species conversion. It is improbable, however, that *B2* would function merely as a passive carrier. It is therefore suggested that *B2* is a gene itself, probably a *M. phlei* gene, part of the chromosomal segment which is lacking in *M. smegmatis*. It follows from this reasoning that *B2* could not have originated in the soil from which it was isolated; it had to emerge in the mycobacterial cultures with which the soil samples were periodically enriched before the isolation of mycobacteriophages.

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Note added in proof. Since this manuscript was submitted for publication the experiments reported were repeated by the replica plating method. Colonies identified as *M. phlei* lysogenic for *B2h.F89* on the masterplate gave rise to intermediates and finally to *M. smegmatis* colonies when serially replica plated on nutrient agar plates.

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Trace Element Requirements of *Lactobacillus acidophilus*

THE most difficult aspect of determining the specific "trace element" requirements of micro-organisms is the preparation of a medium free of these factors. With some micro-organisms, EDTA chelates form a reserve from which cations may be drawn[1], but we have found that *L. acidophilus* cannot utilize EDTA chelates. When the trace elements are tied up by EDTA, a medium in which *L. acidophilus* normally grows well no longer supports growth. Moreover, the EDTA portion of the molecule is not toxic *per se*, because the addition of excess cations provides the usual growth.

Iron, calcium, magnesium, manganese, zinc, copper, cobalt, and molybdenum are commonly associated with growth requirements[2]. Manganese, magnesium and iron are generally believed to be required for *L. acidophilus* (Weinberg, E. D., personal communication).

Ten strains were used in this study: five (*E*, *P*, *A*, *W* and *D*) were isolated from commercial products, three (*F*, F_2 and *H*) from human faeces, and two (832 and 314) were received from the American Type Culture Collection. All were classified by Wheater's method[3] and met the requirements for classification as *L. acidophilus*. The medium was prepared as shown in Table 1. Tubes containing 20 ml. were inoculated with a standard 5-mm loop of 24-h culture, and incubated for 7 days at 37° C. Growth was indicated by colour changes due to the production of acid.

When 0·5 mg/ml. EDTA was added there was no growth. When $FeSO_4.7H_2O$ or $MnSO_4.H_2O$ in concentrations of 0·2 mg and 0·3 mg/ml., respectively, were added singly or together there was no growth. The addition of 1 mg/ml. $MgSO_4.7H_2O$ allowed the growth of two strains and the possible growth of three others. The further addition of 0·2 mg/ml. $FeSO_4.7H_2O$ provided normal growth in four, growth in two, and possible growth in four. Further addition of 0·3 mg/ml. $MgSO_4.7H_2O$ provided normal growth for all ten strains. Also, the combination of magnesium and manganese in the given concentrations gave normal growth for all ten strains.

Table 1. MEDIUM USED TO GROW *L. acidophilus*

Item	Per litre
Bactopeptone	5·0 g
Difco yeast extract	3·0 g
'Tween 80'	1·0 g
Sodium acetate anhydrous	5·0 g
Dextrose	5·0 g
Chlorphenol red	50 mg
*p*H	6·6

The results are shown in Table 2.

Table 2

Strain	Medium containing 0·5 mg EDTA per ml. Fe	Mn	Mg	Fe + Mn	Fe + Mg	Mn + Mg	Mn + Mg + Fe
E	−	−	−	−	+	+ +	+ +
P	−	−	−	−	±	+ +	+ +
F_2	−	−	±	−	+	+ +	+ +
A	−	−	−	−	±	+ +	+ +
832	−	−	±	−	+ +	+ +	+ +
314	−	−	+ +	−	+ +	+ +	+ +
W	−	−	+ +	−	+ +	+ +	+ +
F	−	−	−	−	±	+ +	+ +
D	−	−	+	−	+ +	+ +	+ +
H	−	−	−	−	±	+ +	+ +

−, No growth. ±, Doubtful growth. +, Growth. + +, Normal growth.

The commercially available complexes of iron, manganese and magnesium with citric and gluconic acids were used normally as were the glutamic acid complexes as prepared in this laboratory[4]. The inability of the organism to metabolize a chelate must therefore be due to the high stability constants of the EDTA complexes[5] rather than chelation *per se*.

These results indicate that manganese and magnesium are the only cations required by *L. acidophilus*. Only qualitative results have been obtained. Quantitative studies are now under way.

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New Dehydroxylation Reaction observed in the Microbiological Degradation Pathway of Cholic Acid

IN continuing our examination of microbiological degradation of bile acid[1–3], we found that when cholic acid (I) was incubated with the same method as described in a previous paper[4], a new metabolite of cholic acid by *Corynebacterium* (*Arthrobacter*) *simplex*, 4α-(2-carboxyethyl)-5-oxo-7aβ,γ-dimethyl-3aα-hexahydroindane-1β-butyric acid (IX), was accumulated in the incubation mixture in parallel with a disappearance of a large number of the cholic acid metabolites, 7α,12α-dihydroxy-3-oxocholanic (II), 7α,12α-dihydroxy-3-oxo-Δ^4-cholenic (III), 12α-hydroxy-3-oxo-$\Delta^{4,6}$-choladienic (IV), 12α-hydroxy-3-oxo-Δ^4-cholenic (V) and 12α-hydroxy-3-oxo-$\Delta^{1,4}$-choladienic (VI) acids reported previously[4], from the incubation mixture.

The structure of the dicarboxylic acid IX corresponds to that of the dehydroxylated derivative of the 12α-hydroxyl group in the cholic acid molecule, and the demonstrated microbiological 12α-dehydroxylation reaction is the first one in the bile acid metabolism by micro-organisms, although the acid IX loses already the structure of an original steroidal nucleus. The constitution of the acid IX (melting point 169° C to 170° C, $[\alpha]_D^{27} + 23{\cdot}9 \pm 2°$ ($c = 1{\cdot}035$, in ethyl alcohol)) was conclusively established by a partial synthesis of this acid as follows: methyl 3-oxocholanate (melting point 130° C) → methyl 3-oxocholanate dimethyl ketal (melting point 100·5° C to 102° C) → 24-hydroxy-3-oxocholane (melting