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Cobalamins in Fibroblasts Cultured from Normal Control Subjects and Patients with Methylmalonic Aciduria

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Extract

The intracellular content and proportional distribution of B_{12} (cobalamin) derivatives in fibroblasts cultured from patients with various forms of methylmalonic aciduria, as well as from normal control subjects, has been determined by a two-dimensional chromatobioautographic technique. Each line of fibroblasts was grown in the presence of four concentrations of cobalamin, ranging from the 0.04–0.07 pmol/ml contained in the basal medium to 74 pmol/ml (100 ng/ml), added in form of hydroxocobalamin (OH-CBl). Control cells grown in the basal medium contained substantial proportions of both methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), with the former predominating. As increasing concentrations of OH-CBl were added to the growth medium, the total cellular cobalamin content increased without marked changes in the relative proportions of MeCbl, AdoCbl, and OH-Cbl. Three different patterns were discernable in the cobalamin distributions of the cells cultured from patients with methylmalonic aciduria (Table 1 and Fig. 1).

Speculation

Information about the cobalamin contents of cultured fibroblasts yields further understanding of cobalamin metabolism. Such information, obtained by the technique of two-dimensional chromatobioautography, permits definition of genetically determined abnormalities in the handling of cobalamins, as well as examination of possible correlations between cellular responses to increased concentrations of these compounds and the effectiveness of cobalamins in the clinical treatment of patients.

In recent years a number of patients have been described who suffer from inborn errors affecting the activity of one or both of the enzymes which in mammals are known to require cobalamin (Cbl) derivatives as coenzymes. Most frequently reported have been patients with methylmalonic aciduria due to deficient activity of methylmalonyl-CoA CoA-carbonylmutase (methylmalonyl-CoA mutase, EC 5.4.99.2). Such patients may either have a defective mutase apoenzyme, or may be unable to accumulate normal amounts of the coenzyme, AdoCbl (20, 28). The latter group has recently been further divided on the basis of whether or not cell extracts are able to carry out normal synthesis of AdoCbl when incubated anaerobically with OH-Cbl, ATP, FAD, and dithiothreitol (15). A less frequently reported group of patients exhibit both methylmalonic aciduria, and homocystinuria due, respectively, to lack of activity of methylmalonyl-CoA mutase and of 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13) (3, 6, 23–25). The latter enzyme requires MeCbl as cofactor. The tissues of these patients accumulate neither AdoCbl nor MeCbl (3, 17, 23). Thus, there is ample evidence that the heterogeneous group of patients with methylmalonic aciduria includes several subgroups with various abnormalities in the metabolic handling of cobalamins.

A newly developed technique which has proven useful for gaining insight into many aspects of cobalamin metabolism is that of two-dimensional chromatobioautography (11, 13, 14). This method permits the sensitive and specific determination of individual cobalamin derivatives in small samples of plasma or tissue. We have applied this technique to the study of cobalamins in fibroblasts cultured from patients with various forms of methylmalonic aciduria. In this paper we report the results of this study, as well as the results of cobalamin analyses of fibroblasts cultured from normal control subjects.

METHODS

Cultures were initiated from skin biopsies and fibroblasts were grown and harvested as described previously (25). The same lot of serum supplement for the culture medium was used throughout. For studies of the effect of added OH-Cbl, cells were grown in complete darkness either in the presence of basal medium (containing 0.04–0.07 pmol/ml Cbl), or in the same basal medium with OH-Cbl added as specified in the individual experiments. The cells were re-fed after 2 and 4 days, and on *day 5* they were harvested under the illumination provided by a 25-watt red bulb (Westinghouse) filtered through a Kodak safelight #1A filter and a Wratten no. 70 filter. Cells were counted by use of a Coulter counter. For estimation of fibroblast cobalamins, the weighed cell pellet was dispersed in 5 ml water, and a 2-ml aliquot was taken for the estimation of AdoCbl, MeCbl, OH-Cbl, and CN-Cbl by a two-dimensional chromatobioautographic method (13, 14). Precuations were taken to avoid exposure of samples to light. Total vitamin B_{12} was estimated on 1-ml aliquots of cell suspension by a radioisotopic assay (19). Total nitrogen was estimated by an automated micro-Kjeldahl method (30) using a continuous digester and a modified digestion mixture of 95% sulfuric acid containing 30 g selenium dioxide and 10 ml perchloric acid/liter (9).

