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MINIREVIEWS

Effect of Host Lactate on Gonococci and Meningococci: New Concepts on the Role of Metabolites in Pathogenicity▽

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In 1988, Britigan and colleagues (4) showed that the lactate in human neutrophils stimulated oxygen consumption by *Neisseria gonorrhoeae* and suggested that this might impair their oxygen-dependent bactericidal mechanisms. This first indication that lactate metabolism might be important in the pathogenicity of *N. gonorrhoeae* has been amply confirmed during the past decade (17, 61, 77), and now lactate has been shown to have important effects on *Neisseria meningitidis* (15, 16). This review uses the work on gonococci as background to describe recent research on the meningococcus, which in turn provided the key to proving the influence of lactate on gonococcal pathogenicity in vivo. Studies on *N. meningitidis* showed that, in addition to a general stimulation of metabolism, lactate promotes the production of specific determinants of pathogenicity. Some differences between meningococci and gonococci in their responses to lactate are discussed, and new concepts of the role of metabolites in pathogenicity arising from the work are summarized. Throughout this review, it should be kept in mind that lactate is present in urogenital and respiratory secretions, blood, phagocytes, and cerebrospinal fluid (CSF), together with glucose and pyruvate (61), two other energy sources used effectively by gonococci and meningococci (37, 42, 43). Hence, in vivo, gonococci and meningococci grow on a mixture of carbon sources. To mimic this situation, most of the studies described deal with the influence of lactate on gonococci and meningococci growing in media containing glucose.

STIMULATION OF GONOCOCCAL METABOLISM BY LACTATE: THE MECHANISM INVOLVED AND RELATION TO PATHOGENICITY

The original observations of the potential importance of lactate on the behavior of *N. gonorrhoeae* were reinforced by the demonstration that lactate in blood cell extracts enhanced the sialylation of gonococcal lipopolysaccharide (LPS) by cytidine-5′-monophospho-*N*-acetyl neuraminic acid (CMP-NANA) (49). Previous observations of gonococci harvested from urethral exudates had shown that host-derived CMP-NANA was incorporated into their LPS, rendering them resistant to killing by complement-mediated lysis in human serum, by phagocytes, and by antibody (24, 60). The enhancing effect of physiological concentrations of lactate on LPS sialylation occurred as gonococci were emerging from lag phase in a medium containing glucose. This resulted from an overall stimulation of metabolism, evidenced by greater LPS production (by 10 to 20%), enhanced protein synthesis (10 to 20%), and larger pentose contents (30 to 60%) (21, 22). Increased LPS sialylation occurs through greater production of both LPS and the sialyltransferase. There was more rapid emergence from lag phase with lactate and a 20% increase in the rate of logarithmic growth compared with glucose alone (22). Lactate was used more rapidly than glucose in a synthetic medium (22), as was seen in the fluid obtained from subcutaneous plastic chambers in guinea pigs that had been infected with gonococci (25).

The mechanisms of metabolic stimulation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of gonococci radiolabeled with either lactate or glucose during growth on single or mixed carbon sources showed that the production of specific proteins was not induced by lactate. Instead, lactate carbon was incorporated preferentially into the fatty acids of membrane lipids and LPS (77). The fate of the lactate carbon was followed by nuclear magnetic resonance analysis of membrane lipids and LPS purified from gonococci grown in a defined medium with labeled glucose alone, with labeled lactate and unlabeled glucose, and with labeled glucose and unlabeled lactate (77, 78). With glucose alone, the label was found in the fatty acids and the ethanolamine/glycerol moieties of the membrane lipids, and also in the fatty acids and sugar moieties of LPS. However, with labeled lactate in the presence of glucose, the label was not present in the ethanolamine/glycerol moieties of the membrane lipids or in the sugar moieties of LPS. With labeled glucose in the presence of lactate, the patterns were similar to those for growth in glucose alone. However, the addition of lactate resulted in detectable differences in the fatty acids of membrane lipids and the carbohydrate moieties of LPS. The cardinal result was that in the presence of glucose, lactate carbon was incorporated into fatty acid moieties and not into the ethanolamine/glycerol of membrane lipids or the carbohydrate of LPS. This revealed the underlying mechanism of metabolic stimulation.

