Polyprenylphosphate-arabinose (in which the polyprenyl unit is found both as decaprenyl and octahydroheptaprenyl) is a donor of mycobacterial cell wall arabinosyl residues. Because of this important role, its biosynthetic pathway, and that of the related lipid, polyprenylphosphate-arabinose, was investigated. Surprisingly, phosphoribose pyrophosphate was shown to be a key intermediate on the pathway to both polyprenylphosphate-arabinose and polyprenylphosphate-5-phospho[14C]ribose. Two additional intermediates, polyprenylphosphate-5-phosphoh[14C]ribose and polyprenylphosphate-5-phosphoh[14C]arabinose, were identified. Further experiments showed that the mature polyprenylphosphate-arabinose is formed from phosphoribose pyrophosphate via a two-step pathway involving a transferase to form polyprenylphosphate-5-phosphoribose and then a phosphatase to form the final polyprenylphosphate-arabinose. Polyprenylphosphate-arabinose is formed by a similar pathway with an additional step being the epimerization at C-2 of the ribosyl residue. This epimerization occurs at either the level of phosphoribose pyrophosphate or at the level of polyprenylphosphate-5-phosphoribose.

The mycobacterial cell wall core consists of a highly impermeable layer of the unique 70–90 carbon mycolic acids covalently attached to an inner peptidoglycan layer by way of the connecting polysaccharide, arabinogalactan. Arabinogalactan consists of three regions: the linker region (1), which is connected to the peptidoglycan, a galactan directly attached to the linker (2), and an arabinan (2), which is directly attached to the galactan. The mycolic acids are attached at the non-reducing end (3) of the arabinan. Since the arabinan is fundamental to the structural integrity of the cell wall, its biosynthesis has recently been studied (4–9) with an ultimate aim of developing new tuberculosis drugs targeted at one or more of the arabinose biosynthetic enzymes.

A major breakthrough (4) in these studies was the isolation of polyprenylphosphate-arabinose in the form of decaprenylphosphate-arabinose and octahydroheptaprenylphosphate-arabinose. Subsequently, it was demonstrated that radioactive decaprenylphosphate-arabinose made chemically (5), or a mixture of radioactive decaprenylphosphate-arabinose and octahydroheptaprenylphosphate-arabinose, isolated from cultures of Mycobacterium smegmatis, could function as arabinosyl donors in the presence of M. smegmatis enzymes to form polymeric arabinan. Hence, determining the pathway of biosynthesis of polyprenylphosphate-arabinose itself becomes important for the ultimate goal of developing new drugs against mycobacteria.

In a related area, mycobacteria have been shown (9) to synthesize polyprenylphosphate-ribose. This compound has been characterized as decaprenylphosphate-ribose (9), although it is likely to exist as octahydroheptaprenylphosphate-ribose as well. The function of this glycolipid is not yet clear. It does not appear to be a biosynthetic precursor of polyprenylphosphate-arabinose because radioactive polyprenylphosphate-ribose is not converted by mycobacterial enzymes to polyprenylphosphate-arabinose (9). Polyprenylphosphate-ribose may function as a ribosyl donor (9) since mycobacteria have been shown to ribosylate certain antibiotics (10).

Polyprenylphosphate sugars are generally synthesized by the transfer of a glycosyl residue from a sugar nucleotide to the phosphate moiety of a polyprenylphosphate (11). Thus, in mycobacteria, the polyprenylphosphate-mannoses (12–14) are formed by GDP-Man reacting, in a reversible fashion (15), with polyprenylphosphate to yield polypropenylphosphate-mannose plus GDP. If a similar pathway is used for polyprenylphosphate-arabinose synthesis, the sugar nucleotide of arabinofuranose needs to be clearly identified. One report has suggested the possibility of GDP-Ara (15) in mycobacteria, and a second report has suggested the possibility of UDP-Ara (6) in mycobacteria. However, the status of such arabinosyl nucleotides in mycobacterial arabinan biosynthesis remain to be clearly established.

