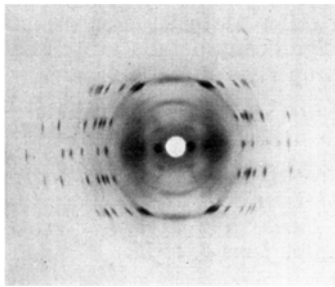
addition compound of collagen. A search of the literature showed that the unit cells of the two patterns agreed closely with those of sodium carbonate monohydrate (Na_2CO_3, H_2O) and sodium sesqui-carbonate (Na_2CO_3, $NaHCO_3$, $2H_2O$) respectively[1]. Thus, it was established that no addition compounds were formed, but that the phenomenon was one of oriented crystallization. Similarly, by keeping collagen in a solution of potassium hydroxide exposed to air, oriented crystallization of $KHCO_3$ has been achieved.



X-ray pattern of collagen in which Na_2CO_3,H_2O has crystallized with its *b*-axis parallel to the fibre axis, taken with a rotation camera 3 cm radius with the fibre axis kept parallel to the axis of rotation of the camera, using CuKα radiation. Photograph reduced by ½ in reproduction.

The specimens containing the crystallized salts are extremely stable, if kept dry, and the x-ray pattern is reproducible even after a month. However, if the specimen is dipped in distilled water for a few seconds and taken out, the salt pattern disappears.

If collagen is kept in freshly prepared sodium hydroxide solution in a full well-stoppered bottle, the above phenomenon was not found, indicating that carbonate produced by contact with air was necessary. So also, if the collagen fibre was kept in a strong solution of sodium carbonate in water, the salt did not enter and crystallize in the collagen. However, if the solution was made alkaline by adding a small amount of sodium hydroxide, then the specimen on drying contained oriented crystals of sodium carbonate. In fact, oriented crystallization of $NaHCO_3$ and Na_2SO_4 has also been achieved by using alkaline solutions of these salts.

It has not yet been possible to explain why the alkali is required. It apparently helps by swelling the collagen and thus enabling the salt to get in. An interesting fact observed with regard to the various salts which crystallized in collagen is that the unit cell dimension along the fibre axis always had a value between 3·2 and 3·75 A or a multiple of this.

A fuller discussion of the experiments will be published in the Proceedings of the Indian Academy of Sciences.

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Zusammenfassung

Wenn gestreckte Kollagenfasern in einer alkalischen Lösung gewisser anorganischer Salze gelassen und dann getrocknet werden, so kristallisieren die Salze mit einer ihrer Achsen in paralleler Orientierung zur Faserachse. Die Periode parallel dieser Achse liegt zwischen 3,2 und 3,75 Å oder einem Vielfachen dieser Werte.

[1] R.W. G. WYCKOFF, *Crystal Structures* (Interscience Publishers 1951), Vol. II, Ch. X, p. 3. – *Structure Reports*, N.V.A. Oosthoek's Uitgevers Mij, Vol. 12, p. 238 (1949).

The Effect of Vitamin B_{12} on the Coenzyme A Content of Normal Strains of *Escherichia coli*[1]

In an earlier investigation, the author observed that the addition of vitamin B_{12} to cultures of "wild" strains of *Escherichia coli* caused an inhibition of the synthesis of free pantothenic acid. It was the author's object in the present report to investigate whether there is any relationship between Vitamin B_{12} and coenzyme A in such strains of *E. coli* as give a positive reaction in the above-mentioned respect. BOXER and his coworkers[2] have previously investigated the relation between vitamin B_{12} and coenzyme A in rat liver cells. They found a definite interrelationship; the coenzyme A content in the liver cells of rats on a vitamin B_{12} deficient diet was considerably higher than the corresponding value of rats receiving additional vitamin B_{12} in the diet.

In his experiments, the author at first isolated from normal human faeces some strains of "wild" *E. coli* and tested the effect of vitamin B_{12} on the pantothenate synthesising ability of these strains. The methods used in these experiments are described in an earlier paper (JÄNNES[3]). The author only modified the culture medium used in this work in the following way: the earlier medium used contained as source of organic nitrogen seven amino acids: Tryptophane, proline, arginine, histidine, glycine, glutamic acid and asparagine. Instead of these, a solution of vitamin-free casamino acids (Difco) with addition of tryptophane and asparagine was used.

The composition of the medium was as follows: K_2HPO_4, 10 g; KH_2PO_4, 4·5 g; sodium citrate 0·75 g; $(NH_4)_2SO_4$, 1·5 g; $MgSO_4 \cdot 7\ H_2O$, 0·15 g; glucose, 15 g; l-asparagine, 4 g; vitamin-free casamino acids (10% solution), 30 ml; dl-tryptophane, 100 mg; distilled water to make 1000 ml. The pH was adjusted to 7·0.

The procedure was as follows: immediately after the preliminary experiments which were mentioned above, strains whose free pantothenate synthesis was inhibited by added vitamin B_{12} were cultivated on stab agar cultures and kept in a refrigerator. On the following day, the effect of vitamin B_{12} on the coenzyme A content of these strains was examined. It was noted that three of the seven investigated strains showed definite inhibition.