When lactate is used alone by gonococci, it is oxidized to

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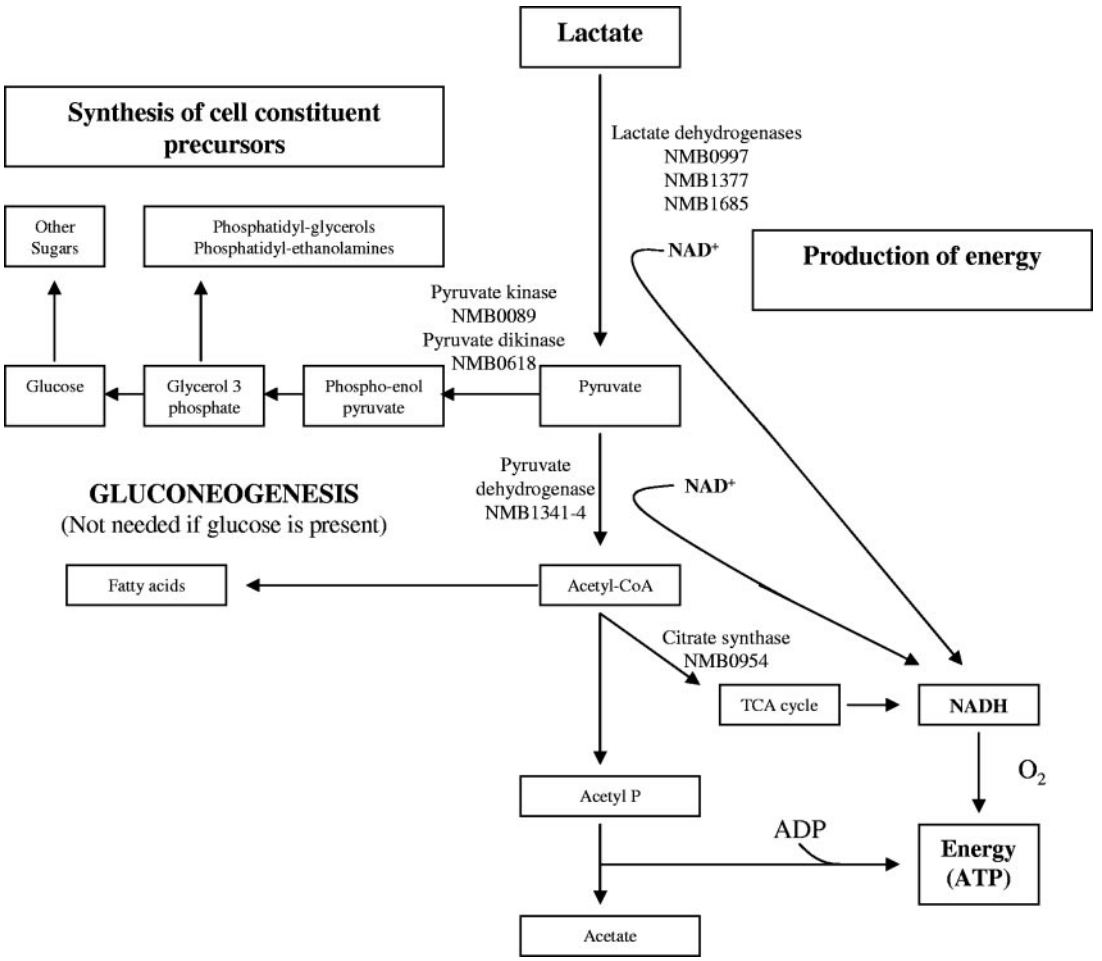

FIG. 1. Utilization of lactate by gonococci for energy production and synthesis of cell constituent precursors. If glucose is present, gluconeogenesis from lactate is shut down. Relevant enzymes are shown and annotated according to the serogroup B *N. meningitidis* genome sequence (http://www.tigr.org).

pyruvate and then fulfils two functions: (i) gluconeogenesis to produce sugar, glycerol, and ethanolamine moieties of gonococcal constituents and (ii) formation of acetyl-coenzyme A (CoA), the precursor of fatty acid synthesis, and constituents of the tricarboxylic acid (TCA) cycle (Fig. 1). Energy (via NADH and ATP) comes from the initial oxidation of lactate to pyruvate and acetyl-CoA and from the TCA cycle. Also, some acetyl-CoA is converted to acetate via acetyl phosphate, providing further energy (37). When glucose is present, gluconeogenesis from lactate does not take place, indicated by the absence of lactate carbon in ethanolamine, glycerol, or sugar moieties of membrane lipids and LPS. Instead, lactate is used solely as a source of additional energy, explaining the stimulation of metabolism in the presence of glucose (Fig. 1).

