As deduced in our earlier papers[1], the valence of cobalt in vitamin B_{12} is probably three or possibly two with attached molecular oxygen. The two-electron reductions observed in the polarograms of B_{12} and $B_{12}CN^-$ then probably involve the reduction of the cobalt from the trivalent to the univalent stage.

The shift of the half-wave potential of B_{12} from $-1{\cdot}12$ to $-1{\cdot}33$ V toward the saturated calomel electrode on formation of the cyanide compound is in the direction expected as the result of more extensive complex formation by the oxidized than by the reduced form of the couple.

Acknowledgement

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Department of Chemistry, Iowa State College, Ames, Iowa, August 28, 1950.

Zusammenfassung

Das Halbwellenpotential der polarographischen Reduktion des $B_{12}CN$-Anions an der Hg-Tropfelektrode beträgt –1,33 Volt (gemessen gegen eine gesättigte Kalomelelektrode); bei der Reduktion sind zwei Elektronen beteiligt. Die als Lösungsmittel verwendete KCN-Lösung muß jeweils frisch bereitet werden. Sie darf insbesondere mit Quecksilber erst bei der Messung in Berührung kommen.

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Cytological Analysis of Bacteria in Growing Cultures

A number of observations have been published in recent years on the morphology of chromatinic bodies within the bacterial cell. The whole topic of bacterial cytology has been recently reviewed by BISSET[1]. The general conclusion is that several chromatic structures can be observed in the same strain.

We have tried to develop a simple quantitative technique to ascertain whether the different cytological patterns, as described by ROBINOW[2], are present with different frequencies in the successive growth phases of the bacterial cultures.

The present investigation was made on a strain of *Escherichia coli* of rather large size[3]. The technique used for the quantitative study of the growth curve, as well as for detecting the structural details, has been the following.

Agar plates were inoculated with suspension of bacteria from a 24 hour culture on agar slant. After plating, Petri dishes were kept at 35°, and every thirty minutes the bacteria were examined by means of the impression technique, osmic acid fixation, HCl hydrolysis, and Giemsa staining. The increase in the number of cells was controlled by washing the plates with physiological solution, and by counting the living bacteria by dilution and plate insemination.

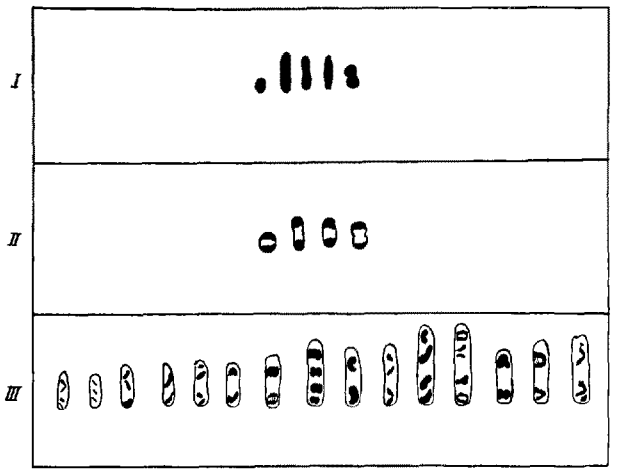

Fig. 1. – Patterns of different bacteria belonging to the three forms described.

The various cell patterns can be grouped in three forms, according to the chromatic microscopical structure:

(1) *Undifferentiated form.* Bacterial cells appear uniformly and rather intensely stained; no nuclear area is differentiated.

(2) *Intermediate form.* Chromatin appears condensed into two small masses at the cell poles. As compared with the first form, the cell volume is slightly larger.

(3) *Differentiated form.* Chromatin appears differentiated into 2 or 4 short, thick rods. The three forms (fig. 1) can be easily distinguished. Counting of their frequencies repeated on the same slide by different observers gave very similar results.

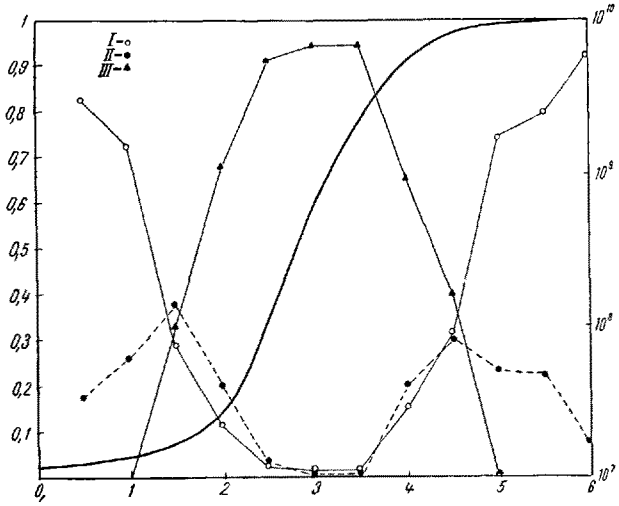

Fig. 2. – Distribution of the different chromatin structures against the time. Abscissa: time in hours. Ordinates: on the left, frequency in decimals of the three forms of cells. On the right, no. of bacteria per cm^3 after washing the plate with 5 cm^3 of physiological solution: ○ undifferentiated form; ● intermediate form; ▲ differentiated form. The sigmoid curve is the growth curve of the culture, from which the samples were taken for the cytological examination.

