Biosynthesis of the O Antigen from *Citrobacter* 139

R. YUAN and B. L. HORECKER

Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Received for publication 21 March 1968

The biosynthesis of the O antigen of *Citrobacter* 139 (*Escherichia coli* 3 Zurich 4, 5, 12:Z90) was shown to proceed through a series of lipid-linked intermediates, similar to those involved in O-antigen synthesis in *Salmonella*. Galactose was the first sugar incorporated, followed by rhamnose and mannose. Abequose was incorporated from cytidine diphosphate (CDP)-abequose only when all three of the other nucleotide sugars (uridine diphosphate galactose, guanosine diphosphate mannose, and thymidine diphosphate rhamnose) were present. Rhamnosyl-galactosyl 1-phosphate and mannosyl-rhamnnosyl-galactosyl 1-phosphate were identified as the products of mild alkaline hydrolysis of the lipid-linked intermediates.

The incorporation of galactose, mannose, and rhamnose into O-antigen repeating units has been reported for a guanosine diphosphate (GDP)-mannose-deficient mutant of *Salmonella typhimurium* by Zeleznick et al. (12), for a thymidine diphosphate (TDP)-rhamnose-deficient mutant of *S. typhimurium* by Nikaido (5), and for wild-type *S. anatum* by Robbins (7). Weiner and co-workers (8, 9) and Wright et al. (11) have obtained evidence for a series of lipid-linked oligosaccharide intermediates in the formation of the O-antigen repeating units in both *S. typhimurium* and *S. newington*. Anderson et al. (1, 2) have shown that similar lipid-linked intermediates are formed in the biosynthesis of cell wall glycopeptide in *Staphylococcus aureus* and *Micrococcus lysodeikticus*.

The antigen carrier lipid of *S. anatum* has recently been identified by Wright et al. (10) as a C36 polyisoprenoid phosphorylated alcohol, and a similar or identical lipid has been shown by Higashi et al. (3) to be involved in peptidoglycan synthesis in *M. lysodeikticus*. The oligosaccharide intermediates are linked to the lipid by pyrophosphate bridges. In each case, the phospholipid is different from the main phospholipid components of the cells.

The O antigen of *Citrobacter* 139 (*Escherichia coli* 3 Zurich 4, 5, 12:Z90) cross-reacts with that of *Salmonella* group B. All of the O-antigen preparations from *Salmonella* group B are of the chemotype XIV, which contains the following sugar components: glucosamine, heptose, 3-deoxyoctulosonate, galactose, glucose, mannose, rhamnose, and abequose (4). It may be assumed that the O antigen from *Citrobacter* 139 has the same chemotype and probably a very similar structure.

The experiments described here were carried out with wild-type *Citrobacter* 139; lipid-linked intermediates have been identified and implicated in the biosynthesis of the O antigen in this organism.

**Experimental**

Enzymatic incorporation of O-antigenic side chain sugars into the cell envelope fraction. Mannose, rhamnose, and galactose were incorporated into the cell envelope fraction when all three nucleotide sugars were present (Table 1). Optimal incorporation of mannose required the presence of all three nucleotide sugars, whereas that of rhamnose required only the presence of uridine diphosphate (UDP)-galactose. However, galactose incorporation which was significant in the absence of the other nucleotide sugars did not increase when these sugars were added, and in some cases decreased slightly. In this respect, it differed from the galactose incorporation reported by Weiner et al. (9) for a galactose-negative strain of *S. typhimurium* deficient in UDP-galactose-4-epimerase.