Possible effects on gonococcal pathogenicity. Lactate enhancement of LPS sialylation by CMP-NANA is the attribute most likely to affect pathogenicity in vivo, since this is observed in gonococci obtained from urethral exudates. The serum resistance of strains is lost on subculture in vitro but regained on incubation with blood-derived CMP-NANA and is increased by the presence of exogenous lactate (59, 60). This so-called "unstable" resistance should be distinguished from "stable" resistance that is not lost on subculture and characterizes strains that cause disseminated disease. The latter is determined by C4b binding protein attaching to porins (52) and the expression of porin 1A, which binds factor H, the major negative regulator of the alternative complement pathway (53). The "unstable" type of resistance is also determined by the recruitment of factor H, the binding of which is modified by sialylated LPS (27, 40, 54). Available lactate could be beneficial for the gonococcus after the initial invasion of mucosal surfaces by enhancing serum resistance. Also, lactate may reduce ingestion and killing by inflammatory polymorphonuclear leukocytes (PMNs) by increasing LPS sialylation (24); it is known that gonococci can survive and grow within human PMNs (67). In addition, at this stage, the gonococci may have to grow under anaerobic conditions, and this would increase serum resistance due to CMP-NANA-induced sialylation of LPS (19). However, there may be an adverse effect on the initial invasion of human cells. Sialylation inhibits the binding of LPS to asialoglycoprotein on urethral cells (44) and Opa protein-mediated entry into epithelial cells (69). Finally, in relation to invasion of previously infected people, increased

LPS sialylation induced by lactate could also interfere with the bactericidal action of antibody and complement (24).

There are other effects of lactate that might benefit gonococci during the primary colonization period. Rapid growth promoted by lactate would provide an advantage to gonococci exposed to host defenses. The increased oxygen consumption caused by lactate might impair oxygen-dependent killing mechanisms of phagocytes (4) when gonococci survive and grow within human PMNs (6, 58, 67). Also, there is an increase in the production of protein virulence determinants, such as type IV pili (for adherence) and Opa proteins (for cell entry) (69), sialylated LPS (21), and porin 1B, an inhibitor of PMN action (2). Turning to inflammation, the main harmful effect of gonorrhea, enhanced LPS production due to lactate could increase the induction of inflammatory cytokines (50) and promote GroEl production, which might have the same effect (8, 47).

Clearly, lactate metabolism could increase gonococcal pathogenicity in vivo. Final proof would require the demonstration that a mutant unable to use lactate is attenuated in a relevant animal model, as has recently been shown for the relevance of LPS sialylation to pathogenicity (76). However, similar evidence was lacking for the influence of lactate until a solution emerged from studies on meningococci.

THE EFFECT OF LACTATE METABOLISM ON MENINGOCOCCAL PATHOGENICITY

Interest in examining the effect of lactate on meningococci arose from the isolation of an attenuated mutant lacking a putative lactate permease (63). This mutant was used in pathogenicity studies with striking effect.

Early metabolic studies on *N. meningitidis*, like those on *N. gonorrhoeae*, involved either glucose or lactate, but not mixtures of the two. Lactate dehydrogenases (LDHs) (13) were recognized, together with pyruvate dehydrogenase (30) and all the TCA cycle enzymes (33). Later, analysis of available genome sequences (48, 65) indicated the presence of enzymes needed for gluconeogenesis and energy production by lactate and, like gonococci, for deriving energy from glucose by the Entner-Doudoroff pathway. A metabolic effect of lactate on meningococci similar to that on gonococci was therefore possible.

A lactate permease-deficient mutant of *N. meningitidis*. Signature-tagged mutagenesis using the infant rat model identified a putative lactate permease-deficient mutant of a serogroup B *N. meningitidis* strain (63). The mutation affected an open reading frame (NMB0543) (http://www.tigr.org) with a predicted product that shared 21% amino acid identity with a characterized lactate permease from *Escherichia coli* (10). Uptake of lactate by an NMB0543 mutant was insignificant compared with the rapid uptake by the wild-type strain (16), and unlike the latter, the mutant could not grow with lactate as the sole carbon source. Polar effects were excluded when growth on lactate alone was restored by complementation with a single copy of the gene (16). Hence, the gene product was designated a lactate permease (LctP).

As for the gonococcus, when millimolar quantities of lactate were added to *N. meningitidis* growing in a medium containing glucose, more rapid emergence from lag phase occurred, together with a higher rate of growth in early log phase, while the *lctP* mutant failed to respond to added lactate (16).

