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Research Article

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Phagocyte-derived Lactate Stimulates Oxygen Consumption by *Neisseria Gonorrhoeae*

An Unrecognized Aspect of the Oxygen Metabolism of Phagocytosis

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Abstract

O_2 consumption resulting from interaction of *Neisseria gonorrhoeae* and human neutrophils represents a composite of O_2 consumed by the two cell systems. Experiments studying the relative contribution of each system suggested the possibility that gonococci increased their metabolic activity in response to interaction with neutrophils. This hypothesis was confirmed by demonstrating that undifferentiated HL-60 cells, which are unable to undergo a respiratory burst, induce a two- to threefold increase in gonococcal O_2 consumption. Gonococcal capacity to adhere to HL-60 cells did not correlate with extent of metabolic stimulation. Stimulatory activity was demonstrable in cell-free supernatant from neutrophils or HL-60 cells, and increased with duration of incubation. Supernatant applied to a G-15 Sephadex column yielded fractions that stimulated gonococcal O_2 consumption. Elution profiles were similar for HL-60 cells, neutrophils, and a stimulatory factor previously isolated from pooled human serum. This stimulatory factor(s) failed to adhere to DEAE or C-18 HPLC columns. Stimulatory activity release from myeloid cells was inhibited by incubation at 4°C or in the presence of NaF, indicating a critical role for glucose metabolism. Lactate, the principal product of resting neutrophil glucose catabolism, was demonstrable in cell-free supernatants after incubation at 37°C. Lactate accumulation was inhibited by NaF and decreased temperature of incubation. Lactate at levels present in cell-free supernatant increased gonococcal O_2 consumption twofold and restored stimulatory activity to dialyzed serum. Live, but not heat-killed gonococci eliminated lactate released from neutrophils during phagocytosis. Gonococci are able to utilize host-derived lactate to enhance their rate of O_2 metabolism.

Introduction

Human mucosal surfaces are the sole natural reservoir for *Neisseria gonorrhoeae.* Survival demands that the organism acquire substrates critical for metabolism and growth while avoiding or combatting local host defenses. Although much is known about the metabolism and physiology of *N. gonorrhoeae* (1), the relationship between these characteristics and pathogenesis has received remarkably little attention. Most studies have focused on the role of gonococcal outer membrane components in this process (reviewed in reference 2). These studies have generally been performed with strains of *N. gonorrhoeae* cultivated in vitro on laboratory media. However, several investigators have shown that cultivation of *N. gonorrhoeae* under conditions that more closely approach those encountered in vivo induce alterations in growth rate (3, 4), metabolism (3–5), and outer membrane phenotype (6–10). These data suggest that microenvironmental conditions may profoundly influence gonococcal pathogenesis.

Anaerobic organisms are the principal flora of mucosal surfaces, showing that O_2 is in limited supply (11, 12). Optimal microbicidal activity of human neutrophils is dependent on their reduction of ambient O_2 to superoxide and other reactive oxygen intermediates (13). O_2 also plays an integral role in the metabolism of *N. gonorrhoeae,* being utilized as a terminal electron acceptor under both aerobic and microaerophilic conditions (1, 14). Under some experimental conditions, gonococci compete effectively with neutrophils for ambient O_2, resulting in diminution of neutrophil formation of reactive oxygen intermediates (15).

N. gonorrhoeae stimulates neutrophil O_2 reduction in the absence of serum-derived opsonins (16, 17). We have previously noted a discrepancy between O_2 consumption rates of gonococcal/neutrophil mixtures, and the apparent contribution of the individual components (17). Since gonococcal O_2 utilization is stimulated by exposure to a low molecular weight component of human serum and mucosal secretions (4, 15, 17, 18), it seemed possible that interaction with neutrophils induced a similar effect. In the present work we confirm this hypothesis and demonstrate that extracellular lactate, released as a consequence of neutrophil glycolysis, enhances gonococcal electron transport and O_2 consumption. Potential implications of this observation to bacterial pathogenesis are discussed.