SUBJECTS

The control lines of fibroblasts used were obtained from healthy individuals free of known metabolic abnormalities. These fibroblasts had been included as normal controls in previous studies of the rates of conversion of [1-^{14}C]propionate to $^{14}CO_2$ by intact cells, and of the activities of 5-methyltetrahydrofolate-homocysteine methyltransferase in cell extracts (25) and were comparable with other control cell lines in these respects.

Cells were studied from six patients with methylmalonic aciduria. The available evidence suggests that these patients fall into at least three groups classified as follows.

GROUP a: DEFECTIVE METHYLMALONYL-CoA MUTASE APOENZYME

MO, male, died at 5 months. Cultured fibroblasts had a normal activity of 5-methyltetrahydrofolate-homocysteine methyltransferase, but failed to convert [1-^{14}C]propionate to $^{14}CO_2$ at a normal rate (present studies). Morrow and coworkers (21) have shown that extracts of cultured fibroblasts and of liver have little or no detectable activity of methylmalonyl-CoA mutase even in the presence of added AdoCbl (21).

GROUP b: FAILURE TO ACCUMULATE AdoCbl

KB was a female who died at 5 weeks. Morrow *et al.* (21) have demonstrated that cultured fibroblasts metabolize propionate abnormally slowly. Extracts of fibroblasts and liver have low activities of methylmalonyl-CoA mutase in the absence of added AdoCbl, but normal activities in the presence of added AdoCbl. The cultured cells fail to accumulate normal amounts of [^{57}Co]-AdoCbl when grown in the presence of [^{57}Co]OH-Cbl (21). Fibroblast extracts fail to synthesize AdoCbl when incubated in an H_2 atmosphere with ATP, $MgCl_2$, OH-Cbl, dithiothreitol, and FAD. Under these conditions extracts of control fibroblasts form AdoCbl (15).

GROUP c: FAILURE TO ACCUMULATE EITHER AdoCbl OR MeCbl

EM, a male, died at 7.5 weeks with methylmalonic aciduria and homocystinuria (10). Cultured fibroblasts have defects in propionate oxidation and homocysteine methylation which are (at least partially) repaired by growth in the presence of high concentrations of OH-Cbl. 5-Methyltetrahydrofolate-homocysteine methyltransferase activity in fibroblast extracts was very low in the absence of added MeCbl, but almost normal in the presence of added MeCbl (25). Liver and kidney contained abnormally low concentrations of AdoCbl (23). Fibroblasts grown in the presence of [^{57}Co]OH-Cbl failed to accumulate normal concentrations of either [^{57}Co]AdoCbl or [^{57}Co]MeCbl (17).

MM, a female child, died at 7 years with methylmalonic aciduria and evidence of disturbed homocysteine metabolism. Liver extracts converted methylmalonyl-CoA to succinate abnormally slowly unless AdoCbl was added. 5-Methyltetrahydrofolate-homocysteine methyltransferase activity in fibroblast extracts was very much below normal unless MeCbl was added. Liver, kidney, spleen, and brain contained abnormally low concentrations of all forms of cobalamins (3).

JR, a male, now 19 years, had methylmalonic aciduria and homocystinuria. No defect in propionate oxidation was demonstrable in intact cultured fibroblasts (6, 8). 5-Methyltetrahydrofolate-homocysteine methyltransferase activity in fibroblast extracts was abnormally low without added MeCbl, but virtually normal with added MeCbl (6).

MR, a male child, now 8 years, had methylmalonic aciduria and homocystinuria, and was a brother of *JR*. Findings with cultured fibroblasts were essentially the same as with fibroblasts of *JR* (6).

RESULTS

For the present studies, each line of fibroblasts was grown in the presence of four concentrations of cobalamin, ranging from the 0.04–0.07 pmol/ml contained in the basal medium, to 74 pmol/ml (100 ng/ml), added in the form of OH-Cbl. Each cell preparation was analyzed for total colbalamin content, and, by two-dimensional chromatobioautography, for the specific derivatives, AdoCbl, MeCbl, OH-Cbl, and cyanocobalamin (CN-Cbl). The results, expressed as cobalamin content per 10 mg cellular nitrogen, are presented in Table 1. In this table, the indivudual compounds are given also as percentages of total cellular cobalamin. For some cell lines, more than one experiment was carried out. The values entered are the means of the individual experiments. Three lines of fibroblasts from normal control subjects were studied. The values are listed as means ± 1 SD.