Experiments attempting to convert radioactive polyprenylphosphate-arabinose into a sugar nucleotide of arabinose by incubating polyprenylphosphate-[14C]arabinose and a variety of nucleotide diphosphates (including UDP and GDP) with enzymatically active membranes prepared from M. smegmatis were unsuccessful, although such experiments succeeded with...
polypropenylphosphate-mannose (13). Because of this result, we sought to identify an alternative pathway used by mycobacte-
ria to synthesize polypropenylphosphate-arabinose. Recent labeling experiments using [14C]glucose and cultures of M. smeg-
matis have shown that the carbon atoms of arabinose present in cell wall arabinan are formed by the non-oxidative pathway of the pentose shunt (7). Additionally, the obvious route from the pentose shunt to the arabinose carbon skeleton via arabi-
nosephosphate isomerase (the enzyme converting ribulose-5-
phosphate to arabinose-5-phosphate) was shown not to occur (7). We therefore considered other possible routes from the pentose shunt to an activated arabinose phosphate. We report here the results of studies using p[14C]Rpp as a biosynthetic precursor of polypropenylphosphate-arabinose and polypropenyl-
phosphate-ribose.

EXPERIMENTAL PROCEDURES

Enzymes and Biochemicals—All enzymes, biochemicals, and growth media were obtained from Sigma unless otherwise noted. Radioactive N-[U-14C]glucose (>100 µCi/mmol) was obtained from American Radio-
abeled Chemicals, Inc. (St. Louis, MO).

Preparation of Polypropenylphosphate—Typically, 100 µCi (100 µCi/mmol) of uniformly labeled N-[U-14C]glucose (1 µmol) was dried in a tube, and 800 µl of a buffer containing 50 mM HEPES, 2 mM MgCl2, and 0.5 mM MnCl2, at a pH of 7.6, were then added. 10 units of hexokinase, 1 µmol of ATP, and 4 µmol of β-NADP were then added, and the sample was incubated at room temperature for 2 min. Then, sequentially, 10 units of glucose-6-phosphate dehydrogenase, 2 units of 6-phosphogluconate dehydrogenase, 10 units of phosphoribosylase, and 1 unit of phosphoribosyl pyrophosphate synthetase were added. The reaction was incubated at 37 °C for 30 min. Then, a total of 1.5 µmol of additional ATP was slowly added over 60 min. This resulted in p[14C]Rpp with a radiopurity of over 85% as analyzed by HPLC. The protein was removed by centrifugation through a 10-kDa cutoff micro-
con "microconcentrator" (Amicon, Inc.). For most experiments the p[14C]Rpp was further purified by preparative Dionex chromatography. The entire sample (∼1 ml) was injected onto a "semi-preparative" Dionex CarboPak PA1 column (9 × 250 cm) and eluted with a linear gradient beginning with 0.05 M sodium acetate in water and increasing to 1 M sodium acetate in water over 30 min. The flow rate was 3 ml/min, 1.5-ml fractions were collected, and a small aliquot of each fraction was counted. The p[14C]Rpp elutes at about 27 min, and fractions containing p[14C]Rpp were combined. Attempts to desalt HPLC-purified p[14C]Rpp resulted in substantial decomposition of the p[14C]Rpp. However, since the sodium acetate did not have a deleterious effect on the M. smegma-
tis enzymatic activities described below, the p[14C]Rpp was used with-
out removing the salt. The purified material was frozen in aliquots and typically contained 10,000 cpm/µl with a sodium acetate concentration of approximately 0.7 M.

M. smegmatis Membrane Enzyme Preparation—M. smegmatis (strain MC155) was grown to mid-log in nutrient broth, harvested by centrifugation, and transferred into buffer A, which consists of 50 mM MOPS, 5 mM MgCl2, and 0.5 mM dithiothreitol, at a pH of 7.8. The cells, suspended in buffer A, were disrupted by sonication at 4 °C with a 4710 series Cole Parmer ultrasonic homogenizer equipped with a microtip. The cell homogenate was then centrifuged at 12,000 × g for 10 min, and the resulting supernatant was measured for protein concentration us-
ing the Bio-Rad Protein Assay (Bio-Rad) and shown to be typically around 10 mg/ml. The supernatant was then ultracentrifuged at 100,000 × g for 1 h, and the resulting membrane-enriched pellet was then removed and homogenized in a small amount of buffer A. Its protein concentration was adjusted to approximately 4 mg/ml with buffer A, unless noted otherwise. This was then used as the "M. smegmatis membranes."

