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Note

Overestimation of cyanocobalamin due to coelution of sulfitocobalamin on SP-Sephadex C-25

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Tortolani *et al.*[1] have described a column chromatographic technique utilizing SP-Sephadex C-25 for the separation of the following cobalamins (Cbls): methylcobalamin (Me-Cbl), 5-deoxyadenosylcobalamin (Ado-Cbl), cyanocobalamin (CN-Cbl) and hydroxocobalamin (OH-Cbl). This method gives excellent separation of all four Cbls. Subsequently, this technique has been used to study the conversion of radioactive CN-Cbl (CN[^{57}Co]Cbl) to coenzyme forms by cells[2] and cell organelles[3] *in vitro*. In addition others have used it to identify the distribution of endogenous Cbls in serum[4] and tissues of the central nervous system[5]. During a study of the conversion of CN[^{57}Co]Cbl to coenzyme forms by cells in tissue culture, we observed a subtle non-symmetry of the CN[^{57}Co]Cbl and the marker (100 μg non-radioactive CN-Cbl). A modification of the collection procedure resolved this radioactive peak into two distinct peaks, one which eluted prior to the marker CN-Cbl and one at the same time. We have identified this pre-CN-Cbl radioactive peak as sulfitocobalamin (SO_3-Cbl). Unless investigators using this method of separation are aware of the presence of SO_3-Cbl in this region, significant errors can result.

MATERIALS AND METHODS

SP-Sephadex C-25 (Pharmacia, Uppsala, Sweden) was prepared as described[1] and packed by gravity to a height of 20 cm in 10-ml plastic pipettes with an I.D. of 0.8 cm.

Cell uptake studies were performed as described[6], the cells sonified and the lysate extracted with hot ethanol[7] and applied to columns in a volume of 1 ml. The collection procedure was modified such that the first 10 ml were collected in twenty 0.5-ml volumes. The remainder of the column was collected in 2.0-ml fractions as described[1]. Paper electrophoresis was performed as described by Ertel *et al.*[8].

CN-Cbl, Me-Cbl, Ado-Cbl and OH-Cbl were purchased from Sigma (St. Louis, Mo., U.S.A.), dissolved in glass-distilled water and the concentration of each determined spectrophotometrically at 367.5 nm in 0.1 *M* KCN using a molar extinction coefficient of 30,800/*M*·cm (ref. 9). SO_3Cbl was prepared as described by Hill *et al.*[10]. Radioactive cyanocobalamin (CN[^{57}Co]Cbl) was purchased from Philips-Duphar (Petten, The Netherlands).

RESULTS AND DISCUSSION

Fig. 1 shows the radioactive cobalamins in Hela cells (B) and RPMI 6410 lymphocytes (C) following a 24 h uptake of human TC II-CN[^{57}Co]Cbl. Marker cobalamins are shown in A. Fractions 1–5, although all points are not shown, actually consisted of twenty 0.5-ml sub-fractions. As can be seen, there are two radioactive peaks which elute off the column in the first 10 ml, the first being SO_3-Cbl followed by CN-Cbl. When 2.0-ml fractions are collected in this region[1] the SO_3-Cbl elutes too close to CN-Cbl and can be easily mistaken for it. This can lead to underestimation of the amount of CN[^{57}Co]Cbl converted to coenzyme forms[2,3] as well as overestimation of the amount of endogenous Cbl which exists as CN-Cbl[4,5]. Fig. 2 shows the radioactive counts obtained by paper electrophoresis of the SO_3[^{57}Co]Cbl

