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Bacteriostatic Action of Progesterone on Staphylococci and Other Microorganisms

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ABSTRACT

YOTIS, WILLIAM (Loyola University, Hines, Ill.), AND RONALD STANKE. Bacteriostatic action of progesterone on staphylococci and other microorganisms. J. Bacteriol. 92:1285–1289. 1966.—Progesterone has been examined in vitro for antibacterial activity against 10 microorganisms. Turbidimetric and manometric techniques were used to assay the antibacterial activity of progesterone. The organisms tested consisted of *Staphylococcus aureus*, *S. epidermidis*, *Gaffkya tetragena*, *Bacillus subtilis*, *Listeria monocytogenes*, *Candida albicans*, *Escherichia coli*, *Aerobacter aerogenes*, *Salmonella paratyphi*, and *Proteus vulgaris*. Antibacterial action was shown by progesterone only against the gram-positive microorganisms when they were grown in tryptic soy broth containing 10 to 20 μg of progesterone per ml. Pregnenolone, 4-pregnen-20β-ol-3-one, and 5α-pregnane also possessed antistaphylococcal properties, whereas pregnanolone, pregnandione, 11α-hydroxyprogesterone, and 17α-hydroxyprogesterone did not. The bacteriostatic action of progesterone on staphylococci was exerted primarily during the first 8 hr of incubation, and it was reduced in the presence of oxygen. In the presence of 20 μg of progesterone per ml, there was significant reduction in the oxidation by resting staphylococcal suspensions or utilization by staphylococci of pyruvate as an energy source during growth.

There is explicit recognition that the reactivation of an infectious staphylococcal process can be affected by the secretions of the endocrine system (11, 12). This situation becomes apparent during infancy, puberty, menopausal and postmenopausal years, and pregnancy. In attempts to determine the mechanism of an altered response of the host to staphylococci, consideration must be given to the possible effect of hormones directly on staphylococci (4). Most studies concerned with the effects of hormones on infectious agents have been performed with the adrenal cortical hormones (8). The present study was undertaken to determine whether hormones which are known to have physiological effects on animal metabolism (1, 3, 13) had any recognizable action on the growth of staphylococci in vitro.

MATERIALS AND METHODS

Cultures. The following microorganisms were used: *Staphylococcus aureus*, serotypes I, II, III (ATCC 12598–12600); *S. aureus* G. F. Gauze 209 (ATCC 13679); *S. aureus* Lederle Labs. strain Rose (ATCC 14154); *S. aureus* FDA strain Wood 46 (ATCC 10832); *S. epidermidis* (ATCC 12228). *S. aureus* 296, phage type 29/52/52A, *S. aureus* 52298 phage type 52/52A/80, and *S. epidermidis* strain 12 and 246 were obtained from the Hines Veterans Administration Hospital, and represented recent isolates during routine bacteriological examination of clinical specimens. They were tested for glucose and mannitol utilization under anaerobic conditions according to the method of Hugh and Leifson (7) with phenol red (0.001%) as an indicator, and for gelatin liquefaction, pigmentation, and both free and bound coagulase, by use of accepted microbiological methods. All the other cultures were obtained from stock culture collections maintained at Stritch School of Medicine, and were originally obtained from the American Type Culture Collection. Cultures were kept in stock at 4 C on tryptic soy agar (Difco) slants in screw-capped tubes. Transfer to a new slant was made every 4 weeks.

Hormone solutions. Crystalline progesterone was obtained from Calbiochem. Limited quantities were also received from Eli Lilly & Co., Indianapolis, Ind., and from the Schering Corp., Bloomfield, N.J. Fresh solutions of the desired concentrations were obtained by dissolving a weighed amount (5 to 20 mg) of progesterone in 10 ml of 95% ethyl alcohol. A 0.5-ml sample of this solution was added to 49.5 ml of growth medium in a 300-ml nephelometric flask, yielding final concentrations of 5 to 20 μg of pro-

gesterone per ml of medium. Control flasks received 0.5 ml of 95% ethyl alcohol. Both sets of media were then sterilized by autoclaving. The hormone intermediates were obtained from Mann Research Laboratories, Inc., New York, N.Y. Solutions of these hormones were prepared the same way as the progesterone solutions.

Growth studies. Cells for growth studies were grown in 10 ml of tryptic soy broth (Difco) or in the synthetic medium, at 37 C for 24 hr, and harvested by centrifugation. A smooth suspension was prepared by manual shaking for 5 min in a 125-ml Erlenmeyer flask containing 30 glass beads, each having a diameter of 5 mm, and was adjusted with sterile distilled water to 10 Klett units by use of a no. 42 filter of a Klett-Summerson photoelectric colorimeter. This suspension contained approximately 7.0×10^6 viable cells per milliliter. Each nephelometric flask was inoculated with 1 ml of the above cell suspension, and was placed on a rotary shaker in an incubator room at 37 C. Turbidity determinations were performed in the incubator room by use of a Klett-Summerson photoelectric colorimeter equipped with a blue filter (no. 42).