Treatment of p[14C]Rpp with Membranes Prepared from M. smegma-
tis Membranes—Aliquots of HPLC-purified p[14C]Rpp (200 µCi/µl of buffer A and contained 470,000 cpm of p[14C]Rpp) were incubated with 250 µl of the M. smegmatis membranes and 450 µl of buffer A for 35 min at 37 °C (Fig. 1A, lane 1). A second reaction was done in the same fashion except that 0.8 µmol of ATP and 0.8 µmol of NADP were included (Fig. 1A, lane 2). After the incubation, the reaction mixtures were distributed into an organic layer and an aqueous layer by the addition 1.33 volumes of methanol and 2.67 volumes of chloroform and briefly centrifuged at 2,000 × g. The organic (bottom) layer contained the newly formed polypropenyl-
phosphate-[14C]pentoses.

TLC Analysis of Lipid Products and Autoradiography—Samples were loaded on an aluminum-backed silica gel (60 F254, E. Merck, Darmstadt, Germany) TLC plate (10 × 20 cm) and run in the solvent

CHCl3:CH3OH:7 M NH4OH in H2O 65:25:4. Autoradiography was car-
ried out by exposing X-OMAT AR film (Kodak) to the TLC overnight at
-70 °C. Samples were removed from the TLC by scraping the band into a test tube and extracting with the same solvent used to develop the TLC.

HPLC Analyses for Monosaccharides, Pentose-5-phosphates, and pRpp—Monosaccharide analysis was as described using Dionex HPLC (7) except that when the released sugars were suspected to be 5-phos-
phorylated, the dry hydrolysate was then taken up in 90 µl of buffer A containing 1 unit of alkaline phosphatase to remove the phosphate groups. Radioactivity eluting from the Dionex HPLC was either moni-
tored by the collection of 30 1-min fractions and counting them on a liquid scintillation system or via direct detection of the column effluent using a β-RAM on-line radioactivity HPLC detector scintillation system (Inus Systems, Tampa, FL).

The detection of pentose-5-phosphates, the samples were hydro-
lyzed for 30 min with 0.1 M trifluoroacetic acid at 120 °C. The 5-phos-
phate group is stable to the acid in these conditions, whereas the phosphorylase at carbon 1 is lost. The samples were then injected onto the Dionex-PAL column and eluted with a gradient of 0.1 M sodium acetate in 2 mM NaOH to 0.8 M sodium acetate in 2 mM NaOH over 50 min.

For the detection of pRpp, the sample was injected onto the PA-1 column and eluted with a gradient from 50 mM to 1 M sodium acetate in water over 60 min. Note that for elution of pRpp, no base is present in the eluent.

Treatment of p[14C]Rpp with Membranes Prepared from M. smegma-
tis in the Presence of Various Additives—Six treatments are reported (Fig. 2). All treatments were done in a total volume of 140 µl of buffer A and contained 400,000 cpm of p[14C]Rpp (non-HPLC purified), 14 µg of ethambutol (to minimize polymerization of any polypropenylphosphate-arabinose formed), and 100 µl of M. smegmatis membranes (15 mg/ml protein). For treatment 1, the membrane preparation was boiled 10 min before use; for treatment 2, there were no additional reagents; and for treatment 3, 25 µg of decaprenylphosphate were added. For treatment 4, the membrane preparation was boiled for 10 min before use, and 1.4 mg of CHAPS and 25 µg of decaprenylphosphate were added. For treatments 5 and 6, 1.4 mg of CHAPS and 25 µg of decaprenylphosphate were added. When used, decaprenylphosphate was dried in the tube beforehand and solubilized by a brief pan sonication in the MOPS buffer system. The tubes were incubated and extracted as described above and an aliquot of the organic phases was counted (Fig. 2).

Preparation of Polypropenylphosphate-pentose Standards—Decapre-
nylphosphate-[14C]arabinose was chemically synthesized as described previously (5). Decaprenylphosphate-[14C]arabinose (9) was prepared by treating growing cultures of M. smegmatis with [14C]glucose and no ethambutol, followed by isolation of the radioactive polypropenylphosphate-pentose by preparative TLC. Sugar analysis of this material showed the presence of 70% [14C]arabinose and 30% [14C]arabinose; the contaminating decaprenylphosphate-[14C]arabinose is apparent in Fig. 3B.

