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Effect of Testosterone on the Growth and Virulence of Staphylococcus aureus

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Effect of Testosterone on the Growth and Virulence of *Staphlococcus aureus*

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LIFE

Joseph L. Waner was born on February 4, 1942 in Detroit, Michigan. He was graduated from Lane Technical High School in Chicago, Illinois in 1959 and entered the University of Illinois, Chicago campus, the same year. In 1961, he entered Loyola University and was graduated with a Bachelors of Science in 1964. After graduation he worked as a research technician at Presbyterian-St. Luke's Hospital, Chicago, Illinois. He began his graduate studies at Loyola University in 1964.

ABSTRACT

The effect of testosterone on the in vitro growth and virulence properties of S. aureus was studied. There was no apparent effect on growth in defined media or in commercially available media at concentrations of 5 ug. and 20 ug. per ml. Uptake of O_2 in a Warburg apparatus by S. aureus in the presence of testosterone when brain heart infusion broth or pyruvate were used as substrates did not differ. Isotope experiments indicated that there was no metabolic breakdown of the hormone molecule and no association with the cellular portion of broth cultures. Glutamic dehydrogenase activity was increased two-fold in the presence of testosterone as evidenced by the reduction of methylene blue in Thunberg tubes. Fewer lesions resulting from intradermal staphylococcal injections occurred in animals treated with testosterone and erythema surrounding the sites of injection was concomitantly smaller. A supression of the inflammatory response was indicated by this. In addition, animals treated with testosterone and injected intradermally with crude preparations of alpha toxin showed smaller necrotic areas than animals that had not been treated. Studies on the growth of staphylococci in serum from animals treated with testosterone and serum from control animals proved inconclusive.

INTRODUCTION

The anabolic role that testosterone exerts on tissue has been known for some time. The nitrogen retaining effect of testosterone was first demonstrated in castrated dogs (Kachakian, 1935). Metabolic effects on tissues include increased fructose utilization by seminal plasma with enhancement of aldose reductase and ketose reductase activity. Amino acid incorporation into prostatic tissue proteins of the rat is increased with testosterone administration (White, Handler, Smith, 1964). Stimulation of citric acid and fatty acid synthesis by prostatic tissue has also been reported (Eder, 1958). Testosterone has a marked effect on erythrocyte production (Menger, 1955). This is considered to be direct evidence of the anabolic role of this hormone in the synthesis of new cells.

In spite of the extensive literature pertaining to the anabolic properties of testosterone on mammalian tissue, little is known regarding its effect on the various groups of microorganisms. An increase in the rate and extent of proliferation of influenza virus in mice treated with testosterone has been reported (Tepperman et al., 1950). No effect on the survival time of mice treated with testosterone and infected with S. aureus was reported by Ghione (Ghione, 1958). Casas-Campillo and Bojalil (1961) reported that certain progesterone analogues have an inhibitory effect in vitro on the growth of filamentous fungi and gram-positive bacteria; deoxycorticosterone has the same effect (Lester, Hechter, 1958). No comparable information on the effect of testosterone could be found in the literature.

Some mention must be made regarding the vast amount of work done implicating testosterone with acne formation. Hamilton observed clinically

that androgens may predispose human skin to acne (Hamilton, 1941). Later, it was found that injections of testosterone produce sebaceous activity with subsequent acne (Hamilton, 1960). However, other authors have observed a beneficial effect of testosterone in controlling acne (Kalz, 1958). A very recent study has shown that testosterone levels in the serum of people suffering from acne are the same as those in normal patients (Pachi, et al., 1965). The controversy continues but most explanations of acne revolve about some sort of androgen-estrogen-progesterone imbalance (Appelzweig, 1962).

Steroids are known to influence inflammation by surpression. Most of the work in this field has been done with the corticosteroids and, especially hydrocortisone (Dougherty, 1953). No studies implicating testosterone with inflammation have been reported. Beneficial effects of testosterone on granulation tissue response to injury where subcutaneous wounds were inflicted have been reported (Taubenhaus, 1949). The ability of testosterone to increase synthesis of the mucopolysaccharides of connective tissue is also known (Dorfman and Schiller, 1958). Both of these observations could account for a beneficial effect on the host when subjected to intradermal infection.

Staphylococcal alpha toxin possesses the classical biological effects of lethality, dermonecrosis, and <u>in vitro</u> hemolysis of appropriate species red blood cells (Bernheimer, 1965). Virtually all investigators who have recently studied the various effects of alpha toxin agree with Burnet (Burnet, 1929) that they are manifestations of a single toxin. No studies on the influence of testosterone on alpha toxin have been reported.

MATERIALS AND METHODS

S. aureus strains:

Two different S. aureus, coagulase positive, strains were used in this work. Strain 296 was used in the growth studies and for lesion production. Strain G61-60 was used exclusively for alpha toxin production. Stock cultures were maintained on Tryptic Soy Agar (DIFCO), transferred periodically every month, and stored at 4°. Both strains were tested periodically for their ability to coagulate plasma, ferment mannitol and liquify gelatin. Colonies on nutrient agar were yellow to orange in color.

Testosterone solutions:

Testosterone was obtained commercially from the California Corporation for Biological Research, Los Angeles, California. Desired concentrations of testosterone in solution were obtained by dissolving a weighed amount of testosterone in 10 ml. of 95% ethanol. The amount dissolved was such that a 1:100 dilution of the ethanol produced the desired final concentration of testosterone. For the growth studies, the dilution was made directly in the growth media. For injection purposes, the dilution was made in sterile saline. All control solutions contained 1% ethanol.