The presence of cytidine diphosphate (CDP)-abequose had little effect on the incorporation of galactose or rhamnose (Table 2). On the other hand, the incorporation of abequose was stimulated by the presence of UDP-galactose, TDP-rhamnose, and GDP-mannose.
Table 1. Incorporation of mannose, rhamnose, and galactose into the cell envelope of Citrobacter 139

<table>
<thead>
<tr>
<th>Radioactive nucleotide sugar added</th>
<th>Nonradioactive nucleotide sugar added</th>
<th>Incorporation (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-galactose-14C</td>
<td>None</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>GDP-mannose</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>TDP-rhamnose</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>GDP-mannose + TDP-rhamnose</td>
<td>0.80</td>
</tr>
<tr>
<td>GDP-mannose-14C</td>
<td>None</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>TDP-rhamnose</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose + TDP-rhamnose</td>
<td>0.75</td>
</tr>
<tr>
<td>TDP-rhamnose-14C</td>
<td>None</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>GDP-mannose</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose</td>
<td>4.64</td>
</tr>
<tr>
<td></td>
<td>GDP-mannose + UDP-galactose</td>
<td>4.29</td>
</tr>
</tbody>
</table>

* Each reaction mixture contained 0.1 M tris(hydroxymethyl)aminomethane (Tris)-chloride buffer (pH 8.4), 0.01 M MgCl₂, 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.9 mg of cell envelope protein in a total volume of 0.25 ml. The sugar nucleotides were present in the following concentrations: GDP-mannose, 0.1 mM; TDP-rhamnose, 0.11 mM; UDP-galactose, 0.14 mM. The specific activities of the radioactive substrates were: GDP-mannose, 925 counts/min per μmole; TDP-rhamnose, 1,480 counts/min per μmole; UDP-galactose, 830 counts/min per μmole. After incubation at 37°C for 20 min, each reaction was stopped by the addition of 8 volumes of cold 0.1 M acetic acid. The resulting precipitate was collected and washed twice with 0.1 M acetic acid, suspended in 50% ethyl alcohol containing 1% NH₃, plated, dried, and counted in a windowless gas-flow counter.

Table 2. Incorporation of galactose, rhamnose, and abequose into the cell envelope of Citrobacter 139

<table>
<thead>
<tr>
<th>Radioactive nucleotide sugar added</th>
<th>Nonradioactive nucleotide sugar added</th>
<th>Incorporation (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-galactose-14C</td>
<td>None</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>TDP-rhamnose + GDP-mannose</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>TDP-rhamnose + GDP-mannose + CDP-abequose</td>
<td>0.67</td>
</tr>
<tr>
<td>TDP-rhamnose-14C</td>
<td>None</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose + GDP-mannose</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose + GDP-mannose + CDP-abequose</td>
<td>0.24</td>
</tr>
<tr>
<td>CDP-abequose-14C</td>
<td>None</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose + TDP-rhamnose</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>UDP-galactose + TDP-rhamnose + GDP-mannose</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Each reaction mixture contained 0.1 M Tris-chloride buffer (pH 8.4), 0.01 M MgCl₂, 0.001 M EDTA, and 0.9 mg of cell envelope protein in a total volume of 0.25 ml. The sugar nucleotides were present in the following concentrations: UDP-galactose, 0.14 mM; TDP-rhamnose, 0.09 mM; GDP-mannose, 0.10 mM; CDP-abequose, 0.10 mM. The specific activities of the radioactive substrates were: UDP-galactose, 830 counts/min per μmole; TDP-rhamnose, 872 counts/min per μmole; CDP-abequose, 525 counts/min per μmole. After incubation at 37°C for 20 min, the reactions were stopped by the addition of 8 volumes of cold 0.1 M acetic acid and were analyzed as described in Table 1.

Relationship of the product to O-antigen. Three identical large-scale reaction mixtures were prepared, each containing one 14C-labeled nucleotide sugar in the presence of the other two 14C-nucleotide sugars, according to the procedure of Zeleznick et al. (12). Since the O antigen should contain repeating trisaccharide units composed of galactose, mannose, and rhamnose, partial hydrolysis of the enzymatically synthesized product should lead to the formation of oligosaccharides...
composed of these sugars. Partial hydrolysis was carried out with 60% formic acid, followed by reduction of the oligosaccharide mixtures with sodium borohydride. The products of hydrolysis were characterized by paper chromatography in ethyl acetate-acetic acid-water. Two labeled oligosaccharides were isolated; a disaccharide containing mannose and rhamnitol, and a trisaccharide containing galactose, mannose, and rhamnitol. These experiments showed that the cell envelope fraction is capable of synthesizing a polysaccharide containing galactose, mannose, and rhamnose. Isolation of a trisaccharide containing the presumed structure α-galactosyl-mannosyl-rhamnose provided evidence that these reactions are involved in the biosynthetic pathway for the production of the O-antigen side chains. These results are similar to those reported by Zeleznick et al. (12) with a mutant of S. typhimurium which was deficient in the synthesis of GDP-mannose. On the basis of their results and because the Citrobacter antigen is known to cross-react with antibody to type B lipopolysaccharide, it may be concluded that the product obtained in the present work also contained the linear galactosyl-mannosyl-rhamnose sequence.