These control cells, when grown in the basal medium, contained substantial proportions of both MeCbl and AdoCbl, with the former predominating. As increasing concentrations of OH-Cbl were added to the growth medium, the total cellular cobalamin content increased progressively, up to approximately 20-fold the concentration under basal conditions. The changes in the individual cobalamins were approximately commensurate, so that the percentage distributions of MeCbl, AdoCbl, and OH-Cbl did not change markedly. Under no circumstances were significant amounts of CN-Cbl detected in the control cells. When the data were expressed in terms of cell number, rather than on the basis of cellular nitrogen (Fig. 1), the patterns of cobalamin concentration were similar. The slight differences which were present do not affect any of the substantive conclusions arrived at subsequently in this presentation.

Three different patterns of response were discernable in the cells cultured from patients with methylmalonic aciduria, as can be seen in Table 1 and Figure 1, and described as follows.

FIBROBLAST LINES

Group a. One fibroblast line, that from *MO*, had no apparent abnormality in cobalamin accumulation. Total Cbl, MeCbl, AdoCbl, and OH-Cbl were essentially within normal limits, and increased normally as OH-Cbl was added to the growth medium.

Group b. One line of cells, that from *KB*, contained virtually no AdoCbl under any of the cultural conditions. In these cells the MeCbl contents and proportions were higher than normal, the OH-Cbl below normal, but the total cobalamin concentrations were in the normal range.

Group c. Four cell lines, those from *EM*, *MM*, *JR*, and *MR*, had multiple abnormalities. Each of these lines had decreased concentrations and decreased proportions of MeCbl, although this trend was less marked for cells from *JR* and *MR*, especially when the cells were grown at low concentrations of cobalamin. As OH-Cbl was added to the growth medium, MeCbl concentrations increased. The proportions of this compound remained approximately the same. Thus, cells grown at the highest concentration of OH-Cbl contained as much, or more MeCbl than did normal control cells grown in the basal medium.

Similar patterns were seen for the cellular concentrations of AdoCbl. For this compound, the deficits in concentrations appeared to be less severe, and the proportions were on the whole normal. Again, the cellular AdoCbl concentrations rose as OH-Cbl was added to the growth medium. In three instances, a medium concentration as low as 7.4 pmol/ml led to cellular concentrations higher than the mean for control cells grown in the basal medium.

These cell lines had low or low normal concentrations of OH-Cbl. The net results of all these changes were decreased cellular concentrations of total Cbl.

It was only in cells among this group (and in single samples from *KB* and *MO*) that significant amounts of CN-Cbl were detected. In no instance was the proportion of this compound more than 13% of the total Cbl (*MR* cells grown in basal medium). The presence of CN-Cb was not observed consistently in repeated experiments.

DISCUSSION

The results reported here are, to our knowledge, the first complete analyses of cobalamin concentrations and distributions in cultured human fibroblasts. For control cells grown in our basal medium we find mean concentrations of 0.67 pmol MeCbl/10^8 cells and 0.34 pmol AdoCbl/10^8 cells. Since approximately 2 × 10^8 cells comprise 1 g wet weight, these values are equivalent for MeCbl and AdoCbl, respectively, to 1.34 and 0.68 pmol/g wet weight cells. Addition of 0.74 pmol/ml OH-Cbl to the growth medium brought about rises in these concentrations to 2.24 and 1.66 pmol/g. These values may be compared with those reported by Mahoney and coworkers using a radioisotopic technique (16, 17). For control human fibroblasts grown in basal medium

Table 1. *Cobalamins in cultured fibroblasts from control subjects and patients with methylmalonic aciduria: Effect of hydroxocobalamin added to growth medium*[1]

