TABLE II

FRACTIONATION OF PNEUMOCOCCAL DNA

Fraction number	31	33	36	38	*Unfract. DNA*
a. *Cells transformed* by 10^{-2} dilution	—	489	86	61	
b. *Cells transformed* by 10^{-3} dilution	154	51	8	4	163
c. *DNA content*	17.6	10.6	7.53	7.40	29.9
d. *Specific transforming activity*	43.7	24.0	5.72	4.13	27.3

to that of guanine + cytosine. In view of the close relationship between the chromatographic and the simple dissociation processes, it may be presumed that the same change in ratio of the bases obtains from the front to the rear of the chromatographic zone. Nevertheless, the evidence is insufficient to favor the simple hypothesis that each genetic unit of DNA reaches a characteristic chromatographic position because of a particular and characteristic ratio of bases in its molecule. It is also possible that the position and base ratio reflects: differences in some as yet uncharacterized state of various types of DNA; differences in degree of degradation; differences in extent of irreversible association among the genetic units (varying lengths of chromosome segments). Experiments designed to clarify some of these possibilities are in progress.

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Received May 26th, 1955

* Contribution No. 32.

The biosynthesis of methionine from homocysteine and methylmethionine sulfonium salt*

The microbiological activity of methylmethionine sulfonium salt (MMS) has been reported by MCRORIE *et al.*[1]. It was found that this compound can replace the methionine requirement of some strains of methionine auxotrophs of *E. coli*. Since the sulfonium derivative was as much as three times as effective as methionine in supporting growth of these mutants, it was suggested that MMS serves a role more complex than simple conversion to methionine. SCHLENK AND DEPALMA[2] have shown that MMS in combination with homocysteine supports greater production of methylthioadenosine in *Torulopsis utilis* than could be accounted for by simple conversion to methionine. It seemed of interest, therefore, to test the ability of MMS and homocysteine to replace the methionine requirement of auxotrophs of *Aerobacter aerogenes* which can utilize methylthioadenosine in the biosynthesis of methionine[3,4]. Methylmethionine sulfonium iodide was prepared according to the procedure of TOENNIES AND KOLB[5]. All other compounds used were commercial products. Both homocysteine and MMS were sterilized by filtration.

A summary of typical results is presented in Table I. It can be seen that MMS alone would not support growth of either methionine auxotroph 62 or 68. Homocysteine permitted less than half maximal growth of both cultures. However, the combination of MMS and homocysteine permitted growth nearly equal to that of equimolar concentrations of methionine. It is interesting that both cultures responded well to the two compounds although only auxotroph 68 can utilize methylthioadenosine as well as methionine. Prolonged incubation up to 96 hours or the use of very heavy inocula did not affect the pattern of results. No growth occurred in the presence of cysteine and MMS. This is in contrast to the experiments of STEKOL[6] who found that this combination would permit growth of rats on a methionine-deficient ration.

* Work performed under the auspices of the U.S. Atomic Energy Commission.

TABLE I

GROWTH RESPONSE (PER CENT LIGHT TRANSMISSION) OF METHIONINE AUXOTROPHS OF *Aerobacter aerogenes* TO VARIOUS COMPOUNDS AFTER 18-HOURS' INCUBATION WITH CONSTANT AERATION AT 30° C

Supplement (0.14 micromoles per ml)	*# 62*	*# 68*
None	100	100
DL-Methionine	21	12
Methylthioadenosine	100	13
DL-Homocysteine	75	65
DL-Methylmethionine sulfonium iodide*	100	100
DL-Homocysteine + DL-Methylmethionine sulfonium iodide	31	19

* Parallel experiments showed that growth of wild type *Aerobacter aerogenes* was neither enhanced nor inhibited in the presence of the same amounts of methylmethionine sulfonium iodide.

TABLE II

THE BIOSYNTHESIS OF METHIONINE IN CELL-FREE EXTRACTS OF AUXOTROPH 68 OF *Aerobacter aerogenes**

Substrate (10 micromoles per ml)	*Methionine formed (micromoles per ml)*
None	< 0.07***
DL-Homocysteine	< 0.07
DL-Methylmethionine sulfonium iodide	< 0.07
L-Cysteine + DL-Methylmethionine sulfonium iodide	< 0.07
DL-Homocysteine + DL-Methylmethionine sulfonium iodide**	4.8

* Reaction mixtures adjusted to pH 7.0 and incubated at 30° C for 2 hours. Extract added to give a concentration of 5 mg protein per ml mixture.

** Parallel mixtures incubated with boiled extract failed to yield any detectable methionine.

*** The methods used permit the detection of 0.07 micromoles of methionine per ml of reaction mixture.

Bacterial cell-free extracts which could synthesize methionine were first reported by SCHWARTZ AND SHAPIRO[4]. Using essentially the same methods, cell-free extracts were prepared from cells of auxotroph 68 in order to demonstrate biosynthesis of methionine from MMS and homocysteine. Methionine was isolated and identified from the reaction mixtures by paper chromatography. After elution of methionine areas, quantitative estimation was carried out with a modification of the nitroprusside test[7] and by means of a microbiological assay with a methionine auxotroph. A summary of typical results is presented in Table II. It can be seen that methionine is produced only when both MMS and homocysteine are present. The large amount of methionine produced makes this system promising for studies on the mechanism of the reaction whereby methionine is produced from homocysteine and methylmethionine sulfonium salt.

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Received May 23rd, 1955