Growth studies:

Studies on the growth of S. aureus strain 296 in media containing testosterone were performed using Tryptic Soy Broth (DIFCO) and Gladstone's defined medium (Gladstone, 1937). Ninety-nine milliliters of medium was placed in 300 ml. nephelometric flasks. Testosterone at a final concentration of 5 ug and 20 ug per ml. was obtained by dissolving 5 mg. and 20 mg.

amounts of testosterone, respectively, in 10 ml. of 95% ethanol and adding 1 ml. of this solution to the 99 ml. of the growth medium. Tryptic soy broth sterilized by autoclaving and Gladstone's media was sterilized by Seitz filtration. An inoculum of S. aureus from an 18 hr. broth culture containing approximately 1000 cells was used in these studies. The inoculum was washed once and resuspended in 0.85% saline. Cultures were incubated statically at 37° C.

Growth was followed turbidimetrically in the Klett Summerson colorimeter using the blue filter, and by viable plate counts. A sample (0.5 ml.) of the culture was withdrawn aseptically at the time of the Klett reading and serially diluted in 4.5 ml. of sterile 0.85% saline. One ml. of the final dilution was placed in a sterile petri dish and 15 ml. of nutrient agar (DIFCO) added. Each sample was run in duplicate, incubated at 37° C. and counted in 48 hours.

After completion of an experiment, homogeneity of the culture was tested by gram stains, coagulase and oxidation-fermentation mannitol tests.

Manometric studies:

Oxygen uptake studies were performed in the presence of testosterone using brain heart infusion broth (BBL) and pyruvate as substrates. A Warburg constant volume respirometer was used and measurements were recorded in terms of microliters of oxygen consumed using air as the gas phase. Five microgram per ml. concentrations of testosterone in ethanol were used while control flasks contained 1% ethanol. The contents of the flasks appear in Figs. 1 and 2.

Measurement of dehydrogenase activity:

Using the Thunberg Technique, as described by Pelczar (1955), dehydrogenase activity was measured with glutamic acid as substrate. A tube whose color intensity represented 90% reduction was prepared and used as a standard. The contents of the Thunberg tubes are given in Fig. 3.

Labeled testosterone experiments:

Testosterone-4-C^{14} was obtained from the Nuclear Chicago Corporation. Its specific activity was 111.2uc/mg. and its radiochemical purity greater than 98%.

For $C^{14}O_2$ determinations, previously reported techniques, with slight modifications were employed (Goldman and Blumenthal, 1963, 1964, a, b; Blumenthal, 1965; Montiel and Blumenthal, 1966) to 60 ml. of Tryptic Soy Broth (DIFCO) was added 0.08 ml. testosterone-4-C^{14} which contained approximately 84,000 cpm/ml. Ten milliter portions were added to 50 ml. Erlenmeyer flasks that were fitted with center wells for the housing of a removable tube. Each tube contained a filter paperwick. Each flask was inoculated with 0.1 ml. of an 18 hour broth culture and all flasks were sealed with rubber serum stoppers. One hour before taking a sample, 1 ml. of ethanolamine-ethyleneglycolmonomethylether (Jeffay and Alvarez, 1961) was added to the removable tube using a 1 ml. syringe and 22 gauge needle. The flasks were incubated in a Dubnoff metabolic shaker at 37° C. At desired intervals, a flask was removed from the shaker and its removable tube recovered. The tube, with the filter paper, was added to a 20 ml. scintillation vial containing: 10 ml. of a mixture of toluene, ethylene-glycolomonomethylether. The vial was shaken, cooled to 0° C. and counted in a Packard liquid scintillation spectrometer Model 314EX. Corrections were

made for background radiation. Klett readings were taken simultaneously by placing 5 ml. of the culture in a colorimetric tube at the time a flask was removed.

In other labeling experiments, 0.08 ml. of testosterone-4-C^{14} was added to 100 ml. of Tryptic Soy Broth (DIFCO) contained in a 250 ml. flask. The flask was inoculated with 0.1 ml. of an 18 hour broth culture and incubated statically at 37° C. At 8 and 24 hrs., 5 ml. samples were withdrawn and centrifuged for 30 min. at 8,000 x g. The cells were washed twice with saline and the washings pooled. One ml. portions of the supernatant and the washing along with the cell pellet remaining after centrifugation were prepared for scintillation and counted as described above.

Test animals:

Six-month old, white New Zealand male rabbits were used in this work. Administered in 1 ml. doses, animals received 5, 30, or 60 ug. of testosterone subcutaneously every 2 days. Control animals received saline containing 1% ethanol at the same time and by the same route. The animals were challenged after the last injection.

Lesion production:

An 18 hr. culture of *S. aureus*-296 was centrifuged, the supernatant liquid discarded, and the cells suspended in sterile saline to concentrations of 70 and 110 Klett units. By plate counts, this corresponds to 3×10^6 and 7.5×10^6 viable organisms respectively. Intradermal injections (0.5 ml.) were made into the shaven backs of rabbits using 1 ml. syringes and 26 gauge needles. Multiple injections of both concentrations, usually 4 of each, were given to the rabbits. An injection was scored as a lesion if a

primary pustule or a central necrotic area appeared at the site of injection during the two week observation period.

Measurement of erythema:

Twenty-four hours after intradermal injection, the erythema surrounding the injection site was outlined on transparent paper and the size of the erythema was determined planimetrically.

