ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1980, p. 281-288
0066-4804/80/08-0281/08$02.00/0

Vol. 18, No. 2

Effects of Steroid Hormones on *Neisseria gonorrhoeae*

PAUL G. LYSKO AND STEPHEN A. MORSE*

Department of Microbiology and Immunology, University of Oregon Health Sciences Center, Portland, Oregon 97201

Various steroids were tested for their effects upon gonococcal O_2 consumption and glucose catabolism. The ability to inhibit gonococcal O_2 uptake appeared to be related to the molecular configuration of the steroid. The presence of lipophilic groups enhanced inhibition, whereas the addition of hydrophilic groups markedly diminished inhibition. Steroid inhibition decreased with an increasing number of polar groups. Glucose catabolism was inhibited by steroid hormones, and the degree of inhibition was influenced by pH and medium composition. Changes in growth medium and pH also resulted in differential steroid inhibition of O_2 uptake. Under certain conditions, lactate partially relieved this inhibition. Gonococci that were grown in one environment and shifted to a new environment were inhibited by steroids to the same extent as if they had been originally grown in the new environment. The differential effects of medium and pH upon steroid inhibition may be due to structural rearrangements involving membrane phase transitions or to altered receptor affinity.

The percentage of positive cultures obtained from females with gonorrhea varies with the day of the menstrual cycle (8, 10, 11). Maximum recovery occurs at menstruation, whereas minimum recovery coincides with the peak plasma progesterone levels seen during week 3 of the cycle. Plasma levels of β-estradiol are biphasic, with a peak at midcycle and a secondary peak during week 3 (24, 25).

Previous reports from our laboratory have indicated that *Neisseria gonorrhoeae* is unusual among gram-negative bacteria in that its growth is inhibited by progesterone (3, 18). Progesterone bound to both protein and lipid components of the cytoplasmic membrane, where it inhibited the activities of respiratory enzymes (14, 18).

This study investigates the inhibitory activities of various steroids and their derivatives on gonococcal respiration to ascertain the molecular characteristics necessary for inhibition. In addition, environmental parameters which might influence the outcome of steroid-cell interactions were examined.

MATERIALS AND METHODS

Organisms. *N. gonorrhoeae* CS-7 was used throughout this study. Isolation, characteristics, and maintenance of this strain have been described previously (16, 19). Strains 1384 and 2024 are auxotrophic for arginine, hypoxanthine, and uracil (AHU strains) and were isolated from disseminated infections (17). Strain F62, colony types 1 and 4, was obtained from the Neisseria Reference Laboratory, Public Health Service Hospital, Seattle, Wash.

Medium and growth conditions. Gonococci were grown in either a complex medium (CM; 19) lacking soluble starch or in a defined medium (DM) supplemented with hypoxanthine and uracil (17). Both CM and DM were buffered with 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and adjusted to the desired pH with NaOH or HCl. Liquid media were inoculated with cells obtained by suspending the growth on corresponding solid agar medium after incubation overnight at 37°C with increased CO_2 (4% CO_2). All liquid cultures were incubated at 37°C with shaking. Turbidity was measured at 540 nm with a Klett-Summerson colorimeter.

Chemicals and radioisotopes. Steroid hormones were obtained from Sigma Chemical Co., St. Louis, Mo. [1-^{14}C]glucose (specific activity, 9.6 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass. All other reagents were of analytical grade.

O_2 uptake. O_2 uptake was measured with a Yellow Springs Instrument Co. model 53 oxygen monitor attached to a circulating water bath adjusted to 37°C. Exponential-phase cultures of gonococci were diluted with fresh medium, sparged, and added to the electrode chambers at a cell density of ca. 2.0×10^8 in a volume of 4 ml. The number of cells was determined by visual counts with a Petroff-Hausser counting chamber. Diplococci were counted as two cells. When CM was used for diluent, the growth factor supplement (19) was omitted since its presence caused O_2 uptake. When DM was used for diluent, solution III was omitted. Each diluent contained 5 mM glucose.

When the rate of O_2 uptake was linear, steroids dissolved in absolute ethanol were added to the chambers to a final concentration of 40 μg per 5×10^7 cells. Inhibition was recorded as the percentage of the original rate of O_2 uptake 10 min after steroid addition. With β-estradiol diacetate, inhibition was biphasic and was recorded from the linear portion of each curve regardless of the time. The final concentration of

ethanol was <0.4% and did not inhibit growth or O_2 uptake.

The relative concentrations of O_2 in each medium at each pH were determined by comparison of air-saturated media to air-saturated 0.02 M sodium phosphate buffer (pH 7.4). The absolute concentration of O_2 in the phosphate buffer was determined by the method of Robinson and Cooper (23). Absolute concentrations of O_2 in the growth media could not be directly determined by this method. Relative O_2 concentrations in each medium were within 2% of each other.