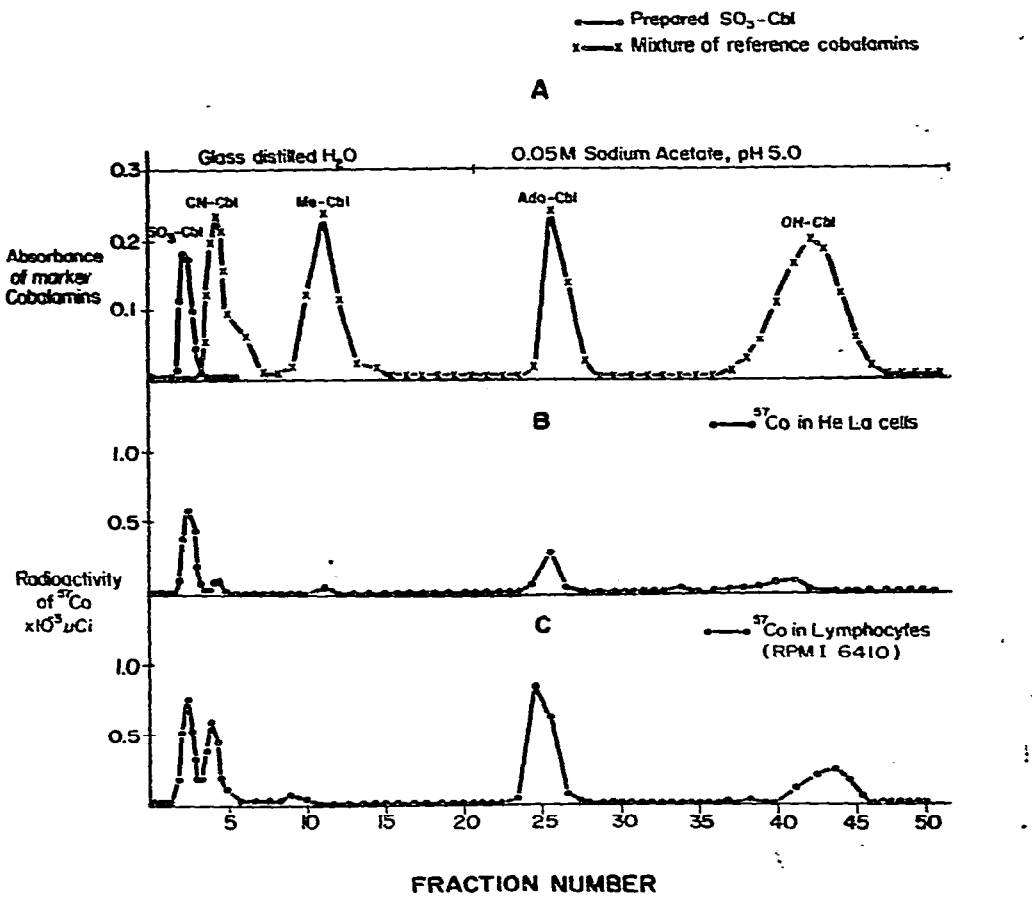

Fig. 1. SP-Sephadex C-25 column chromatography. Fractions 1–5 were collected in twenty 0.5-ml volumes instead of five 2.0-ml volumes[1]. Fractions 6–50 were the usual 2.0-ml volume. A, marker cobalamins, at approximately 100 μg each; B, radioactive cobalamins in HeLa cells following 24-h uptake of CN[^{57}Co] Cbl bound to human Transcobalamin II (TC II); C, radioactive cobalamins in lymphocytes (RPMI 6410) following 24-h uptake of CN[^{57}Co] Cbl bound to human TC II. Collection of 0.5-ml volumes through the CN-Cbl region resolves the radioactive peak into 2 peaks, SO_3 [^{57}Co]Cbl followed by CN[^{57}Co]Cbl.

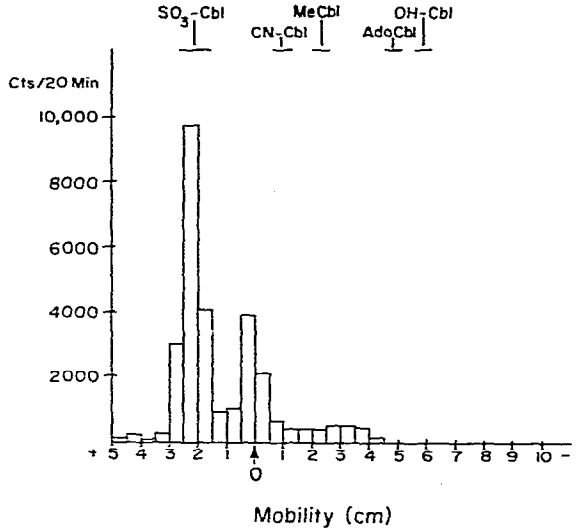

Fig. 2. Paper electrophoresis of the SO_3[^{57}Co]Cbl peak from Fig. 1B. Marker Cbls were included in the same run. The radioactive strip was cut into 0.5-cm segments and counted for 20 min each.

peak from 1B. Although SO_3-Cbl may not be in all samples, we recommend the above modification for the routine separation of Cbls by this method.

The presence of SO_3-Cbl in biological materials may mean that it was actually present or derived from OH-Cbl artifactually[11,12]. A simple modification of collection allows SO_3-Cbl to be easily separated from CN-Cbl on SP-Sephadex C-25 and eliminates errors of overestimating the amount of CN-Cbl present. Further studies on the biological significance of SO_3-Cbl are needed.

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