Alpha toxin:

Alpha toxin was prepared according to the method of Bernheimer (Bernheimer, 1963). No steps beyond dialysis were carried out, however, and the contents of the dialysis bag were used as testing material. A 2 l. flask containing 500 ml. of yeast extract media was inoculated with 0.1 ml. of an 18 hour culture of S. aureus G61-60 and incubated on a rotary shaker for 72 hrs. at 37° C. The cells were separated by centrifugation and the clear supernatant fluid was saturated with $(NH_4)_2SO_4$. The saturated solution was placed in the cold at 4° C. where all the subsequent operations were performed. After 24 hours the $(NH_4)_2SO_4$ precipitate was removed by centrifugation. 0.46% powdered cellulose was added to the supernatant fluid with constant stirring. The precipitate was separated by centrifugation, and added to the original $(NH_4)_2SO_4$ precipitate. Five extractions of these pooled precipitates were then made using 10 ml. volumes of borate buffer, 0.03 M, pH 8.6. The extracts were combined and 4.2 g of solid $(NH_4)_2SO_4$ added for each 10 ml. with constant stirring. The precipitate was removed by centrifugation and stored overnight. Five extractions were then made with 10 ml. volumes of 0.4 saturated $(NH_4)_2SO_4$ allowing 30 minutes for each extraction. The extracts were pooled and 1.4 g solid $(NH_4)_2SO_4$ dissolved for

each 10 ml. of fluid. This was allowed to stand for 25 minutes with occasional stirring. The precipitate that formed was removed by centrifugation and dissolved in 3 ml. of 0.03 M borate buffer, pH 8.3. This solution was dialysed against 6 l. of distilled water for 3 days, changing the water each day. After dialysis, the volume in the dialysis sack usually, approximated 7 ml. Any precipitate in the sack was removed by centrifugation and discarded.

The alpha toxin preparation was injected intradermally, in 0.25 cc. amounts, into the shaven backs of rabbits that had been previously treated with 30 ug. of testosterone. In 24 hours, measurements of the resulting necrotic areas were made as described above for erythema.

Hemolysis tests:

Rabbit red blood cells were used exclusively for hemolysis tests. They were obtained by arterial ear puncture using 2% sodium citrate as an anticoagulant, washed in phosphate buffer, pH 7.2, and stored in a 10% solution in the phosphate buffer. The reaction mixture contained a 0.5 ml. of 1% red blood cells and 0.5 ml. of the various dilutions of alpha toxin. The tubes were incubated for 1 hour in a 37° C. water bath. Hemolysin titres were read as the highest dilution giving complete hemolysis.

Effect of testosterone on alpha toxin:

To 2 ml. of the alpha toxin 0.2 ml. of saline containing 20 ug. of testosterone was added and the mixture incubated for 1 hour at 37° C. Controls contained 2 ml. alpha toxin and 0.2 ml. of saline containing 1% ethanol. Following incubation the toxin mixtures were divided into two portions. The first portion was used for the performance of the usual hemolysis test *in vitro*. From the second portion 0.25 ml. was injected

intradermally into the shaven backs of normal 6-month old male rabbits. The necrotic areas were measured planimetrically.

Serum studies:

After a series of injections totally 30 ug. of testosterone had been administered to a rabbit, and before the animal was challenged with s. aureus or alpha toxin, a serum sample was obtained by arterial ear or cardiac puncture. One ml. was incubated with approximately 400 to 600 cells in 0.1 ml. saline or 7000 cells in 1 ml. of saline. The serum cell mixtures were placed in a water bath and incubated for 6 hrs. at 37° C. Plate counts were performed at 0 time and at the end of incubation. Comparable controls were run simultaneously using serum from animals that received 1% ethanol injections.

RESULTS

Effect of testosterone on growth in vitro:

Testosterone, in concentrations of 5 ug. and 20 ug. per ml. in Gladstone's synthetic medium and in Tryptic Soy broth did not alter either the content or rate of growth of *S. aureus*. In Table I the averages of five typical growth curves using Gladstone's medium are presented. Table II presents similar results, using Tryptic Soy broth. Strain 296 was used in all growth experiments. In no instance did the average Klett reading, at any given time differ more than 10 Klett units from that of the controls. The richer Tryptic Soy medium produced an average total growth greater than Gladstone's medium and in one-half the time. Plate counts taken of cultures during growth showed no effect of the hormone on the viable cell numbers.

Manometric studies:

Fig. 1 shows the effect of testosterone on the oxidation of brain heart infusion broth. The lines in the graph represent average values of five experiments. Testosterone at a concentration of 5 ug/ml. did not alter the oxidation of brain heart infusion broth by *S. aureus*.

Fig. 2 shows the effect of testosterone on the oxidation of pyruvate. As with brain heart infusion, the values plotted are averages of five experiments. The amount of O_2 consumed in the preparation containing 5 ug/ml. of testosterone was virtually identical to that consumed by the control.

Dehydrogenase activity:

The effects of testosterone on glutamic dehydrogenase is shown in Fig. 3. It can be seen that testosterone at a concentration of 5 ug/ml. was

capable of stimulating glutamic dehydrogenase activity of s. aureus.

Fate of labeled testosterone:

Fig. 4 presents graphically the quantity of $C^{14}O_2$ evolved in a culture containing C^{14} testosterone. Plotted in the same figure is the turbidimetric growth of the culture. The values given are averages of five experiments. The individual counts of $C^{14}O_2$ are presented in Table 3. When 83,000 cpm. of C^{14} testosterone were present in the growth medium, only an average of 60 cpm were recovered as $C^{14}O_2$, indicating that the testosterone molecule was not being metabolized to CO_2.

From Table 4 it can be seen that a culture medium containing 2,364,000 cpm retained 2,240,000 in the supernatant; 79,000 cpm. were found in cell washings; and 23,700 cpm. were associated with the cell mass.

Lesion production:

The effects of testosterone on the production of lesions in rabbits is shown in Table 5. Given under each experimental group is the percentage of injections that became lesions. Between 3 to 7 rabbits and from 15 to 27 injections were used in each group. Animals treated with testosterone showed fewer pus forming lesions than the controls. The occurrence of lesions progressively decreased as testosterone concentration was increased and when the inoculum of 110 Klett units was employed. The protective effect of testosterone on lesion production was more pronounced when an inoculum adjusted to a turbidity of 70 Klett units (3×10^6 colony forming units) was used as compared to the 110 Klett unit inoculum (7.5×10^6 colony forming units).