Respiratory quotients (QO_2) were determined for cells under each set of conditions and are expressed as microliters of O_2 consumed per minute per 10^8 cells.

Radiorespirometry. Cultures were harvested during the exponential phase of growth (120 Klett units), centrifuged, and resuspended in the appropriate medium to a cell density of 5×10^8 cells per ml. The techniques for measuring CO_2 release from radiolabeled glucose have been previously described (19). Samples of respiratory $^{14}CO_2$ were collected every 10 min for 80 min. At 30 min, steroid hormones dissolved in absolute ethanol were added to the cell suspensions to a concentration of 60 μg per 5×10^8 cells. An identical amount of ethanol was added to a cell suspension not receiving hormones.

RESULTS

Effect of various steroids on O_2 uptake. A spectrum of inhibitory activity was observed when various steroids were tested for their ability to inhibit O_2 uptake of exponential-phase gonococci (Table 1). Among the steroid hormones, the estrane derivatives exhibited the least inhibition. Notable exceptions were ethynyl estradiol and its 3-methyl ether, norethynodrel, and norethindrone acetate, all of which are used as estrogenic components of birth control pills. Norethindrone acetate possesses both an acetyl chain and an α-ethynyl group (—C≡CH) at C-17 (Fig. 1). Norethynodrel and ethynyl estradiol and its 3-methyl ether, which possess the α-ethynyl group but lack the acetyl group, were far less inhibitory toward gonococci than was norethindrone acetate. β-Estradiol diacetate was unusual in that its inhibition was biphasic; the dipropionate derivative did not exhibit biphasic inhibition.

Progesterone was the most inhibitory pregnane derivative tested. The addition of hydroxyl or acetoxy groups at C-11 or C-17 reduced the inhibition. The addition of a methyl group at C-6 and an acetoxy group at C-17 (medroxyprogesterone acetate) completely abolished inhibition. 5β-Pregnan-3,20-dione, which closely resembles progesterone, lacking only the double bond between C-4 and C-5, was half as inhibitory as progesterone. Pregnenolone, an intermediate in progesterone synthesis, and its acetate derivative exhibited no inhibitory effects. The adrenocorticosteroids tested were not very inhibitory. Cortisone and hydrocortisone had no effect on O_2 uptake. Corticosterone and deoxycorticosterone were only slightly inhibitory, which is interesting since deoxycorticosterone only differs from progesterone by the presence of a hydroxyl group at C-21.

Inhibition by testosterone was enhanced by the addition of acetate or propionate groups; the hemisuccinate form was not very inhibitory. The C-19 methyl group was apparently important for inhibition, since 19-nortestosterone did not inhibit O_2 uptake. Androstandione, androstendiol, and dehydroisoandrosterone, which lack the C-4,5 double bond and have altered polarity, were less inhibitory.

Cholesterol, the only sterol tested, had essentially no inhibitory effect. Diethylstilbestrol, a synthetic estrogen, was markedly inhibitory and was the most inhibitory compound tested.

TABLE 1. *Effect of various steroids on O_2 uptake of N. gonorrhoeae CS-7*[a]

Steroid	% Original O_2 uptake[b]
Estrane derivatives	
β-Estradiol	81
α-Estradiol	85
β-Estradiol diacetate	56–94[c]
β-Estradiol dipropionate	96
Ethynyl estradiol	71
Ethynyl estradiol-3-methyl ether	47
Norethynodrel	72
Norethindrone acetate	14
Pregnane derivatives	
Progesterone	25
11α-Hydroxyprogesterone	81
17α-Hydroxyprogesterone	95
11α-Acetoxyprogesterone	77
5β-Pregnan-3,20-dione	54
Pregnenolone	100
Pregnenolone acetate	98
Medroxyprogesterone acetate	100
Cortisone	100
Hydrocortisone	100
Corticosterone	81
Deoxycorticosterone	88
Androstane derivatives	
Testosterone	64
Testosterone acetate	15
Testosterone propionate	19
Testosterone-17β-hemisuccinate	86
19-Nortestosterone	96
Androstandione	84
Androstendiol	79
Dehydroisoandrosterone	84
Miscellaneous	
Cholesterol	95
Diethylstilbestrol	4

[a] Gonococci were grown in a complex liquid medium (pH 7.2) with glucose at 37°C with shaking. Cells were diluted with fresh medium to approximately 5×10^7 cells per ml. Steroids were added to a final concentration of 40 μg per 5×10^7 cells.

[b] Determined from the linear rate 10 min after steroid addition.

[c] Inhibition was biphasic.

