (10). The exact role of TC III vitamin B_{12} in plasma is still uncertain. Chanarin and co-workers have recently shown that following oral administration of ^{57}Co B_{12}, the labelled vitamin is taken up simultaneously by all 3 serum binders (7, 11). These authors also showed immunological identity of TC I and TC III. However, the ability of TC III to deliver vitamin B_{12} to cells is still unknown.

Hoffbrand *et al.* (12) used phytohemagglutinin (PHA)-transformed lymphocytes as a model cell system to investigate uptake of serum bound radioactive vitamin B_{12} by normal proliferating human hemopoietic cells. In the present study we have used this system to compare the ability of human TC III with that of TC I and TC II to deliver labelled vitamin B_{12} to human cells.

Materials and Methods. Preparation of vitamin B_{12} binders. This has been reported in detail elsewhere (13). Thirty ml of normal serum were used in each column chromatographic separation. Serum used in each experiment was drawn from an individual donor considered to be hematologically normal. Five hundred pg of ^{57}Co B_{12} (specific activity 120 mCi/mg; purchased from the Radiochemical Centre, Amersham, Amersham, England) were added per ml to 30 ml of serum. The solution was allowed to stand at 37° for 20 min, then dialysed at 4° against 4 litres of 0.0175 *M* sodium phosphate buffer, pH 6.3, for 48 hr. The buffer was changed twice during this period.

DEAE-cellulose (Schleicher and Schuell, No. 70, of ion exchange capacity between 0.90 and 0.95 mEq/g) was packed after

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preparation, into 3 × 60 cm columns (ea). The following buffers were used for elution at 4° with flow rate of 30 ml/hr: 0.0175 *M* sodium phosphate buffer, pH 6.3 (600 ml); 0.04 sodium phosphate buffer, pH 5.9 (1,000) ml; 0.1 *M* sodium phosphate buffer, pH 5.8 (500 ml); and 0.4 *M* sodium phosphate buffer, pH 5.2 (700 ml). Buffer solutions were made in distilled water containing 0.09% methylparaben and 0.01% propylparaben (Tenneco Chemicals). TC II was eluted with the 0.04 *M* buffer, TC III with the 0.1 *M* buffer and TC I with 0.4 *M* buffer. Eluates containing binders were dialyzed and freezed dried. No further attempt at purification was made.

Phytogemagglutinin (PHA)-transformed lymphocytes were prepared by the method of Hoffbrand *et al.* (12) with minor modifications. Approximately 50 ml of venous blood were collected in heparinized tubes and thoroughly mixed. Blood was spun for 10 min at 3,000 rpm. Supernatant plasma was removed and preserved. TC 199 (Burroughs-Wellcome) was added in a volume equal to the underlying red cells, the tubes inverted several times, and the contents layered on a Triosil-Ficoll mixture. This was centrifuged at 3,000 rpm for 15 min. White cells were removed from the junction of the mixture, washed with TC 199 3 times, then diluted with TC 199 so that the suspension contained about 3 × 10^6 lymphocytes/ml. Autologous plasma (1 ml), TC 199 (0.9 ml) and PHA (0.1 ml) were added and entire mixture incubated at 37° for 72 hr. At the end of this period, cells were washed 3 times with TC 199 and resuspended in TC 199 (3 ml) to which labelled transcobalamins were added. Cultures were incubated for one hour at 37°, washed 3 times with phosphate-buffered saline, and the radioactivity of button of cells counted in an automatic gamma-counter.

In one experiment free ^{57}Co B_{12} and TC II ^{57}Co B_{12} were added to the mixture of lymphocytes and PHA, prior to incubation for 72 hr at 37°. After this period cells were washed three times with phosphate-buffered saline, and radioactivity of the button of cells counted.

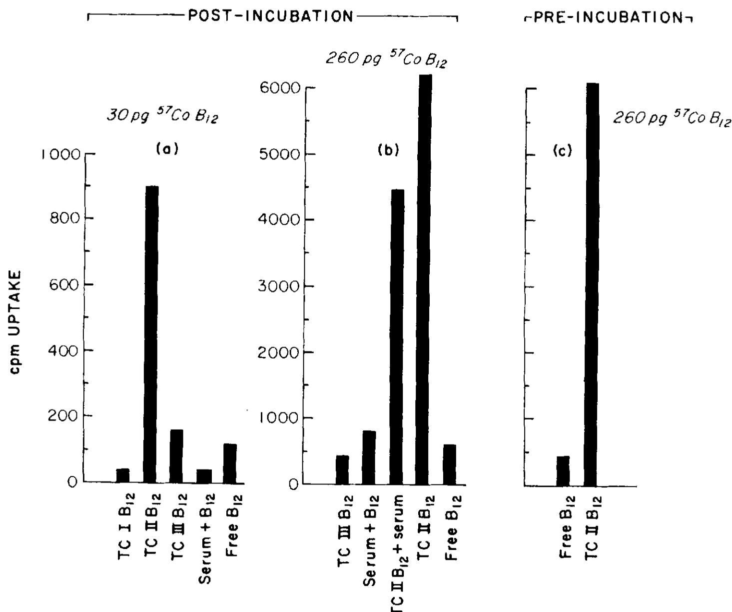

FIG. 1. (a & b) Uptake of free and bound vitamin B_{12} by PHA stimulated lymphocytes, using 30 and 260 pg ^{57}Co B_{12}. (c) Uptake of free and TC II bound B_{12} added to lymphocytes prior to stimulation with PHA.