Manometric studies. Cells for the Warburg experiments were grown in 100 ml of tryptic soy broth at 37 C for 18 hr, harvested by centrifugation, and washed twice with distilled water; a smooth suspension was prepared by manual shaking for 3 to 5 min with glass beads. The turbidity of the suspension was then adjusted with distilled water to a reading of 250 Klett units, and contained 0.4 to 0.6 mg (dry weight) per ml. In a typical experiment, 0.5 ml of 0.1 M potassium phosphate buffer (*p*H 7.0), containing 2 mg of sodium pyruvate, 2 μg of nicotinic acid, and 2 μg of thiamine hydrochloride, and 1 ml of cell suspension were added to each Warburg flask. A 0.2-ml amount of 40% KOH and a filter-paper wick were placed in the center wells. Rates of oxygen uptake in air were determined for 180 min by conventional manometric techniques at 37 C.

Synthetic medium. The chemically defined method described by Fildes et al. (6) was used for the growth of staphylococci, with the following modifications. The staphylococcus factor was replaced by 0.01 g of thiamine hydrochloride and 0.01 g of nicotinamide per liter; ferrous ammonium sulfate and dithiodiglycollic acid were omitted; and sodium pyruvate was substituted for glucose. All chemicals were chromatographically pure, and were purchased from Calbiochem.

RESULTS

Effect of progesterone on various strains of S. aureus. Six strains of *S. aureus* were prepared for growth studies as described. Three separate experiments were performed with each strain, and the mean values obtained are shown in Table 1. Progesterone exerted an inhibitory action on the growth of the six strains of staphylococci used in this study. From the results, it appeared that progesterone slows the rate of transfer of cells from the lag phase into the exponential phase, thereby causing a lengthened lag phase and a resultant decrease in growth during the exponential phase as compared with controls. By the 7th hr of growth, the degree of inhibition among the six strains of *S. aureus* ranged between 32 and 61%, whereas at the 8th hr of growth it ranged between 25 and 55%.

Quantitative aspects of the antibacterial action of progesterone. The amount of progesterone necessary to affect the growth of staphylococci was determined. Standard suspensions of *S. aureus* serotypes I, II, III, *S. aureus* ATCC 13679, and *S. aureus* ATCC 14154 were inoculated into flasks containing 0 to 20 μg of progesterone per ml of medium, and the growth was assayed turbidimetrically. Increasing the amount of progesterone in the growth medium resulted in a progressive retardation of the growth of staphylococci (Table 2). However, after growth for 24 hr, both the control and experimental cultures reached the same turbidity. Identical results were

TABLE 1. *Effect of progesterone on the growth of various strains of Staphylococcus aureus*[a]

S. aureus strain	Klett reading at various times (hr)									
	Control					Progesterone (20 μg/ml)				
	0	4	6	7	8	0	4	6	7	8
Serotype I	0	0	11	75	185	0	0	0	35	103
Serotype II	0	0	15	81	198	0	0	0	31	113
Serotype III	0	4	38	114	242	0	0	19	61	169
G. F. Gauze 209	0	36	91	193	310	0	19	56	130	214
52298	0	13	33	92	187	0	0	12	48	83
296	0	25	81	204	330	0	3	25	95	206

[a] The system contained 49.5 ml of tryptic soy broth in a 300-ml nephelometric flask, progesterone at a final concentration of 20 μg per ml of broth dissolved in 0.5 ml of 95% ethyl alcohol, and 7.0×10^6 viable staphylococci. Flasks were incubated for 8 hr on a rotary shaker in an incubator room at 37 C. Three separate experiments were performed with each strain, and the mean values obtained were recorded.

obtained when the inoculum consisted of staphylococci grown in the presence of hormone.

Action of progesterone on other microorganisms. To determine the specificity of this action, the effect of progesterone on the growth of various microbial species was determined. Cell suspensions, adjusted with distilled water to 10 Klett units, of *S. epidermidis*, *Aerobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans*, *Gaffkya tetragena*, *Listeria monocytogenes*, and *Salmonella paratyphi* were prepared, and 1 ml of the suspension was inoculated into appropriately labeled nephelometric flasks containing 50 ml of tryptic soy broth and 20 μg of progesterone per ml. Turbidity determinations were performed as previously described.

As seen in Table 3, a survey of the effect of progesterone on the growth of various microorganisms indicated that progesterone exerted an inhibitory effect on the growth of all the gram-positive organisms tested, but it did not influence any of the gram-negative organisms. In harmony with *S. aureus* but in contrast to the other gram-positive microorganisms tested, *S. epidermidis* was able to overcome the effect of progesterone within 24 hr and arrive at equal turbidity values with its respective controls. It appears that progesterone retards the transition of cells from their lag phase to their logarithric phase of growth, thereby causing a lengthened lag phase and a resultant decrease in growth during the exponential phase as compared with controls.