Erythema:

The size of erythema resulting from the injection of staphylococci

into rabbits treated with testosterone is shown in Table VI. Between 3 and 7 rabbits were used to obtain the measurements given in each column. A statistical analysis is presented at the bottom of each column. The measurements of the animals receiving testosterone were compared with those of the controls as to the T-values and P values. Significant t-values, greater than 2, were obtained for each experiment where testosterone treatment was analysed except the one where staphylococcal inoculums of 110 Klett units were employed to infect rabbits which had been treated with 60 ug. of testosterone. Corresponding P values demonstrated similar statistical significance.

Effect of testosterone on alpha toxin:

The effect of testosterone on hemolysis of rabbit red blodd cells by alpha toxin in vitro is presented in Table 7. Three experiments were performed using three different alpha toxin preparations. The titre of alpha toxin incubated with 20 ug. of testosterone was in every experiment equal to that of the control.

The effect of incubating 20 ug. of testosterone with alpha toxin demonstrated in vivo, is shown in Table 8. The three alpha toxin preparations used were the same as those appearing in Table 7. It can be seen that incubation of alpha toxin with testosterone and subsequent intradermal injection into normal rabbits did not alter the potency of the alpha toxin. Necrotic areas of comparable size occurred in rabbits treated with testosterone and in rabbits that received no treatment.

Effect of alpha toxin on animals treated with testosterone:

Table 9 presents data on the size of necrotic areas resulting from intra-

dermal injections of alpha toxin in animals that received 30 ug. of testosterone. Three different rabbits were used to obtain the measurements presented in each column. Statistical evaluation in terms of mean values, standard errors of deviation, and t-values are presented at the bottom of each column. A t-value of 45 was obtained when the sizes of necrotic areas in testosterone treated animals were compared with those in the controls. It would appear then, that treatment of rabbits with testosterone will partially protect the animals from the dermonecrotic effect of partially purified staphylococcal alpha toxin.

Serum growth studies of S. aureus in normal and testosterone treated rabbits:

The effect of testosterone on the antistaphylococcal activity of serum is presented in Table 10. Two different inoculums of staphylococci were analysed. Serum was tested so that one sample from a control animal was assayed simultaneously with one from a rabbit treated with testosterone. All samples were tested in duplicate and the numbers presented are averages. With an inoculum of 400-600 cells one half of the testosterone samples had greater killing power and one half had less than the samples from the control rabbits. In 4 of 5 experiments performed with an inoculum of 7000 cells, the serum from testosterone treated rabbits had less antistaphylococcal activity than the controls.

DISCUSSION

Effect of testosterone on the metabolism of S. aureus would be reflected in rate and quantity of growth, and oxidation of substrate. To exert such influences, it is probable that the hormone molecule or some portion of it would be absorbed by the microorganism. Hormones work by derepressing genes previously repressed, thus causing synthesis of messenger RNA molecules not made in the absence of the hormone and thus in turn causing the synthesis by the tissue or organ of new kinds or of increased amounts of enzyme molecules. (Bonner, J., 1965) The results of the isotope experiments in the present study indicate that testosterone was not associated with the cells during growth. Nor did it appear that the molecule was broken down and metabolized to CO_2. It must be pointed out, however, that judgments on the entire molecule are made based upon observation of a single, labeled carbon atom. Utilization of any other carbon atom would pass undetected.

Staphylococci can oxidize pyruvate to CO_2 using it as a sole energy source. There appears to be no effect by testosterone on the oxidation of pyruvic acid. The studies on rate and amount of growth, measured turbidimetrically, further indicate an absence of an effect by testosterone.

The reduction of methylene blue occurred two times faster in the presence of testosterone than in its absence when glutamate was the substrate. This indicates a stimulatory effect on the activity of glutamic dehydrogenase. It has been reported that this is the second most active amino acid dehydrogenase present in Staphylococci (Butler, 1947). A doubling of it's activity could result in significant changes in metabolism. Glutamic acid

dehydrogenase catalyses the reaction by which glutamic acid is formed by reductive amination of alpha ketoglutaric acid, an important intermediate of the tricarboxylic acid cycle. This is a reversible reaction that can be used as an energy source when glutamate is available in the medium. Therefore, an important energy producing metabolic pathway could be influenced. The tricarboxylic acid cycle usually uses O_2, as the ultimate electron acceptor, however, and from the O_2 uptake studies discussed above, there appeared to be no effect when testosterone was present.

By transamination, the alpha-amino group of glutamic acid can be transferred to other alpha-keto acids with the subsequent production of other amino acids. If increases in the amounts of amino acids resulted, it is conceivable that an increase in protein synthesis might occur. This would be reflected in the volume of cells in a culture. No such effects were observed, possibly because any increase in protein synthesis was too small to be reflected in growth studies.

The absence of an effect by testosterone on the growth of S. aureus in vitro and alpha toxin indicates that the smaller erythema sizes, smaller necrotic sizes, and smaller incidence of lesion production observed is testosterone treated animals than in the controls reflects an effect of the hormone on the animal. Injection of microorganisms induces local changes at the site of invasion. These changes, termed the inflammatory response, are important for the host's defense. Erythema, the "redness" appearing at the site of injection, is the first reaction noticed. It is caused by vaso-dilation of the local capillaries. Following erythema, an increased permeability of the vessels causes edema. Finally, a cellular

infiltration of the injured area leads to pus formation. Certain steroids, such as hydrocortisone, are known to suppress inflammation on the basis of their effect on vascular tone and permeability. A similar effect by testosterone could explain the smaller erythema sizes. The occurrence of a few pustules reflects the overall suppression of the inflammatory response. These observations were in agreement with work showing that a testosterone derivative was beneficial in hastening the healing time of cutaneous nocardiosis (Ghione, 1958).

Statistically, the decreased sizes of erythema in animals treated with testosterone are significant. Greater statistical importance could be obtained if each experiment was analysed individually. Presented as they are, the results obtained emphasize the variables of animal susceptibility and experimental technique.

