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Growth Factors for *E. coli* 113—3, other than the Vitamin B₁₂-Group or Methionine

L.-E. ERICSON and A. G. M. SJÖSTRÖM

Division of Food Chemistry, Royal Institute of Technology, Stockholm, Sweden

Methionine seems to be the only compound that has hitherto been reported as replacing the vitamin B₁₂-group as a growth factor for *E. coli* 113—3 under aerobic conditions¹⁻⁶. 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 B₁₂.

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 B₁₂ 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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