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Delivery of ^{57}Co B_{12} to Lymphoblasts Derived from Mice with Transplanted 1210 Ascites Tumor Cells by Transcobalamins I, II, and III[1] (38415)

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It has been shown in this and other laboratories that uptake of radioactive vitamin B_{12} from serum in various cell systems is mediated through a protein identified as transcobalamin II (TC II). Two other binders in serum, TC I and TC III, do not provide this function (1–8). With the availability of BDF1 mice with transplanted L1210 ascites tumor cells in this laboratory, an attempt was made to determine whether lymphoblasts from the abdominal cavity of these animals would accept radioactive B_{12} from any of the labeled transcobalamins derived from human serum. A recent report with these same fractions utilizing PHA-stimulated human lymphocytes indicated that only TC II ^{57}Co B_{12} was capable of accomplishing this function (7).

Materials and Methods. Preparation of vitamin B_{12} binders. This has been reported in detail elsewhere (9). Thirty milliliters 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 picograms of ^{57}Co B_{12} (specific activity 120 mCi/mg; purchased from the Radiochemical Centre, Amersham, England) were added per milliliter to 30 ml of serum. The solution was allowed to stand at 37° for 20 min, dialysed at 4° against four liters 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 mequiv/g) was packed after 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 *M* 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 0.04 *M* buffer, TC III with 0.1 *M* buffer, and TC I with 0.4 *M* buffer. Eluates containing binders were dialyzed and freeze-dried. No further attempt at purification was made.

L1210 cells were collected in normal saline from abdominal cavities of mice, washed three times in 0.02 *M* tris–normal saline, and suspended in tris saline 0.01 *M* $CaCl_2$. An aliquot containing 250 μl (10^6 cells) of 4% suspension was prepared. The frozen transcobalamins were thawed and suspended in tris calcium saline, centrifuged at 12,000 rpm for 30 min, and supernatant retained. Supernatants (each containing 5000 cpm) and suspension of prepared tumor cells were incubated at 37° for 2 hr, centrifuged, washed with normal saline three times, and the pellets counted in an automatic gamma counter (Nuclear Chicago, Model 1185). All uptake studies were prepared in duplicate. ^{57}Co B_{12} in tris buffer bound to normal serum served as controls.

Results and Discussions. It is apparent from Fig. 1 that following addition of TC II-bound ^{57}Co B_{12} to lymphoblasts removed from BDF1 mice with transplanted L1210 ascites tumor cells, uptake of radioactivity is clearly elevated above values compared to TC I B_{12} and TC III

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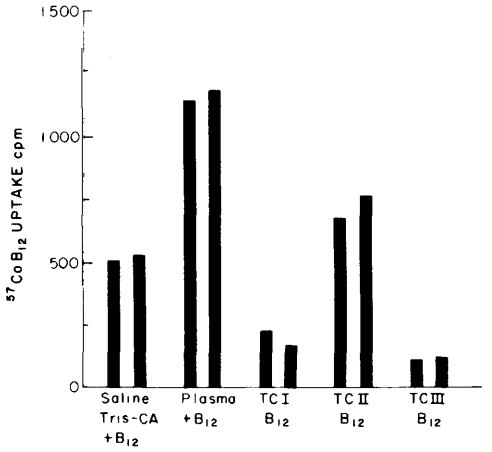

FIG. 1. ^{57}Co B_{12} uptake from labeled transcobalamins I, II, and III by lymphoblasts from mice transplanted with L1210 ascites tumor cells.

B_{12}. Cells exposed to the latter two fractions (labeled with ^{57}Co B_{12}) disclosed radioactivity levels which are only slightly above background. Similar results have been reported using mouse tumor cells, PHA-stimulated lymphocytes, and other cell systems (1–8). Negligible amounts of free labeled B_{12} are taken up by the cells. The ability of ^{57}Co B_{12} bound plasma to deliver the vitamin in the same cell system is clearly evident. This is undoubtedly due to TC II content of plasma. Work in this laboratory also suggests that the whole complex of TC II B_{12} enters the cell because binder B_{12} complex can subsequently be found in the soluble phase of subcellular fractions of the tumor cells (8).

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