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The Effect of Progesterone on the Growth and Virulence of Staphylococcus aureus

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THE EFFECT OF PROGESTERONE ON THE GROWTH AND VIRULENCE OF *STAPHYLOCOCCUS AUREUS*

by

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LIFE

Ronald T. Stanke was born on October 20, 1942, in Chicago, Illinois. He was graduated fron St. Procopius High School in Lisle, Illinois in 1960. He entered St. Procopius College in Lisle, Illinois, in 1960 and received a Bachelor of Science Degree in Biology in 1964. He began his graduate studies at Loyola University in 1964.

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STATEMENT OF THE PROBLEM

Many investigators have shown that hormones can alter an animals susceptibility to a variety of disease states. Most of the work concerning the effect of hormones on infection has been done with those hormones arising from the adrenal cortex. Sex hormones have also received a lot of attention, especially the estrogens, since there exist numerous instances where the female species has been shown to be more resistant to certain diseases.

The aim of the present work is a study of the effects progesterone, hormone of the corpus luteum, has on *Staphylococcus aureus* *in vitro* and the infections it causes *in vivo*.

INTRODUCTION

Differences in age, sex, and various periods within the life cycle of an individual have been known to influence an individuals ability to resist infection. The effect of age was studied in part by Dingle(1936) who noticed that immature rats and rabbits did not form as high a titer of agglutinating antibodies as did mature animals of the same species. Investigating the possibility that hormones might influence the agglutinating antibody titer he found that gonadotropin, prolan, theelin, or pituitary implant did not increase the young animals ability to form antibody.

Kemp(1937), investigating the effect of pregnency on experimental syphilis, found that the infection was milder in pregnent animals than in normal controls. He also noticed that the infection was less severe in normal female rabbits than in normal males.

Sprunt et al.(1938) exploring the relationship between resistance to vaccinia virus and the hormones estrogen and gonadotropin, found that estrogen, and possibly gonadotropin, increased the resistance of castrated rabbits to vaccinia. Concomittant with this observation was the fact that these two hormones also inhibited the spread of India Ink through the skin.

Weinstein(1939a), utilizing a variety of hormone preperations, found that insulin, estrin, and progesterone did not alter the course of experimental anthrax infections in mice. Prolactin and gonadotropin were highly effective in protecting mice whereas thyroxine and testosterone were only moderately protective. The effects of these hormones were shown to be dose dependent since

small amounts of gonadotropin were inactive while large doses were protective. The opposite effect was noticed with testosterone, smaller doses being more beneficial than larger ones.

The effects of estrogen on adult male and female rabbits was studied by Weinstein(1939b) who demonstrated that estrogen produced an increase in the amounts of circulating agglutinins for Escherichia coli and hemolysin for sheep erythrocytes. The amount of increase in antibody was directly related to the dose of hormone administered.

Investigating the effects of various sex hormones upon experimental pneumococcal infections in mice, Van Haam and Rosenfeld(1942) showed that progesterone exerted no influence on the course of the infection. Estrone, when administered one week prior to challenge, had a protective effect, while testosterone, given three days before infection, had a mild protective effect. Stilbesterol failed to modify the course of the infection. From these results they concluded that not only the dose of hormone but also the time interval between hormone administration and bacterial challenge determined if the hormone would exert any effect on the course of the infection.

Foley and Aycock(1944), treating mice with a single dose of stilbesterol and then challenging them with virulent streptococci, showed that stilbesterol had a protective effect in mice challenged with streptococci. They found at post-mortem bacteriological examination that the spread of the streptococci was retarded in the stilbesterol treated mice. From this they concluded that stilbesterol induced some factor which retarded the spread of streptococci in mice.

In a review on the relationship of adrenocortical hormones to infection Kass and Finland(1953) pointed out that these hormones may depress resistance by inhibiting the inflammatory response and antibody production. These

compounds may also activate latent infections or render animals susceptable to normal inhabitants of the respritory or intestinal tract.

Fungus infections and their relationship to hormones was studied by Mankowski and Zbigniew (1954) who demonstrated that progesterone had no effect on the development of infection in mice due to Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, or an Aspergillus species. Testosterone protected mice slightly against experimental aspergillosis while estradiol shortened the survival time of infected animals.