Lactate metabolism induces resistance to complement-mediated serum killing: the multifactorial mechanisms involved. The main innate host defenses against the meningococcus are complement-mediated extracellular lysis and probably ingestion and killing by phagocytes. We deal here with resistance to the first of these defenses. Notably, after growth for 2 h in a medium containing lactate, glucose, and CMP-NANA, the survival of the *lctP* mutant was only 16% of that of the wild type in the presence of normal human serum (16). Consistent with this greater sensitivity to serum, the mutant bound more complement component C3 than the wild-type strain.

Clearly, lactate metabolism enhances meningococcal resistance to killing by complement. What mechanisms are involved? For gonococci, the enhanced resistance is due to lactate increasing the amount of sialylated LPS. Meningococci also contain sialylated LPS, but in contrast to gonococci, serogroup B and C meningococci synthesize their own CMP-NANA (40). Strains can also utilize exogenous CMP-NANA to increase LPS sialylation (14). In the LPSs of both meningococci and gonococci, the sialic acid is linked to the terminal galactose residue of the carbohydrate side chain lacto-*N*-neotetraose epitope (60, 62).

Observations of the lactate permease-deficient *N. meningitidis* demonstrated that lactate increases sialylation of meningococcal LPS. When bacteria were grown with lactate, glucose, and CMP-NANA, immunoblotting of whole-cell extracts showed that the LPS from the LctP-deficient mutant was less sialylated, while the complemented mutant had levels of sialylation similar to those of the wild-type strain (16). Thus, the increase in meningococcal serum resistance induced by lactate could be due to the enhanced LPS sialylation. However, the situation is not as clear as for gonococci due to the expression of a polysialic acid capsule by seroroup B and C *N. meningitidis*, which also mediates resistance to complement-mediated killing (23, 34, 39, 73).

The relative importance of capsular polysialic acid and sialylated LPS in overall resistance remains a matter of debate. Estabrook et al. (14) examined serogroup C meningococci from healthy carriers and found that endogenous and exogenous LPS sialylation are associated with serum resistance. Kahler et al. (34) looked at mutants of an invasive serogroup B meningococcus with altered capsule, LPS, and sialylation; they stated that capsulation is essential for serum resistance but that LPS structures and sialylation also contribute. Vogel et al. (70, 71) studied isogenic α2-3 sialyltransferase (*lst*) mutants of three encapsulated clinical isolates. The mutants were more serum sensitive than the wild types, but only at high serum concentrations. They concluded that LPS sialylation was of minor importance for serum resistance. Sialylation of meningococcal LPS does not increase binding of factor H, as happens for gonococci (56), so the mechanism of its role is unknown. In reviewing the situation, Vogel and Frosch (72) cautioned that factors other than capsulation and LPS sialylation could influence serum resistance and that their relative importance could vary from strain to strain. For instance, glycosylation of pili affects the serum resistance of some strains (28), and Opa proteins may reduce serum resistance mediated by LPS sialylation by being an additional target for C4 (A. Prasad, U.

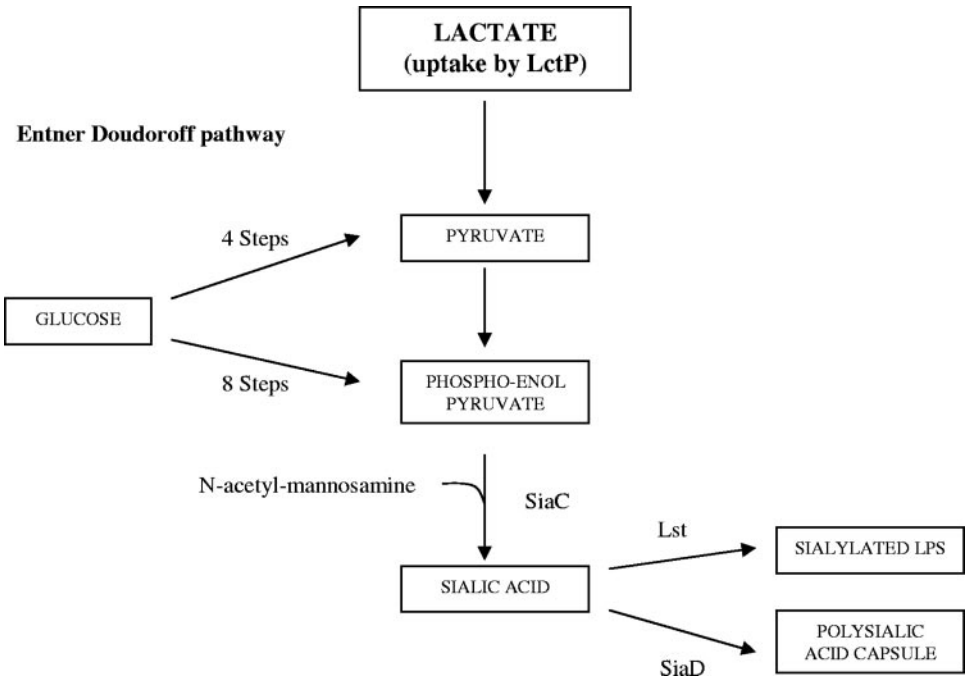

FIG. 2. Metabolic pathways by which sialic acid, sialylated LPS, and capsular polysialic acid are formed by meningococci from lactate and glucose, with the enzymes involved.