Results and Discussion. Figure 1a shows results obtained when 30 pg of ^{57}Co B_{12} free, bound to isolated transcobalamins, or bound to whole serum, were introduced to the cell system after 72 hr of lymphocyte culture. Uptake of ^{57}Co B_{12} from its complex with TC II was substantially higher than from complexes with other binders, whole serum, or from free ^{57}Co B_{12}. In particular, uptake from TC III was similar to uptake from TC I. Uptake of ^{57}Co B_{12} bound to serum was not significantly greater than from free ^{57}Co B_{12}.

Figure 1b shows uptake of vitamin B_{12} when 260 pg of ^{57}Co B_{12} (free or bound) were introduced to cell preparations after 72 hr of culture. Uptake of ^{57}Co B_{12} from TC I was not tested at ths level, since supply of this binder was exhausted. Normal serum was added to the TC II ^{57}Co B_{12} complex to compare with the uptake from TC II ^{57}Co B_{12} alone. There was a high uptake of ^{57}Co B_{12} from TC II-^{57}Co B_{12}, a lower uptake from ^{57}Co B_{12}—TC II complex when serum was also present, and a relatively poor uptake from serum plus ^{57}Co B_{12}. Uptake of ^{57}Co B_{12} from its combination with TC III was less than uptake of ^{57}Co B_{12} from the free labelled vitamin.

Figure 1c shows uptake of ^{57}Co B_{12} by human lymphocytes incubated for 72 hr with PHA and with free ^{57}Co B_{12} or TC II ^{57}Co B_{12}. The amount of vitamin B_{12} taken up from ^{57}Co B_{12} TC II after 72 hr incubation with the cells (Fig. 1c) is similar to the amount taken up when TC II B_{12} is added for only 1 hr to 72 hr cultures of PHA-stimulated lymphocytes (Fig. 1b).

Figure 2a shows uptake of ^{57}Co B_{12} by PHA-stimulated lymphocytes from 200 pg of the vitamin, free or bound to each of three binders which were prepared from serum of another donor. As in the results shown in Fig. 1a, labelled vitamin was taken up from complex with TC II to a greater extent than from the other binders. In this experiment, serum bound ^{57}Co B_{12} was not taken up as effectively as TC II bound ^{57}Co B_{12} but uptake from serum was greater than from free ^{57}Co B_{12}. TC I and TC III B_{12} were equally ineffective. At the 600 pg level using free ^{57}Co B_{12}, TC II ^{57}Co B_{12} and TC II ^{57}Co B_{12} in the presence of serum, the highest uptake again occurred from TC II B_{12} alone, less uptake when serum was added to ^{57}Co B_{12}—TC II and none with free ^{57}Co B_{12} (Fig. 2b).

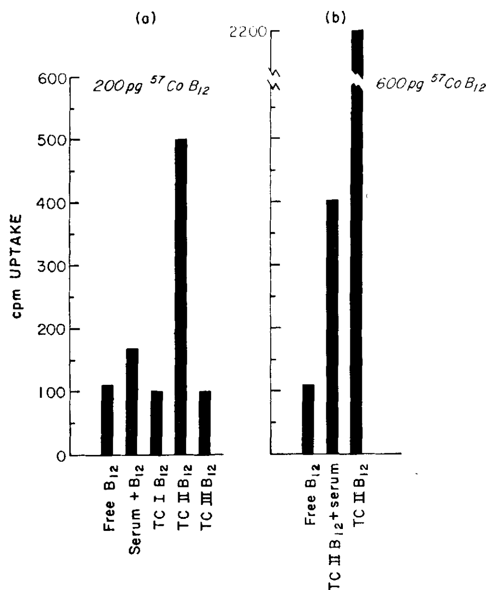

FIG. 2. Uptake of free and bound vitamin B_{12} by PHA stimulated lymphocytes using 200 and 600 pg ^{57}Co B_{12}.

The results of these experiments show that TC III like TC I does not promote uptake of vitamin B_{12} by human hemopoietic cells and confirms, using PHA-transformed lymphocytes, that TC II considerably enhances uptake of the vitamin. The lower uptake of ^{57}Co B_{12} from its complex with TC II in the presence of added serum may be due to competition for same binding sites on the surface membranes of lymphocytes between labelled binder and native binders present in serum. An alternate explanation is that uptake of endogenous vitamin B_{12} from serum decreased percentage uptake of TC II ^{57}Co B_{12}.

Summary. ^{57}Co B_{12} bound to TC II promoted uptake by PHA-stimulated lymphocytes, while TC III and TC I bound B_{12} had no such effect. TC III like TC I appears to be a storage protein in blood rather than a means of transferring vitamin B_{12} selectively to hematopoietic cells.

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