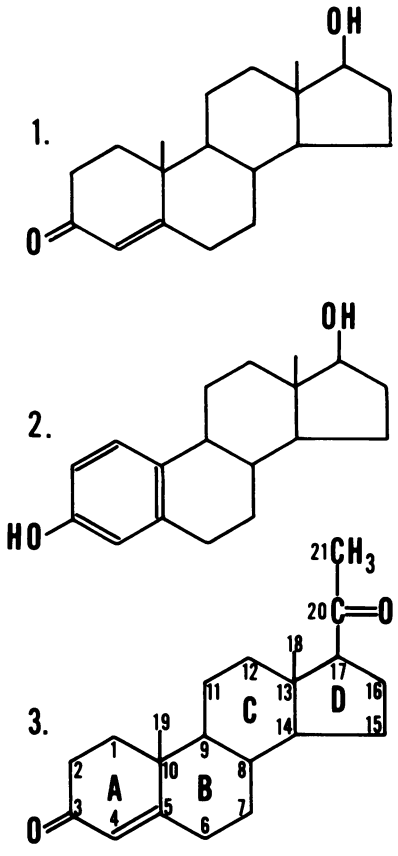

FIG. 1. *Structures of some common steroid hormones: (1) Δ^4-androsten-17β-ol-3-one (testosterone); (2) $\Delta^{1,3,5,(10)}$-estratriene-3,17β-diol (estradiol); (3) Δ^4-pregnen-3,20-dione (progesterone). The ring designations of progesterone are indicated by capital letters. The carbon atoms are numbered. Carbons at C-18 and C-19 are methyl groups in a β-configuration which rise toward the viewer. Groups with an α-designation recede into the page.*

Inhibition of glucose metabolism. Selected steroid hormones were tested for their ability to inhibit the release of $^{14}CO_2$ from [1-^{14}C]glucose by gonococci grown in two types of media at three different pH values. The rates of $^{14}CO_2$ production were lower in DM than in CM at each pH examined. Figure 2 shows that, when gonococci were grown in CM at pH 7.2, glucose catabolism was inhibited in an increasing order of effectiveness by β-estradiol diacetate, progesterone, and testosterone acetate. Increasing the pH to 8.0 decreased the inhibition, whereas decreasing the pH to 6.2 increased the inhibition. Increasing the pH of DM from 7.2 to 8.0, or decreasing the pH to 6.2, reduced the rates of $^{14}CO_2$ production by untreated cells. However, when gonococci were grown in DM at pH 7.2, inhibition by steroids was not as marked as in CM; in fact, β-estradiol diacetate did not inhibit $^{14}CO_2$ release at all at pH 7.2 or 8.0. When grown in DM, gonococci were inhibited by β-estradiol diacetate only at pH 6.2. Contrary to the results obtained in CM, inhibition by progesterone and testosterone acetate was greater for cells grown in DM at pH 8.0 than at pH 7.2. Inhibition by these two steroids was greatest when gonococci were grown in DM at pH 6.2.

Effects of medium and pH on steroid inhibition of O_2 uptake. Changes in the growth medium and pH resulted in differential effects of steroid hormones on O_2 uptake by exponential-phase gonococci (Table 2). Gonococci grown in CM were inhibited least at pH 6.2; this inhibition was partially reversed by the addition of 5 mM L-lactate. Gonococci grown in CM at either pH 7.2 or 8.0 were inhibited to the same extent by the testosterones and progesterones. The estradiols were more inhibitory at pH 8.0. L-Lactate did not increase O_2 uptake of either treated or untreated cells grown at pH 7.2 or 8.0 in CM. When L-lactate was added to untreated cells at pH 6.2, the rate of O_2 uptake increased by 14%. When L-lactate was added to steroid-treated gonococci at pH 6.2, the rate of O_2 uptake increased to the same extent as the untreated control. This effect was also seen with gonococci grown in DM.

The O_2 uptake of gonococci grown in DM was inhibited to a greater extent at pH 6.2 and 8.0 than at pH 7.2. With the exception of testosterone acetate, little inhibition was evident at pH 7.2. In general, inhibition was greatest at pH 6.2, exactly the opposite result from that of cells grown in CM. As seen with CM, the inhibition of β-estradiol diacetate was greatest at pH 8.0 and was generally less than that observed with progesterone or testosterone acetate. Overall, the inhibition of O_2 uptake of DM-grown gonococci was less than that of CM-grown cells at each pH tested. The addition of 5 mM L-lactate increased the O_2 uptake of DM-grown cells at each pH; the extent of lactate oxidation increased with a decrease in pH.

The decreased inhibition of O_2 uptake with DM-grown cells was not due to a dilution effect of steroid action by an increased content of respiratory components. Table 3 shows that the QO_2 of DM-grown cells was less than that of CM-grown cells and that it decreased with pH. However, since inhibition of DM-grown cells was greater at pH 6.2, and inhibition of CM-grown cells was least at pH 6.2, no correlation of QO_2 with steroid inhibition could be made.

