Summary

Rabbits and chicken which easily get experimental arteriosclerosis, have very low values of serum mucoproteins. High values are found in rats, where they are significantly higher than in men.

Elaboration of Vitamin B_{12} by *Escherichia coli*[1]

Uncertainties regarding the role played by cyanocobalamin (B_{12}) and methionine in the metabolism of microorganisms induced us to study the elaboration of B_{12}-active material by *Escherichia coli* which would satisfy the B_{12}-requirement of *Euglena gracilis*, *Ochromonas malhamensis*, and *Lactobacillus leichmannii*. For this purpose we used the mutant strain, *E. coli* 113-3, its parent strain (ATCC No. 9637) and a wild type *E. coli* isolated from normal human feces.

Since the mutant *E. coli* 113-3 has a nutritional requirement alternatively satisfied by B_{12} or methionine[2], the experiments reported below were designed to permit inferences as to the role of methionine in biosynthesis of B_{12}-like metabolites.

Methods: *E.coli*. For the experiments reported here we used a wild type *E. coli* ATCC 9637, the parent of 113–3, which served as prototrophic control for the mutant *E.coli* 113-3. These organisms were grown for 3 days in Burkholder's medium[3]; the cells were collected by centrifugation, resuspended in distilled water, and lyophilized. Supernatants from each organism were concentrated 10-fold under reduced pressure. Their B_{12} content was assayed.

B_{12} assays: Methods for the determination of B_{12} using *E. gracilis Z.*, *O. malhamensis*, and *L. leichmannii* as test organisms have been outlined[4]. Samples of the supernatants and lyophilized organisms were autoclaved at pH 4·5 with metabisulfite to liberate and stabilize B_{12}. This hydrolysis is insufficient to break down deoxyribosides.

Results: The concordant low values by the *Ochromonas* and *Euglena* assays (Table), show little assayable B_{12} in the bodies and the supernatant of methionine-grown *E. coli* 113-3; the high initial values obtained with *L. leichmannii* for the culture fluids of methionine-grown *E. coli* 113-3 are almost certainly attributable to deoxyribosides. We assume that the *O. malhamensis* assay yields values closest to the true B_{12}-content[4].

Very little B_{12} was found in both wild type *E. coli* strains grown with neither methionine nor B_{12}. The stimulation by methionine of B_{12}-production (see supernatant values Table) in wild type *E. coli* (from feces) supports BRAY and SHEMIN's[5] finding that most of the angular methyl groups of B_{12} can originate from methionine. Not all *E. coli* strains show this effect, as demonstrated by the failure of methionine to stimulate B_{12}-production in *E. coli* 9736 and its mutated strain.

When B_{12}-activity of cells, as assayed with either *O. malhamensis* or *E. gracilis*, is deducted from the *L. leichmannii* results, the difference may be attributed to the presence of deoxyribosides. Supplmentation for all three *E. coli* strains with B_{12}, but not with methionine, resulted in striking increases of deoxyribosides.

The low content of assayable B_{12} in supernatants from *E. coli* 113-3 cells grown on methionine, implies that B_{12}-by-passing factors for the assay organisms are absent here. Further analysis of the B_{12}-by-passing problem may

Vitamin B_{12}-content of *Escherichia coli*.
'B_{12}' of the culture medium = cyanocobalamin; 'meth.' = methionine. B_{12} content is expressed as µµg/ml for culture supernatants and µµg/mg for dried cells

A. Dried Cells (µµg/mg)

Organism	Metabolite added/liter	Assay System: *L. leichmannii*	*O. malhamensis*	*E. gracilis*
E. coli	no B_{12}, no meth.	16	0·6	0·65
wild-type	meth. 0·1 g	7	10	10
isolate	B_{12} 0·1 µg	600	50	26
E. coli	meth. 0·1 g	39	3	3
113–3	B_{12} 0·1 µg	850	130	306
E. coli	no B_{12}, no meth.	27	3	6·6
9736	meth. 0·1 g	30	3·7	4·0
	B_{12} 0·1 µg	300	75	70

B. Supernatants (µµg/ml)

Organism	added/liter	*L. leichmannii*	*O. malhamensis*	*E. gracilis*
E. coli	no B_{12}, no meth.	104	6	1
wild-type	meth. 0·1 g	298	200	235
isolate	B_{12} 0·1 µg	1500	463	800
E. coli	meth. 0·1 g	563	10	9
113–3	B_{12} 0·1 µg	740	175	604
E. coli	no B_{12}, no meth.	100	20	6
9736	meth. 0·1 g	60	6	10
	B_{12} 0·1 µg	130	2	10

require the use of natural B_{12}-free materials, prepared biologically, perhaps by microorganisms. These compounds may be of restricted distribution and could embody 1-carbon fragments such as the methyl purines and methyl pyrimidines recently described by LITTLEFIELD and DUNN[6]. The lack of B_{12} activity in yeast and higher plants originally signalled that B_{12} has a restricted distribution compared with other B vitamins; the present results suggest that B_{12} by-passing compounds are even more restricted in distribution.

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Résumé

Les auteurs ont constaté l'absence de la vitamine B_{12} dans le corps 1° de *E. coli* 113-3, 2° de son parent prototrophique et 3° dans la souche sauvage. Le titre de B_{12} dans le milieu de culture s'est trouvé fortement augmenté par la méthionine, dans le cas de la souche sauvage uniquement. En ajoutant de la cyanocobalamine on a obtenu dans les trois cas une augmentation de désoxyribosides.

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