Methods

Bacteria. Neisseria gonorrhoeae strains F62 (serum sensitive, serotype IB, pro-) and FA1090 (serum resistant, serotype IB, prototrophic) were maintained at 37°C, 5% CO_2 on gonococcal base (GCB) agar (Difco Laboratories, Detroit, MI) containing 1 and 0.5% Kellogg defined (19) supplements 1 and 2, respectively. Colony morphology was defined as described by Swanson and maintained by selective colony passage (20, 21).

Neutrophils. Neutrophils were separated from heparinized whole blood via erythrocyte sedimentation using Plasmagel (Roger Bellon, Neuilley, France) followed by sedimentation of the leukocyte fraction

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through Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) as previously described (17). Contaminating erythrocytes were removed by hypotonic lysis and the resulting neutrophils suspended in Hanks' balanced salt solution (HBSS). Cell concentration was determined using an automated blood cell counter (model D2N; Coulter Electronics, Inc., Hialeah, FL). Giemsa stain showed that > 98% of the cells were neutrophils and > 95% were viable based on exclusion of Trypan blue dye.

Cell lines. The human promyelocytic HL-60 cell line (22) was obtained from the Lineberger Cancer Research Facility of the University of North Carolina. HL-60 cells were maintained in their undifferentiated form by continuous suspension culture in Dulbecco's minimal essential media (DMEM-F12)[1] supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), ±5% heat inactivated FCS. Before use cells were pelleted (250 *g* for 10 min), washed three times in HBSS, and resuspended in HBSS at the desired concentration as determined by Coulter Counter or direct enumeration. Experimental results were identical regardless of whether or not DMEM-F12 was supplemented with FCS (data not shown).

Oxygen consumption. O_2 consumption rates were measured in a Clark type O_2 electrode (Yellow Springs Instrument Co., Yellow Springs, OH) as previously described (17). In experiments utilizing bacteria, 18–24 h old gonococcal GCB colonies were scraped into GCB broth, washed with HBSS, and resuspended in HBSS at the desired concentration. Gonococcal concentration was adjusted using a Klett-Summerson colorimeter (Klett Manufacturing, Inc., New York) to determine turbidity, which had been previously correlated with direct enumeration or colony forming units (10). O_2 consumption rates were measured at 37°C using a 1-ml vol of HBSS containing gonococci (10^9/ml), ±eukaryotic cells (5×10^6/ml), ±1 mM KCN. Results were expressed as the maximal rate of O_2 consumption recorded over a 5-min observation period. Unless otherwise specified a piliated opaque colony variant of strain F62 was used. Although O_2 consumption rates of each F62 colony phenotype suspended in HBSS (baseline) varied, all colony variants exhibited a similar proportional increase in maximal O_2 consumption when exposed to neutrophils or undifferentiated HL-60 cells. Substitution of strain FA1090 for F62 yielded similar results. In some experiments reaction mixtures also contained D(−) or L(+) lactate (Sigma Chemical Co., St. Louis, MO) with pH readjusted to 7.0–7.4 via NaOH titration.

Association of N. gonorrhoeae with HL-60 cells. Gonococci were labeled with [^{14}C]adenine (New England Nuclear, Boston, MA) and association with HL-60 cells determined as previously described for neutrophils (10), except that the reaction mixture also contained 0.6% BSA to limit nonspecific gonococcal adherence. Reaction mixtures contained ^{14}C-labeled gonococci (5×10^7) and HL-60 cells (5×10^6) in a 1-ml volume of HBSS/BSA. 13,015±4,070 and 7,497±1,966 (mean±SD) CPM were incorporated in 5×10^7 piliated and nonpiliated bacteria, respectively. Results were expressed as the percentage of bacterial inoculum associated with the myeloid cell at defined time points.

Serum. Serum was collected as previously described (17) from volunteers who did not have a history of gonococcal infection. Serum was pooled from six to eight donors, filtered, and stored at −70°C until usage.

Collection of cell-free supernatant. Neutrophils or HL-60 cells were washed and resuspended in fresh HBSS (1 ml) at a concentration of 5×10^6/ml. Cell suspensions were incubated at 37°C or on ice (4°C) for defined time periods. Cells were pelleted (250 *g* for 10 min) and supernatant saved. In some cases incubation mixtures contained NaF (Fisher Scientific Co., Fair Lawn, NJ; 10 mM), cycloheximide (Sigma Chemical Co.; 25 mM), or *N. gonorrhoeae* (strain F62, 10^8/ml) that were viable or had been killed by heating at 100°C for 15 min. For experiments in which neutrophil lysis was desired, cell suspensions were frozen (−70°C) and thawed three times. All supernatants were stored at 4°C until use.