[1] K. A. BISSET, *The Cytology and Life-history of Bacteria* (E. and S. Livingstone Edinburgh, 1950).

[2] C. F. ROBINOW, Proc. Roy. Soc. [B] *30*, 299 (1942); and addendum in: R. J. DUBOS, *The bacterial cell* (Harvard University Press, 1947), p. 355.

[3] Kindly provided by Dr. J. HEDÉN of the Karolinska Institute for Cell Research, Stockholm, to whom we express our gratitude.

Results of one experiment are shown in fig. 2; comparable results were obtained in a series of similar experiments. While the form I prevails at the beginning and at the end of the considered growth period, reaching a minimum during the most active cell reproduction, the form III prevails in the middle of the logarithmic phase. The two-peaked curve of the form II may indicate that this form represents an intermediate stage between the forms I and III.

It can be concluded that there is a relationship between the appearance of a given structural form and a particular phase of the growth curve. In the logarithmic growth phase, the culture is composed almost exclusively of differentiated forms.

The fact that quantitative relationships can be shown between age of the cultures and frequency of the different chromatic forms shows that, by this method, investigations can be developed on the cytological make-up of bacterial cells under different growth conditions.

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Zusammenfassung

In der vorliegenden Arbeit wurde eine Untersuchung über den Zusammenhang zwischen Chromatinstrukturveränderungen und Wachstumskurve bei *Escherichia coli* ausgeführt.

Durch mit ROBINOWS Technik gefärbte Proben, die an den charakteristischen Punkten der Wachstumskurve von der Kultur entnommen wurden, wurde gezeigt, daß die Bakterienteilung mit der Erscheinung von mitoseähnlichen Figuren gebunden ist, wo Chromatinmassen zustande kommen, die mit Chromosomen vergleichbar scheinen.

The Synthesis of the Purine Nucleus by *Escherichia coli*, a Study on the Mode of Action of Sulfa-Drugs

It appears to be an established fact that in higher animals the synthesis of the purine system in uric acid (as in xanthine I) involves glycine (positions 4, 5, 7), formate or the β-carbon atom of serine (positions 2, 8) and CO_2 (position 6)[1]; a similar mechanism applies to hypoxanthine[2]. Also the guanine contained in yeast nucleic acid is constructed in the cell by an analogous process[3].

Whether this mechanism applies also to bacterial cells, is not yet evident. The observation of SHIVE[4] that sulfa-inhibited *Escherichia coli* produces 4-amino-imidazole-5-carboxamide (II), an obvious intermediary in the synthesis of the purine derivative xanthine (I), tends to show that the *mechanisms*, by which C^2 and C^8 are incorporated into the final structure, are not identical: the sulfa-inhibition involves only C^2 not C^8. It is not even clear whether the *source* of C^2 and C^8 is identical.

The compound (II) is an ideal starting point for an investigation of the origin of C^2 in *E. coli*, of the role which *p-aminobenzoic acid* (PABA) plays in catalysing the incorporation of this lone carbon atom in (II), and of the mechanism by which sulfa-drugs interfere with the action of PABA[1].

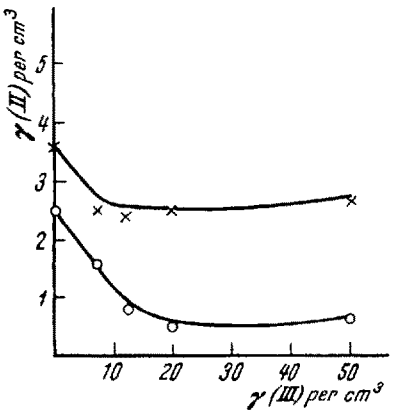

Fig. 1. – Influence of *DL*-methionine (III) on the formation of (II): xxx in presence of 5 γ sulfadiazine per cm^3; ooo the same with 0·025 γ PABA per cm^3.

It has now been established that neither choline nor betaine *nor serine* decrease markedly the amount of (II) formed by sulfa-inhibited *E. coli;* nor does the combination of any of these three substances with PABA exert a significant influence on the formation of (II). Also methionine (III) has only a weak effect. The simultaneous administration of methionine and of catalytic quantities of PABA, however, has a very strong effect, provided that not too much of the sulfa-drug is present (fig. 1). The effect of this combination on the *growth* of the bacterium, equals only about that of methionine. It appears, therefore, that in *E. coli the source of* C^2 *in the purine nucleus is methionine and that PABA acts as the transfer agent of a lone carbon atom from methionine to* (II).

Additional evidence has been sought for this conclusion, which shows a difference in the biosynthesis of the purine nucleus in higher animals and in *E. coli*.

(*a*) It has been known[2] that 2-chloro-4-aminobenzoic acid is an inhibitor of methionine formation in *E. coli*. It has now been found that this antagonism expresses itself in an increased formation of (II), if the 2-chlorinated PABA is added to the culture medium of *E. coli*.

(*b*) In view of existing data[3], it seemed possible that ethionine (IV) would also act as an antagonist of methionine, and increase the amount of (II) formed by the bacterium: ethionine is known as a synergist of sulfa-drugs[4], and this was confirmed in our case, if ethionine *alone* was incorporated in the culture medium. However, the combination of ethionine with small quantities of PABA has the same effect as the system methionine-PABA: the amount of (II) decreases considerably (fig. 2).

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