Inhibition of Growth of *Tetrahymena piriformis* by Certain Steroids

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SUMMARY: The growth of *Tetrahymena piriformis* *W* is inhibited by several steroids of mammalian origin. The degree of inhibition is related to the molecular configuration. All of the effective growth inhibitors were of the carbon-21 or pregnane series. Hydroxyl substitution at carbon-11 enhanced inhibition, while hydroxyl substitution at carbon-17 lowered the potency of inhibition. The inhibition of growth induced by these steroids was antagonized by stigmasterol or cholesterol. The mode of action of these compounds is discussed with regard to possible competitive inhibition with some metabolite essential for this organism.

The growth of *Tetrahymena piriformis* *W* has been reported to be inhibited by a number of compounds which are antagonized by stigmasterol or cholesterol (Conner, 1957; Conner & Nakatani, 1958; Dewey, Heinrich & Kidder, 1958). An understanding of how the inhibition of such unrelated compounds as 2:4-dinitrophenol (Conner, 1957), colchicine, deoxycorticosterone (Conner, & Nakatani 1958) and 6-methyl purine (Dewey *et al.* 1958) is antagonized by certain sterols has not yet been reached. It was noticed, however, that the various steroids differed in their ability to inhibit the growth of this organism, and the greater the degree of inhibition the higher the concentration of stigmasterol required to annul this effect. This led to a study of the molecular configuration of different steroids with regard to potency of biological action.

METHODS

Tetrahymena piriformis *W* was grown in a modified chemically defined medium by techniques based on those developed by Kidder *et al.* (1951) and Dewey *et al.* (1951). The composition of the medium is given in Table 1.

The steroids were suspended in warm Tween 80 by the aid of a motor-driven Teflon homogenizer in preparation for testing. These solutions were sterilized separately and added aseptically while still warm. All the steroids tested were soluble to *c.* 10^{-3} M in the culture medium used. The following steroids were tested at final concentrations of 1, 2.5, 5, 7.5 and 10×10^{-4} M: two preparations of deoxycorticosterone, two samples of progesterone, two samples of cortisone, one of 11-dehydrocorticosterone, corticosterone, 17-hydroxydeoxycorticosterone, 9- α -fluorocortisone, 17-hydroxycorticosterone, 1-dehydrocortisone, 1-dehydro-17-hydroxycorticosterone, diethylstilbestrol, testosterone, estradiol (Merck, Sharp and Dohne, Inc., given by Dr L. Sarret, Rahway, New Jersey, U.S.A.).

The sterols stigmasterol (from Dr O. Behrens, Eli Lilly Co., Indianapolis,

Table 1. Composition of medium used for the growth of *Tetrahymena piriformis* W.

Compound	($\mu\text{g./ml.}$)	Compound	($\mu\text{g./ml.}$)
DL-Alanine	110.0	MgSO ₄ .7H ₂ O	100.0
L-Arginine	206.0	Fe(NH ₄) ₂ (SO ₄) ₂ .6H ₂ O	25.0
L-Aspartic acid	122.0	MnCl ₂ .4H ₂ O	0.50
Glycine	10.0	ZnCl ₂	0.05
L-Glutamic acid	233.0	CaCl ₂ .2H ₂ O	50.00
L-Histidine	87.0	CuCl ₂ .2H ₂ O	5.00
DL-Isoleucine	276.0	FeCl ₃ .6H ₂ O	1.25
L-Leucine	344.0	K ₂ HPO ₄	1000.0
L-Lysine	272.0	KH ₂ PO ₄	1000.0
DL-Methionine	248.0		
L-Phenylalanine	160.0	Guanylic acid	30.0
L-Proline	250.0	Adenylic acid	20.0
DL-Serine	394.0	Uracil	10.0
DL-Threonine	326.0	Cytidylic acid	20.0
L-Tryptophan	72.0		
DL-Valine	162.0	Glucose	2500.0
Calcium pantothenate	0.10	Sodium acetate	1000.0
Nicotinamide	0.10		
Pyridoxine hydrochloride	1.0	Tween 80	10,000.0
Pyridoxal hydrochloride	0.10		
Pyridoxamine hydrochloride	0.10	DL-Thioctic acid	0.002
Riboflavin	0.10		
Pteroylglutamic acid	0.01	Disodium ethylenediamine tetraacetic acid (EDTA)	20.0
Thiamine hydrochloride	1.00		
Biotin (free acid)	0.0005		
Choline chloride	1.00		

Indiana, U.S.A.) and cholesterol (Nutritional Biochemicals, Cleveland, Ohio, U.S.A.) were added as emulsions prepared as described previously (Conner, van Wagtenonk & Miller, 1953). These were tested at final concentrations ranging from 1×10^{-9} to 1×10^{-4} M.

All tubes were inoculated with 0.1 ml. of a 1/10 dilution of a 72 hr. culture of *Tetrahymena piriformis* W. The growth period was 96 hr. and the amount of growth was estimated as an increase in optical density measured in a Lumitron colorimeter with a 650 m μ filter.

All concentrations of each steroid were tested in triplicate and each experiment was repeated at least three times.