Nature of the reaction product formed with all three nucleotide sugars. Mild acid hydrolysis with acetic acid was used to separate the polysaccharide portion from the lipid moiety. Gel filtration of the water-soluble polysaccharide product indicated that it was macromolecular (Fig. 1). The three polysaccharide products were then subjected to high voltage electrophoresis at pH 3.5; three similar electropherograms, two of which are illustrated in Fig. 2, were produced. The bulk of the radioactivity remained at the origin, suggesting that most of it was present as neutral polysaccharide.

Formation of a disaccharide-lipid intermediate. Three reactions were run in parallel with the cell envelope fraction: the first contained UDP-galactose-14C and TDP-rhamnose, the second contained TDP-rhamnose-14C and UDP-galactose, and the third contained both nonradioactive nucleotide sugars (Table 3, incubation I). After the first incubation with these sugar nucleotides, 63.5% of the rhamnose-14C incorporated was present in the chloroform-methanol extractable fraction. A significant portion of the galactose-14C was also chloroform-methanol soluble, although most of it remained in the particulate fraction. Paper chromatography of the lipid-linked intermediates in two different systems indicated that most of the radioactivity is present as a single substance. These compounds were converted to the disaccharide-phosphate by mild alkali hydrolysis (see below).

Conversion of the disaccharide-lipid intermediate to trisaccharide-lipid. Portions of each of the reaction mixtures from incubation I (Table 3) were washed free of the nucleotide sugar substrates by centrifugation, and the cell envelope fractions containing the primary reaction products were incubated at 10°C with unlabeled GDP-mannose ( Tubes 1 and 2) and GDP-mannose-14C (tube 3). Mannose was incorporated rapidly (incubation II, Table 3), and approxi-
mately 56% of the radioactivity incorporated was present in a product soluble in chloroform-methanol. The ratio of galactose-rhamnose-mannose in the CHCl₃-methanol fraction was 1:1.2:1.1.

When the temperature of the reaction mixtures was increased to 37°C (incubation III, Table 3), there was no decrease in the solubility of the products in chloroform-methanol. Analysis of the products demonstrated the presence of large amounts of disaccharide- and trisaccharide-lipid intermediates. Thus, the polymerization reaction in Citrobacter 139 appears to be much slower than in the galactose-negative mutant of S. typhimurium (9). Moreover, in both this and other experiments, there was a lack of stoichiometry in the ratios of the sugars incorporated into the lipid-soluble fraction, the reasons for which remain unknown. In these experiments, substantial losses of radioactivity were observed; these losses may be due to the instability of the sugar-phospholipid linkage.

Characterization of the oligosaccharide components of the chloroform-methanol-soluble intermediates. (i) Disaccharide phosphate. Portions of the products resulting from incubation I, containing either galactose-¹⁴C or rhamnose-¹⁴C, were hydrolyzed with mild alkali. High voltage electrophoresis of the hydrolysates yielded a single anionic peak which was identical in the experiments with both labeled sugars. The mobility relative to that of glucose-6-phosphate of the labeled product was 0.76 (Fig. 3). When treated with alkaline phosphatase followed by electrophoresis, the radioactivity in both samples remained at the origin (Fig. 4). The dephosphorylated product was oxidized with hypoiodite and hydrolyzed; galactonate-¹⁴C and rhamnose-¹⁴C were the only radioactive products obtained.