Factor purification and characterization. Dialysis of HL-60 or neutrophil supernatants was performed in 1,000-mol wt membrane exclusion tubing overnight against HBSS (4). Column chromatography was performed using G-15 Sephadex. 3 ml of cell-free supernatant, DMEM-F12, or serum was applied to the G-15 column, eluted with sterile deionized H_2O and fractions (40 drops/fraction) collected. Fractions were assayed for ability to stimulate gonococcal metabolism by adding 80% (vol/vol) to HBSS (1 ml) containing 10^9 gonococci and recording the rate of gonococcal O_2 consumption in the Clark electrode. G-15 fractions demonstrating capacity to stimulate gonococcal O_2 consumption were then applied to a DEAE column and eluted with Tris buffer (pH 8.2) containing increasing concentrations of NaCl (0–0.2 M). Fractions (40 drops/fraction) were collected and assessed for gonococcal stimulatory activity as described for the G-15 fractions.

Lactate concentration. Lactate concentrations were measured by the technique of Alderman and Cross (23).

Statistical analysis. The paired Student's *t* test was used for all comparisons. Results were considered significant at $P < 0.05$.

Results

Effect of interaction with neutrophils on gonococcal oxygen metabolism. N. gonorrhoeae strain F62 suspended in HBSS (10^9/ml) exhibited a maximal O_2 consumption rate of 16.8±1.6 nmol/min (mean±SEM, Fig. 1). When neutrophils (5×10^6/ml) were added to the gonococcal suspension, a maximal O_2 consumption rate of 38.5±5.1 nmol/min was observed, representing the sum of O_2 consumed by neutrophils and gonococci (Fig. 1). Inclusion of 1 mM KCN in the incubation mixture reduced the maximal O_2 consumption rate to 10.4±5.1 nmol/min (Fig. 1). This should equal neutrophil O_2 consumption resulting from stimulation by *N. gonorrhoeae* since KCN totally inhibits gonococcal but minimally alters stimulated neutrophil O_2 consumption (24, 25). Theoretically, addition of the individual O_2 consumption rates of gonococci and neutrophils should equal the rate actually measured. Yet using this approach we were able to account for only 70% (27.2±2.7 nmol/min) of the O_2 consumption measured (Fig. 1, $P < 0.02$). One explanation for these data was that gonococcal O_2 consumption increased as a consequence of interaction with neutrophils and this possibility was examined.

Stimulation of gonococcal oxygen metabolism by the undifferentiated HL-60 promyelocytic cell line. To eliminate neutrophil O_2 consumption and the impact of KCN on this process, experiments were repeated using undifferentiated HL-60 cells. In their undifferentiated state, HL-60 cells are unable to

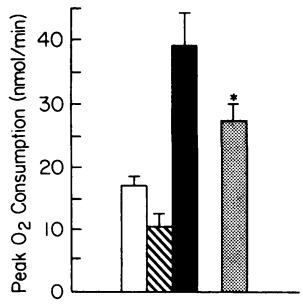

Figure 1. Maximal O_2 consumption rates (mean±SEM) observed following the incubation of *N. gonorrhoeae* (10^9/ml) alone (□) or in the presence of human neutrophils (5×10^6/ml) with (▨) and without (■) 1 mM KCN ($n = 5$). Since KCN inhibits gonococcal O_2 metabolism (but minimally effects that of stimulated neutrophils), experiments done in the presence of KCN presumably reflect gonococcal stimulation of neutrophil O_2 consumption. The sum of the apparent individual rates of gonococcal and stimulated neutrophil O_2 consumption (□) failed to account for the magnitude of O_2 consumption observed for the gonococcal/neutrophil mixture (*$P < 0.02$).

1. *Abbreviations used in this paper:* DMEM, Dulbecco's minimal essential medium; LDH, lactate dehydrogenase; PMA, phorbol myristate acetate.