Studying the effect of hormones on microorganisms in vitro Osborne and Bourdeau (1955) found that progesterone and testosterone stimulated the growth of Vibrio fetus. Lester and Hechter (1958), testing deoxycorticosterone, found that this compound inhibited the growth of a wide range of gram positive but not gram negative bacteria. Casas-Campillo (1959) found that 21, 21, dimethoxy, 21, 21, diethoxy and 21, 21, diisopropyl progesterone inhibited a variety of fungi and also that 21, 21, dimethoxy progesterone inhibited the growth of gram positive bacteria including Mycobacteria and Nocardia species. In a more extensive investigation of the effect of 21, 21, dimethoxy progesterone on mycobacteria in vitro Casas-Campillo (1961) found that this compound inhibited the growth of 43 different strains of mycobacteria. Moskal and Boucek (1964) showed that radioactive estrone, estradiol and estriol became loosely associated with Staphylococcus aureus and did not penetrate beyond the cell membrane.

Hulka et al. (1965), studying the effect of hormones on allograft rejection rates and antibody formation in rabbits, found that estradiol, progesterone, 17, hydroxyprogesterone and medroxyprogesterone had no effect on

graft survival while cortisone treated animals tolerated grafts at least twice as long as the control group. Progesterone, medroxyprogesterone and cortisone suppressed antibody production to bovine serun albumin while estradiol and 17, hydroxyprogesterone were without effect. These workers demonstrated that progesterone was without effect on the cellular elements concerned with graft rejection but that it did inhibit the humoral mechanisms of antibody formation.

In conclusion, then, hormones can either have a beneficial or detrimental effect on an animals resistance. Some of the ways they have been shown to be beneficial is by either inhibiting the spread of the invading organism or by increasing antibody production. The detrimental effects of hormones include a suppressed inflammatory response, a decreased antibody production and an increased susceptability to normally non-pathogenic inhabitants of the respritory or intestinal tract.

In many instances investigators have noticed that hormones can exert opposing effects depending upon the dose administered. Small amounts of some compounds increased resistance while larger doses decreased resistance with the opposite effect also found. The time interval between hormone administration and challenge with an infectious agent determined if the compound exerted any effect on the disease.

Progesterone was shown to be without effect on a variety of bacterial and fungal infections. Note should be taken that mice were the only animals used in testing the effect of progesterone on infections. Thus if progesterone does not effect mice or if mice differ in their metabolism of progesterone from rabbits or humans, the reason for a lack of an effect is readily apparent.

MATERIALS AND METHODS

Cultures: All cultures were obtained from stock culture collections maintained at Stritch School of Medicine, Loyola University, Department of Microbiology, Hines, Illinois. Ea ch culture was checked with respect to its gram reaction, motility, nitrate reduction, methyl red and Voges-Proskauer reactions. Carbohydrate oxidation or fermentation was determined using phenol red (.001%) as an indicator in the O-F medium of Hugh and Leifson (1953). Each coccus was also tested for its ability to clot citrated plasma (Difco). The results of these morphological and physiological characters were compared to those listed in Bergey's Manual (1957) for the particular organism being studied. Cultures were maintained on brain heart infusion agar slants (Difco) in the refrigerator and transferred every three weeks.

Hormone: Crystalline progesterone in 25 mg. ampules was obtained from the California Company for Biological Research, Los Angeles, California. Fresh stock solutions of progesterone were prepared by dissolving the hormone in 95% ethanol prior to each experiment.

Effect of Progesterone on the Growth of Various Bacteria: One ml. of a stock solution of progesterone containing 2 mg. of hormone per ml. was added to 99 ml. of tryptic soy broth (Difco) to yield a final hormone concentration of 20 ug. per ml. Control flasks received only 1 ml. of 95% ethanol. Both sets of media were then sterilized by autoclaving. Each flask, equipped with a side arm for direct insertation into the Klett Colorimeter, was then inoculated

with 0.1 ml. of a suspension of the organism adjusted to a reading of 20-30 Klett units. The flask s were then placed either on a rotary shaker or incubated statically at 37 °C. The growth was followed by means of the turbidity measurements as described below.