Compound analyzed	OH-Cbl added, pmol/ml	Cellular cobalamin concentration, pmol/10 mg cellular N						
		Normal controls	*MO*	*KB*	*EM*	*MM*	*JR*	*MR*
MeCbl	0	2.1 ± 1.3 (37 ± 23)[2]	1.9 (38)	6.2 (96)	0.1 (6)	0.1 (10)	0.6 (19)[3]	0.7 (23)[3]
MeCbl	0.74	3.8 ± 2.5 (34 ± 22)	9.2 (32)	18.7 (80)	0.1 (3)[3]	0.3 (14)	0.6 (9)[3]	0.8 (11)[3]
MeCbl	7.4	7.6 ± 1.6 (34 ± 7)	6.2 (26)[3]	29.6 (83)	0.1 (1)[3]	0.8 (13)	2.0 (19)[3]	1.4 (11)[3]
MeCbl	74	26.8 ± 6.5 (21 ± 5)	34.3 (38)	26.2 (58)[3]	3.2 (4)	3.8 (9)	10.7 (19)[3]	14.1 (14)[3]
AdoCbl	0	1.0 ± 0.3 (18 ± 5)	1.1 (22)	0.1 (2)	0.4 (25)	0.3 (30)	0.8 (25)	1.0 (32)
AdoCbl	0.74	2.5 ± 0.7 (22 ± 6)	7.8 (28)	0.8 (3)	0.8 (28)	0.5 (24)	0.8 (12)	0.7 (10)
AdoCbl	7.4	5.7 ± 0.9 (25 ± 4)	8.5 (36)	0.3 (1)	2.3 (25)	1.7 (27)	2.9 (27)	1.4 (11)
AdoCbl	74	42.3 ± 8.1 (34 ± 6)	22.1 (24)	0.4 (1)	21.3 (28)	11.8 (28)	17.4 (30)	20.7 (20)
OH-Cbl	0	2.5 ± 1.7 (45 ± 30)	2.0 (40)	0.1 (2)	1.1 (69)	0.6 (60)	1.7 (53)	1.0 (32)
OH-Cbl	0.74	5.0 ± 2.4 (44 ± 21)	11.3 (40)	3.9 (17)	1.9 (66)	1.3 (62)	5.2 (78)	5.4 (74)
OH-Cbl	7.4	9.3 ± 4.4 (41 ± 19)	8.8 (37)	5.6 (16)	6.6 (71)	3.8 (60)	5.7 (53)	9.9 (77)
OH-Cbl	74	56.3 ± 9.5 (45 ± 8)	34.9 (38)	17.6 (39)	52.5 (68)	27.0 (63)	28.8 (50)	68.0 (65)
Total Cbl	0	5.6 ± 2.1	5.0	6.4	1.6	1.0	3.2	3.1
Total Cbl	0.74	11.3 ± 4.4	28.3	23.4	2.9	2.1	6.7	7.3
Total Cbl	7.4	22.6 ± 3.3	23.6	35.5	9.3	6.3	10.7	12.8
Total Cbl	74	125 ± 17.4	91.3	45.3	77.0	42.6	57.1	104

[1] Fibroblasts were grown as described under *Methods*. After 5 days of growth in the dark in the presence of the specified added concentrations of hydroxocobalamin (OH-Cbl), the cells were harvested in red light and analyzed for cobalamins. The growth medium without added OH-Cbl contained 0.04–0.07 pmol/ml cobalamin. MeCbl: methylcobalamin; AdoCbl: adenosylcobalamin.

[2] Mean (for three cell lines) ± 1 SD. Values in parentheses are percentages of total cobalamin values.

[3] In these experiments, the sums of the individual components are less than the totals. The differences were contributed by cyanocobalamin.

(containing 0.02 pmol cobalamin/ml, with [^{57}Co]OH-Cbl added to 0.10–0.15 pmol/ml), these workers found concentrations of MeCbl and AdoCbl of 0.44 and 0.16 pmol/g wet weight of cells. As pointed out in the original description of the radioisotopic method, these values represent only *newly synthesized* coenzymes, and on this account would have underestimated total cellular coenzyme concentrations by as much as twofold under the conditions used, since the unlabeled cobalamins present in the cells at the start of these experiments would have gone undetected. Furthermore, it is possible the cells used the unlabeled cobalamin endogenously present in the growth medium (and probably bound to proteins) in preference to the exogenously added (possibly unbound) [^{57}Co]OH-Cbl. Such preferential use of the endogenous cobalamin would have led to additional underestimation of MeCbl and AdoCbl synthesis (16). If these factors are taken into consideration, the two methods give estimates of total cellular MeCbl and AdoCbl which are in reasonable agreement. Both values for AdoCbl are below the concentration of 3.7 pmol/g wet weight of fibroblasts, obtained by Rosenberg *et al.* (28) using an enzymic assay with extracts of cells grown in a medium containing 0.02 pmol/ml cobalamin. The reason or reasons for the discrepancy between our results and those of Rosenberg *et al.* are not clear. Poor recovery of AdoCbl is not likely to the responsible, since the yields of tissue cobalamins analyzed by the present method are 80% or better (87% ± 4.2 SEM).

