

(W-3096)<sup>6</sup>, which by itself did not show any detectable transferase activity but is reported to contain abundant epimerase<sup>7</sup>, was able to allow the reaction to completion with the catalytic amount of uridine diphosphoglucose, if combined with the extract of *M* cells. (But the former loses its catalytic activity when treated for a few minutes at 100°C.). Thus it seems now obvious that *M* cells have a block at the epimerase level. This is in contrast to the transferaseless mutants of *E. coli*, which are reported to show marked bacteriostasis but not lysis in the presence of galactose<sup>7</sup>. We were very recently informed by Dr. H. M. Kalekar<sup>8</sup> that he also had independently demonstrated by his more specific method of assay, that the metabolic block of one of our *E. coli M* mutants lies at the level of epimerase.

Since epimerase is believed to be responsible also for the biosynthesis of galactose, the sugars in the cell wall hydrolyzate were analyzed by paper chromatography. It was found that wild-type cells contain a large amount of galactose in addition to glucose and rhamnose; but *M* cells did not contain galactose and rhamnose at all. This is in agreement with the recent report of Kalekar and Kurahashi<sup>7</sup> that their *E. coli* mutant W-3099, lacking epimerase, transferase and galactokinase, does not possess galactose and rhamnose in its polysaccharides. In the light of this finding, some peculiar features of *M* cells become intelligible. *M* cells form somewhat rough colonies, they have greatly altered susceptibility to phages. In the transduction using temperate phage *PLT-22* and *M* mutants of *Salmonella typhimurium LT-2* and *LT-7*, these various characteristics behaved all together with sensitivity to and non-fermentation of galactose. These characteristics had been interpreted as the pleiotropic expression of a single gene mutation, but they can now be considered as solely due to the abnormal composition of the cell wall induced by the primary defect in epimerase, and it serves to demonstrate how far-reaching the effect of a single enzymatic defect could be.

The mechanism of lysis has not yet been elucidated. But considering the results<sup>9</sup> which show that the synthesis of neither cell wall lipocarbohydrate nor cell wall protein is quantitatively impaired by the presence of galactose, the simple inhibition of cell wall synthesis<sup>1</sup> seems rather unlikely. In the *M* mutants of *S. typhimurium LT-7*, which cannot adsorb phage *PLT-22* in contrast to wild-type cells, galactose appears to induce the de novo formation of 'normal' phage receptors<sup>9</sup>. It might be considered that the 'incompatibility' between the newly formed 'normal' cell wall and the pre-existing 'abnormal' one might be the direct cause of lysis by galactose.

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### Factors in Forest-Tree Litter Extracts affecting the Growth of Soil Micro-Organisms

It is well known that beech litter is less susceptible to decomposition than is the litter of many other species including maple. One reason for this may be the presence or absence of certain factors inhibiting or stimulating microbial growth. For example, factors inhibitory to various fungi have been shown to occur in leaf exudates of certain plants<sup>1</sup> and in many plant extracts<sup>2,3,4</sup> whereas factors stimulating certain mycorrhizal and saprophytic Hymenomycetes have also been observed<sup>5</sup>. Antibacterial factors have been demonstrated in extracts of oak and maple leaves<sup>5</sup>, spruce needles<sup>6</sup>, and in other species<sup>7</sup>. Autoclaving of the extract has been shown to increase the inhibition of fungi<sup>3</sup> and bacteria<sup>5</sup> under the experimental conditions used.

Rather different properties of inhibition and stimulation were observed in the following study in which newly fallen beech (*Fagus grandifolia*) and maple (*Acer saccharum*) leaves were extracted with cold water. The dried leaves were milled, homogenized with ten times their weight of cold water, filtered and then centrifuged to remove suspended organic material. The pH was adjusted to 6.8, and half of the extract sterilized by Seitz-filtration and the remainder by autoclaving. Medium consisting of equal quantities of Difco nutrient broth and leaf extract was then inoculated with each test organism (Table 1). Fungi were incubated for 20 days and growth determined by dry-weight measurements. Bacteria were incubated for two days and growth estimated by plate counts. The results are shown in Table 1.

The fungi showed similar growth responses as also did the bacteria but the two groups differed from each other. Thus the fungi alone were inhibited by the filtered extract but only that prepared from beech leaves was active in this way. The bacteria, however,

Table 1. GROWTH OF FOUR MICRO-ORGANISMS IN NUTRIENT BROTH CONTAINING TREE LEAF LITTER EXTRACTS STERILIZED IN TWO WAYS.

	Beech		Maple		
	Control filtered	Seitz- Auto- claved	Seitz- Auto- claved	Auto- claved	
<i>Rhizopus nigricans</i> mgm./25 ml.	4.1	1.1	24.4	8.2	15.7
<i>Aspergillus niger</i> mgm./25 ml.	7.4	1.7	31.4	41.1	38.7
<i>Azotobacter</i> sp. No. × 10 <sup>6</sup> /ml.	67	325	1	286	0
<i>Pseudomonas fluorescens</i> No. × 10 <sup>6</sup> /ml.	179	515	14	435	0

were inhibited strongly by both autoclaved extracts which were stimulatory to both fungi tested. The significance and mechanism of the apparently separate bacterial and fungal inhibitors must await further investigation, but it is conceivable that the fungistatic activity of the filtered beech extract may have ecological significance in the field.

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