Effect of Progesterone on the Growth of Staphylococcus aureus: One ml. of a progesterone stock solution or one ml. of 95% ethanol was added to 99 ml. of tryptic soy browth to yield a final hormone concentration of from 2.5-20 ug. per ml. or a 1% ethanol solution. All flasks were then sterilized by autoclaving. To each side arm flask was added 0.5 ml. of a 1:1000 dilution of a 20 Klett unit suspension prepared from an 18-24 hr. culture of S. aureus. Growth was followed by plate counts and turbidity.

Measurement of Bacterial Growth: Growth was measured by the measurement of turbidity or by plate counts. Turbidity determinations were performed using the Klett-Summerson Photoelectric Colorimeter equipped with a blue (420 mμ.) filter. Plate counts were made by aseptically withdrawing 0.5 ml. aliquots and making serial ten-fold dilutions in sterile 0.9% saline. Pour plates were made with 0.5 ml. of appropriate dilutions using trypticase soy agar (BBL) and placed in a 37 °C incubator for 48 hrs. after which time the number of colonies were counted.

Animals: All mice and rabbits were obtained fron Abrams Small Stock Breeders, Chicago, Illinois. The mice were maintained on a diet of Rockland Mice Pellets while the rabbits were maintained on Dixie Rabbit Pellets.

Administration of Progesterone to Rabbits: One month and/or six month old New Zealand female rabbits were used in all experiments. Each six month

old animal was shaved on the left side and injected subcutaneously either with 1 ml. of the hormone in saline preparation (5-20 ug./ml.) or 1 ml. of a 1% ethanol in saline solution. Each rabbit received four preparatory doses, one every four days, totaling from 20-80 ug. of progesterone or 4 ml. of the alcohol in saline solution. One month old rabbits were prepared in the same manner except that the hormone was given every other day for a total of four injections. All animals were injected intracutaneously with either Staphylococcus aureus strain 296 or India Ink (see below) 24hrs. after the last hormone injection.

Administration of Progesterone to Mice: Six week old female Swiss mice, 18-23 gm., were injected intraperitoneally with 0.25 ml. of a progesterone in saline or a 1% ethanol in saline solution. Each animal received four injections, one every four days, totaling from 20-80 ug. of progesterone or 1 ml. of the ethanol in saline solution. The mice were then challenged intracerebrally with S. aureus (Towler strain) 24 hrs. hours after the 1 ast hormone injection.

India Ink Spreading: Higgens non-waterproof black India Ink (2ml.) was diluted with saline (4ml.) and autoclaved. The left sidw of each of the hormone treated rabbits and controls was then injected intracutaneously with 0.75 ml. of this suspension. The spread of the ink was measured 24 hrs. later by drawing its outline on tracing paper and measuring the area with a planimeter.

Preparation of S. aureus for Animal Injection: The growth from a 24 hr. brain heart infusion agar slant culture os S. aureus strain 296 was suspended in ten ml. of sterile sal ine. This suspension was then transferred to a

small bottle containing a few glass beads and shaken manually to obtain a homogeneous suspension which was then adjusted to an optical density of 0.800 with sterile saline. The right side of each of the hormone treated and control rabbits was inlected intracutaneously with 0.5 ml. of this suspension, containing 4.7-8.4 x 10^8 colony forming units per 0.5 ml. The size of the lesion was determined by drawing its outline on tracing paper and measuring the area with a planimeter.

The cells from an 18-24 hr. tryptic soy broth culture of S. aureus (Towler strain) were washed once with saline, honogenized manually with glass beads as described above, and adjusted to an optical density of 0.120. Each group of mice was injected intracerebrally with 0.03 ml. of this suspension. The mice were observed for four days and the number of deaths recorded each day.

RESULTS

Cultures: The results of the morphological and biochemical characteristics of each organism listed in Table I were compared to those given in Bergey's Manual (1957). From this data it was concluded that all the organisms were as labelled in the stock cultures.