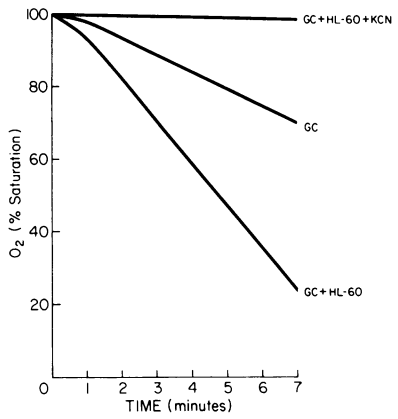

Figure 2. Representative (n = 6) Clark electrode tracing showing the marked increase in gonococcal (10^9/ml) O_2 consumption following exposure to undifferentiated HL-60 cells (5 × 10^6/ml). Essentially no O_2 consumption was observed when 1 mM KCN was added to the HL-60/GC mixture. HL-60 cells incubated in the absence of gonococci yielded curves identical with that seen for KCN (data not shown).

respond to membrane stimuli with a "respiratory burst" (Fig. 2, 22). As shown in Fig. 2, the O_2 consumption rate of gonococci (10^9/ml) suspended in HBSS increased more than twofold with the addition of undifferentiated HL-60 cells (5 × 10^6/ml). Gonococci and HL-60 cells incubated in the presence of 1 mM KCN or HL-60 cells in the absence of bacteria consumed no O_2 (Fig. 2).

Relationship between adherence to myeloid cells and stimulation of gonococcal metabolism. It seemed possible that adherence of the bacteria to the myeloid membrane provided the stimulus for enhanced gonococcal O_2 metabolism. Expression of pili (19, 20) or colony opacity protein II (P.II; 26) by *N. gonorrhoeae* has been suggested to play an important role in gonococcal adherence to neutrophils (2, 16). Piliated and nonpiliated opaque colony variants of strain F62 were radiolabeled with [^{14}C]adenine and their association with HL-60 cells determined. The piliated variant exhibited a much greater association with HL-60 cells (Fig. 3 *A*). In spite of this difference

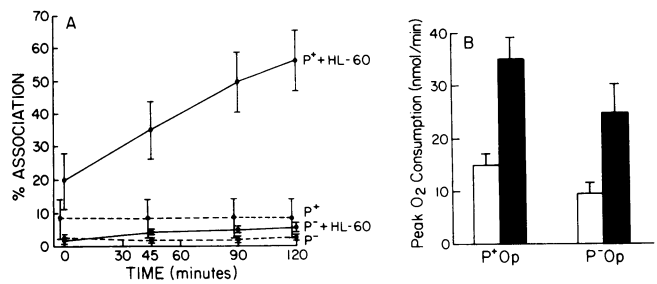

Figure 3. Effect of gonococcal pilus expression (P^+) or nonexpression (P^-) on the percentage of the initial inoculum of ^{14}C-labeled GC that associated with HL-60 cells over time (mean±SEM, n = 3). Incubation mixtures consisted of gonococci (5 × 10^7/ml) and HL-60 cells (2.5 × 10^6/ml) in 0.6% BSA. Controls were obtained in the absence of HL-60 cells for each gonococcal phenotype to assess nonspecific association. Each of the two phenotypic variants exhibited an opaque colony morphology. (*B*) Maximal O_2 consumption rates (mean±SEM, n = 4) observed after the incubation of the piliated-opaque and nonpiliated opaque gonococcal strains (10^9/ml) used in Fig. 3 *A* alone (□) or in the presence (■) of undifferentiated HL-60 cells (5 × 10^6/ml). Although the O_2 consumption rates of the two phenotypes vary when suspended in HBSS, the magnitude of increase is similar in the presence of HL-60 cells. P^+, piliated, P^-, nonpiliated, Op, opaque.

in adherence, the two phenotypes exhibited insignificant differences in metabolic stimulation in response to HL-60 cells (Fig. 3 *B*).