The concentrations of MeCbl and AdoCbl in fibroblasts grown in our basal medium are not particularly high when compared with the adult human tissues richest in these compounds. For example, liver, kidney, spleen, brain, and pituitary contain from 5–35 pmol MeCbl/g wet weight, and from 17–414 pmol AdoCbl/g (12). In the cultured fibroblasts, MeCbl is present at higher concentrations than is AdoCbl. In this respect, these cells differ in general from adult human tissues (in which AdoCbl is the major cobalamin (11)), but resemble fetal tissues. MeCbl is the major cobalamin in fetal liver, kidney, spleen, and brain (11). It should be realized, also, that is is extremely difficult to relate the cobalamin concentrations found in the present experiments to the concentrations which might exist in fibroblasts *in vivo*. Not only is the concentration of cobalamin in the interstitial fluid unknown, but cellular uptake of cobalamins *in vivo* may well be markedly facilitated by binding proteins not present in the growth medium used in the present experiments (1, 2, 4, 7, 26, 27, 32, 33).

In gel-filtered extracts of normal control human fibroblasts grown in our basal medium, about 50% of the enzyme, 5-methyltetrahydrofolate-homocysteine methyltransferase, is found as an apoenzyme activatable by *in vitro* addition of MeCbl, and about 50% in the form of a holoenzyme (25). The latter may already be complexed to a cobalamin, either MeCbl or a demethylated derivative which would be recovered in our assay as OH-Cbl. The present results indicate that under basal conditions cellular Cbl may be present in such low concentrations as to be limiting in holoenzyme formation. The 10^8 fibroblasts which contain 0.67 pmol MeCbl and 0.88 pmol OH-Cbl (Fig. 1) would yield approximately 15 mg soluble protein (31). Even if the methyltransferase has a molecular weight as high as 10^6, and represents no more than one part in 10^4 of the soluble cellular protein, this enzyme would be present in amounts stoichiometric with MeCbl + OH-Cbl. If the enzyme is smaller, or if it represents a higher portion of the protein, the methyltransferase will exceed these cobalamins. This line of reasoning suggests that most, perhaps all, of the fibroblast MeCbl will normally be complexed to methyltransferase apoenzyme. Perhaps a major portion of the material recoverable as OH-Cbl will be complexed also (34). Limitation of holoenzyme formation by available Cbl is compatible with the observations that as OH-Cbl is increased in the growth medium, and brings about a 22-fold increase in cellular MeCbl + OH-Cbl (Table 1, Fig. 1) holoenzyme comes to represent more than 90% of the total 5-methyltetrahydrofolate-homocysteine methyltransferase activity. Concomitantly there is a 2.5-fold increase in the total amount of functional protein of this enzyme (25).

For fibroblasts cultured from patients with methylmalonic aciduria the present results for each patient are in agreement with the variety of evidence (reviewed above) which indicates that: (*1*) *MO* had a defect in formation of methylmalonyl-CoA mutase