Effect of Progesterone on Various Organisms: A survey of the effect of progesterone on the growth of various bacteria in vitro revealed that progesterone exerted an inhibitory effect on the growth of all of the gram positive organisms studied but that it did not influence any of the gram negative organisms with the possible exception of Proteus mirabilis (Table II). The turbidity after 24-27 hrs. amoung the organisms was approximately equal in the gram negative group while the hormone suppressed growth in most of the gram positive group. Staphylococcus aureus and S. epidermidis were able to overcome the effect of progesterone and arrive at nearly equal turbidity values with their respective controls. Even though S. epidermidis grown in the presence of hormone was able to reach a final turbidity comparable to its control there was an exceptionally long lag phase in the cultures containing hormone. From the results shown with Gaffkya tetragena and S. epidermidis it appears that progesterone slows the rate of transfer of cells from the lag phase into the log phase thereby causing a lengthened lag phase and a resultant decrease in growth during the exponential phase as compared to controls.

Effect of Progesterone on S. aureus: The effect of progesterone (20 ug.)

on the growth of three strains of S. aureus was studied and strain 296 was randomly selected for further work. Progesterone exerted an inhibitory effect on the growth of all three strains tested.

The results of increasing quantities of progesterone on the growth of S. aureus strain 296 is preasented in Table III. Increasing the amount of progesterone in the growth medium resulted in a slower rate of growth of the organism although the final turbidity was equal.

Effect of Progesterone on Skin Permeability: The effect of progesterone treatment in rabbits upon the intracutaneous spread of India Ink is presented in Table IV. Although some sex hormones have been shown to alter skin permeability (Sprunt et al., 1938), progesterone did not effect the permeability of the skin in these experiments. This was manifested by the equal spread of India Ink in the skin of the progesterone treated and control rabbits.

Effect of Progesterone on Gross Lesion Formation in Rabbits: The use of the term lesion here is confined to that area of the skin which appears red and swollen. This swollen area reached a maximum in 24-48 hrs. and discharged pus 48-72 hrs. after inoculation. Prior treatment of six month old rabbits with progesterone resulted in a marked reduction in the size of the lesions formed. (Table V). The average area of the control group is nearly twice that of the hormone treated rabbits. The relative rates of appearance and disappearance of the lesions measured over a period of six days was not affected by prior hormone treatment. All lesions reached a maximum in 24-48 hrs. and then regressed to a point at which after six days the lesion size in both groups of animals was approximately equal. The large increase in the size of the lesions

between 24 and 48 hrs. in the control group is misleading since an unusually large increase in the first two control rabbits raised the average of the entire group to a point where it appears that the lesions of all the rabbits are increasing in size. There also appeared to be no difference between the rate of pus formation or time of discharge.

Progesterone was without effect on the size of the lesions formed in immature rabbits (Table VI). There was no difference in the rate of lesion formation or regression measured over a period of five days. There also seemed to be no difference in the rate of pus formation or time of discharge.

Effect of Progesterone on the Intracerebral Challenge of Mice with S. aureus (Towler strain): Table VII shows that prior treatment of mice with from 20-80 ug. of hormone exerted no effect on the outcome of the intracerebral infection of mice with S. aureus (Towler strain). Although this type of infection is far from the normal type of staphylococcal disease the use of a second animal host was desired in testing the in vivo effect of progesterone. No increase in the numbers of survivors could be detected nor was there any difference in the death rates between the various groups of mice, the majority of deaths occuring within 24 hrs. after challenge

Although the amount of hormone administered to both the rabbits and mice was equal it should be noted that there is a great difference in the size of these two animals so that 20 ug. in a one kilogram rabbit is not equal to 20 ug. in a 20 gm. mouse.

DISCUSSION

Progesterone, in microgram amounts, was shown to exert an inhibitory effect on the growth of gram positive organisms. Species susceptibility to this compound is indicated by the large difference in the degree of sensitivity between S. aureus and S. epidermidis.