Release of a metabolic stimulatory factor by myeloid cells. Since phagocyte adherence did not appear necessary for stimulation of gonococcal O_2 consumption, it seemed likely that the myeloid cells released a stimulatory factor into the supernatant. Neutrophils (or HL-60 cells) were washed, resuspended in fresh HBSS (5 × 10^6/ml) at 37°C, and the effect of cell free supernatant on gonococcal O_2 consumption determined (Table I). After 30 min of neutrophil incubation, stimulatory activity was demonstrable in the buffer. Activity continued to increase over the 120-min incubation period. Similar results were obtained using HL-60 cells (data not shown).

DMEM-F12 lacking FCS also acquired the capacity to stimulate gonococcal O_2 metabolism after supporting HL-60 growth for 3 d (Table I). Placement of this material in 1,000-mol wt membrane exclusion tubing followed by overnight dialysis against HBSS eliminated its stimulatory capacity (Table I). We had previously noted that a serum factor(s) that stimulated gonococcal metabolism was removed following the same dialysis protocol (4), suggesting a relationship between the two factors.

Purification and comparison of the cell- and serum-derived stimulatory factor(s). To further characterize and compare

Table I. Effect of Neutrophil Cell-Free Supernatants on the Rate of N. gonorrhoeae O_2 Consumption

Buffer system	Maximal oxygen consumption rate
	nmol/min
HBSS (Control)	25.0±2.5
Neutrophil supernatant	
37° × 0′	22.4±2.1
37° × 30′	39.1±4.9*
37° × 120′	52.8±7.0‡
4° × 120′	24.9±2.5
37° × 120′ + cycloheximide	52.2±8.7§
37° × 120′ + NaF	29.9±4.6
Freeze/Thaw	25.5±2.6
DMEM-F12	59.4±5.6‡

Maximal rates of O_2 consumption (mean±SEM, n = 3–5) of *N. gonorrhoeae* (10^9/ml) suspended in HBSS in the absence (control) or presence of cell-free supernatant obtained after neutrophil incubation (5 × 10^6/ml) in HBSS at 37° or 4°C for varying time periods. For some experiments neutrophil incubation was performed in the presence of NaF (10 mM), cycloheximide (25 mM), or neutrophil suspensions were subjected to repetitive freeze-thawing to achieve cell lysis. Also shown is the rate of gonococcal O_2 consumption in the presence of 10% (vol/vol) serum-free DMEM-F12 that had supported growth of HL-60 cells for 3 d. Gonococcal O_2 consumption was not different from control when exposed to the same DMEM-F12 that was subsequently dialyzed in 1,000-mol wt membrane exclusion tubing against HBSS or when exposed to DMEM-F12 that had not supported HL-60 growth.

Statistically significant increase in gonococcal O_2 consumption relative to control.

* $P < 0.02$.

‡ $P < 0.01$.

§ $P < 0.05$.

these cell- and serum-derived stimulatory factor(s), serum DMEM-F12 (FCS-free), which had supported HL-60 growth, and HBSS in which neutrophils had incubated (120 min, 37°C) were applied to a G-15 Sephadex column and eluted with water. Fractions that exhibited the capacity to increase gonococcal O_2 consumption were similar for serum, DMEM-F12, and HBSS, eluting immediately before salt (Fig. 4). G-15 fractions possessing stimulatory capacity were in turn applied to a DEAE column and eluted with .015 M Tris buffer (pH 8.2) containing increasing NaCl concentrations or to an HPLC C-18 column. Regardless of the original source of the material, stimulatory activity eluted from each of these columns in the first several fractions, indicating failure to adhere. These data suggested that the serum- and cell-derived stimulatory factors were the same or closely related and are consistent (although not specific) for a small organic acid.

Identification of the stimulatory factor. Accumulation of a stimulatory factor in supernatant following myeloid cell incubation could arise by a number of mechanisms. First it could leak or be intentionally released by the phagocyte from an intracellular storage pool. To address this possibility, neutrophils were suspended in fresh HBSS and immediately disrupted by repetitive freezing and thawing. The resulting supernatant failed demonstrate stimulatory activity (Table I). Factor accumulation could also result from the extracellular release of a product of phagocyte metabolism. Cellular metabolism is markedly reduced at 4°C and no stimulatory activity was detectable in cell-free supernatant derived from cells incubated 120 min at this temperature (Table I). Cycloheximide, an inhibitor of protein synthesis, had no impact on factor accumulation (Table I). On the other hand, NaF, an inhibitor of unstimulated neutrophil glucose metabolism, reduced factor recovery (Table I). At the concentration employed, NaF had no effect on gonococcal O_2 consumption (data not shown). Supernatant activity also decreased markedly if neutrophils or HL-60 cells were suspended in a glucose free buffer (data not shown).