Vogel, J. Ngampasutadol, S. Gulati, S. Getzlaff, P. A. Rice, and S. Ram, presented at the 14th International Pathogenic Neisseria Conference, Milwaukee, WI, 5 to 10 September 2004).

There are several mechanisms by which lactate could affect production of sialylated LPS and capsular polysialic acid. General stimulation of metabolism would produce more of both in a shorter time. Lactate may be the predominant carbon source for the biosynthesis of capsular and LPS sialic acid, which is formed from phosphoenol pyruvate (PEP) and *N*-acetyl mannosamine (20). The PEP can be derived from glucose via pyruvate and glycerol-3-phosphate formed by the Entner-Doudoroff pathway, but this involves four and eight metabolic steps, respectively (Fig. 2). Production of PEP from lactate is more direct, involving only two steps (Fig. 2). Finally, lactate might induce changes in the LPS structure that affect killing by complement, either directly by changing LPS moieties that interact with complement components (e.g., phosphoethanolamine residues [51]) or indirectly by affecting the degree of sialylation.

Attempts were made to identify which of the mechanisms outlined above predominate, using a series of meningococcal mutants (16). The *siaD* gene encodes the polysialyl-transferase required for capsule synthesis (12), and mutants lacking this gene show a marked increase in serum sensitivity compared with wild-type strains in 50% serum (Table 1). However, as observed by others (40), the strain retained some resistance to 3% serum. This resistance was further reduced when an *lctP* mutation was inserted (Table 1), indicating that part of the serum resistance induced by lactate was mediated by influences other than capsular polysialic acid. When the *lctP* mutation was introduced into a double *siaD*- and *lst*-negative background, there was no significant reduction in resistance (Table 1). Hence, lactate contributes to resistance by affecting LPS sialylation. Finally, the *lctP* mutation was introduced into a strain lacking *siaC*, which codes for the enzyme catalyzing the synthesis of sialic acid from PEP and *N*-acetyl mannosamine (Fig. 2); again, this strain retained some resistance to 3% serum. When the *lctP* mutation was introduced into a *siaC*-negative background, there was no significant alteration in serum resistance (Table 1). Therefore, sialic acid synthesis from lactate plays a critical role in determining serum resistance due to LPS sialylation in unencapsulated strains. Other aspects of lactate metabolism that could also influence LPS sialylation are an increased amount of available LPS and a possible change in LPS structure, which reduces sialylation. Nevertheless, the main role of lactate in inducing the resistance of unencapsulated mutants to killing by 3% serum (Table 1) is probably to provide a ready source of PEP, and therefore sialic acid, for

TABLE 1. Increase in sensitivities of mutants to complement-mediated killing by human serum

Mutant examined	Increase in serum sensitivity compared with[a]:			
	MC58 (50%)	MC58Δ*siaD* (3%)	MC58Δ*siaD*Δ*lst* (3%)	MC58Δ*siaC* (3%)
MC58Δ*lctP*	+++			
MC58Δ*siaD*	+++			
MC58Δ*siaD*Δ*lst*	+++			
MC58Δ*siaC*	+++			
MC58Δ*siaD*Δ*lctP*		++		
MC58Δ*siaD*Δ*lst*Δ*lctP*			+/−	
MC58Δ*siaC*Δ*lctP*				+/−

[a] The concentrations of serum used in the assays are shown in parentheses. +++, >75%; ++, >50%; +/−, insignificant.

sialylation of LPS. The same mechanism could apply for the production of capsular polysialic acid. The influence of lactate on capsule production is strongly suggested by substantially reduced resistance of the *lctP* mutant to 50% serum at a level comparable to reductions caused by mutations that remove the capsule (*siaD* and *siaC*) (Table 1).