Effect of environmental shift. Gonococci were grown in one environment and shifted to a new environment to determine whether the dif-

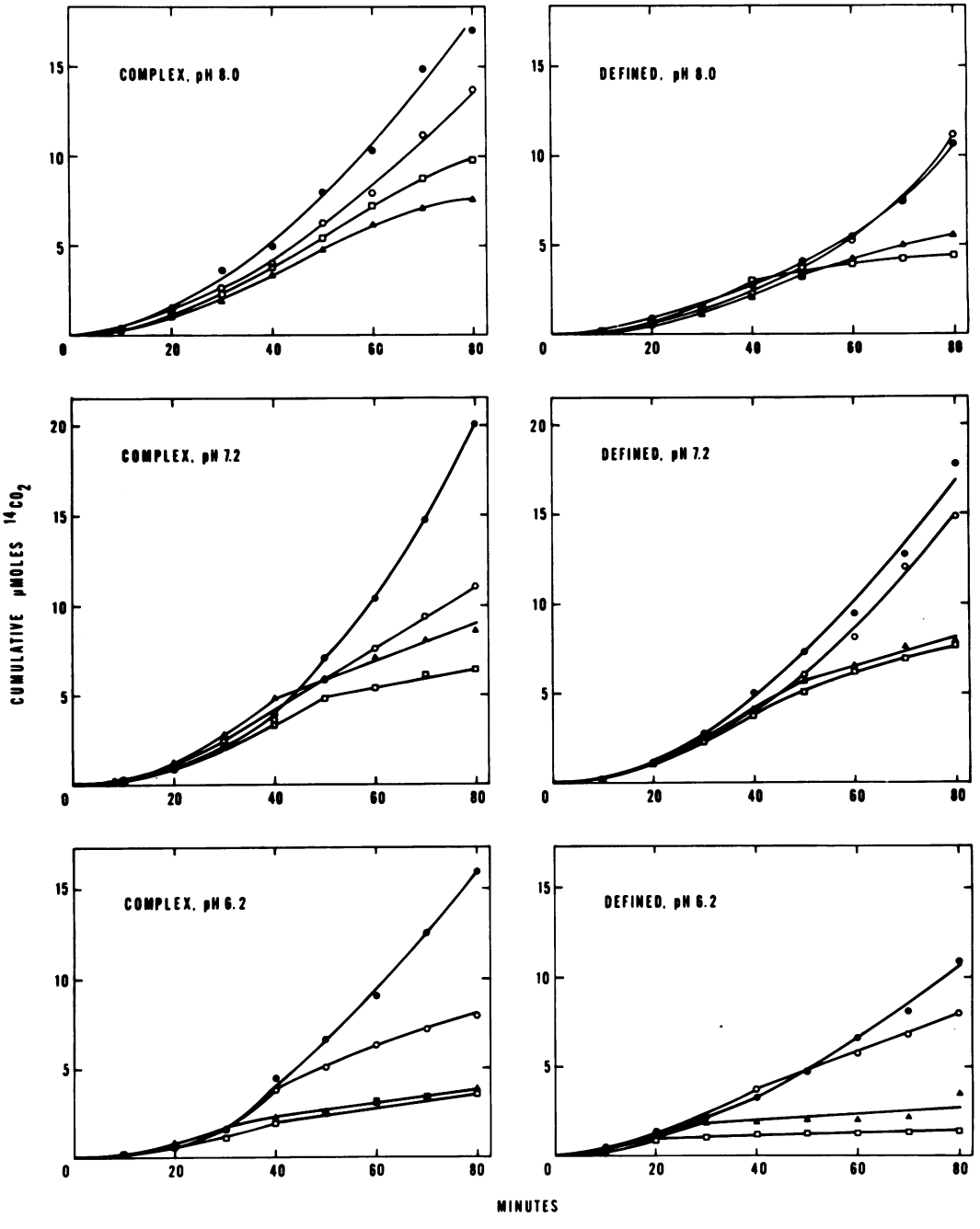

FIG. 2. *Inhibition by steroids of the utilization of glucose by N. gonorrhoeae strain CS-7 while growing under different conditions of medium and pH. Steroids were added after 30 min to a concentration of 60 μg per 5 × 10⁸ cells. Symbols:* (●) *flasks receiving only absolute ethanol, no steroids;* (○) *flasks receiving β-estradiol diacetate;* (△) *flasks receiving progesterone;* (□) *flasks receiving testosterone acetate.*

ferential effects of steroid hormones upon O_2 uptake were due to phenotypic modification. The results are shown in Table 4. β-Estradiol diacetate was used because the biphasic nature of its inhibition gave two points of reference. When gonococci were grown under one set of conditions and shifted to either a different growth medium or a different pH, the degree of