The injection of alpha toxin caused complete necrosis of the affected area of rabbit skin. The absence of inflammatory cells and circulation at the necrotic site for at least twenty four hours (Thal, 1954) indicates that inflammation played a small part in the host's defense. In dealing with necrotic lesions induced in animals that had received testosterone, the ability of this hormone to stimulate formation of granulation tissue could be of great significance (Appelezweig, 1962). Testosterone increases the rate of hyaluronic acid synthesis, an important component of the overall mucopolysaccharide composition of animal tissue. A "sound" tissue structure in treated animals might restrict the action of the staphylococcal alpha toxin.

It should be noted that good results were obtained when statistical analysis was applied to the necrotic sizes. A P-value of .001 was obtained.

The same toxin preparation was used for all injections and these were done in the same lot of rabbits. This procedure eliminated the two important variables that existed in the experiments on the effect of testosterone on erythema.

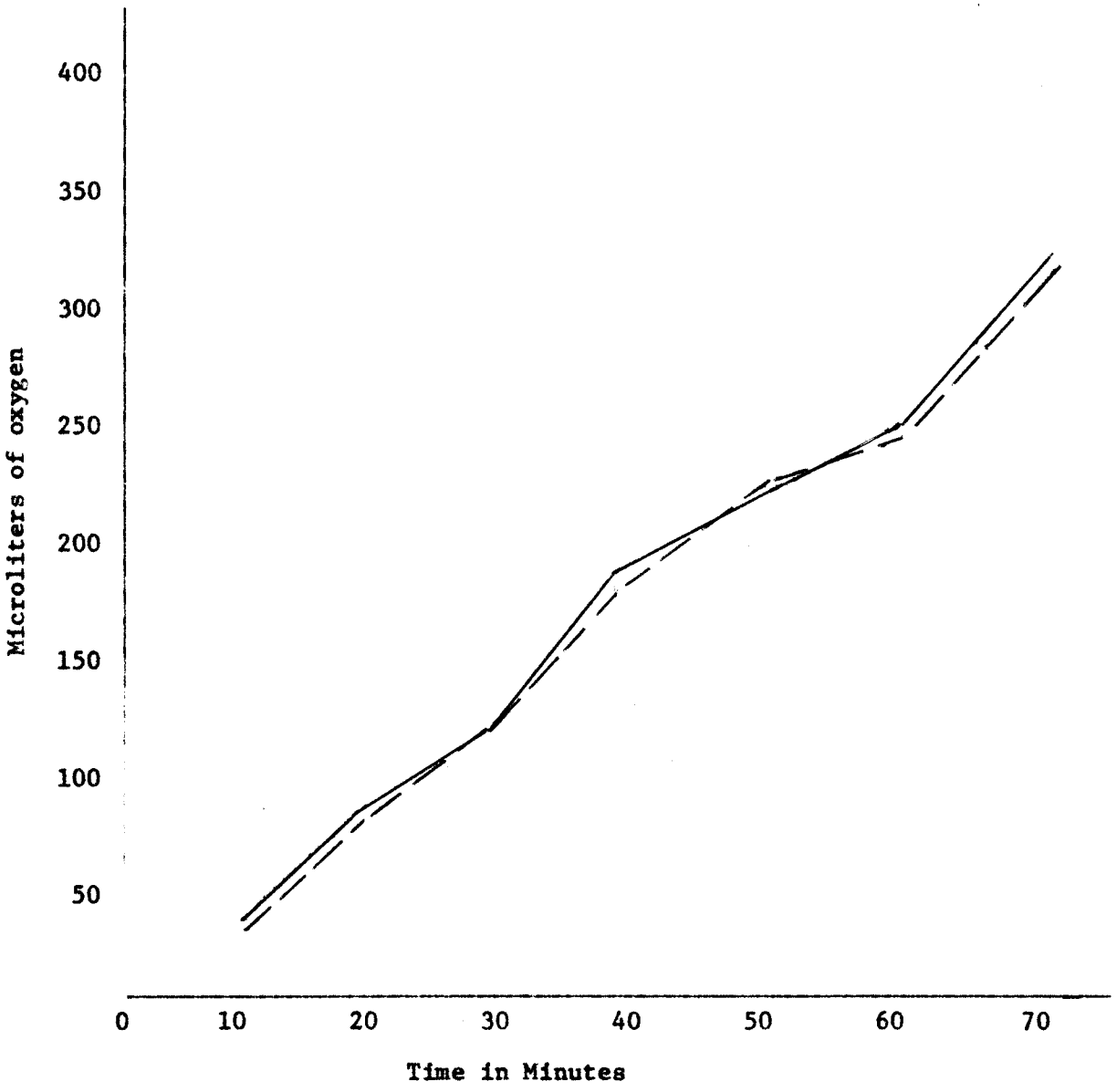

Fig. 1. Effect of testosterone on the oxidation of brain heart infusion broth by S. aureus suspensions.

Contents of Flasks

1 ml. 200 Klett unit cell suspension
0.5 ml. Sodium phosphate buffer, 0.1 M, pH 7.0, 5 ug. testosterone
0.5 ml. BHI broth
0.2 ml. 40% KOH in center well