Treatment of p[14C]Rpp with M. smegmatis Membranes in the Pres-
ence of Pentadecaprenylphosphate—This treatment was done as de-
scribed above for decaprenylphosphate except that pentadecapre-
nylphosphate was used as the lipid.
Conversion of Polyprenylphosphate-5-phospho-C14-ribose to p-C14-Rpp by Membranes Prepared from M. smegmatis—Four aliquots of 13.050 cpm of the polyprenylphosphate-5-phospho-C14-ribose prepared in the presence of CHAPS and Na2WO4 were resuspended in buffer A (50 μl). One of the aliquots received PPI so that the final concentration was 4 μM, a second 2 μM, and the third and fourth received no PPI. To tubes 1, 2, and 3, 92 μl of active M. smegmatis membrane preparation (6.3 mg/ml protein) were added; to tube 4, 92 μl of boiled membrane preparation were added. After incubation for 30 min at 37 °C, the samples were extracted, and the aqueous and organic layers were counted.

A large scale experiment was done by suspending 200,000 cpm of the polyprenylphosphate-5-phospho-C14-ribose into 100 μl of buffer containing 1% CHAPS. One- and two-tenths μmol of PPi, in 50 μl of buffer were added along with 150 μl of active membrane. After incubation for 30 min at 37 °C, the material was extracted, and the aqueous phase was analyzed by Dionex HPLC for p-C14-Rpp (Fig. 8).

RESULTS

Conversion of p-C14-Rpp into Decaprenylphosphate-C14-arabinose, Octahydroheptaprenylphosphate-C14-arabinose, and Decaprenylphosphate-C14-ribose by Mycobacteria Enzymes—p-C14-Rpp was incubated with crude M. smegmatis membrane preparations for 35 min at 37 °C, after which the reaction mixtures were separated into organic and aqueous soluble fractions by extraction with CHCl3/CH3OH/H2O. Approximately 1% of the starting radioactivity was converted into organic soluble material as compared to less than 0.1% in an enzyme boiled control. The organic soluble material from two reactions using active membranes was run on TLC, and the resulting autoradiogram is presented in Fig. 1A. The materials with Rf 0.43 and 0.50 were removed separately from the TLC, hydrolyzed, and analyzed by HPLC (Fig. 1B and C) for C14-sugars. The material with a Rf 0.5 was mostly arabinose, and the material with Rf 0.43 was about an equal mixture of arabinose and ribose. Previous studies (4, 9) have shown that of the polyprenylphosphate-pentoses, decaprenylphosphate-arabinose runs the fastest on TLC, with octahydroheptaprenylphosphate-arabinose and decaprenylphosphate-ribose migrating nearly together (9) and slightly slower than decaprenylphosphate-arabinose. This information, combined with the sugar analyses (Fig. 1B and C) allowed for the material with Rf 0.5 to be identified as decaprenylphosphate-C14-arabinose and the material with Rf 0.43 to be identified as a mixture of decaprenylphosphate-C14-ribose and octahydroheptaprenylphosphate-C14-arabinose (see below for further substantiation of these identifications).

The Effect of Various Additives on the Conversion of p-C14-Rpp into Organic Soluble Material—Since the conversion of p-C14-Rpp into polyprenylphosphate lipid catalyzed by the M. smegmatis membranes was low, the effect of various additives on this conversion was investigated (Fig. 2). Most striking was the dramatic stimulation of activity by the detergent CHAPS (Fig. 2, treatment 5). Also important was the stimulation of activity by decaprenylphosphate, which could be seen both in presence and absence of the detergent (Fig. 2, treatments 6 and 3). TLC analysis of the material treated with both CHAPS and decaprenylphosphate gave a profile similar to that shown in Fig. 1 except that sugar analysis of the faster migrating material yielded only ribose. These results led to the conclusion that the conversion of pRpp to polyprenylphosphate-pentose was strongly stimulated by the detergent, but only polyprenylphosphate-ribose was formed. Clearly, one of the enzymes involved in the arabinose lipid formation fails to act in the presence of CHAPS.

Evidence for the Nature of the Lipid Group Present on the Glycerolipids Formed from p-C14-Rpp—As noted above, the TLC migration times strongly suggested that the lipid moieties of
this expectation was realized with a faster migrating band (pentadecaprenylphosphate. Examination of Fig. 4 shows that migrating bands would be evident due to the chain length of an acceptor to form pentadecaprenylphosphate-pentoses, faster. It was expected that if this exogenously added lipid was used as the presence and absence of pentadecaprenylphosphate (C75).