TABLE 2. *Differential effects of steroid hormones at three pH values in two types of growth media*[a]

Medium	Steroid	% of original oxygen uptake					
		pH 8.0		pH 7.2		pH 6.2	
		After 40 μg/ 5 × 10^7 cells	After 5 mM L-lactate[b]	After 40 μg/ 5 × 10^7 cells	After 5 mM L-lactate	After 40 μg/5 × 10^7 cells	After 5 mM L-lactate
CM	None	100	NE[c]	100	NE	100	114
	β-Estradiol	69	NE	81	NE	91	104 (114)[d]
	β-Estradiol diacetate	13–46[e]	NE	50–89	NE	58–88	96 (109)
	Progesterone	30	NE	27	NE	63	72 (114)
	11α-Hydroxy-progesterone	80	NE	81	NE	81	98 (121)
	Testosterone	67	NE	64	NE	89	102 (115)
	Testosterone acetate	17	NE	15	NE	27	33 (122)
DM	None	100	150	100	162	100	180
	β-Estradiol	98	136 (139)	108	189 (175)	74	138 (186)
	β-Estradiol diacetate	27–64	76 (119)	87–108	173 (160)	72–89	136 (153)
	Progesterone	63	69 (110)	91	104 (114)	55	105 (191)
	11α-Hydroxy-progesterone	98	138 (141)	96	161 (168)	56	136 (243)
	Testosterone	98	143 (146)	95	139 (146)	86	133 (155)
	Testosterone acetate	23	27 (117)	66	99 (150)	48	90 (188)

[a] Results represent the average of at least two separate determinations.
[b] Added to the same electrode chamber 15 min after steroid addition.
[c] NE, No effect.
[d] Percentage of the inhibited rate is given in parentheses.
[e] Inhibition by β-estradiol diacetate was biphasic.

TABLE 3. *Growth of N. gonorrhoeae strains at three different pH values in two types of growth media*[a]

Strain	Medium	pH	QO_2[b]	Generation time (h)
CS-7	CM	8.0	0.402	1.5
CS-7	CM	7.2	0.358	1.5
CS-7	CM	6.2	0.312	2.25
CS-7	DM	8.0	0.286	1.24
CS-7	DM	7.2	0.232	1.25
CS-7	DM	6.2	0.186	2.5
F62 (T1)	CM	7.2	1.65	2.25
F62 (T4)	CM	7.2	0.820	1.25
1384	CM	7.2	0.394	1.9
2024	CM	7.2	0.340	1.4

[a] Values represent averages of at least two determinations.
[b] Expressed as microliters of O_2 consumed per minute per 10^8 cells.

steroid inhibition was as if the cells had been grown in the new environment. The best correlation was observed when the medium remained constant and the pH was shifted.

Effects of steroids on various strains of *N. gonorrhoeae*. All previous results were obtained with strain CS-7. We examined other strains to determine if the inhibition of O_2 uptake by steroid hormones was a strain-related phenomenon. The data presented in Table 5 indicated that there was strain variation in susceptibility to steroid inhibition. Within the same strain, there appeared to be some variation between colony types, as shown for strain F62. However, this observation was hampered by the tendency of cells from T1 colonies to clump, thereby lending inaccuracy to Petroff-Hausser cell counts. Experiments with the T1 cells were standardized by adding steroids relative to the rates of original O_2 uptake, and not solely relative to the cell number counted. It is quite possible that the number of cells present was underestimated, resulting in an erroneously high QO_2 for T1 cells (Table 3). If this were true, the steroid-to-cell number ratio would be lower for T1 than for T4 cells. This would mean that T1 cells are more susceptible to steroid inhibition than T4 cells.

All of the strains listed in Table 5 were inhibited to a much greater extent with β-estradiol diacetate and to a lesser extent by progesterone than was strain CS-7. Between the AHU strains, strain 2024 was much more susceptible to β-estradiol diacetate, whereas inhibition of O_2 uptake by the other steroids was comparable.

TABLE 4. *Effects of β-estradiol diacetate on gonococci grown and diluted into the same or a different medium*

Diluted into the same medium		Diluted into different medium or pH[a]		
Growth medium, pH	% Original O_2 uptake	Growth medium, pH	Dilution medium, pH	% Original O_2 uptake
CM, 8.0	13–46[b]	DM, 8.0	CM, 8.0	9–43
CM, 7.2	50–89	DM, 7.2	CM, 7.2	23–66
CM, 6.2	58–88	CM, 8.0	CM, 6.2	57–88
DM, 8.0	27–64	DM, 6.2	DM, 8.0	20–65
DM, 7.2	87–108	CM, 7.2	DM, 7.2	99–112
DM, 6.2	72–89	DM, 7.2	DM, 6.2	75–85

[a] Dilutions were at least 1:20 from an exponential-phase culture.
[b] Inhibition was biphasic.