Curve: A ⬌ Control
B = Testosterone

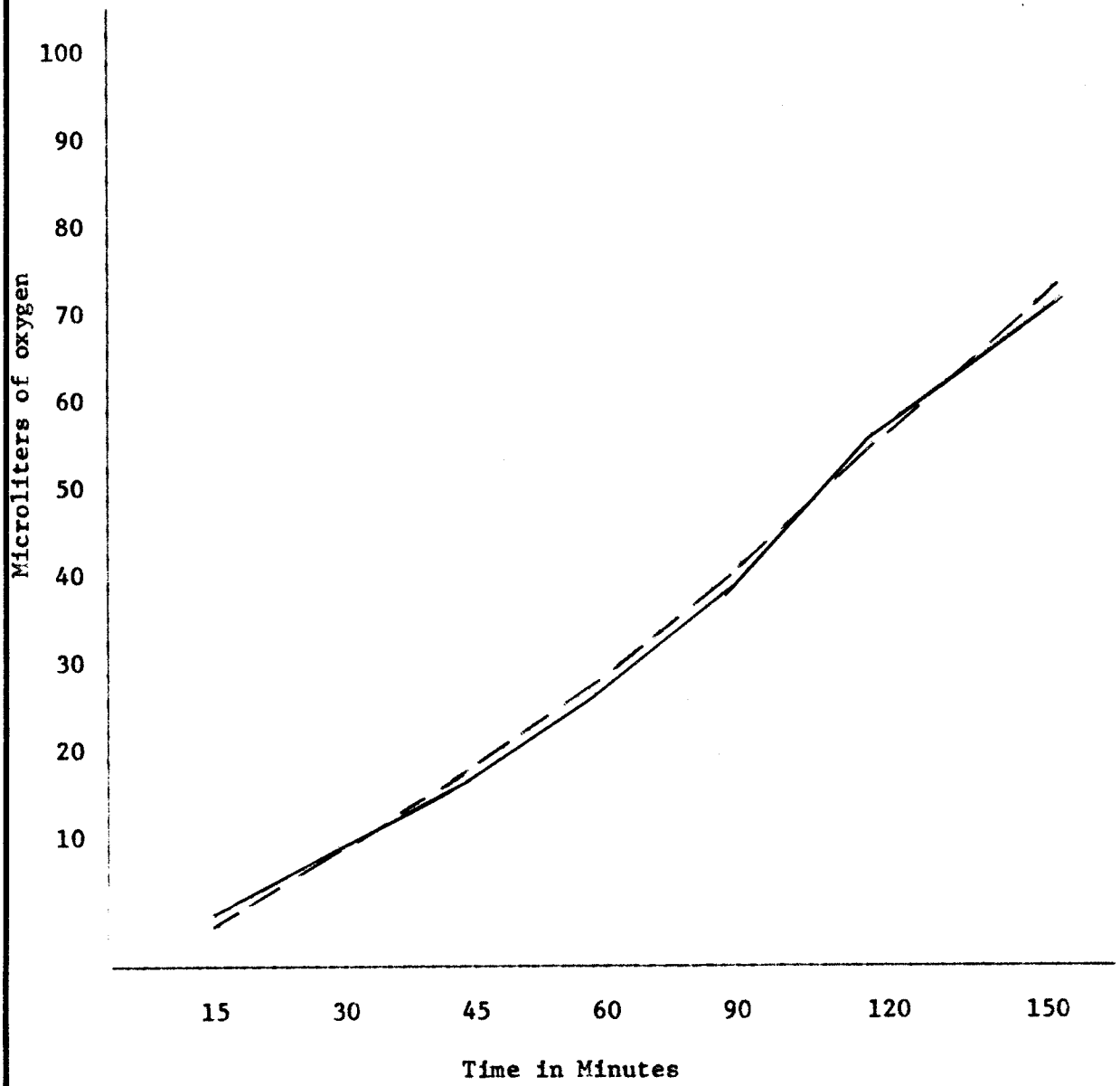

Fig. 2. Effect of testosterone on the oxidation of pyruvate.

Contents of Flasks

A. Klett unit cell suspension (0.4 mg. dry wt. of cells)
B. 1 ml. Sodium phosphate buffer 0.1 M, pH 7.0 containing:
   1. 0.2 M pyruvate
   2. 5×10^{6} M thiamin
   3. 1×10^{5} M niacin
   4. 5 ug. testosterone 1% ethanol
C. 0.2 ml. 40% KOH in center well

Curve: A = Control
B = Testosterone

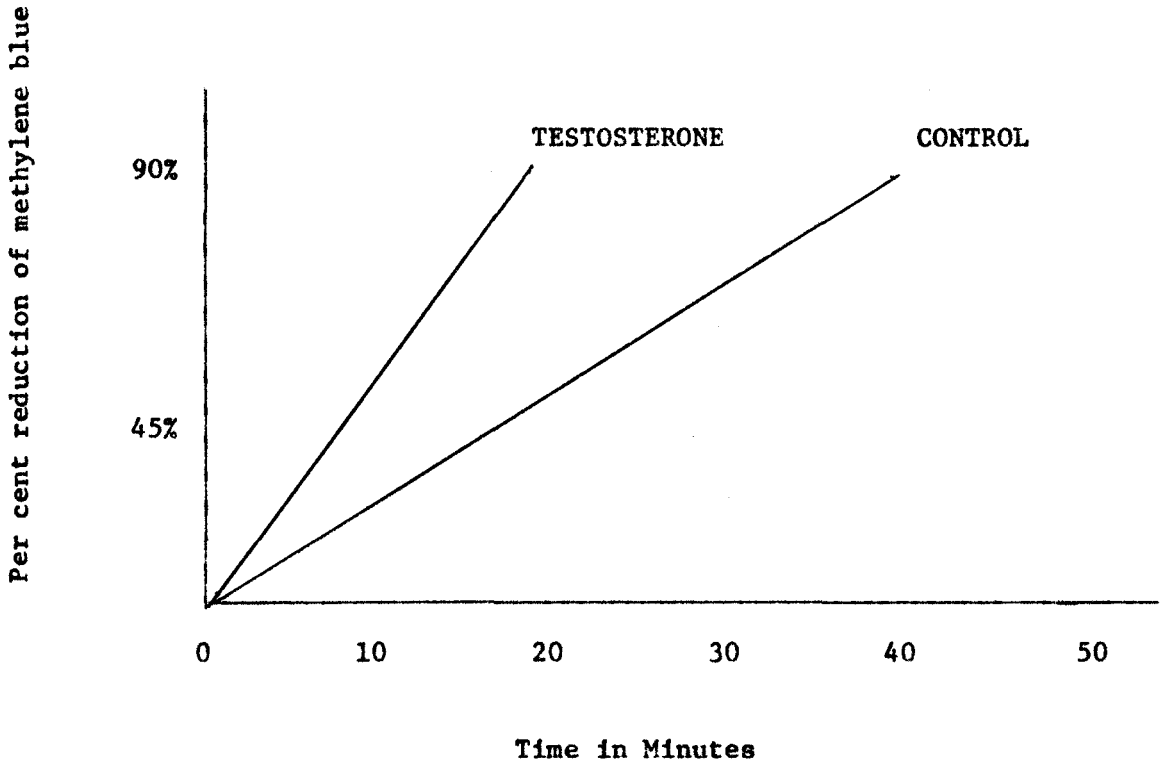

Fig. 3. Effect of testosterone on glutamic dehydrogenase.