RESULTS

The steroids tested were readily divided into active and inactive groups. Inactive steroids were: testosterone, diethyl-stilboesterol, oestradiol, 1-dehydrocortisone and 1-dehydro-17-hydroxycorticosterone. The steroids which inhibited the growth of *Tetrahymena piriformis* W could be placed in a series ranging from slightly inhibitory to highly inhibitory. Table 2 gives the percentage and range of inhibition of growth for given concentrations of steroids.

Fig. 1. Skeletal structures of the steroids.

The growth inhibition induced by progesterone and by corticosterone, respectively, and its annulment by stigmasterol is shown in Figs. 2 and 3.

Cholesterol seemed to be nearly as effective as stigmasterol in annulling growth inhibition in most experiments. Oestradiol had no effect.

Table 2. *Relative activity of various steroids in the inhibition of growth of Tetrahymena piriformis* W

Compound	Concentration (M)	Inhibition (range)* (%)
Progesterone	1×10^{-3}	20 (18–24)
9 α -fluorocortisone	1×10^{-3}	18 (10–25)
Cortisone†	1×10^{-3}	64 (50–72)
11-ketoprogesterone	1×10^{-3}	67 (60–80)
17-hydroxydesoxycorticosterone	1×10^{-3}	67 (54–78)
17-hydroxy corticosterone	1×10^{-3}	75 (60–90)
Desoxycorticosterone	7.5×10^{-4}	97 (90–100)
Corticosterone	2.5×10^{-4}	100
11-dehydrocorticosterone	2.5×10^{-4}	100

* Percentage inhibition is the average of nine determinations.

† Cortisone values given here are somewhat higher than reported previously (Conner & Nakatani, 1958). This difference is attributed to the sample of steroid used.

Fig. 2

Fig. 3

Fig. 2. Growth response of *Tetrahymena piriformis* in presence of progesterone (1×10^{-3} M) with increasing concentrations of stigmasterol. With stigmasterol alone (O); with progesterone + stigmasterol (x).

Fig. 3. Growth response of *Tetrahymena piriformis* in presence of corticosterone (2.0×10^{-4} M) and stigmasterol. With stigmasterol alone (O); with corticosterone + stigmasterol (x).

DISCUSSION

Comparison of the active and inactive steroids indicates the following specificities of effect:

(1) A side chain (C-20, -21) was necessary for growth inhibition. Progesterone (see Fig. 1 for structure of compounds) was inhibitory, while testosterone and estradiol were not.

(2) A hydroxyl grouping at C-21 enhanced inhibition. Progesterone was less inhibitory than deoxycorticosterone.

(3) Substitution of a keto or hydroxyl grouping at C-11 enhanced inhibition. Corticosterone was more inhibitory than deoxycorticosterone, 11-dehydrocorticosterone more than deoxycorticosterone, 11-keto progesterone more than progesterone.

(4) Substitution of a 17- β -hydroxy grouping lowered the inhibitory response. 11-Dehydrocorticosterone was more inhibitory than cortisone, and deoxycorticosterone more than 17- β -hydroxydeoxycorticosterone. 17- β -Hydroxycorticosterone was not available in amounts sufficient for testing to compare with corticosterone.

(5) Further unsaturation of the A ring lowered potency. 1-Dehydrocortisone and 1-dehydro-17- β -hydroxycorticosterone were less inhibitory than cortisone.

(6) Substitution in the B ring at position 9 lowered potency. 9- α -Fluorocortisone is less inhibitory than cortisone. *Tetrahymena piriformis* W has been reported to contain steroid-like materials (McKee, Dutchen, Groupé & Moore, 1947; Seaman, 1950); from McKee's data it would appear this material is not cholesterol or stigmasterol. Perhaps these two sterols are converted to some physiologically active form. This supposition is strengthened by the findings of Holtz (1957) that a species of *Tetrahymena* required a sterol for growth, a requirement satisfied by cholesterol, β -sitosterol or stigmasterol. These organisms then differ from *Paramecium aurelia* which is unable to utilize cholesterol (Conner & van Wagtenonk, 1955). This may represent a greater metabolic lesion in the synthetic capacity of *P. aurelia*. The steroid isolated from *Tetrahymena piriformis* supported the growth of *P. aurelia* (Conner, 1957). Thus it would seem that a common metabolite or physiological form of the steroid exists in this group of protozoans.

The growth inhibition induced by certain of the steroids appears to be annulled by stigmasterol in a competitive fashion. Perhaps the configuration of the steroid inhibitor is related to the configuration of the steroid which is produced by the organism. It is striking that a carbonyl or hydroxyl grouping at carbon-11 greatly enhanced the degree of inhibition, while a carbon 17- β -hydroxyl substitution decreased it.

An action of steroids on metabolism has been reported for many types of organisms, organs, tissues, cells and cellular preparations. However, the probable *in vivo* mode of action for only one steroid, estradiol, has been elucidated (Talalay, Hudock & Williams-Ashman, 1958). The protozoans may provide further information about this. Investigations are at present being carried out to determine the mode of action of the inhibitory steroids on metabolism both *in vivo* and *in vitro*.

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