TABLE 2. *Quantitative aspects of the antibacterial action of progesterone*[a]

Time	Klett reading with various hormone concentrations (μg/ml)			
	0	5	10	20
hr				
0	0	0	0	0
4	7	6	0	0
5	26	24	15	11
6	77	70	56	27
7	175	160	121	87
8	335	320	285	203
24	590	610	595	590

[a] The experimental conditions were the same as those cited in Table 1. However, various concentrations of progesterone were employed.

Effect of progesterone intermediates. The finding that progesterone had a bacteriostatic effect on the growth of *S. aureus* served as a stimulus to determine whether progesterone intermediates had any antibacterial activity. To this end, experiments were performed with the six strains of staphylococci in which pregnenolone, pregnanolone, pregnandione, 11α-hydroxyprogesterone, 17α-hydroxyprogesterone, 5α-pregnane, and 4-pregnen-20β-ol-3-one were added to tryptic soy broth at a final concentration of 20 μg per ml of broth, and assayed for antibacterial activity as previously described. As seen in Table 4, at the 6th hr of the growth cycle 4-pregnen-20β-ol-3-one, pregnenolone, and 5α-pregnane showed a 70, 50, and 50% inhibition, respectively, in the growth of the seven strains of *S. aureus* included in this study. By the 8th hr of growth, the degree of inhibition by these steroids dropped to 42, 33, and 29%, respectively. Under identical conditions, progesterone inhibited the growth of *S. aureus* 64% after 7 hr and 37% after 8 hr. Pregnenolone, pregnandione, 11α-hydroxyprogesterone, and 17α-hydroxyprogesterone did not demonstrate any antibacterial activity.

TABLE 3. *Action of progesterone on other microorganisms*[a]

Microorganism	Klett reading at various times (hr)									
	Control					Progesterone (20 μg/ml)				
	0	6	8	15	24	0	6	8	15	24
Gram-positive										
Staphylococcus epidermidis	0	91	201	410	580	0	0	57	103	560
Bacillus subtilis	0	0	0	148	510	0	0	0	79	315
Candida albicans	0	0	23	85	650	0	0	11	49	510
Gaffkya tetragena	0	0	0	60	560	0	0	0	2	401
Listeria monocytogenes	0	12	30	344	704	0	0	0	194	508
Gram-negative										
Aerobacter aerogenes	0	12	87	460	700	0	15	92	450	700
Escherichia coli	0	180	320	500	700	0	178	320	500	700
Proteus vulgaris	0	30	76	440	700	0	31	88	440	700
Salmonella paratyphi	0	30	144	597	647	0	34	164	597	659

[a] Footnotes same as in Table 1. Various microorganisms were employed as inoculum.

TABLE 4. *Effect of progesterone intermediates on the growth of Staphylococcus aureus*[a]

Hormone	Klett reading at various times (hr)				
	0	6	7	8	9
None	0	48	127	230	348
Progesterone	0	17	55	143	209
Pregnenolone	0	24	66	152	281
Pregnanolone	0	61	147	256	362
4-Pregnen-20β-ol-3-one	0	14	54	132	219
Pregnandione	0	52	139	257	384
5α-Pregnane	0	24	70	163	270
11α-Hydroxyprogesterone	0	43	128	235	363
17α-Hydroxyprogesterone	0	44	124	224	335

[a] Experimental conditions similar to those cited in Table 1. Progesterone intermediates replaced progesterone in the experimental system.

TABLE 5. *Enhancement of the antibacterial activity of progesterone by anaerobiosis*[a]

Staphylococcus aureus strain	Klett reading at 24 hr with various hormone concentrations (μg/ml)			
	0	5	10	20
Serotype I	202	145	23	0
Serotype II	152	150	101	21
G. F. Gauze 209	128	128	71	0
FDA strain Wood 46	147	84	47	14
Lederle Labs. strain Rose	162	160	94	0

[a] The system contained 49.5 ml of tryptic soy broth in a 300-ml nephelometric flask; progesterone at a final concentration of 0, 5, 10, and 20 μg/ml dissolved in 0.5 ml of 95% ethyl alcohol; and 7.0×10^6 viable staphylococci. Flasks were incubated for 24 hr in an anaerobic Precision Thelco incubator at 37 C. Three separate experiments were performed with each strain, and the mean values obtained were recorded.