These results suggested that glucose catabolism was intimately involved in factor production and/or release. Almost all glucose metabolized by unstimulated neutrophils is via the Embden-Meyerhoff pathway (27, 28). Stimulation of the neutrophil respiratory burst leads to a marked increase in glucose utilization, almost all of which enters the hexose monophosphate shunt (25, 29). Since the factor accumulated in the absence of neutrophil stimulation (and was not enhanced by it, Table I) attention was focused on the Embden-Meyerhoff pathway. Approximately 85% of glucose consumed by resting neutrophils is converted to lactate (28), suggesting this compound might accumulate over time. As shown in Fig. 5, lactate markedly increased O_2 consumption rates of gonococci suspended in HBSS. Stimulation was detectable at a (L+)-lactate concentration of 0.5 mg/dl, with maximal activity at 2.0 mg/dl. L(+)-lactate exhibited somewhat greater activity than the D(−) isomer (Fig. 5).

Lactate was detectable in cell free neutrophil supernatants and increased in proportion to duration of incubation (Fig. 6). Lactate levels closely paralleled the ability of the cell free supernatants to stimulate gonococcal O_2 consumption (Table I). Minimal lactate was detected in supernatants derived from neutrophils incubated at 4°C or in the presence of NaF (Fig. 6). The presence of metabolically active gonococci depleted lactate levels after only 30 min of incubation. The elution profile of lactate applied to G-15 was identical to those described earlier for the serum and cell-derived stimulatory fac-

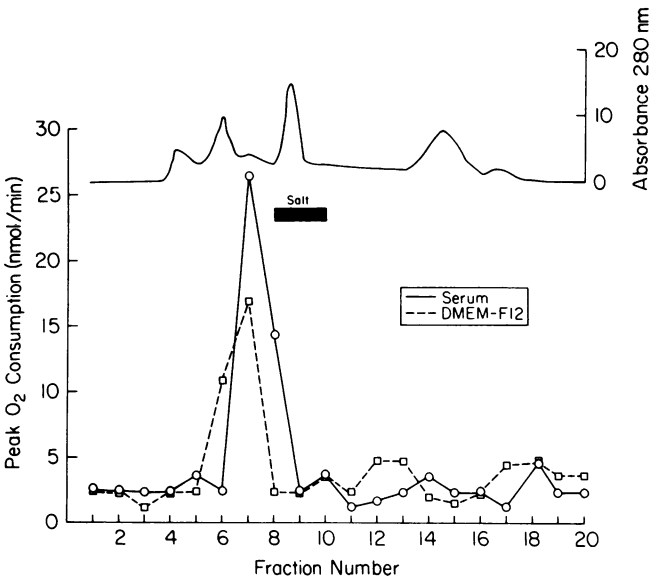

Figure 4. Representative demonstration (n = 3–5) of the ability of G-15 fractions of serum and DMEM-F12 (FCS free) that had supported HL-60 growth to stimulate gonococcal O_2 consumption. The left vertical axis shows the maximal O_2 consumption observed as a result of the metabolic activity of gonococci (10^9/ml) exposed to an 80% (vol/vol) concentration of the column fraction noted by number on the horizontal axis. Separate tracings are shown for serum and DMEM-F12. Also indicated are the fractions containing salt. The top of the figure shows the 280-nm absorbance tracing for the same DMEM-F12 fractions. A similar elution profile was obtained with G-15 fractions obtained following application of cell-free HBSS supernatant derived following incubation of neutrophils (5×10^6/ml) at 37°C for 120 min.