(ii) Trisaccharide-phosphate. The chloroform-methanol extracts from incubation II were evaporated to dryness and hydrolyzed with 0.05 N KOH; the hydrolysates were subjected to electrophoresis at pH 3.7. The resulting electropherograms (Fig. 5) show the bulk of the radioactivity migrating as a peak with a mobility relative to that of glucose-6-phosphate of 0.56 to 0.62; the peak was identified as mannosyl-rhamnosylgalactosyl-1-phosphate. Treatment with alkaline phosphatase resulted in the loss of electrophoretic mobility. Approximately 50% of the galactose-¹⁴C was recovered as galactonate after oxidation of the dephosphorylated disaccharide, followed by hydrolysis. The sequence was determined by partial acid hydrolysis followed by reduction with

**Fig. 2. Electrophoresis of enzymatically produced polysaccharide.** The mannose-¹⁴C-labeled compound isolated in the experiment described in Fig. 1 was spotted on Whatman no. 1 paper. It was then subjected to high voltage electrophoresis at pH 3.5, 80 v/cm for 2 hr.
The GDP-mannose, were envelope cell counting, and chloride buffer were: UDP-galactose, in pendled with the 14C to brought as sis,ing and tion of ml of 0.05 acid, 3. FIG. has shown was R. and sodium borohydride. The resulting disaccharide was shown to be mannosyl-rhamnitol.

Data to be published elsewhere (M. J. Osborn and R. Yuan, in preparation) show that, in the galactose-lipid intermediate, the phosphate is linked to the C-1 position of galactose. These data also indicate that the electrophoretic mobilities and other characteristics of the disaccharide-phosphate and trisaccharide-phosphate are identical to those of the mutant strain of S. typhimurium. Therefore, it may be concluded that the disaccharide phosphate and trisaccharide-phosphate also contain phosphate linked to the C-1 position of galactose.

### TABLE 3. Sequential incorporation of nucleotide sugars into the cell envelope fraction

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Radioactive nucleotide sugar added</th>
<th>Nonradioactive nucleotide sugar added</th>
<th>Incorporationa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CHCL-methanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Percentage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lipid soluble</td>
</tr>
<tr>
<td>I</td>
<td>#1 UDP-galactose-^14C</td>
<td>TDP-rhamnose</td>
<td>67.0</td>
</tr>
<tr>
<td></td>
<td>#2 TDP-rhamnose-^14C</td>
<td>UDP-galactose</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>#3 --</td>
<td>TDP-rhamnose + UDP-galactose</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63.5</td>
</tr>
<tr>
<td>II</td>
<td>#1 --</td>
<td>GDP-mannose</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>#2 --</td>
<td>GDP-mannose</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>#3 GDP-mannose-^14C</td>
<td>--</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55.8</td>
</tr>
<tr>
<td>III</td>
<td>#1 --</td>
<td>--</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>#2 --</td>
<td>--</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>#3 --</td>
<td>--</td>
<td>132.0(?)</td>
</tr>
</tbody>
</table>

* The pellets were collected by centrifugation and washed twice with 0.1 M acetic acid. They were suspended in water, and the pH was adjusted to 7.0 with 2 M Tris base; a small sample was removed for counting, and the remainder was extracted three times with 8 volumes of chloroform-methanol (3:1). The combined organic phases were filtered, and samples were dried and counted. The specific activities were: UDP-galactose, 3,320 counts/min per mmole; TDP-rhamnose, 3,540 counts/min per mmole; GDP-mannose, 2,310 counts/min per mmole.

b Each tube contained 0.1 M Tris-chloride buffer (pH 8.4), 0.01 M MgCl₂, 0.001 M EDTA,(204) mg of cell envelope protein, 0.14 mM UDP-galactose, and 0.12 mM TDP-rhamnose in a total volume of 5 ml. After incubation at 37 C for 20 min, the reaction mixtures were diluted with 12.5 ml of cold 0.05 M Tris-chloride buffer (pH 9.0), and the particulate fraction was collected by centrifugation and washed once with the same buffer. The pellets were suspended in 5.0 ml of the Tris-MgCl₂-EDTA mixture and were brought to 10 C. A sample (0.5 ml) was taken into 3.0 ml of cold 0.1 M acetic acid for subsequent washing and analysis.

c A sample of each residue (4.5 ml) was added to tubes containing GDP-mannose or GDP-mannose-^14C to give a final concentration of 0.11 mM. After 5 min at 10 C, 3.5-ml samples were removed for analysis, as described in footnote a.

d The reaction mixtures were placed in a 37 C bath for 10 min. The reaction was stopped by the addition of 6 volumes of cold 0.1 M acetic acid.