TABLE 5. *Effects of steroid hormones on different strains of N. gonorrhoeae*[a]

Steroid[b]	% Original O_2 uptake			
	AHU 1384	AHU 2024	F62 (T1)	F62 (T4)
β-Estradiol	89	85	75	70
β-Estradiol diacetate	16–50[c]	6–13	2–21	10–33
Progesterone	45	40	49	56
11α-Hydroxyprogesterone	100	82	98	80
Testosterone	78	82	81	81
Testosterone acetate	18	19	17	15

[a] Cells were grown and tested in CM, pH 7.2.
[b] Steroids were added to a final concentration of 40 μg per 5×10^7 cells.
[c] Inhibition was biphasic.

DISCUSSION

The inhibition of O_2 uptake appears to be related to the molecular configuration of the steroid (see Fig. 1). The progesterone molecule loses inhibitory activity when its planarity and polarity are altered. The *trans* arrangement of the A-B ring junction helps the molecule to form a flat plane; the presence of a 5β-hydrogen results in a *cis* arrangement. The latter produces an altered conformation of the A-ring, destroying planarity and apparently modifying the mode of action, as seen with 5β-pregnan-3,20-dione. The addition of various groups at C-6 or C-11 apparently causes steric interference (26), resulting in diminished inhibitory activity in our system as well as in a mitochondrial system (26). Similar structural requirements have been found to be important for such diverse biological functions as sterol metabolism in yeast (20), estrogenicity, and androgenicity (20) and for steroid inhibition of reduced nicotinamide adenine dinucleotide oxidase activity of heart muscle mitochondria (26). The latter has an electron transport system similar to that of the gonococcus (29).

The increase in polarity resulting from the addition of hydroxyl groups at C-11, -17, or -21 may account for the lack of inhibitory activity of the adrenocorticosteroids. According to the polarity rule, the binding affinity between a protein and a steroid decreases with an increasing number of polar groups (28). Similarly, the alterations in polarity among testosterone derivatives substituted at C-3 and C-17 may account in part for the differences in activity seen here and with heart muscle sarcosomal fragments (26).

Progesterone was the best inhibitor among the naturally occurring steroid hormones tested for their ability to inhibit gonococcal O_2 uptake. The steroid hormone derivatives that had the greatest inhibitory effect were norethindrone acetate, testosterone acetate, and testosterone propionate. What these molecules have in common is the presence of less polar, more lipophilic groups in the β position at C-17: —CO—CH_3 for progesterone, —O—CO—CH_3 for norethindrone acetate and testosterone acetate, and —O—CO—CH_2—CH_3 for testosterone propionate. The more hydrophobic acetate and diacetate derivatives have also been found to be more inhibitory for mitochondrial reduced nicotinamide adenine dinucleotide oxidase (26). Previous studies have shown that bound progesterone associates with protein- and lipid-containing cell fractions of *N. gonorrhoeae* (14, 18). Thus, the presence of these additional lipophilic groups would augment the transmembrane diffusion of steroid molecules through the hydrocarbon interior of the outer membrane (21).

Glucose catabolism was inhibited by lower concentrations of steroids than were needed to inhibit O_2 uptake. However, comparison may be difficult because of the difference in experimental conditions. O_2 uptake experiments utilized growing cells diluted with fresh media to a suitable concentration. Radiorespirometric experiments required the centrifugation, resuspension, and starvation of the cells to remove exogenous and endogenous glucose before the addition of radiolabeled substrate. Gonococci may have been stressed during this manipulation, resulting in an increased sensitivity to steroids. However, steroid inhibition of glucose catabolism was always compared to $^{14}CO_2$ release from the same cells receiving ethanol, but not steroids. The mechanism by which steroids inhibit glucose catabolism is probably indirect. Previous results showed that the reduced nicotinamide adenine dinucleotide oxidase and cytochrome *b* L-lactate dehydrogenase activities of gonococcal membrane preparations were inhibited by progesterone (18). Recently, it was observed that gonadal steroid hormones had no effect upon the activity of partially purified gonococcal glucose 6-phosphate dehydrogenase (A. F. Cacciapuoti and S. A. Morse, Can. J. Microbiol., in press). Our finding that steroids inhibit the respiration of growing gonococci supports the conclusion that the binding of steroids to the cytoplasmic membrane perturbs electron transport (14, 18). The inhibition of energy generation may therefore affect related phenomena, such as active transport, or the recycling of reduced pyridine nucleotides which regulate glucose catabolism.