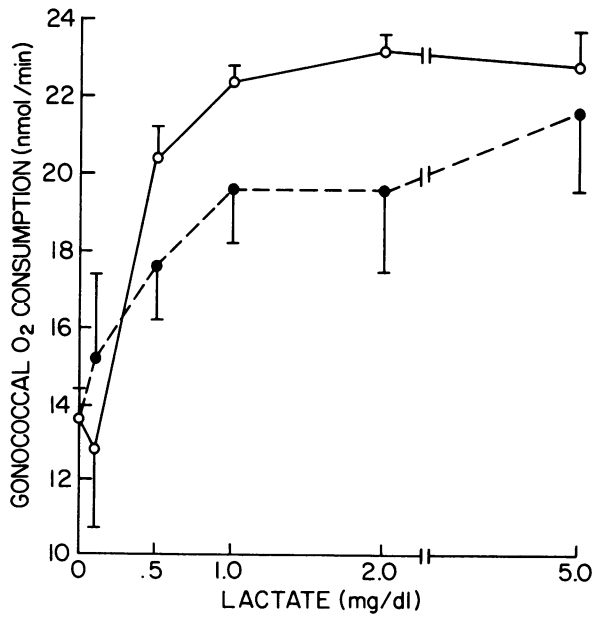

Figure 5. Dose-response curves of the effect of L(+) (——) and D(−) (– – –) isomers of lactic acid on the maximal O_2 consumption rate of gonococci (10^9/ml) suspended in HBSS (n = 3).

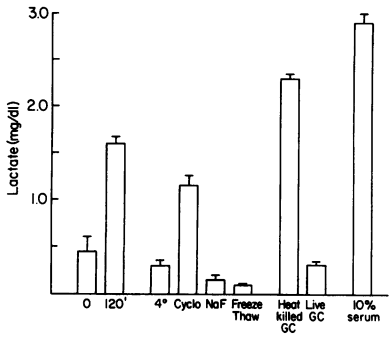

Figure 6. Lactate concentration (mean±SEM, single experiment performed in triplicate) of cell-free HBSS in which neutrophils (5×10^6/ml) had been incubated under the conditions noted. 0 = immediately repelleted; 120′ = 37°C × 120 min; 4° = 4°C × 120 min; Cyclo = 37°C × 120 min in the presence of cycloheximide (25 mM); NaF = 37°C × 120 min in the presence of NaF (10 mM); Freeze thaw, neutrophils immediately frozen at −70°C and lysed by repetitive freezing and thawing; heat killed GC, incubated in the presence of heat killed gonococci (10^8/ml) × 120 min at 37°C; Live GC, incubated at 37°C × 120 min and for the final 30 min of incubation live gonococci (10^8/ml) were also present. Also shown for comparison is the lactate concentration of 10% normal pooled human serum.

tor(s) (data not shown). G-15 fractions derived from HL-60 supernatant and serum that possessed stimulatory activity contained 4 and 10 mg/dl of lactate, respectively. Lactate also partially restored the capacity of dialyzed serum to stimulate gonococcal O_2 consumption. Dialyzed serum (10% vol/vol) to which L-lactate (2 mg/dl) had been added increased the O_2 consumption rate of *N. gonorrhoeae* from 12.8±0.8 to 30.5±4.3 nmol/min ($n = 3$, $P < 0.025$). However, this increase was significantly less than that with 10% normal serum that stimulated a gonococcal O_2 consumption rate of 40.0±3.8 nmol/min ($P < 0.05$).

Discussion

O_2 consumption resulting from neutrophil stimulation by metabolically active *N. gonorrhoeae* was significantly greater than could be accounted for by the apparent contributions of the two components (Fig. 1, 17). These observations were unexpected since inhibition of bacterial O_2 consumption has been utilized to assess the antibacterial activity of the Cl^--H_2O_2-myeloperoxidase system (30).

Several possibilities could have accounted for the observation. Neutrophil stimulation by heat killed bacteria (e.g., *Pseudomonas aeruginosa*) is only one-third of that with live organisms (31), suggesting that "metabolically inactive" (KCN treated) gonococci represent an inadequate stimulus. In fact, we have noted a 50% decrease in gonococcal adherence to neutrophils in the presence of KCN (32). However, maximal O_2 consumption resulting from neutrophil stimulation with phorbol myristate acetate (PMA, 100 ng/ml) was similar to that with KCN-treated gonococci.