The effect of this hormone is most pronounced in its action on S. epidermidis where it greatly increases the lag phase as compared to controls. During this period the bacterial cells are forming the enzymatic machinery which will enable them to metabolize the growth medium. The transfer of cells from a primary to a secondary medium of the same composition guarantees that all of the enzymes necessary for growth are present and all that is now needed is the substrate. The facts that progesterone lengthens the lag phase in S. epidermidis and that estradiol and estrone can penetrate no further than the cell membrane in S. aureus (Moskal and Boucek, 1964) and are found loosely associated with it and also that deoxycorticosterone inhibits the uptake of certain nutrients into fungi (Lester, 1958) suggest that progesterone may act to coat the cell membrane and thus limit the uptake of nutrients. This decreased nutrient uptake would account for the extended lag period noted in S. epidermidis.

That a decreased nutrient uptake is the means by which progesterone exerts its effect is substantiated by the fact that both progesterone and deoxycorticosterone have the same range of activity which is associated with the type of gram reaction. Both compounds are inhibitory to gram positive

organisms and are generally without effect on gram negative bacteria (Lester and Hechter, 1958; Casas-Campillo, 1959, 1961).

An alternative explanation for the reduced growth of gram positive organisms was demonstrated in part by Yotis and Stanke (1966) who,showed that progesterone inhibited the oxidation of pyruvate using manometric techniques. This is significant since progesterone has been shown to be a powerful inhibitor of DPNH-oxibase (Yielding et al., 1960). Thus, if the respritory enzymes are located on the bacterial cell membrane, progesterone may act to inhibit the flow of electrons in the cytochrome system resulting in a decrease in availa ble energy to the cell. This decrease in energy would result in a lessened growth.

The inhibitory effect of progesterone and deoxtcorticosterone on gram positive and not gram negative bacteria may be related to differences in the permeability of the cell wall. The routine use of laboratory media containing bile acids, which are structurally related to these and many other steroids, for the selective isolation of gram negative bacteria and inhibition of gram positive organisms again points out the fact that there is a difference in the susceptibility to these types of compounds between these two broad groups of bacteria. Thus the resistance of gram negative organisms to progesterone is probably due to a difference in the permeability of the cell wall. This hormome probably does not penetrate the cell wall of gram negative bacteria and therefor it cannot exert its inhibitory effect.

The ability of progesterone to reduce the size of staphylococcal lesions in adult rabbits and not in the small sample of immature rabbits tested is interesting since it was thought that the effect of progesterone should be

more pronounced in the young animals due to a lack of endogenous progesterone. That this is not the case was evidenced by the inability of this compound to reduce the lesion size in treated one month old animals. A comparison of the size of the lesions formed in the two age groups of rabbits showed that the area of the lesions in the young animals was much smaller than in the adults. If this small sample was representative of this age group the ability of progesterone to decrease staphylococcal lesion size in rabbits may depend not only on this compound but upon some other factors (possibly hormonal) that arrive with sexual maturity. In the rabbit sexual maturity is reached when the animals are approximately six months of age.

The observation that progesterone decreased the area of lesions formed in mature rabbits was interesting since systemic progesterone treatment has been shown to exert no appreciable anti-inflammatory effects (Krohn, 1954; Glen, 1964; Hulka et al., 1965). The possibility that progesterone may act directly on the staphylococci causing a slower growth rate and thus making it easier for the host to eliminate them was unlikely since the amounts of hormone administered were so small. There was no reduction in lesion size when increasing amounts of progesterone were administered in vivo while in vitro the effect of this compound in increasing the lag phase increased with increasing concentrations of hormone.

The results of the India Ink spreading experiments presented in this paper showed that progesterone did not alter the permeability of the skin. Therefore if these non-specific factors can be ruled out it may be possible that progesterone caused the release or accumulation of some factor or factors that influenced the course of staphylococcal infection.

Margulus et al. (1965) have demonstrated that cyclic treatment of women with progestational agents caused an increase in the mean platelet count. This may be of importance since Donaldson and Jenson (1965) have shown that B-lyson, active against gram positive organisms and derived from the platelets, entered the inflammatory area at a faster rate than other serum proteins. Thus an increase in a relatively non-specific antibacterial factor could explain the effect of progesterone.