The roles of lactate metabolism in pathogenicity at successive stages in meningococcal infection. In environments that contain glucose, such as sites infected by meningococci in vivo (61), lactate has two potentially important effects on pathogenicity, namely, the stimulation of growth and the induction of resistance to killing by complement and possibly phagocytes. These effects are now considered in relation to progressive stages of meningococcal disease, colonization of the nasopharynx (79), occasional bloodstream invasion, and growth in CSF (66).

During the first stage of colonization, initial attachment to nasopharyngeal epithelial cells is determined by type IV pili for encapsulated strains and by Opa and Opc proteins for nonencapsulated strains (1, 45, 69). The next step is multiplication in the underlying tissue; meningococci are seen in the nasopharynx internal to the epithelial layer (57). To investigate the role of lactate during colonization, infection of human nasopharyngeal mucosa explants from resected adenoids was used (15). The explants contain lactate and glucose at levels similar to those in serum (0.6 to 2.4 mM and 3.9 to 5.8 mM for lactate and glucose, respectively). At 4 h after inoculation, the populations of the wild-type and lactate permease-deficient strains were the same as those at 0 h, but from 4 to 18 h, the numbers of wild-type bacteria increased 10-fold whereas levels of the mutant were static. Possible explanations for the colonization defect of the *lctP* mutant are reduced adherence to epithelial cells, susceptibility to host defenses, and growth retardation due to inability to use lactate. There was no detectable change in type IV pili, Opa, and Opc, and the mutant adhered to Chang epithelial cells better than the wild type, probably because of its lower degree of LPS sialylation (9). As for resistance to host defenses, there is little inflammation during meningococcal colonization of the nasopharynx (5) and complement levels are negligible in healthy individuals (36). Furthermore, the mutant and wild-type strains survived equally during the first 4 h in the explants, indicating that there was no significant role for resistance to host defenses. Hence, lactate stimulation of the growth is the most likely explanation for the enhanced colonization of the wild-type strain, showing that lactate has an important role in the first stage of pathogenesis (15).

A relevant model of bloodstream infection in humans by meningococci is systemic infection of infant rats, and both sialylated LPS and the polysialic acid capsule are virulence determinants in this model (73). In the model, the *lctP* mutant was significantly attenuated compared directly with wild-type bacteria in competitive infections (16). The competitive index (CI, the ratio of the numbers of mutant and wild type recovered from the animals divided by the ratio in the inoculum) was 0.108 for the lactate permease-deficient mutant (a CI of 1 indicates no attenuation). This could have resulted either from a lower growth rate or a decrease in resistance to killing by complement and/or by phagocytes. Experiments using complement-deficient rats were not possible, so a murine model was used. The CI of the mutant in systemic infection of mice (0.15) was similar to that in rats. However, in congenic C3-negative mice, the CI was 0.8, i.e., the virulence of the *lctP* mutant was restored in mice lacking intact complement. Thus, during systemic spread, lactate influences the virulence of the meningococcus by preventing complement-mediated killing and possibly increasing complement-dependent opsonophagocytosis, not by stimulating growth.

Turning to the influence of lactate on the final stage of meningococcal infection, wild-type bacteria grew faster than the *lctP* mutant in human CSF and reached a higher final population (16). Nuclear magnetic resonance analysis showed that *N. meningitidis* utilized glucose and lactate simultaneously but that lactate was used more rapidly. This result is similar to that when gonococci grow in guinea pig chamber fluid (25). Clearly, lactate stimulation of meningococcal growth in CSF may be relevant to the development of meningitis, particularly as CSF lactate levels rise during meningitis while glucose concentrations fall (11).

Differences between meningococci and gonococci in response to lactate. Lactate stimulates metabolism and growth of both gonococci and meningococci in media containing glucose, and the mechanism of growth stimulation for gonococci probably applies to meningococci. However, the species differ in the fact that unlike gonococci, some meningococci can synthesize their own sialic acid and CMP-NANA. This difference between the species affects the mechanism of increase in sialylated LPS and serum resistance induced by lactate. For gonococci, general stimulation of metabolism by lactate, producing more LPS and sialyltransferase, is predominant (22). For meningococci, the major role of lactate appears to be in promoting sialic acid production for LPS sialylation and incorporation in capsular polysaccharide.