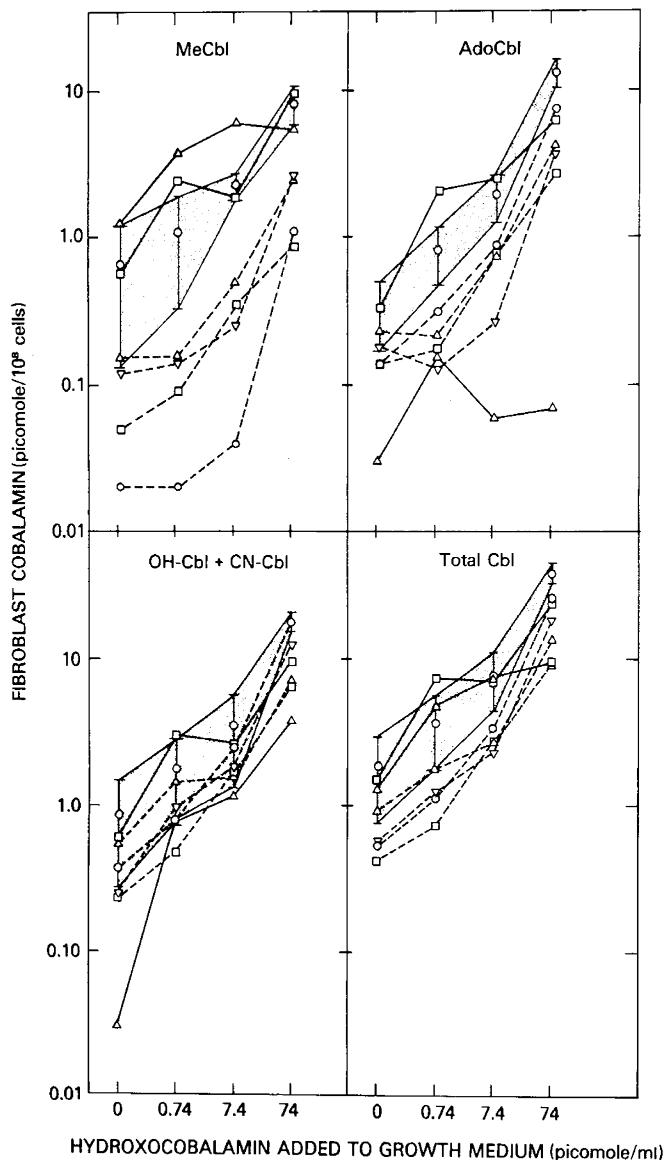

Fig. 1. Effect of hydroxocobalamin in growth medium upon cellular cobalamins of cultured fibroblasts. See legend to Table 1 for description of experimental conditions. *Solid lines:* O, mean value for three control lines (±1 SD, indicated by vertical bars and shaded areas; □, *MO;* Δ, *KB*. *Dashed lines:* O, *EM;* □, *MM;* Δ, *JR;* , *MR*. Because the ordinate is logarithmic, the plotted arithmetic means are not midway in the bars indicating standard deviations. *MeCbl:* methylcobalamin; *AdoCbl:* adenosylcobalamin; *OH-Cbl:* hydroxocobalamin; *CN-Cbl:* cyanocobalamin *Cbl:* cobalamin.

apoenzyme but no abnormality of cobalamin metabolism; (*2*) *KB* was unable to form AdoCbl, but could form at least normal amounts of MeCbl; (*3*) *EM*, *MM*, *JR*, and *MR* were unable to accumulate normal amounts of either MeCbl or AdoCbl. This is the first direct demonstration of such defects in *JR* and *MR* cells. Each of these four cell lines presumably has a lesion affecting a step in the cellular uptake or early metabolism of cobalamin which is involved in the formation of both MeCbl and AdoCbl. However, as has been pointed out previously, the available evidence does not permit exact definition of the specific lesion in any single case. Furthermore, it is possible that not each of these cell lines is affected at the same metabolic site (3).

One of the purposes of growing the fibroblasts at a range of Cbl concentrations was to investigate whether any particular Cbl concentration was especially useful in bringing out the metabolic abnormalities present in the mutant cell lines. In these experiments, deviations from normal appeared most strikingly in the mutant cells grown in medium containing 0.74–7.4 pmol OH-Cbl/ml. However, the most convincing results for any given cell line were obtained by examining the patterns of cellular cobalamins over the entire range investigated.

The data on the cobalamin contents of the four lines of fibroblasts comprising *group c* suggest that in none of this group of patients was the metabolic block complete. For each cell line, the addition of OH-Cbl to the growth medium led to rises of cellular MeCbl and AdoCbl to concentrations which were comparable with those found in control cells grown in the presence of lower amounts of cobalamin. These results correlate with the clinical observation that the methylmalonic aciduria of *JR* was greatly decreased after treatment with high doses of OH-Cbl (5). It has also been shown that fibroblasts from *EM* grown in the presence of high concentrations of OH-Cbl metabolized propionate at almost a normal rate and possessed a small amount of N^5-methyltetrahydrofolate-homocysteine methyltransferase holoenzyme (25).