The course of intracerebrall infection in mice with S. aureus was not affected by prior hormone treatment. Although on a weight-weight basis the mice were given far more progesterone than were the rabbits no increase in the number of survivers could be detected. This was not surprising since the organisms were introduced into a highly critical site. The use of another route of infection would perhaps yield useful data.

SUMMARY

The effect of progesterone on the growth of various microorganisms was studied *in vitro* and the effect of progesterone on infections due to *Staphylococcus aureus* was studied *in vivo*. The results of these experiments can be summarized as follows:

1) Progesterone inhibited the growth of gram positive but not gram negative bacteria.

2) Progesterone lengthened the lag phase of gram positive bacteria resulting in a decreased growth during the exponential phase.

3) Progesterone was without effect on the course of staphylococcal lesion development in immature female rabbits.

4) Progesterone reduced the size of lesions formed in adult female rabbits due to *S. aureus*.

5) Progesterone exerted no effect on the outcome of intracerbral infection of mice with *S. aureus*.

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ORGANISM	GRAM STAIN	MOTILITY	GLUCOSE O	GLUCOSE F	MANNITOL O	MANNITOL F	MR	VP	NITRATE	UREA	COAGULASE
Proteus mirabilis	G-rod	Motile	AG	AG	X	X	P	P	P	P	X
Bacillus subtilis	G+rod	Motile	A	A	X	X	N	P	P	X	X
Salmonella paratyphi	G-rod	Motile	AG	AG	X	X	P	N	P	N	X
Serratia marcescens	G-rod	Motile	A	A	X	X	N	P	P	N	X
Listeria monocytogenes	G+rod	Motile	A	A	X	X	P	P	N	X	X
Gaffkya tetragena	G+coccus	Non-motile	A	A	N	N	N	N	N	X	N
Staphylococcus aureus(Towler) 53/77	G+coccus	Non-motile	A	A	A	A	X	X	P	X	P
Staphylococcus aureus(296) 29/52/52A	G+coccus	Non-motile	A	A	A	A	X	X	P	X	P
Staphylococcus epidermidis(246)	G+coccus	Non-motile	A	A	N	N	X	X	P	X	N

TABLE I. PHYSIOLOGICAL CHARACTERISTICS OF THE TEST MICROORGANISMS.
A- ACID ONLY AG- ACID PLUS GAS P- POSITIVE N-NEGATIVE X- NOT DONE

AGE OF CULTURE IN HOURS	ORGANISMS							
	Proteus mirabilis		Salmonella paratyphi		Serratia marcescens		Listeria monocytogenes	
	H	C	H	C	H	C	H	C
0	.000	.000	.000	.000	.000	.000	.000	.000
5	.000	.000	.000	.000	.086	.074	.000	.002
5.5	.038	.048	.034	.030	----	----	----	----
6.5	----	----	----	----	.324	.326	.006	.012
7	.208	.256	.164	.144	----	----	----	----
7.5	----	----	----	----	.524	.504	.008	.014
8.5	.488	.538	.438	.402	----	----	----	----
9	----	----	----	----	.654	.654	.004	.030
9.5	.610	.630	.626	.620	----	----	----	----
10.5	----	----	----	----	----	----	.050	.114
11	.740	.766	.746	.740	----	----	----	----
11.5	----	----	----	----	----	----	.112	.218
12	.820	.840	.776	.780	----	----	----	----
12.5	----	----	----	----	----	----	.194	.344
13	.890	.910	.812	.810	----	----	----	----
14	----	----	----	----	1.194	1.194	.328	.484
15	----	----	----	----	----	----	.468	.554
24.5	.960	.960	.963	.940	----	----	----	----
27.5	----	----	----	----	1.318	1.294	.518	.704

TABLE II. EFFECT OF PROGESTERONE ON THE GROWTH OF VARIOUS BACTERIA.