The meningococcal lactate permease is an immunogen. Bacterial permeases are located on inner bacterial membranes and therefore are unlikely to have immunizing activity. The following observations (64) were therefore surprising. Recombinant LctP produced in an *E. coli* vector was purified and used to immunize mice by the subcutaneous route on two occasions. A week later, the animals were challenged with live bacteria. In two experiments, mice receiving LctP were partially protected against live challenge, in contrast to nonimmunized animals. This significant but incomplete protection was comparable to that produced by meningococcal PorA. Although it is not predicted to be on the outer membrane, LctP was detected on intact *N. meningitidis* by fluorescence-activated cell sorter analysis and enzyme-linked immunosorbent assay (64). This is similar to some periplasmic proteins of meningococci that elicit high levels of serum bactericidal antibodies (26). The mechanism of protection induced by LctP is unknown, as it evoked only low levels of serum bactericidal antibodies (64). Antibody neutralization of lactate permease activity may lead to a lower growth rate or lower resistance to killing by serum or opsonophagocytosis.

PROOF THAT LACTATE METABOLISM IS RELEVANT TO THE PATHOGENICITY OF GONOCOCCI IN VIVO

As stated above, final confirmation of lactate's importance in gonococcal pathogenicity required virulence tests in vivo on

strains specifically unable to use lactate. Potentially, LDH-deficient mutants could be used, but gonococci and meningococci have at least three different LDHs, making it difficult to construct mutants unable to utilize lactate by this approach (18). The identification of the meningococcal LctP mutant provided the solution.

Searches of the *N. gonorrhoeae* genome sequence with the deduced amino acid sequence of the meningococcal LctP revealed an identical sequence (NGO1449), except that Val^{167} in the meningococcal sequence is an Ala in the gonococcus. The sequence is predicted to encode a protein with 14 transmembrane domains, consistent with an inner membrane permease. A deletion mutant of NGO1449 was constructed and shown to be defective for uptake of exogenous lactate and growth in defined media with lactate as the sole carbon source; complementation of the mutant reverted these phenotypes to the wild type (17). Therefore, NGO1499 was assigned as the gonococcal LctP.

In vitro studies related to pathogenicity of the lactate permease-deficient gonococcal mutant. Since emergence from lag phase and rapid initial growth could benefit gonococci in the initial phases of infection, the growth of the mutant was examined in a defined medium with physiological concentrations of glucose and lactate (10 and 2 mM, respectively). The growth of the mutant was significantly less than that of the wild type and the complemented mutant under these conditions (17). Furthermore, the *lctP* mutant was more sensitive than the wild type to complement-mediated killing by human serum; fluorescence-activated cell sorter studies showed that the mutant was less sialylated than the wild type, whereas the results for the complemented mutant were similar to those for the wild type (17). This decrease in LPS sialylation probably accounts for the enhanced sensitivity of the mutant to complement-mediated killing by serum. However, changes other than LPS sialylation may have a role. Indeed, the addition of lactate to gonococcal cultures containing physiological concentrations of glucose is associated with changes in the fatty acid and carbohydrate composition of LPS (77, 78). Alterations of both lipid and carbohydrate components of LPS have been associated with changes in serum resistance of pathogenic neisseria (72, 73).

In vivo studies on the pathogenicity of the lactate permease mutant. The ability of the *lctP* mutant to colonize the murine vagina was compared with that of the wild-type strain. In this model of gonorrhea (31, 32), 4- to 6-week-old BALB/c mice are treated with 17-β estradiol and streptomycin to promote gonococcal infection of the vagina. The antibiotic reduces the commensal flora that can inhibit the gonococcal infection, and lactate and glucose concentrations in the mouse vaginal fluid were similar to those reported in human vaginal secretions (17). The *lctP* mutant demonstrated a dramatic disadvantage in this model compared with the parent strain. On days 4, 6, and 8 after inoculation, the CI of the mutant fell to 0.2, 0.04, and 0.02, respectively. In contrast, the complemented strain did not display a colonization defect throughout the course of infection.

This result means there is no doubt that lactate aids gonococcal infection of the mouse vagina. However, the underlying reasons for this are not clear. Lactate enhancement of LPS sialylation (thereby increasing factor H deposition) may be the reason for the greater resistance of the wild type to killing by human serum compared with the *lctP* mutants. However, this cannot apply to the murine results, because murine factor H does not bind to the gonococcus (46). Recent observations of a sialyltransferase-deficient mutant are pertinent (76). Wild-type bacteria passaged in the murine vagina for up to 9 days were highly resistant to killing by normal human serum, but not after incubation with neuraminidase or a single passage in vitro in the absence of CMP-NANA. In contrast, the *lst*-deficient mutant was sensitive to killing by human serum throughout the 9-day period. Thus, LPS sialylation occurs in the murine vagina and renders gonococci resistant to killing by human serum. The effect of LPS sialylation on resistance to murine serum cannot be tested, as it does not kill gonococci in vitro (76). Hence, the possibility of sialylation affecting killing by complement-mediated mechanisms was examined by competitive infection of the *lst* mutant in C5-deficient mice. The mutant was as attenuated in the C5-deficient mice as in normal mice (76), indicating that interference with complement-mediated lysis is not the explanation for the enhancement of vaginal infection by LPS sialylation. Thus, it is likely that lactate enhancement of vaginal infection is not due to changes in LPS sialylation.