Effect of anaerobiosis. The ability of progesterone to inhibit the growth of five strains of *S. aureus* under anaerobic conditions was tested at 37 C, in a Precision Thelco anaerobic incubator. After a 24-hr anaerobic incubation in the presence of 20 μg of progesterone per ml, all of the strains were severely inhibited and three of the five failed to grow at all (Table 5). Progesterone at a concentration of 10 μg/ml produced a 33 to 88% inhibition in the growth of the five staphylococcal strains.

Effect of progesterone on the oxidation of pyruvate. Attempts were made to detect inhibition in the rate of oxidation of chemically defined substrates by staphylococci in the presence of various concentrations of progesterone. To this end, pyruvate was chosen as substrate, and the oxidation of this compound by six strains of staphylococci was determined in the presence and absence of progesterone by use of manometric techniques. There was an increasing reduction in oxygen consumption of *S. aureus*, ranging from 17 to 71%, when resting cell suspensions supplemented with thiamine and nicotinic acid were allowed to oxidize pyruvate in the presence of concentrations of progesterone ranging from 2.5 to 20 μg/ml (Table 6). A further attempt was then made to demonstrate the suppressive effect of progesterone on the utilization of pyruvate by using this compound as the main carbon energy source in a synthetic medium. Standardized cell suspensions of *S. aureus*, grown previously in the

TABLE 6. *Effect of progesterone on the oxidation of pyruvate*[a]

Progesterone concn	Q_{O_2}
μg/ml	
0	90 ± 20
2.5	74 ± 13
5	58 ± 11
10	39 ± 17
20	26 ± 8

[a] The flask contents were: 1 ml of cell suspension (0.6 mg, dry weight), 0.5 ml of 0.1 M potassium phosphate buffer (*p*H 7.0), containing 2 mg of sodium pyruvate, 2 μg of nicotinic acid, 2 μg of thiamine hydrochloride, and various progesterone concentrations. A 0.2-ml amount of 40% KOH and a filter-paper wick were placed in the center wells. Oxygen uptake in air was determined at 37 C.

TABLE 7. *Progesterone and growth of Staphylococcus aureus in synthetic medium with pyruvate as the carbon source*[a]

Age of culture	Klett reading	
	Control	Progesterone
hr		
0	0	0
24	16	0
48	65	0
96	112	28
120	194	63

[a] The system contained 49.5 ml of synthetic medium with sodium pyruvate as the carbon source, progesterone at a final concentration of 20 μg/ml dissolved in 0.5 ml of 95% ethyl alcohol, and 7.0×10^6 viable cells of six strains of staphylococci grown previously in the synthetic medium. Flasks were incubated statically at 37 C for various time intervals.

synthetic medium with glucose as the main carbon and energy source, were prepared as previously described and used to inoculate nephelometric flasks containing 50 ml of synthetic medium, in which sodium pyruvate replaced glucose and 20 μg of progesterone per ml. The flasks were incubated statically at 37 C. Progesterone at a concentration of 20 μg/ml was able to produce a marked supression in the growth of *S. aureus* (Table 7).

DISCUSSION

The data presented in this paper indicate that progesterone, in physiological concentrations, had an inhibitory effect on the growth of staphylococci and all other gram-positive organisms included in this study. The effect of progesterone on the multiplication of staphylococci showed that progesterone actually exerted a definite inhibitory action only during the first 8 hr of incubation. Subsequently, the treated staphylococcal cells seemed to grow at a rate comparable with that of the control cells. Since identical results were obtained when staphylococci grown in the presence of progesterone were used as inocula, the transient nature of the initial growth inhibition could not be accounted for by adaptation. The possibility exists that the cessation of the growth-inhibitory action of progesterone after 8 hr of incubation might conceivably be accounted for by inactivation or transformation of progesterone. Fabian (5) has shown that staphylococci reduced 4-androstene-3,17-dione, a steroid which can be derived from 17-hydroxyprogesterone, to testosterone in cultures of staphylococci grown in nutrient broth for 24 hr on a reciprocal shaker. It is worthy of note that hydroxyprogesterone did not have any inhibitory action on the growth of staphylococci.

Interference with membrane permeability and active transport may be offered as an alternative explanation for the antibacterial action of progesterone. It has been shown in this study that progesterone inhibited the oxidation of pyruvate. Progesterone has been shown to be a powerful inhibitor of reduced nicotinamide adenine dinucleotide ($NADH_2$) oxidase (14). Thus, progesterone may act to inhibit the flow of electrons in the cytochrome system, resulting in a decrease of the available energy to the cell, and this decrease in energy would result in a lessened growth. However, progesterone also inhibits anaerobically where no $NADH_2$ oxidase is operative. That interference with membrane permeability may be a means by which progesterone exerts its effect is substantiated by the observation that deoxycorticosterone, an intermediate steroid in the metabolism of progesterone, inhibited the uptake of nutrients in fungi (10), and that both compounds are inhibitory to gram-positive organisms but not to gram-negative bacteria (2, 9).

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