The organic soluble glycolipid were polyphenylphosphate. To compare TLC migrations directly, preparations of the polyphenylphosphate-pentoses synthesized from p[14C]Rpp were isolated, and their TLC mobility was compared with standards. Thus, a mixture of decaprenylphosphate-[14C]arabinose and decaprenylphosphate-[14C]ribose was synthesized using membranes and p[14C]Rpp in the absence of detergent. The polyphenylphosphate-pentose region of the TLC was eluted and shown by sugar analysis to be 50% arabinose and 50% ribose. This material was then compared by TLC to a chemically synthesized standard of decaprenylphosphate-[14C]arabinose. This material was then compared by TLC to a chemically synthesized standard of decaprenylphosphate-[14C]arabinose (Fig. 3B). As expected (9), the decaprenylphosphate-[14C]ribose synthesized enzymatically from p[14C]Rpp ran more slowly on TLC than chemically synthesized decaprenylphosphate-[14C]arabinose. However, it ran with the same mobility as the decaprenylphosphate-[14C]ribose standard.

Further conformation of the nature of the lipid and of the fact that a polyphenylphosphate was utilized as a substrate by the membrane enzymes was obtained by performing incubations in the presence and absence of pentadecaprenylphosphate (C75).

It was expected that if this exogenously added lipid was used as an acceptor to form pentadecaprenylphosphate-pentoses, faster migrating bands would be evident due to the chain length of pentadecaprenylphosphate. Examination of Fig. 4 shows that this expectation was realized with a faster migrating band (Rf 0.52) in the polyphenylphosphate-pentose region and a faster band (Rf 0.09) in the “slowly migrating” region of the TLC.

Characterization of the Slowly Migrating (Rf 0.07–0.14) 14C-Labeled Lipids—The slowly migrating lipids (Fig. 1, Rf 0.14) were suspected to be a mixture of polyphenylphosphate-5-phosphoarabinose and polyphenylphosphate-5-phosphoribose. Consistent with this hypothesis, acid hydrolysis followed by alkaline phosphatase (to remove the phosphate from the 5 position) released both arabinose and ribose from these lipids as shown in Fig. 5A. Similar treatment of the analogous glycolipid formed from p[14C]Rpp in the presence of detergent yielded only [14C]ribose (Fig. 5B). In a different analysis, the material at Rf 0.14, which contained both arabinose and ribose (Figs. 1 and 5A), was treated with mild acid to remove the expected pentose-5-phosphates from the lipid in an intact form. The resulting sugar phosphates were analyzed by HPLC. The results (Fig. 5C) showed the presence of both ribose-5-phosphate.
and arabinose-5-phosphate. As anticipated, similar analysis of the slowly migrating lipid formed in the presence of detergent revealed only ribose-5-phosphate (results not presented). Finally, treatment of the slowly migrating ribose glycolipid with alkaline phosphatase converted it to mature polyprenylphosphate-ribose (Fig. 6, lane 3 before treatment and lane 2 after treatment). Therefore, the structures of the slowly migrating glycolipids were assigned to be polyprenylphosphate-5-phospho[14C]ribose and polyprenylphosphate-5-phospho[14C]arabinose.

Evidence for the Conversion of Polyprenylphosphate-5-phosphoribose to Polyprenylphosphate-ribose as Part of the Polyprenylphosphate-pentose Biosynthetic Pathway in Mycobacteria—The polyprenylphosphate-5-phosphopentoses, due to their structures, are very likely to be the direct biosynthetic precursors of the mature polyprenylphosphate-pentoses. Direct evidence of such was obtained for the ribo compounds by using known inhibitors of phosphatases. Thus, Na2WO4, NH4VO3, and NaF were added, in the presence of CHAPS, to the p[14C]Rpp and M. smegmatis membranes. After incubation and extraction, the organic soluble materials were run on TLC as shown in Fig. 7. As is evident, the formation of mature polyprenylphosphate-5-phosphoribose strongly inhibited by the tungstate (94%), partially inhibited by the vanadate (32%), and partially inhibited by the fluoride (30%). In a more direct approach, polyprenylphosphate-5-phosphoribose was isolated and then re-incubated with M. smegmatis enzymes. Such treatment converted the polyprenylphosphate-5-phosphoribose to polyprenylphosphate-ribose (Fig. 6, lane 3 before re-incubation and lane 1 after re-incubation).