There are two other mechanisms that might account for lactate promoting vaginal infection. First, the effect of lactate in a rapid emergence from lag phase and early growth may be crucial in the early stages of infection. Second, increased LPS sialylation induced by lactate may increase resistance to killing by PMNs (24). The latter explanation is supported by recent findings with the *lst*-deficient mutant (76). LPS sialylation not only rendered bacteria more resistant to killing in vitro by mouse PMNs, it also promoted less association of the bacteria with PMNs and a weaker respiratory burst. Thus, LPS sialylation enhances the pathogenicity of the gonococcus in the murine model by increasing resistance to killing by PMNs. This is consistent with the report (4) that lactate promotes excess oxygen usage by gonococci within phagocytes with a consequent reduction in oxygen-dependent killing mechanisms. Clearly, the *lctP*-deficient mutant could be investigated in similar experiments. The mouse model results have obvious implications for the role of lactate in human gonorrhea and warrant testing of the mutant in human volunteers.

NEW CONCEPTS OF THE ROLE OF METABOLITES IN PATHOGENICITY ARISING FROM STUDIES ON GONOCOCCI AND MENINGOCOCCI

It is recognized that the ability to grow in host tissues is a fundamental aspect of bacterial pathogenicity without which virulence determinants responsible for the inhibition of host defenses and causing damage to the host cannot be produced (60). This is underlined by the finding that many of the virulence genes necessary in vivo, revealed by signature-tagged mutagenesis and in vivo expression technology, have metabolic functions (59). However, up to the present, metabolites that either are required for bacterial growth or stimulate it have been considered individually and with respect to their influence on overall growth in the tissues. An example is erythritol, a growth stimulant for *Brucella* spp. that promotes massive bacterial replication in the placenta and fetal fluids of ungulates, leading to abortion (75). Another is urea, a growth stim-

ulant for *Proteus mirabilis* that causes severe kidney infections (3, 38). It is also recognized that lack of certain nutrients can induce bacterial mechanisms for acquiring them, e.g., production of siderophores and cell wall receptors for iron-bearing transferrins by gram-negative bacteria when they lack available iron (74). However, new concepts about the role of metabolites have now been revealed by studies on the influence of lactate on gonococcal and meningococcal pathogenicity.

The effects of metabolites in combination must be considered, as pathogens grow on mixtures in vivo. Like the individual metabolites mentioned above, the role of lactate in gonococcal pathogenicity lies in stimulating overall metabolism and growth. However, its dramatic effect is dependent on the presence of glucose, as occurs in vivo. The mechanism of this stimulation has been elucidated, and it may apply to other pathogens that occupy sites in vivo containing lactate and glucose, because they have the enzymes needed for it to operate (61). Clearly, lactate stimulates the metabolism of *N. meningitidis* in media containing glucose. Similar studies on the influence of lactate on the pathogenicity of *Haemophilus influenzae* (35) have been supported recently by the identification of an attenuated putative lactate permease-deficient mutant (29).

Antigens involved in metabolism may contribute to vaccines. Until now, most vaccines have been based on virulence determinants, such as toxins, adhesins, and capsules. The surprise discovery that meningococcal LctP has immunizing ability shows that antigens connected with metabolism should also be considered. Obvious candidates are the products of genes with metabolic functions that have been revealed by in vivo expression technology, STM, or directed approaches to operate in vivo and affect virulence (43, 59).

Growth-stimulating metabolites can influence the production of specific virulence determinants. For meningococci, lactate not only stimulates growth, it is a more direct source than glucose for producing PEP needed for sialic acid biosynthesis and hence two virulence determinants, sialylated LPS and the polysialic capsule. Furthermore, these two effects can influence different stages in pathogenesis. For meningococci, growth stimulation promotes initial colonization and survival in cerebrospinal infection, while enhanced expression of virulence determinants and inhibition of host defenses contributes to spread in the bloodstream. The two roles of lactate might also operate for *E. coli* K-1 and group B streptococci, because like *N. meningitidis*, they also synthesize sialic acid (7, 68). We are not aware of any other metabolites, such as glutamate (41), that have this dual role either on their own or in combination with another metabolite, but the observations of meningococci may be relevant to research on other organisms.

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