Note on Vitamin B_{12b}

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Vitamin B_{12b} was first isolated from *Streptomyces aureofaciens* fermentation broth by Pierce *et al.*[1]. The vitamin was believed to be identical with vitamin B_{12a} obtained by catalytic hydrogenation and subsequent exposure to air of vitamin B_{12}[2,3]. However, the opinion has been expressed that vitamin B_{12b}[1] and vitamin B_{12a}[2] should not be identical [4,5].

It can be shown in different ways that vitamin B_{12b} from *Streptomyces aureofaciens* (Lederle 7—9125) is not a homogeneous compound. Ionophoresis on paper in a acetate buffer containing 0.13 mg NaCN/ml has revealed eight different compounds active for *E. coli* 113—6 [6]. One of these is probably cyanocobalamin (obtained even when no cyanide was added to the buffer) as judged from chromatographic, ionophoretic and spectrophotometric examinations. One of the main compounds of the vitamin B_{12b} used, appears to be identical with the major compound of factor A[7]. It showed maxima at 2 780, 3 615 and 5 520 Å, which changed to maxima at 2 780, 3 685, 5 450 and 5 820 Å on addition of an excess of cyanide. Another factor obtained from "vitamin B_{12b}" appeared to be identical with the major component of pseudovitamin B_{12}[7,8]. A compound with chromatographic and ionophoretic properties identical with those of factor B[7] was also detected.

Besides the described factors, four others — all moving towards the anode — were detected. It appears likely that two of these are identical with the factors C_1 and C_2[9]. Vitamin B_{12s}[10] that also moves towards the anode seems to be one of the negatively charged factors for *E. coli* present in the "vitamin B_{12b}". The most predominant of these four factors which was not vitamin B_{12s} showed maxima at about 2 650, 3 600 and 5 500 Å (the preparation used was obtained from an ionophoretic experiment with borate buffer).

A yellow compound was also observed in the „vitamin B_{12b}" employed. Its spectrum was not similar to that of the vitamin B_{12} group of factors, and showed a plateau between 4 400 and 4 650 Å which changed to a maximum at 4 850 Å on addition of cyanide. This as well as its chromatographic behaviour in a mixture of *n*-butanol, acetic acid and water (80 : 15 : 29)[11] suggests that this yellow compound is similar to or identical with the yellow compound observed in a B_{12}-preparation by Schmid *et al.* [11]. The yellow compound obtained from "vitamin B_{12b}" showed activity for *E. coli* but this seems to be due to the presence of cyanocobalamin.

Vitamin B_{12} (Merck & Co., Inc.) also contains several vitamin B_{12}-factors but in this case cyanocobalamin is by far the main compound.

A growth factor for *Lb. lactis* Dorner and *Lb. leichmannii* occurring in Normocytin (Lederle), Reticulogen (Lilly & Co.) and in a *Streptomyces griseus* fermentation broth showing ionophoretic properties different from cyanocobalamin and hydroxycobalamin was reported in a previous paper [12]. A factor with a similar ionophoretic behaviour can also be obtained from "vitamin B_{12s}" provided no cyanide is added to the buffer. It appears to be the aquocomplex of the major component of factor A.

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Growth Factors for *E. coli* 113—3, other than the Vitamin B_{12}-Group or Methionine

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Methionine seems to be the only compound that has hitherto been reported as replacing the vitamin B_{12}-group as a growth factor for *E. coli* 113—3 under aerobical conditions [1-6]. We would like to report the occurrence of other substances stimulating the growth of *E. coli* 113—3 utilized in the agar cup plate method, in media [6,7] that are used for estimation of vitamin B_{12}.

A growth substance for *E. coli*, showing an R_F-value of 0.14 when chromatographed in water-saturated sec. butanol containing 3 % acetic acid and 25 mg/l KCN, was found in insects, *e.g.* ants, grasshoppers and millipedes, in normal human blood and (in higher concentrations) in blood from a patient suffering from leukemia, as well as in mushrooms. Ant eggs were found to be a good source of the factor. Ionophoresis on paper, of a concentrate of this substance obtained from ant eggs, revealed the presence of both acidic and basic groups. Autoclaving this concentrate in 1 *N* HCl or 1 *N* NaOH at 120° C for 1 hour did not decrease its microbiological activity. When hydrolysed in 6 *N* HCl at 100° C for 24 hours the factor disappeared and two new growth factors for *E. coli* with R_F 0.28 and 0.49 appeared. Acid hydrolyses of a sample of autolysed blood from the above mentioned patient with leukemia destroyed the factor having the R_F 0.14 and gave rise to three other factors, two with R_F-values 0.28 and 0.49 respectively, *i.e.* the same values as in the case of ant eggs, the third having R_F 0.67. Methionine has an R_F-value of 0.43 in this solvent. The type of growth caused by these four new factors for *E. coli* is similar to that obtained with methionine and is clearly different from that caused by the vitamin B_{12} group of factors. This indicates that they are active only in comparatively high concentrations. None of these new factors was found in casein hydrolysate nor could they be identified with any of twenty common amino acids. The factor with R_F 0.14 may be a peptide.

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