The Effect of Steroids on Microbial Growth[1]

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The importance of hormones in the regulation of growth has long been known. Only in recent years have these compounds been available in sufficient quantity for animal investigation and have been shown to be functional in important physiological processes. To date, however, the role which hormones may play in many of these processes is undefined.

Information concerning the effects of steroids and related compounds on microbial growth, or possible functions of these compounds in microbial metabolism is very limited. Stilbestrol-type compounds have been reported to be bactericidal against gram-positive *Staphylococcus* and *Streptococcus* (1), while vitamin D, ergosterol, and cholesterol have been reported to inhibit growth of both gram-positive and gram-negative organisms (2). The initial oxidation of cholesterol and hydroxysteroids as the sole source of carbon apparently was accomplished by the *Proactinomyces* (5, 7), while steroids lacking the C_{17} side chain were utilized by certain gram-negative bacteria (3,6). Others have reported that attempts to grow bacteria with cholesterol or other steroids as a sole source of carbon were unsuccessful (4).

It would seem logical that since these compounds are important in animal processes, they might also function in some capacity in microorganisms. Studies were undertaken to determine the effects of steroids and related compounds on carbohydrate utilization in microorganisms. This paper reports some of the preliminary results obtained while studying the growth and utilization of various carbohydrates by *Aerobacter aerogenes*.

MATERIALS AND METHODS

Growth experiments were conducted using a synthetic medium containing a single carbohydrate as the source of carbon and energy. The synthetic medium had the following basal composition: NaCl, 0.2 gm.; KH_2PO_4, 0.32 gm.; K_2HPO_4, 0.42 gm.; NH_4Cl, 0.1 gm.; and distilled water, 100 ml. To this was added 0.1 ml. of a mineral salts solution consisting of $MgSO_4.7H_2O$, 5.0 gm.; $MnSO_4$, 0.1 gm.; $FeCl_3$, 1.0 gm.; $CaCl_2$, 0.5 gm.; in distilled water, 100 ml.

The cell suspension used as the inoculum was grown in the synthetic medium with 0.1 per cent glucose as the carbon source. The culture was incubated 20 hours at 37 C. with constant shaking. Aliquots were removed, washed three times with an M/200 phosphate buffer of pH 7.0, and resuspended in the buffer to give approximately 10 per cent light transmittance at 420 mu in a Spectronic 20 colorimeter. One half ml. of the standardized cells was used as the inoculum in all experiments.

The carbohydrates were used in a final concentration of 0.1 per cent with the exceptions of starch which was used in a concentration of 0.05 per cent. All sugars were sterilized separately and added to the synthetic medium just prior to use.

The steroid or steroid-type compounds were added to tubes containing 10 ml. of synthetic medium prior to the addition of the carbohydrate and inoculum. The final concentration of all steroid compounds was 1 mg. per ml. The tubes were incubated at 37 C and growth determined by measuring the per cent transmittance at 420 mu. Appropriate controls were run where necessary. Since the steroid compounds are only slightly soluble in water, the tubes were read both before and after shaking to facilitate accurate growth determinations.

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The results from the turbidity experiments were correlated with plate counts. Aliquots were removed from the tubes at various time intervals and total counts made on nutrient agar.

RESULTS

The hydrolysis of starch by *A. aerogcnes* was studied in the presence of several steroid compounds, and also in the presence of stilbestrol, a related compound. Figure 1 shows the results obtained from one of several experiments. The data indicate that stilbestrol, estradiol, and progesterone apparently enhance the growth of *A acroyenes* when starch is present as a source of carbon and energy. Cholesterol, estrone, and testosterone did not significantly affect the rate of growth in these experiments. Stilbestrol demonstrated the greatest growth enhancement followed by progesterone and estradiol in this order.

The lag period appears to be considerably shorter when stilbestrol was present, while the initiation of growth in the presence of progesterone and estradiol occurred at about the same time as in the control.

The stimulation of growth by stilbestrol was studied by varying the concentration and comparing the rate of growth in standing and shaking cultures. Table I shows the results obtained in this experimentation. The degree of stimulation appears to be dependent on the concentration since 2 mg. per ml. of stilbestrol shows only a small degree of stimulation while a final concentration of 4 mg. definitely enhance the rate of growth. Although the effect was observed in both the standing and shaking cultures, the phenomenon was more pronounced in the latter.

Deviations from the normal procedure were used to determine if the steriod compounds exerted an effect on starch hydrolysis when the growth medium of the inoculum was varied. The cells were grown in synthetic medium containing starch and 1.0 ml. of the actively growing culture placed directly into the tubes containing starch and the steroid compounds. Progesterone and estradiol were the only compounds which stimulated growth under these conditions.

The same compounds were also testsd using glucose, maltose, or raffinose as the source of carbon and energy. The results indicate the steroid compounds do not exert any significant effect on growth of *A aerogenes* in these sugars.

Simultaneous studies revealed that *A. aerogenes* apparently was unable to oxidize the steroid compounds or stilbestrol as a sole source of energy.