OPTICAL DENSITY DETERMINATIONS AT 420 mu.
H - TRYPTIC SOY BROTH PLUS 20 ug./ml. OF PROGESTERONE IN ETHANOL
C - TRYPTIC SOY BROTH PLUS ETHANOL

AGE OF CULTURE IN HOURS	Bacillus subtilis		Gaffkya tetragena		Staphylococcus aureus (Towler)		Staphylococcus epidermidis	
	H	C	H	C	H	C	H	C
0	.000	.000	.000	.000	.000	.000	.000	.000
5	.004	.000	----	----	----	----	----	----
5.5	----	----	----	----	.022	.036	----	----
6.5	.010	.002	----	----	----	----	----	----
7	----	----	----	----	.100	.190	----	----
7.5	.004	.008	----	----	----	----	----	----
8.5	----	----	----	----	.344	.526	.000	.028
9	.016	.032	----	----	----	----	----	----
9.5	----	----	.000	.000	.570	.758	.000	.094
10.5	.072	.146	.000	.000	----	----	----	----
11	----	----	.000	.010	.818	.918	.000	.254
11.5	.186	.302	----	----	----	----	----	----
13	----	----	.002	.060	.998	.988	.006	.594
14	.588	.674	----	----	----	----	----	----
15	.708	.754	----	----	----	----	----	----
24.5	----	----	.802	1.158	1.018	1.018	.972	.924
27.5	.870	.914	----	----	----	----	----	----

TABLE II. (cont.) EFFECT OF PROGESTERONE ON THE GROWTH OF VARIOUS BACTERIA.

OPTICAL DENSITY DETERMINATIONS AT 420 mμ.
H- TRYPTIC SOY BROTH PLUS 20 ug./ml. OF PROGESTERONE IN ETHANOL
C- TRYPTIC SOY BROTH PLUS ETHANOL

AGE OF CULTURE IN HOURS	MICROGRAMS OF PROGESTERONE PER ML.				
	2.5	5.0	10.0	20.0	0
	OPTICAL DENSITY AT 420 mu.				
0	.000	.000	.000	.000	.000
9	.014	.012	.012	.004	.024
10.25	.032	.032	.032	.012	.048
11.25	.070	.068	.068	.068	.088
12	.098	.094	.094	.066	.126
14.50	.236	.236	.220	.164	.276
15	.264	.262	.256	.204	.316
26	.620	.642	.648	.622	.618
	VIABLE CELLS PER ML.				
0	1.4×10^2	1.6×10^2	1.7×10^2	1.1×10^2	1.8×10^2
3	3.0×10^3	1.2×10^3	1.6×10^3	8.0×10^2	1.6×10^3
5.5	3.0×10^4	4.6×10^4	3.8×10^4	2.0×10^4	8.6×10^4
6.75	3.2×10^5	3.4×10^5	3.0×10^5	2.0×10^5	6.6×10^5
7.75	14×10^5	14×10^5	12×10^5	8.0×10^5	30×10^5
9	8.4×10^6	11×10^6	8.8×10^6	2.8×10^6	20×10^6
10.25	4.8×10^7	3.2×10^7	4.0×10^7	2.0×10^7	5.4×10^7
13	1.1×10^8	1.9×10^8	1.2×10^8	1.1×10^8	1.2×10^8
26	9.2×10^8	8.2×10^8	9.6×10^8	10×10^8	9.4×10^8

TABLE III. EFFECT OF PROGESTERONE ON THE GROWTH OF STAPHYLOCOCCUS AUREUS (STRAIN 296).

RABBIT NUMBER	AMOUNT OF PROGESTERONE ADMINISTERED 0 ug.	20 ug.
	AREA OF SPREAD OF INDIA INK IN SQUARE INCHES	
1	1.50	1.44
2	1.25	1.66
3	2.22	1.67
4	1.61	1.95
5	1.14	1.47
AVERAGE $\pm$ S.D.	1.54 $\pm$ .38	1.64 $\pm$ .13
P VALUE	---	$<$ .20

TABLE IV. EFFECT OF PROGESTERONE ON SKIN PERMEABILITY AS MEASURED BY THE SPREAD OF INDIA INK.