Contents of Tubes

500 Klett unit cell suspension
1 ml. methylene blue (1:10 dilution in standard)
2 ml. phosphate buffer 0.01 M; pH 7.0
Glutamic acid M/50

AGE OF CULTURE (HRS.)	CONTROL (KLETT UNITS)					AVG.	TESTOSTERONE (KLETT UNITS)					AVG.
0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
8	3	0	0	0	0	.6	1	0	0	0	0	.2
10	12	21	18	18	13	16	11	25	18	18	15	17
14	24	30	26	30	26	27	22	35	26	27	30	28
18	34	42	35	41	42	38	31	49	34	35	47	39
22	52	60	49	56	63	56	47	57	50	49	68	54
28	92	77	65	72	85	78	89	85	61	67	91	78
32	115	127	103	115	121	116	109	135	100	103	131	115
40	261	240	191	163	183	207	256	246	178	151	191	204
44	285	276	230	220	233	248	280	285	221	208	241	247
50	285	280	238	225	233	252	280	296	230	212	241	251

TABLE 1.

LACK OF EFFECT OF TESTOSTERONE ON GROWTH OF S. aureus, STRAIN 296, IN GLADSTONE'S MEDIUM

AGE OF CULTURE (HRS.)	CONTROL (KLETT UNITS)					AVG.	TESTOSTERONE (KLETT UNITS)					AVG.
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	8	7	15	18	17	13	4	5	19	14	15	11
6	15	16	27	29	25	22	11	12	29	22	29	21
7	30	31	40	44	37	36	24	26	40	33	35	31
8	46	45	51	56	48	49	38	40	52	56	46	46
9	69	68	65	76	65	68	46	59	68	58	60	58
10	101	100	72	95	88	91	86	88	75	84	90	84
11	133	131	88	128	119	119	114	116	90	115	125	112
12	167	164	103	160	152	149	150	152	107	144	162	143
13	202	196	162	186	174	184	180	182	165	174	186	177
18	245	241	240	249	236	242	215	220	241	235	241	230
22	286	280	260	261	255	268	255	278	260	256	259	261
25	308	308	278	276	267	287	306	313	282	284	277	212
27	308	308	278	276	267	287	306	313	282	284	277	292

TABLE 2.