Differences between the inhibition of O_2 uptake of DM- and CM-grown cells may be the result of media components and not due to intrinsic differences in the composition of the cells. Preliminary results (data not shown) suggest that the fatty acids present in proteose peptone no. 3 may be responsible for the increased inhibition of O_2 uptake of CM-grown gonococci. Fatty acids are inhibitory to the growth of gonococci in vitro (13), and fatty acid acyl coenzyme A derivatives inhibit glucose 6-phosphate dehydrogenase activity (Cacciapuoti and Morse, in press). Fatty acids are present in human vaginal secretions (22) and may potentiate the activity of steroids or cause a more effective partitioning of the steroids into the lipid phase of the gonococcal cell membranes.

O_2 uptake by gonococci grown under one set of conditions and diluted into different conditions was inhibited by steroids to the same extent as when cells had been grown originally in the diluting medium. Therefore, the differential effects were not due to synthesis of new envelope components. The differential effects of pH upon steroid inhibition may involve changes in membrane fluidity due to temperature-dependent phase transitions. These phase transitions can be influenced by growth medium (2, 5, 6), pH (1, 7, 27), and cation concentrations (7, 27; K.A.E. Lysko, Ph.D. thesis, University of Massachusetts, Amherst, 1980). Alternatively, changes in pH could affect the interaction between steroids and the gonococcal cell envelope. Steroid hormones are not charged at physiological pH (28); however, the pH change may affect the affinity between steroids and cell membrane receptors. The affinity of α_1-acid glycoprotein for progesterone decreases with a decrease in pH (4). A similar change in affinity could be responsible for the reduced inhibition of O_2 uptake by progesterone when cells were tested at pH 6.2.

Since L-lactate partially relieved steroid inhibition of O_2 uptake, the oxidation of lactate may have survival value for the gonococcus. Although cytochrome *b* L-lactate dehydrogenase activity in gonococcal membrane preparations was inhibited by progesterone, the addition of lactate to gonococcal growth media partially prevented progesterone inhibition of growth (18). In the present study, we find that L-lactate is serving as an alternate electron donor and that its oxidation is affected by steroid hormones. The addition of L-lactate to steroid-treated gonococci did not increase the rate of O_2 uptake to that of untreated cells, but only increased the rate relative to the degree of inhibition. We suggest that it is the total capacity for O_2 consumption that is diminished and that steroid inhibition of O_2 uptake in the gonococcus is a general phenomenon occurring at the membrane level and not due to action upon a particular component of the respiratory chain.

Progesterone in normal human females reaches a serum level of 10 to 30 ng/ml about 8 days after ovulation (9, 24, 25). The total number of gonococci in the cervicovaginal area of women with gonorrhea has been estimated to average 1.5×10^5 colony-forming units, with a range from 4.0×10^2 to 1.8×10^7 colony-forming units (12). In our experiments, progesterone inhibited O_2 uptake >50% at a concentration of 40 μg per 5 $\times 10^7$ cells. Due to limitations in the sensitivity of the assay, we could not measure O_2 uptake at a 1,000-fold lower cell concentration, where we might have tested for inhibition by in vivo levels of steroid. Therefore, a direct extrapolation from our studies to what might occur under in vivo conditions cannot be made at this time. However, the high surface-volume ratio of gonococci may favor steroid interaction in a dilute system.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI 12928 from the National Institute of Allergy and