TOTAL AMOUNT OF PROGESTERONE ADMINISTERED	AREA OF LESIONS IN SQUARE INCHES PER DAY				
	1	2	3	4	5
0 ug.	0.29	3.32	2.31	----	0.56
0 ug.	0.37	4.21	3.13	----	0.69
0 ug.	3.09	2.44	0.41	0.50	0.59
0 ug.	1.34	1.52	1.56	1.14	0.74
0 ug.	1.76	1.70	1.14	0.74	0.62
0 ug.	3.52	3.20	1.76	1.32	0.94
0 ug.	2.92	3.17	2.86	1.47	0.93
0 ug.	2.43	2.04	2.00	----	----
0 ug.	3.95	3.77	1.74	----	----
AVERAGE ±S.D.	2.19±1.06	2.82±.80	1.88±.62	1.03±.43	.72±.16
20 ug.	0.34	0.36	0.40	----	0.27
20 ug.	0.35	0.67	0.59	----	0.54
20 ug.	0.50	0.48	0.48	0.53	0.30
20 ug.	1.28	1.02	0.76	0.80	0.80
20 ug.	0.93	1.31	1.28	0.39	0.52
20 ug.	1.49	1.39	1.15	0.53	0.36
20 ug.	1.22	1.57	1.37	1.20	0.89
20 ug.	2.29	1.78	0.96	----	----
20 ug.	2.11	1.76	1.45	----	----
AVERAGE±S.D.	1.17±.58	1.15±.51	0.93±.34	0.69±.25	0.52±.20
P VALUE	----	.005	---	---	---
40 ug.	1.44	1.36	1.24	0.50	0.65
40 ug.	1.28	1.73	1.32	0.55	0.74
40 ug.	0.94	1.61	0.59	0.59	0.50
40 ug.	1.79	1.28	0.67	---	---
AVERAGE±S.D.	1.36±.25	1.50±17	0.96±.33	0.55±.03	0.63±.10
P VALUE	---	.05	---	---	---
80 ug.	1.87	1.21	0.77	----	----
80 ug.	1.80	1.23	0.74	----	----
80 ug.	1.61	1.46	0.92	----	----
AVERAGE±S.D.	1.76±.10	1.30±.11	0.81±.07	----	----
P VALUE	---	.02	---	---	---

TABLE V. EFFECT OF PROGESTERONE ON THE SIZE OF SKIN LESIONS IN ADULT RABBITS.

AMOUNT OF PROGESTERONE ADMINISTERED	AREA OF LESIONS IN SQUARE INCHES PER DAY				
	1	2	3	4	5
0 ug.	1.53	0.29	0.37	0.29	0.33
0 ug.	0.94	0.84	0.62	0.28	0.32
0 ug.	0.62	0.49	0.48	0.41	0.30
0 ug.	0.63	0.46	0.44	0.27	0.23
AVERAGE±S.D.	0.93±.37	0.52±.20	0.48±.09	0.31±.06	0.29±.04
20 ug.	0.94	0.63	0.25	0.21	0.24
20 ug.	0.70	0.61	0.60	0.58	0.35
20 ug.	0.68	0.62	0.47	0.41	0.17
20 ug.	0.61	0.22	0.21	0.25	0.21
AVERARE±S.D.	0.73±.13	0.52±.17	0.38±.16	0.36±.14	0.24±.07
P VALUE	<.05	--	--	--	--

TABLE VI. EFFECT OF PROGESTERONE TREATMENT ON THE SIZE OF LESIONS DUE TO STAPHYLOCOCCUS AUREUS (STRAIN 296) IN IMMATURE RABBITS.

AMOUNT OF PROGESTERONE ADMINISTERED	DEATHS PER DAY					
	1	2	3	4	%S	%M
80 ug.	31/50	34/50	35/50	36/50	28	72
40 ug.	25/50	31/50	34/50	35/50	30	70
20 ug.	31/50	34/50	34/50	36/50	28	72
0 ug.	33/50	34/50	37/50	37/50	26	74

TABLE VII. EFFECT OF PROGESTERONE TREATMENT ON THE OUTCOME OF INTRACEREBRAL CHALLENGE OF MICE WITH STAPHYLOCOCCUS AUREUS (TOWLER STRAIN).

APPROVAL SHEET

The thesis submitted by Ronald T. Stanke has been read and approved by three members of the faculty of the Stritch School of Medicine, Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 27, 1966
Date

William Mortis
Signature of Advisor