

microbial metabolism are at least two biological products important in rendering insoluble phosphates available. Research is continuing on other bacterial and fungal isolates.

I wish to thank Mrs. L. E. R. Rogers for taking X-ray patterns and identifying the precipitate from calcium chloride and di-potassium phosphate as very finely crystalline hydroxyapatite.

JOAN I. SPERBER

Microbiology Department, Division of Soils,
Commonwealth Scientific and
Industrial Research Organization,
c/o Waite Agricultural Research Institute,
Private Bag, Adelaide.

¹Gerretsen, F. C., *Plant and Soil*, **1**, 51 (1948).
²Gomori, G., *J. Lab. Clin. Med.*, **27**, 955 (1942).
³Thompson, A. R., *Aust. J. Sci. Res.*, **B**, **4**, 180 (1951).
⁴Ladd, J. N., and Nossal, P. M., *Aust. J. Exp. Biol.*, **32**, 523 (1954).
⁵Barker, J. B., and Summerson, W. H., *J. Biol. Chem.*, **135**, 535 (1941).
⁶Johnson, H. W., *N.Z. J. Sci. Tech.*, **B**, **33**, 436 (1952); **36**, 49, 281 (1954).
⁷Hewitt, H. B., *J. Path. Bact.*, **59**, 657 (1947).

Pathway of the Synthesis of Fucose from Glucose in *Klebsiella aerogenes*

It is possible to obtain evidence for the synthetic pathway of a monosaccharide by determining its isotopic labelling in a suitable polysaccharide which has been produced from a labelled substrate. Experiments were undertaken to determine the synthetic pathway of fucose using a fucose-containing extracellular bacterial polysaccharide which was produced from labelled glucose and methionine as the sole carbon and energy sources.

A washed suspension containing about 0.5 mgm. total cell nitrogen per ml. of a slime-forming strain of type 54 *Klebsiella aerogenes* (A3 'S1') was prepared from a nitrogen-deficient solid medium¹. It was aerated for 6 hr. at 30° in 250 ml. of a medium containing 0.1 per cent glucose, 0.001 per cent methionine, 0.001 per cent magnesium chloride, 0.0001 per cent calcium chloride and 0.2 M sodium phosphate-potassium phosphate buffer pH 7.2. The extracellular type-specific polysaccharide was isolated, purified, hydrolysed and the component sugars (D-glucose, D-glucuronic acid and L-fucose) isolated as described previously². The labelling on each carbon of glucose was determined using the *Leuconostoc mesenteroides* method³ and on C6 of glucuronic acid by decarboxylation with hydrochloric acid⁴. Fucose was degraded by periodate oxidation to give formic acid (C1-4) and acetaldehyde (C5-6). The acetaldehyde was oxidized to acetic acid which was degraded to give C5 and C6 separately⁵.

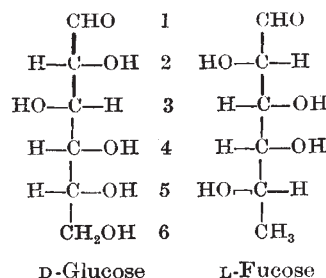
Experiments using unlabelled glucose and ¹⁴CH₃-labelled methionine (100 μc.) as the carbon and energy sources for synthesis showed little radioactivity incorporated into the polysaccharide (less than 0.01 per cent conversion) and no increased labelling of the methyl group of fucose compared with the rest of the polysaccharide. This result indicated that fucose is probably not formed by methylation of the corresponding pentose. Experiments using unlabelled methionine and either glucose-1-¹⁴C or glucose-6-¹⁴C (200 μc.) gave a yield of radioactivity in the purified polysaccharides of 12.1 and 13.2 per

Table 1. PER CENT DISTRIBUTION OF RADIOACTIVITY IN MONOSACCHARIDES DERIVED FROM TYPE 54 *Klebsiella aerogenes* POLYSACCHARIDE AFTER INCUBATION WITH GLUCOSE-1-¹⁴C OR GLUCOSE-6-¹⁴C

Carbon atom	Glucose-1- ¹⁴ C			Glucose-6- ¹⁴ C		
	Glucose	Glucuronic acid	Fucose	Glucose	Glucuronic acid	Fucose
1	68.7	—	—	13.7	—	—
2	1.2	—	—	1.4	—	—
3	5.6	—	—	3.7	—	—
4	3.0	—	—	6.7	—	—
5	1.1	—	1.6	1.2	—	0.9
6	19.0	18.6	20.3	72.9	69.2	71.5
Average 1-4	19.6	—	18.5	6.4	—	5.2

cent respectively. The distribution of carbon-14 in the component sugars is shown in Table 1.

It is evident that the distribution of labelling in the three sugars is very similar, so far as the experimental method can determine. This suggests that glucuronic acid and fucose are probably derived from glucose without a splitting of the carbon chain and without a reversal of the molecule. The result with glucuronic acid was expected from a knowledge of its synthetic pathway⁶ and agrees with previous results obtained with *Streptococcus pyogenes*⁴. However, conversion from D-glucose to L-fucose without splitting or inversion of the carbon chain involves a Walden inversion of three carbon atoms as well as reduction of C6 to a methyl group—a very surprising metabolic pathway.



Similar results showing a conversion of D-glucose to L-fucose without a change in carbon chain have been obtained recently by Heath and Roseman (in the press). They degraded the fucose of the extracellular polysaccharide by periodate oxidation of the benzimidazole compound in order to get C1, 2, 5 and 6 separately. As their results are reported in detail, those presented in this communication will not be published further.

The experiments described above were carried out during 1955 at the Berkeley campus of the University of California. I am indebted to the Rockefeller Foundation for a fellowship during this period.

J. F. WILKINSON

Bacteriology Department,
University of Edinburgh,
Edinburgh.

¹Wilkinson, J. F., Duguid, J. P., and Edmunds, P. N., *J. Gen. Microbiol.*, **11**, 59 (1954).
²Wilkinson, J. F., Dudman, W. F., and Aspinall, G. O., *Biochem. J.*, **59**, 446 (1955).
³Edelman, J., Ginsburg, V., and Hassid, W. Z., *J. Biol. Chem.*, **213**, 843 (1955).
⁴Roseman, S., Ludowieg, J., Moses, F. E., and Dorfman, A., *J. Biol. Chem.*, **206**, 665 (1954).
⁵Strominger, J. L., Kalckar, H. M., Axelrod, J., and Maxwell, E. S., *J. Amer. Chem. Soc.*, **74**, 6411 (1954).