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.

LUISA VISCONTI DI MODRONE BORGHI e NICCOLÒ VISCONTI DI MODRONE

Institute of Genetics, University of Milan, and Institute of General Chemistry, Politechnic School, Milan, June 15, 1950.

*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.

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## 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 $\beta$-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].

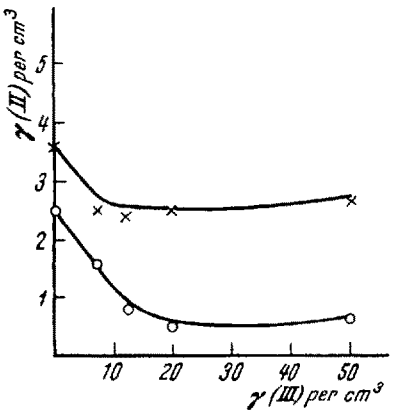


Fig. 1. – Influence of *DL*-methionine (III) on the formation of (II): xxx in presence of 5 $\gamma$ sulfadiazine per cm$^3$; ooo the same with 0·025 $\gamma$ PABA per cm$^3$.

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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This is in keeping with recent observations by STEKOL, WEISS, and WEISS[1]; evidently, in these cases either ethyl transfer takes place or, through a degradation in the course of the transfer process, the ethyl group acts as a source of *one* carbon atom. Thus, our observation can be considered as additional support for the above statement.

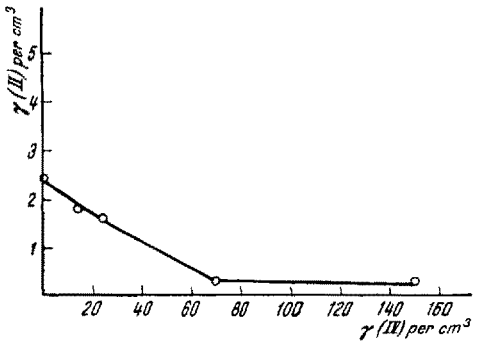
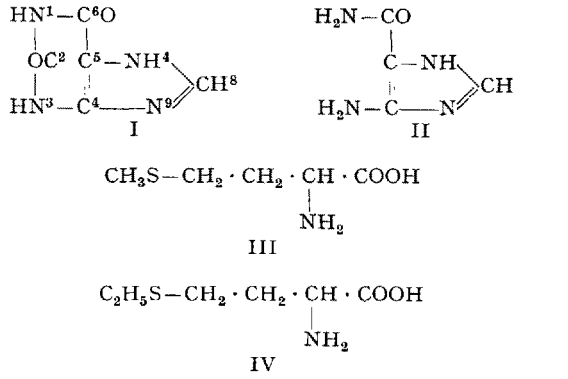



Fig. 2. – Influence of *DL*-ethionine (IV) on the formation of (II), in presence of 5 $\gamma$ sulfadiazine and 0·025 $\gamma$ PABA per cm³.

No evidence has been sought regarding the *form* in which the methyl group is transferred from methionine to (II); MACKENZIE[2] and SAKAMI[3] have assumed that the active agent is formate. This mechanism would be parallel to the *chemical* synthesis of xanthine from (II)[4].

How does PABA participate in this mechanism and how do sulfa-drugs interfere with this participation? It is tentatively suggested that both substances are capable of combining with the "labile methyl group"[5], which dissociates from methionine; however, whilst the derivative formed from PABA is reactive enough to donate the lone carbon atom to (II), the compound into which the sulfa-compounds are transformed[6] are stable and do not transfer the carbon atom acquired from methionine. WINKLER and DE HAAN[7] have shown for the case of *E. coli*, that increasing quantities of sulfa-drugs inhibit the formation of (*a*) methionine, (*b*) xanthine, (*c*) serine, (*d*) pteroylglutamic acid (and thymine); each of these four sulfa-antagonists exhibits its activity only when the lower members of the series are present; in all cases but the last, PABA has to be present. This tends to show, first, that serine does not participate in the biosynthesis of purine (but rather that the reverse could be true), and, second, that the inhibition at least in (*a*), (*b*), (*c*) involves a "methylation" reaction[8]. It is an interesting speculation that also in the biosynthesis of folic acid a lone carbon atom may be involved.




This investigation is part of a thesis submitted by RUTH BEN-ISHAI to the Hebrew University, Jerusalem, in partial fulfilment of the requirements for the degree of Ph. D. The experimental details will be published elsewhere.

RUTH BEN-ISHAI, BENJAMIN VOLCANI, and ERNST D. BERGMANN.

Weizmann Institute of Science, Rehovoth, Israel, August 10, 1950.

*Zusammenfassung*

In Kulturen von *Escherichia coli*, die durch Sulfonamidverbindungen gehemmt sind, wird 4-Amino-imidazol-5-carboxamid (II) gebildet. Diese Substanz ist eine Zwischenstufe in der Biosynthese des Purin-(Xanthin-) Systems. Das fehlende Kohlenstoffatom C² wird durch Methionin (III) geliefert. Der Überträger des Kohlenstoffatoms ist die *p*-Aminobenzoesäure. Cholin, Betain und Serin können das fehlende C² nicht liefern. Äthionin (IV) verhält sich wie Methionin.

## Über von Cu·· abhängige bakteriostatische Wirkungen

Es ist bekannt, daß 8-Oxychinolin eine ausgeprägte bakteriostatische und insbesondere auch tuberkulostatische Wirkung besitzt. Von ALBERT, RUBBO, GOLDACRE und BALFOUR[1] sowie von ALBERT und D. MAGRATH[2] wurde diese Eigenschaft mit der Fähigkeit dieser Verbindung zur Bildung von Metallkomplexen in Zusammenhang gebracht. Wir haben in einer früheren Arbeit[3] durch einen Vergleich des 8-Oxychinolins mit dem isosteren 4-Oxybenzthiazol versucht, Einblicke in diese Beziehungen zu gewinnen.

In einer Reihe von Arbeiten verschiedener Autoren wurden für die Vorstellung, daß an der Beeinflussung des Stoffwechsels der Tuberkelbazillen Metallionen, insbesondere Cu·· beteiligt sind, weitere Hinweise gebracht[4]. Im folgenden wollen wir über eine Beobachtung berichten, die gleichfalls in diesen Zusammenhang gestellt werden kann. 8-Oxychinolin wirkt auf das Oberflächenwachstum von Tbc-Kulturen in *Lockemann*-Nährlösung in einer Konzentration von m/50000 total hemmend. Verwendet man das Cu-Salz des 8-Oxychinolins

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