![Galactose-^14C](Galactose-14C.png)  

**FIG. 3. Electrophoresis of the disaccharide-phosphate.** The chloroform-methanol extracts obtained as described previously were evaporated to dryness. Galactose-^14C-labeled material (3,000 counts/min) was suspended in 0.25 ml of 0.05 N KOH and was hydrolyzed in a boiling water bath for 15 min. The samples were then neutralized with 1 ml perchloric acid, spotted on Whatman no. 1 paper, and subjected to high voltage electrophoresis at pH 3.5, 50 V/cm for 90 min.
DISCUSSION

The experimental evidence suggested that the biosynthesis of O antigen in Citrobacter 139 proceeds via the pathway shown in Fig. 6. Reaction 1 has been described elsewhere (Osborn and Yuan in preparation). Reactions 4, 5, and 6 have not yet been studied in Citrobacter 139.

The incorporation of abequose from CDP-abequose into an acid-insoluble product requires the presence of the three other nucleotide sugars (UDP-galactose, GDP-mannose, and TDP-rhamnose); this suggested that a tetrasaccharide-

![Fig. 4. Electrophoresis of the alkaline phosphatase-treated disaccharide-phosphate. A sample of rhamnose-\(^{14}\)C-labeled disaccharide-phosphate (1,500 counts/min) was dissolved in 0.25 ml of 0.01 M Tris-chloride buffer (pH 7.9), with 0.001 M MgCl\(_2\), and 1 \(\mu\)g of alkaline phosphatase was added. The reaction mixture was incubated at 37°C for 1 hr, spotted on Whatman no. 1 paper, and electrophoresed at pH 3.5, 60 v/cm for 1 hr.]

![Fig. 5. Electrophoresis of trisaccharide-phosphate. Trisaccharide-phosphate containing mannose-\(^{14}\)C was prepared by taking the chloroform-methanol extract from incubation II (Table 3), evaporating it to dryness, suspending it in 1.0 ml of 0.05 N KOH, and hydrolyzing it in a boiling-water bath for 15 min. The hydrolysates were passed through columns of Dowex-50 (H\(^+\)) having a bed volume of 1.5 ml, spotted on Whatman no. 1 paper, and electrophoresed at pH 3.7, 80 v/cm for 1 hr.]

\[
\begin{align*}
\text{UDP-gal} & \quad \text{lipid} \quad \text{(1)} \quad \text{Gal-1-P-"lipid"} \\
& \quad \text{+ UMP} \quad \text{\Rightarrow Rha-Gal-1-P-"lipid"} \\
& \quad \text{TDP-Rha} \quad \text{(2)} \quad \text{GDP-Man} \\
& \quad \text{Man-Rha-Gal-1-P-"lipid"} \\
& \quad \text{Abe} \quad \text{(3)} \quad \text{CDP-Abe} \\
& \quad \text{Man-Rha-Gal-1-P-"lipid"} \\
& \quad \text{Abe} \quad \text{(4)} \quad \text{"lipid"} \\
& \quad \text{Abe} \quad \text{(5)} \quad \text{(Man-Rha-Gal)_\(n\)-Man-Rha-Gal-1-P-"lipid"} \\
& \quad \text{Core LPS} \quad \text{(6)} \quad \text{Complete LPS}
\end{align*}
\]

Fig. 6. Proposed pathway for the biosynthesis of O antigen in Citrobacter 139.
lipid intermediate with the structure abe-mannh-
ra-gal-1-P-"lipid" is formed in this organism, 
similar to that reported by Weiner for the S.
typhimurium mutant (8). Polymerization in the 
Citrobacter system frequently occurs in the ab-
sence of CDP-abequose, and at other times it
does not occur at all. This is reminiscent of the 
results obtained with cell envelope preparations 
of S. typhimurium; polymerization was also in-
dependent of the presence of CDP-abequose. 
However, there is no evidence for in vivo poly-
merization at the trisaccharide level with either 
oranism.