Infectious Diseases. P.G.L. is a recipient of an N.L. Tartar research fellowship and a Venereal Diseases Research Fund fellowship from the American Social Health Association. S.A.M. is the recipient of Public Health Service Research Career Development Award AI 00140 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Brandts, J. F., R. D. Taverna, E. Sadasivan, and K. A. Lysko.** 1978. Calorimetric studies of the structural transitions of the human erythrocyte membrane. Studies of the B and C transitions. Biochim. Biophys. Acta **512**:566–578.
2. **DeKruyff, B., R. A. Demel, and L. L. M. VanDeenen.** 1972. The effect of cholesterol and epicholesterol incorporation on the permeability and on the phase transition of intact *Acholeplasma laidlawii* cell membranes and derived liposomes. Biochim. Biophys. Acta **255**: 331–347.
3. **Fitzgerald, T. J., and S. A. Morse.** 1976. Alteration of growth, infectivity, and viability of *Neisseria gonorrhoeae* by gonadal steroids. Can. J. Microbiol. **22**:286–294.
4. **Ganguly, M., R. H. Carnighan, and U. Westphal.** 1967. Steroid-protein interactions. XIV. Interaction between human α_1-acid glycoprotein and progesterone. Biochemistry **6**:2803–2814.
5. **Huang, L., and A. Haug.** 1974. Regulation of membrane lipid fluidity in *Acholeplasma laidlawii*: effect of carotenoid pigment content. Biochim. Biophys. Acta **352**: 361–370.
6. **Huang, L., S. K. Lorch, G. G. Smith, and A. Haug.** 1974. Control of membrane lipid fluidity in *Acholeplasma laidlawii*. FEBS Lett. **43**:1–5.
7. **Jacobson, K., and D. Papahadjopoulos.** 1975. Phase transitions and phase separations in phospholipid membranes induced by changes in temperature, pH, and concentrations of bivalent cations. Biochemistry **14**: 152–161.
8. **James, J. F., and J. Swanson.** 1978. Studies on gonococcus infection. XIII. Occurrence of color/opacity colonial variants in clinical cultures. Infect. Immun. **19**: 332–340.
9. **Johansson, E. D. B.** 1969. Progesterone levels in peripheral plasma during the luteal phase of the normal human menstrual cycle measured by a rapid competitive protein binding technique. Acta Endocrinol. **61**:592–606.
10. **Johnson, D. W., K. K. Holmes, P. A. Kvale, C. W. Halverson, and W. P. Hirsch.** 1969. An evaluation of gonorrhea case findings in the chronically infected female. Am. J. Epidemiol. **90**:438–448.
11. **Koch, M. L.** 1947. A study of cervical cultures taken in cases of acute gonorrhea with special reference to the phases of the menstrual cycle. Am. J. Obstet. Gynecol. **54**:861–866.
12. **Lowe, T. L., and S. J. Kraus.** 1976. Quantitation of *Neisseria gonorrhoeae* from women with gonorrhea. J. Infect. Dis. **133**:621–626.
13. **Miller, R. D., K. E. Brown, and S. A. Morse.** 1977. Inhibitory action of fatty acids on the growth of *Neisseria gonorrhoeae*. Infect. Immun. **17**:303–312.
14. **Miller, R. D., and S. A. Morse.** 1977. Binding of progesterone to *Neisseria gonorrhoeae* and other gram-negative bacteria. Infect. Immun. **16**:115–123.
15. **Miller, R. D., W. J. Warren, R. C. Sizemore, and S. A. Morse.** 1978. Binding of cholesterol by *Neisseria gonorrhoeae*. Infect. Immun. **22**:698–708.
16. **Morse, S. A., and L. Bartenstein.** 1974. Factors affecting autolysis of *Neisseria gonorrhoeae*. Proc. Soc. Exp. Biol. Med. **145**:1418–1421.
17. **Morse, S. A., and L. Bartenstein.** 1980. Purine metabolism in *Neisseria gonorrhoeae*: the requirement for hypoxanthine. Can. J. Microbiol. **26**:13–20.
18. **Morse, S. A., and T. J. Fitzgerald.** 1974. Effect of progesterone on *Neisseria gonorrhoeae*. Infect. Immun. **10**:1370–1377.
19. **Morse, S. A., S. Stein, and J. Hines.** 1974. Glucose metabolism in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:702–714.
20. **Nes, W. R., and M. L. McKean.** 1977. Biochemistry of steroids and other isopentenoids. University Park Press, Baltimore.
21. **Nikaido, H.** 1976. Outer membrane of *Salmonella typhimurium*. Transmembrane diffusion of some hydrophobic substances. Biochim. Biophys. Acta **433**:118–132.
22. **Preti, G., G. R. Huggins, and G. D. Silverberg.** 1979. Alterations in the organic compounds of vaginal secretions caused by sexual arousal. Fertil. Steril. **32**:47–54.
23. **Robinson, J., and J. M. Cooper.** 1970. Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. Anal. Biochem. **33**:390–399.
24. **Ross, G. T., C. M. Cargille, M. B. Lipsett, P. L. Rayford, J. R. Marshall, C. A. Strott, and D. Rodbard.** 1970. Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. Rec. Prog. Hormone Res. **26**:1–62.
25. **Saxena, B. N., N. Dusitsin, V. Poshyachinda, and I. Smith.** 1974. Luteinizing hormone, oestradiol, and progesterone levels in the serum of menstruating Thai women. J. Obstet. Gynecol. Br. Commonw. **81**:113–119.
26. **Stoppani, A. O. M., C. M. C. de Brignone, and J. A. Brignone.** 1968. Structural requirements for the action of steroids as inhibitors of electron transfer. Arch. Biochem. Biophys. **127**:463–475.
27. **Träuble, H., and H. Eibl.** 1974. Electrostatic effects on lipid phase transitions: membrane structure and ionic environment. Proc. Natl. Acad. Sci. U.S.A. **71**:214–219.
28. **Westphal, U.** 1971. Steroid-protein interactions. *In* Monographs on endocrinology, vol. 4. Springer-Verlag, New York.
29. **Winter, D. B., and S. A. Morse.** 1975. Physiology and metabolism of pathogenic *Neisseria*: partial characterization of the respiratory chain of *Neisseria gonorrhoeae*. J. Bacteriol. **123**:631–636.