Polyprenylphosphate-5-phosphoribose Can Be Converted to p[14C]Rpp in the Presence of Inorganic Pyrophosphate—If polyprenylphosphate-5-phosphoribose is synthesized by an enzyme that transfers a ribose-5-phosphate moiety from pRpp to decaprenylphosphate forming polyprenylphosphate-5-phosphoribose and PPi, the reaction should be reversible, and p[14C]Rpp should be formed by incubating polyprenylphosphate-5-phosphoribose in the presence of PPi and membranes. Hence, polyprenylphosphate-5-phosphoribose was incubated with membranes and with varying amounts of PPi. After 30 min, the reaction mixtures were extracted with CHCl3/CH3OH/H2O to form an aqueous and organic fraction. If formed, p[14C]Rpp should appear in the aqueous fraction. When 0, 2, and 4 mM PPi were present, the percentage of radioactivity in the aqueous fraction was 14, 31, and 68%. This result showed a PPi-dependent conversion of organic soluble...
radioactivity to aqueous soluble radioactivity. The aqueous soluble radioactive material produced in the presence of 4 mM PPi was analyzed by Dionex HPLC and shown to co-elute with p[14C]Rpp (Fig. 8).

**DISCUSSION**

The experiments reported herein allowed for the elucidation of the biosynthetic pathway for the formation of polyprenylphosphate-ribose (Fig. 9). Thus, a 5-phosphoribosyl transferase transfers a ribose-5-phosphate unit from the pyrophosphate moiety of pRpp to the phosphate moiety of decaprenylphosphate to form decaprenylphosphate-5-phosphoribose. This transfer occurs with an inversion of configuration at the 1 position of the ribosyl unit, as pRpp is in the a configuration and decaprenylphosphate-ribose is in the b configuration (9). The reaction is novel but follows the same general principles as those used to form polyprenylphosphate-hexoses. The difference is that with the hexoses, the leaving group is a nucleotide-pyrophosphate (11) rather than inorganic-pyrophosphate.

The second step of the reaction is the dephosphorylation of polyprenylphosphate-5-phosphoribose to form the mature polyprenylphosphate-ribose (Fig. 9). The phosphatase catalyzing this reaction is found in the membrane fraction and is strongly inhibited by tungstate (Fig. 7).

The experiments reported herein show that pRpp is a biosynthetic precursor of polyprenylphosphate-arabinose and that polyprenylphosphate-arabinose is formed via polyprenylphosphate-5-phosphoarabinose. Clearly, an epimerization at C-2 must occur given the ribo stereochemistry of pRpp and the arabino stereochemistry of polyprenylphosphate-arabinose. The evidence for the existence of polyprenylphosphate-5-phosphoarabinose (Fig. 5) suggests that pRpp might be epimerized to form 5-phosphoarabinose pyrophosphate, which then reacts with decaprenylphosphate to form polyprenylphosphate-5-phosphoarabinose (path A, Fig. 9). Alternatively, the epimerization might occur at the lipid level with polyprenylphosphate-5-phosphoribose being epimerized to form polyprenylphosphate-5-phosphoarabinose (path B, Fig. 9). Of these two possibilities, we presently favor the former because of earlier results (16) consistent with the inter-conversion of pRpp and 5-phosphoarabinose pyrophosphate.

Unexpectedly, pentose sugar nucleotides are not involved in the biosynthetic pathway for polyprenylphosphate-pentose formation (Fig. 9). This finding does not preclude the formation of an arabinofuranose sugar nucleotide in a different pathway and its utilization by some mycobacterial arabinosyl transferases as a donor. However, since recent studies have shown that many, if not all of the arabinosyl residues present in mycobacterial arabinan, arise from polyprenylphosphate-arabinose, the distinct possibility exists that mycobacteria synthesize their arabinofuranosyl polymer entirely without the utilization of an arabinofuranosyl sugar nucleotide.

Inhibition of the polyprenylphosphate-5-phosphoarabinose phosphatase should inhibit the formation of polyprenylphosphate-arabinose and, hence, the formation of arabinan and the cell wall. Similarly, inhibition of the enzyme that forms poly-
prenylphosphate-5-phosphoarabinose, whether it is an epimerase or transferase, should inhibit mycobacterial cell wall formation. Inhibitors of these two target enzymes would likely be nontoxic given the lack of D-arabinofuranosyl residues in humans.

The most important unresolved issue concerns the point at which the D-ribo to D-arabino conversion takes place in the formation of polyprenylphosphate-arabinose. Research efforts in this direction are now proceeding.

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