KCN has been reported to either decrease (25) or increase (24) stimulated neutrophil O_2 consumption. We have noted that KCN slightly increases neutrophil O_2 consumption in response to PMA, but decreases it when opsonized zymosan is the stimulus (Thompson, B., G. Sivam, B. E. Britigan, G. M. Rosen, and M. S. Cohen. In press.). The small magnitude of this KCN effect (4.2 nmol/min with PMA) would not account for our results.

Our data suggest that gonococcal O_2 consumption increased as a consequence of interaction with neutrophils. Consistent with this hypothesis, gonococcal O_2 consumption increased two- to threefold following exposure to undifferentiated HL-60 cells. Subsequent data revealed that gonococcal metabolic stimulation resulted from release of a factor from the myeloid cells. Factor production and/or release was dependent on myeloid glucose metabolism, suggesting that lactate (the principal product of this process) could be responsible. We have shown that lactate accumulates extracellularly as a consequence of neutrophil glucose catabolism and the magnitude observed is consistent with earlier studies assessing total lactate formation by unstimulated neutrophils (33, 34).

Lactate concentrations present in serum and phagocyte-derived supernatant stimulated gonococcal O_2 consumption. Chromatographic and other data suggest lactate is responsible for both phagocyte- and serum-mediated stimulation of gonococcal metabolism. However, gonococcal stimulation in response to lactate was less than that with serum (4, 15, 17), suggesting other factor(s) may contribute to the serum-mediated effect (4). Previously, we were unable to restore stimulatory activity to dialyzed serum by simultaneous addition of lactate, cysteine, and pyruvate. The latter compound inhibits lactate oxidation by some organisms (35) and may have masked lactate stimulation of gonococcal O_2 consumption.

The ability of *N. gonorrhoeae* to utilize lactate as a substrate for electron transport has been appreciated for more than 50 years (36, 37). The organism possesses an electron transport linked (NAD-independent) L(+) and D(−) lactate dehydrogenase (LDH) associated with the cytoplasmic membrane (1, 38, 39) as well as a cytosolic NAD-dependent LDH (1). Since lactate derived from mammalian sources is L(+) (40), a gonococcal L(+) LDH is most important in the metabolic responses observed.

Studies of membrane vesicles derived from *Escherichia coli* and *S. aureus* have shown that electron transport-linked LDH plays an integral role in active transport of amino acids (41, 42), sugars (43), and other metabolic substrates (44). The importance of lactate to gonococcal growth and metabolism has received some attention (1, 45–47). We have demonstrated that serum (i.e., lactate) increases gonococcal glucose transport and adenine incorporation (4, 17) suggesting a role for lactate oxidation in gonococcal acquisition of critical substrates.

It is likely that lactate is available in high concentrations in vivo at sites important in gonococcal infection. In vaginal secretions lactate ranges from 0 to 5.0 mg/g secretion, varying with menstrual cycle (48). Gonococcal lactate utilization in vivo has been shown using a guinea pig subcutaneous chamber model (46).

In vitro gonococci decrease neutrophil formation of reactive oxygen intermediates (15) by competing effectively for molecular O_2. Maintenance of this inhibition depends on ongoing bacterial metabolism (15). Within a phagosome, gonococcal access to most metabolic substrates (including mucosal or serum lactate) would be limited. However, lactate derived from neutrophil metabolism is likely to accumulate in the phagosome (34). Gonococcal LDH activity persists over the range of pH encountered in the phagosome (38, 49, 50). The location of the enzyme(s) responsible (1, 38, 39) and its resistance to chemical and temperature stress (36, 37) suggest it could remain active after phagocytosis.

Gonococci are susceptible in vitro to a variety of (O_2 independent) neutrophil granule components (51–53). However,

killing of *N. gonorrhoeae* by acid extracts of neutrophil leukocyte granules and a purified 57K granule protein is greatly reduced under anaerobic conditions (53). Gonococcal depletion of phagosomal O_2 may enhance the organism's resistance to both oxygen-independent and oxygen-dependent killing mechanisms. Many human pathogens possess LDHs capable of utilizing L(+) lactate (35). Serum stimulates *S. aureus* and *E. coli* O_2 consumption and enhances inhibition of neutrophil oxygen reduction (15). This suggests bacterial LDH may be important in the pathogenesis of infection with a number of organisms, and work to explore this hypothesis is ongoing.

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