All observations were confirmed by correlating turbidity experiments with plate counts. Additional studies are now in progress to more clearly elucidate the observed phenomena.

DISCUSSION

The studies were conducted to determine the effects of various steroid and related compounds on microbial growth and utilization of certain carbohydrates. Stilbestrol, progesterone and estradiol appeared to enhance the growth of *A. aerogenes* when starch was present in the medium as a source of carbon and energy. The results indicate that in the case of stilbestrol, the enhancement of growth may be due to the stimulation of the enzyme. This would seem likely since the lag period was shorter in the tubes containing stilbestrol than in the controls. Progesterone and estradiol apparently stimulate the rate of growth, however, the initiation of growth occurred at about the same time as in the controls.

In experiments in which the inoculum had previously been exposed to starch, the only compounds demonstrating an appreciable effect were progesterone and estradiol. Since these compounds showed no significant effect on the length of the lag period, the evidence indicates they stimulate growth by exerting an effect on metabolic processes other than the pro-

duction of the enzyme. Stilbestol did not influence growth when the cells were previously exposed to starch, thus indicating that this compound may exert its activity solely on the enzyme system. As is to be expected these compounds appear to exert their activity on different metabolic systems of the bacterial cell.

The stimulation of starch hydrolysis and growth of *A. aerogenes* has been shown to be dependent on the concentration of stilbestrol present in the medium. The phenomenon was observed both in standing and shaking cultures, however, the shaking cultures definitely accentuated the stimulation.

Stilbestrol, cholesterol, progesterone, estradiol, estrone and testosterone were also studied for any effect on microbial growth when either glucose, maltose, or raffinose was used as a source of carbon and energy. No significant effects were observed in any of these studies.

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SUMMARY

Stilbestrol, cholesterol, progesterone, estradiol, estrone, and testosterone were studied to determine if these compounds affected the growth of *A. aerogenes* when various carbohydrates were present as the source of carbon and energy. No effects were observed when glucose, maltose, or raffinose was used as the carbon source. however, progesterone, stilbestrol, and estradiol stimulated growth when starch was used as the source of energy. The degree of stimulation by stilbestrol appears to be dependent on the concentration. The possible mode of action of these compounds is discussed.

Experiments also showed that *A. aerogenes* was not capable of using any of the steroid compounds as a sole source of energy for growth and reproduction.

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TABLE I

The effect of varied stilbestrol concentrations on the growth of *A. aerogenes* in standing and shaking cultures containing starch as the energy source

Conditions of growth	Per Cent Transmittance at 420 mu Time in Hours											
	0	10	15	20	25	30	35	40	45	50	55	60
Control-stand	70	70	69	69	69	67	65	61	57	52	46	40
Stilbestrol-stand 2 mg.	71	69	70	68	67	64	61	56	51	45	39	32
Stilbestrol-stand 4 mg.	69	70	68	67	65	62	57	52	46	40	33	26
Control-shake	69	69	70	70	69	66	62	58	52	48	41	36
Stilbestrol-shake 2 mg.	70	70	69	68	65	61	56	51	44	37	30	24
Stilbestrol-shake 4 mg.	70	69	67	64	59	53	45	36	30	25	22	20

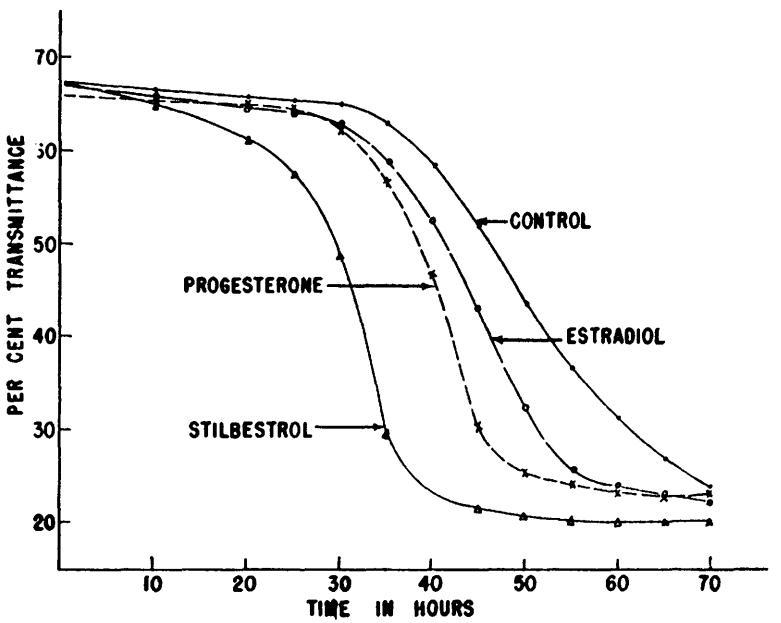

Figure 1 The effect of progesterone, estradiol, and stilbestrol on growth of *A. aerogenes* in medium containing starch as the energy source.