For the assay of coenzyme A, the strain was cultivated in two 2 litre ERLENMEYER bottles, with and without addition of vitamin B_{12}. The same medium as in the preliminary experiment was used. The growth of the bacteria was not in any way affected by the addition of vitamin B_{12}. After 24 h growth the cells were harvested with a Sharpless supercentrifuge and dried in a thin layer over phosphorous pentoxide in a vacuum exsiccator. It was found necessary that the conditions during the drying procedure were absolutely the same for both samples.

Exactly 100 mg of dried bacteria was weighed from each sample and suspended in 3 ml of water. Both suspensions were than boiled in a gaseous flame for exactly the same time.

After centrifugation, the coenzyme A content of the supernatants was determined by the method of LIPMANN and KAPLAN[4]. The "transacetylase enzyme" was pre-

[1] This work has been aided by grants from Emil Aaltonen Foundation and Sigrid Juselius Stiftelse.

[2] G. F. BOXER, W. H. OTT, and O. E. SHONK, Arch. Biochem. Biophys. *47*, 474 (1953).

[3] L. JÄNNES, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

[4] N. O. KAPLAN and F. LIPMANN, J. Biol. Chem. *174*, 37 (1948).

pared from pigeon livers. The only noteworthy modification was that frozen liver pieces were pulverised before addition of cold acetone (JÄNNES[1]).

The "transacetylase enzyme" prepared by the author was able to acetylate at most 65% of sulfanilamide in the reaction mixture when boiling water of yeast or rat liver was used as standard.

The author had no possibility to operate with commercial coenzyme A standards. The following results were obtained in these assays.

Effect of vitamin B_{12} on the pantothenate synthesising ability of *E. coli* strain No. 1.

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·09 γ
0·3 γ	0·04 γ

The effect of vitamin B_{12} on the pantothenate synthesising ability of this strain was very clear.

Assay of coenzyme A content of cells of this strain gave the following results:

Vitamin B_{12} per ml of medium	Sulfonamide acetylation readings with Beckmann Photometer Wavelength 545 mμ					Coenzyme A in Lipmann units per g of dry bacteria
	Blank test	Maximum acetylation	Readings with boiling water from the bacteria			unit
			ml			
			0·05	0·1	0·3	
0·0 γ	0·90	0·36	0·71	0·59	0·39	285
0·3 γ			0·71	0·60	0·42	300

In this experiment, the acetylation readings were almost the same and no notable difference in the amounts of coenzyme A in bacteria cultivated with and without vitamin B_{12} could be detected.

A larger addition of vitamin B_{12} had no effect in this respect:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry bacteria
0·0 γ	290
1·0 γ	280

The author also isolated strain No. 2 of *E. coli*, which had the following properties:

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·06 γ
0·3 γ	0·02 γ

[1] L. JÄNNES, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

The corresponding values for coenzyme A were:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry weight
0·0 γ	305
0·3 γ	310

According to these experiments, the effect of vitamin B_{12} concerns only the synthesis of the amount of pantothenic acid which is liberated by the cells to the medium. It appears probable that the bacterial cells satisfy their own need of pantothenic acid for the synthesis of coenzyme A. The cultivation time in my experiments, however, was short, only 24 h, and data concerning experiments with a longer cultivation time are not available. The growth of the cells was unaffected by the addition of vitamin B_{12}. The observation of MAAS[1] that there exists in *E. coli* an enzyme which is capable of synthesising pantothenic acid from β-alanine and pantoic acid and is not dependent on coenzyme A, is not in disagreement with my results.

SAXENA, GHOTEK, and AGARWALO[2] noted in 1954 that vitamin B_{12} causes a similar effect on the synthesis of thiamine in the metabolism of *E. coli*. These effects have as yet no explanation and therefore deserve further investigation.

J. JÄNNES

Department of Medical Chemistry, University of Helsinki, April 4, 1955.

Zusammenfassung

Auf vitaminfreien Nährböden wurden *Escherichia-Coli*-Stämme kultiviert, die bei Zusatz von Vitamin B_{12} einen deutlichen Rückgang der Abgabe von Pantothensäure in das Nährmedium zeigten.

Die Bestimmung des Coenzym-A-Gehaltes der Bakterien ist mittels der Methode von KAPLAN und LIPMANN durchgeführt worden. Es ergab sich, dass keine grösseren Schwankungen des Coenzym-A-Gehaltes der Bakterien vorkamen, obgleich der Gehalt an freier Pantothensäure in der Nährflüssigkeit deutlich abnahm.

[1] W. K. MAAS, J. Biol. Chem. *198*, 23 (1952).

[2] K. C. SAXENA, S. GHOTEK, and S. C. AGRAWALO, Exper. *10*, 488 (1954).

Ether Soluble Pigments in Interglacial Gyttja

It has been demonstrated that certain circumstances may favour the preservation of plant pigments. Thus TREIBS[1] identified a series of chlorophyll- and haemin-derivates in mesozoic oil-slate, coals etc.; FOX *et al.*[2] showed that carotenoids were present in marine sediments 8000 years old. Recently VALLENTYNE[3] described three chlorophyll degradation products from freshwater sediments aged up to 11,000 years from Canadian lakes.

[1] A. TREIBS, Liebigs Ann. *509*, 103 (1934); *510*, 42 (1934); *517*, 172 (1935); *520*, 144 (1935).

[2] D. L. FOX, D. M. UPDEGRAFF, and D. G. NOVELLI, Arch. Biochem. *5*, 1 (1944).

[3] J. R. VALLENTYNE, Canad. J. Bot. *33* (1955) (in the press).