The product formed in the complete system 
with all three nucleotide sugars was isolated by 
phenol extraction, and, although a certain am-
ount of the radioactivity migrated as an anionically 
charged polysaccharide in high voltage electro-
phoresis, the bulk of it remained at the origin. 
This suggested that most of the material is present 
as lipopolysaccharide. When the lipopolysac-
charide was hydrolyzed with mild acid, a trisac-
charide unit with the structure \( \alpha \)-galactosyl-
mannosyl-rhamnose was formed, and it may 
therefore be concluded that this repeating unit is 
present in the O antigen. This conclusion is also 
supported by the fact that the O antigen from 
Citrobacter 139 cross-reacts with that of type B 
Salmonella.

The existence of disaccharide-lipid and trisac-
charide-lipid intermediates was shown by sequen-
tial addition experiments of the type conducted 
by Weiner et al. (9).

From these studies, it is apparent that the 
Citrobacter system for the biosynthesis of lip-
opolysaccharide is similar to the biosynthetic 
system of S. typhimurium. However, there are 
significant differences, such as the nature of the 
product resulting from the complete system lack-
ing CDP-abequose, and the differences in the 
stoichiometry of the sugars incorporated.

ACKNOWLEDGMENTS

The authors are indebted to M. J. Osborn for her 
interest and many valuable suggestions during the 
course of this work.

This investigation was supported by Public Health 
Service grants GM 11301 and GM 1191 from the 
National Institute of General Medical Sciences 
and National Science Foundation grant GB 1465.

LITERATURE CITED

1. Anderson, J. S., M. Matsushima, M. A. Haskin, 
and J. L. Strominger. 1965. Lipid-phospho-
acylumuramyl-pentapeptide and lipid-phos-
phodiisaccharide-pentapeptide: presumed mem-
brane transport intermediates in cell wall 
889.

2. Anderson, J. S., P. M. Meadow, M. A. Haskin, 
and J. L. Strominger. 1966. Biosynthesis of the 
peptidoglycan of bacterial cell walls. I. Utiliza-
tion of uridine diphosphate acetylmuramyl 
pentapeptide and uridine diphosphate acetyl-
glucosamine for peptidoglycan synthesis by 
particulate enzymes from Staphylococcus aureus 

1967. Structure of a lipid intermediate in cell 
wall peptidoglycan synthesis: a derivative of a 

4. Kauffmann, F. 1961. The bacteriology of Sal-
monella species. Munksgaard, Copenhagen.

of cell wall polysaccharide in mutant strains of 
Salmonella. IV. Synthesis of S-specific side-
19:322–327.

L. Horecker. 1962. Biosynthesis of bacterial 
peripolysaccharide. I. Enzymatic incorporation 
of galactose in a mutant strain of Salmonella. 

1964. Enzymatic synthesis of the Salmonella 
1309.

L. Horecker. 1966. Biosynthesis of O-antigen 

9. Weiner, I. M., T. Higuchi, L. Rothfield, M. 
Saltmarsh-Andrew, M. J. Osborn, and B. L. 
Horecker. 1965. Biosynthesis of bacterial lip-
opolysaccharide. V. Lipid-linked intermediates 
in the biosynthesis of the O-antigen groups of 

10. Wright, A., M. Dankert, P. Fennessey, and P. 
W. Robbins. 1967. Characterization of a poly-
isoprenoid compound functional in O-antigen 
57:1798–1803.

1965. Evidence for an intermediate stage in the 

12. Zelezniak, L. D., S. M. Rosen, M. Saltmarsh-
Andrew, M. J. Osborn, and B. L. Horecker. 
1965. Biosynthesis of bacterial lipopolysaccha-
ride. IV. Enzymatic incorporation of mannose, 
rhamnose, and galactose in a mutant strain of 