LACK OF EFFECT OF TESTOSTERONE ON GROWTH OF S. aureus, STRAIN 296, IN TRYPTIC SOY BROTH

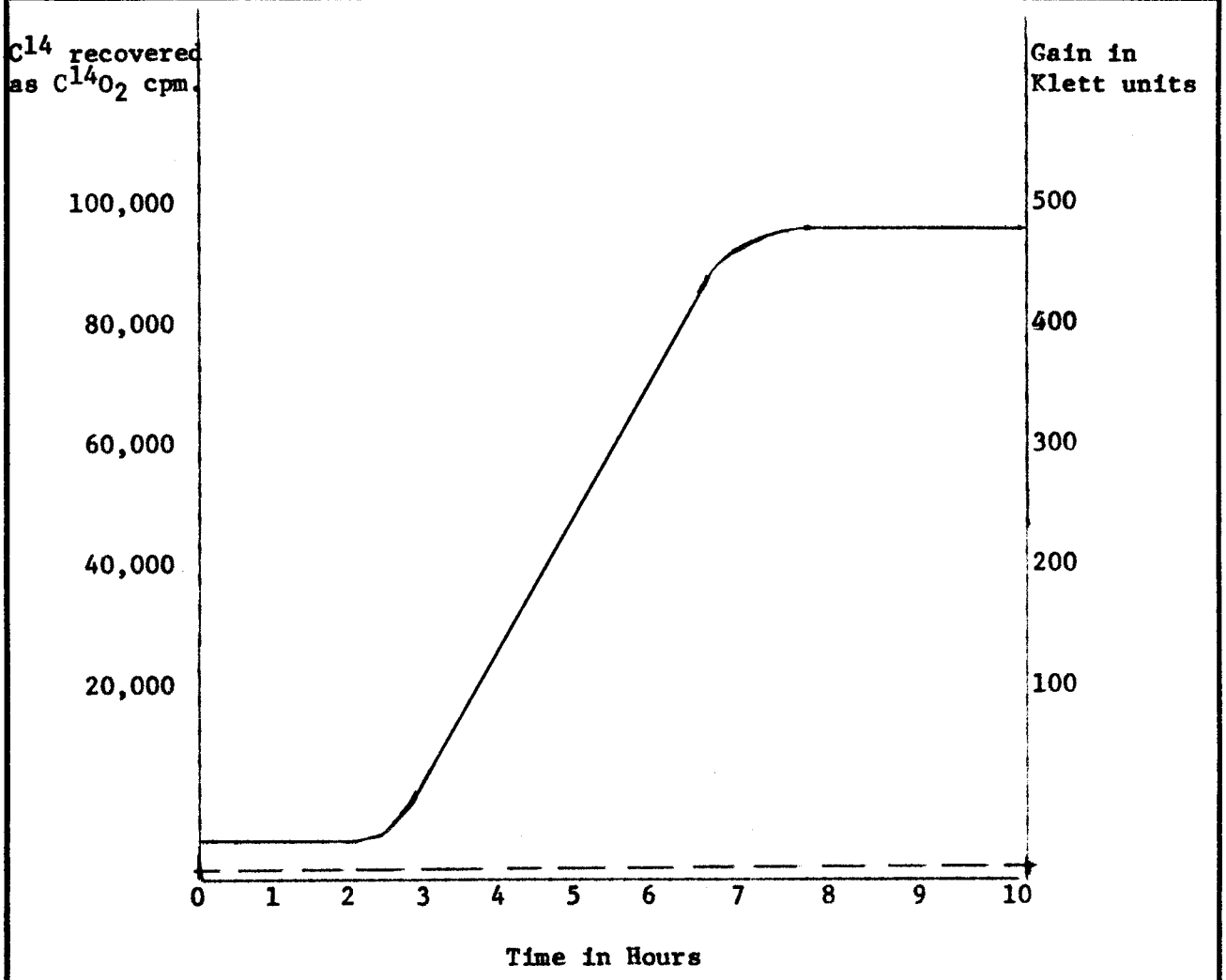

Fig. 4. Evolution of $C^{14}O_2$ as compared to growth. Increase in Turbidity $C^{14}O_2$ Evolution

TIME (HRS.)	1	3	5	7	9	10
CPM $C^{14}O_2$ (minus background)	53	52	72	68	60	55

TABLE 3

EVOLUTION OF $C^{14}O_2$ (83,000 cpm of C^{14} TESTOSTERONE PRESENT IN MEDIA)

TIME IN HOURS	CPM SUPERNATENT	CPM CELL WASHING	CPM/CELLS
0	2,364,000	------	------
8	2,224,000	93,200	23,700
24	2,250,300	65,300	27,200

TABLE 4

DISTRIBUTION OF C^{14} TESTOSTERONE IN S. aureus CULTURE

INOCULUM (KLETT UNITS)	PER CENT CONTROL	INJECTIONS BECOMING LESIONS		
		5 ug	30 ug	60 ug
70	52%	16%	5%	16%
110	84%	66%	57%	33%

TABLE 5

EFFECT OF TESTOSTERONE ON LESION PRODUCTION

INOCULUM (KLETT UNITS) 0.5 cc.	70	70	70	110	110	110
AMOUNT OF TESTOSTERONE ADMINISTERED	0	30 ug	60 ug	0	30 ug	60 ug
MEASUREMENT	2.89	1.53	1.83	3.02	1.89	2.06
OF ERYTHEMA	2.47	1.34	1.67	2.77	1.62	1.96
(sq. in.)	2.31	1.16	1.52	2.76	1.59	1.76
	2.02	1.02	1.44	2.35	1.54	1.56
	1.93	.99	1.33	2.20	1.48	1.45
	1.93	.98	1.31	2.16	1.34	1.44
	1.82	.85	1.04	2.10	1.18	1.44
	1.71	.76	1.04	2.08	1.13	1.40
	1.57	.69	1.04	1.79	.93	1.33
	1.55	.69	1.04	1.76	.92	1.31
	1.42	.50		1.74	.88	
	1.18	.44		1.70	.72	
	1.13			1.44	.52	
	.92			1.43	.52	
	.73			1.39		
	.68			1.37		
	.60			1.18		
	.50			1.13		
t. VALUE	----	3.380	2.409	----	2.66	.789
P. VALUE	----	.005	.050	----	.020	.500

TABLE 6

EFFECT OF TESTOSTERONE ON ERYTHEMA

EXPERIMENT NO.	TITRE	TITRE (1% ETOH)	TITRE (20 ug TESTOSTERONE)
1	1-64	1-64	1-64
2	1-128	1-64	1-64
3	1-128	1-128	1-128

TABLE 7

EFFECT OF TESTOSTERONE ON ALPHA TOXIN HEMOLYSIS IN VITRO

EXPERIMENT NO.	AVERAGE NECROTIC SIZE (1% ETOH)	(20 ug TESTOSTERONE)
1	1.85	1.73
2	3.57	3.66
3	2.67	2.69

TABLE 8

EFFECT OF TESTOSTERONE ON ALPHA TOXIN IN VIVO

	CONTROL	TESTOSTERONE
SIZE OF	2.26	1.85
NECROTIC AREA	2.19	1.77
(sq. in)	2.16	1.62
	1.96	1.59
	1.96	1.56
	2.05	1.46
	2.11	1.41
	1.92	1.34
	1.83	1.33
	1.68	1.22
	1.63	1.15
	1.48	1.09
	1.45	1.02
	1.42	1.32
	1.29	.88
MEAN	1.82	1.37
t. VALUE	----	4.50
P. VALUE	----	.001

TABLE 9

SIZE OF NECROTIC AREAS IN ANIMALS TREATED WITH TESTOSTERONE

INOCULUM	PAIRS TESTED SIMULTANEOUSLY	PLATE COUNTS CONTROL	PLATE COUNTS TESTOSTERONE
400-600 cells in 0.1 ml.	1	10	270
	2	16	14
	3	18	120
	4	51	115
	5	150	128
	6	175	103
7000 cells in 1 ml.	1	2,700	2,400
	2	2,000	2,300
	3	2,200	4,700
	4	3,100	3,500
	5	1,760	2,100

TABLE 10

BACTERIALCIDAL POWER OF SERUM FROM ANIMALS TREATED WITH TESTOSTERONE

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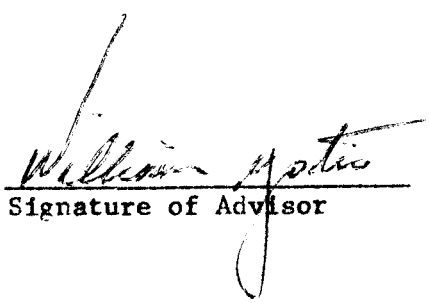
APPROVAL SHEET

The thesis submitted by Joseph L. Waner 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



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