In contrast, the inability of cells from *KB* to accumulate AdoCbl showed no signs of being overcome by OH-Cbl addition. Even at the highest concentration of this compound tested, *KB* fibroblasts contained virtually no detectable AdoCbl (Table 1 and Fig. 1). This child received a large dose of OH-Cbl 12–18 hr before her death, but showed no clinical benefit. Unfortunately, because of the brief time involved, this trial must be regarded as inconclusive (18). Among the group of patients whose fibroblasts (and other tissues) do not accumulate AdoCbl, most are clinically responsive to large doses of cobalamins, but some are not (21). Since clinical responsiveness of a genetically determined metabolic disease to vitamin therapy is likely to require the presence of at least a small residual activity of the affected enzyme (22), it would not be surprising if in some patients the lesion preventing AdoCbl formation was so complete that it could not be alleviated by increasing the available cobalamin substrate. The evidence suggests that *KB* was such a patient.

Taken together, the findings reported here indicate that two-dimensional chromatobioautography is useful in the study of patients with methylmalonic aciduria. Not only does this technique permit direct demonstration of defects in cobalamin metabolism but it allows, in addition, investigation of possible correlations between the responses of whole cells to cobalamin supplementation and the effectiveness of such compounds in the clinical treatment of patients.

SUMMARY

Two-dimensional chromatobioautography has been used to study the cellular contents of OH-Cbl, CN-Cbl, MeCbl, and AdoCbl in several lines of fibroblasts cultured from normal control subjects and grown in the presence of increasing concentrations of OH-Cbl. These cells are unlike most adult human tissues, but similar to fetal tissues, in that MeCbl is the predominant alkylated cobalamin derivative. When grown in the presence of high concentrations of OH-Cbl, these normal fibroblasts were able to form increased amounts of both AdoCbl and MeCbl. The low concentrations of cobalamins under basal conditions may limit the accumulation of 5-methyltetrahydrofolate-homocysteine methyltransferase holoenzyme.

Cobalamin concentrations and distributions were determined also in six lines of fibroblasts cultured from patients with various forms of methylmalonic aciduria. One line had no abnormality of cobalamin metabolism, but was defective in formation of methylmalonyl-CoA mutase apoenzyme. One line accumulated virtually no AdoCbl, but did form at least normal amounts of MeCbl. Four cell lines did not accumulate normal amounts of either AdoCbl or MeCbl. The responses of these mutant cell lines to growth in the presence of high concentrations of OH-Cbl correlate with the clinical responsiveness of at least some of the patients in question to treatment with cobalamins.

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33. A sample of fetal calf serum was found to have a relatively high unsaturated TC O-like cobalamin binding protein, 10 times as much TC I-like protein and one-eighth as much TC II-like protein as compared with normal human serum (J. M. England and M. C. Down, personal communication to the authors).
34. The same conclusion is supported by calculations based on the measured activity of 5-methyltetrahydrofolate-homocysteine methyltransferase. Extracts of normal fibroblasts grown in our basal medium have a mean holoenzyme specific activity at 37° of 4.2 nmol methionine formed/mg soluble protein/hr (17), or 70 pmol methionine/mg soluble protein/min. Taylor *et al.* (29) have pointed out that both the bacterial and the mammalian methyltransferase, assayed under optimal conditions at 37°, have catalytic turnover numbers of 600 900 pmol methionine formed/minute/pmol of enzyme-bound Cbl. Taking a mean turnover number of 750, this suggests that the fibroblast extracts contain at least 70/750 = 0.09 pmol enzyme-bound Cbl/mg soluble protein. As pointed out above, the total MeCbl + OH-Cbl of these cells is (0.67 + 0.88)/(15) = 0.10 pmol/mg soluble protein. Thus, the major portions of these cobalamins may exist in the fibroblasts complexed to the methyltransferase. Taylor and coworkers (29) have recently noted a similar agreement between the Cbl content of human bone marrow cells and the 5-methyltetrahydrofolate-homocysteine methyltransferase activity of these cells.
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