

## Studies on the effect of hormones on the growth of *Aspergillus clavatus*, *A. fumigatus* and *A. niger* in vitro

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In a previous study, REFAI and EL-SHERIF (1974) found that hormones exerted an inhibitory or stimulatory effects on the growth of *Candida albicans* and *Cryptococcus neoformans*. Some of these results could be verified in experimental animals. On the continuations of this work, EL-SHERIF and REFAI (1974) proved that 11 hormonal agents inhibited the growth of 4 dermatophytes, however, the degree of response varied as regard to the different dermatophytes and hormones.

In this respect, MOURSI and REFAI (1968), in a preliminary study, noticed that sex hormones had different effect on the growth of fungi and the responsiveness of each hormone differed according to the dose.

The present work is dealing with investigating the effect of hormones on the growth of *Aspergillus clavatus*, *A. fumigatus* and *A. niger* in vitro.

The eleven hormones and methods of testing and evaluation of the results are the same as previously mentioned in the other 2 papers (REFAI and EL-SHERIF, 1974, and EL-SHERIF and REFAI, 1974).

### Results

1. The effect of hormones on the growth of *Aspergilli* in concentrations comparable to their normal physiological or therapeutic levels in man.

As shown in Table 1 it is clear that *Aspergillus clavatus* was the most sensitive while *A. fumigatus* and *A. niger* resisted the effect of all hormones with the exception of the thyroid gland secretion which exerted a very highly significant inhibition of growth of the 3 species.

*A. clavatus* was very highly significantly inhibited by ACTH, thyroid gland secretion, stilboestrol and norethisterone.

It is interesting to note that the L. H. inhibited the growth of *A. fumigatus* and *A. niger* in a very highly significant manner ( $P < 0.0005$ ) in concentration lower than that of the normal physiological level of this hormone in man; when the concentration of the hormone exceeded 1.0 mcg/ml both *Aspergillus* species grew the same as the control plates and only *A. clavatus* showed reduction in the size of the colony in a graded manner (Table 2-4).

2. The effect of hormones on the growth of *Aspergilli*, in concentrations higher than that of their normal physiological or therapeutic values in man.

*Aspergillus niger* resisted the effect of oestradiol 17 $\beta$ , ethinyl oestradiol and progesterone in concentrations up to 100.0 mcg/ml, stilboestrol in concentrations up to 12.5 mcg/ml and insulin up to 6.4 mcg/ml. ACTH had no effect on it in concentrations up to 0.001 mcg/ml (Table 2) but it inhibited its growth completely in a concentration of 0.05 mcg/ml.

Likewise, testosterone inhibited the growth of *A. niger* in a very highly significant manner in a concentration of 42.0 mcg/ml and more, while lower concentrations had no effect (Table 5).

A very highly significant inhibition of this mould was achieved by the posterior pituitary hormone, thyroid gland secretion and norethisterone.

Table 1: The effect of hormones on the growth of *Aspergilli* in concentrations equivalent to their normal physiological or therapeutic levels

Hormones	Concentration in mcg per ml	<i>A. clavatus</i>	<i>A. fumigatus</i>	<i>A. niger</i>
ACTH	$0.1 \times 10^{-3}$	-65 %	—	—
	$0.15 \times 10^{-3}$	-68 %	—	—
L. H.	5.0	-24 %	—	—
Posterior pituitary	$1.0 \times 10^{-3}$	—	—	—
Thyroid extr.	5.0	-16 %	-29 %	-29 %
	10.0	-30 %	-29 %	-29 %
Insulin	$8-64 \times 10^{-6}$	—	—	—
Oestradiol 17 $\beta$	$1.0 \times 10^{-4}$	—	—	—
Ethinyl oestradiol	$1.0 \times 10^{-3}$	-8 %	—	—
Stilboestrol	$25 \times 10^{-2}$	-72 %	—	—
Progesterone	0.05	-5 %	—	—
Testosterone	$8.4-42 \times 10^{-4}$	—	—	—
Norethisterone	0.5	-23 %	—	—
	1.0	-26 %	—	—

Table 2: The effect of ACTH on the growth of *Aspergilli*

	Concentration in mcg/ml		
	0.0005	0.001	0.05
<i>A. clavatus</i>	-69 %	-72 %	-100 %
<i>A. fumigatus</i>	—	—	-100 %
<i>A. niger</i>	—	—	-100 %

*A. clavatus* resisted the effect of ethinyl oestradiol in concentrations up to 100.0 mcg/ml; stilboestrol inhibited it markedly in concentrations up to 10.0 mcg/ml but it lose its effect completely in higher concentrations (Table 6—8).

Progesterone and norethisterone exerted a triphasic effect on *A. clavatus*, in concentrations comparable to their physiological or therapeutic levels they inhibited its growth, higher concentrations stimulated the growth and more higher concentrations caused inhibition of this mould again (Table 9, 10).

Insulin exerted a diphasic effect consisting of initial stimulation followed by inhibition of *A. clavatus* on increasing its concentration (Table 11, 12).

*A. fumigatus* resisted the effects of oestradiol 17 $\beta$  and stilboestrol. In a concentration of 0.05 mcg/ml the ACTH inhibited its growth completely. Graded type of inhibition was exerted by the posterior pituitary extract, ethinyl oestradiol, progesterone, norethisterone, testosterone, insulin and thyroid gland secretion in a very highly significant manner ( $P < 0.0005$ ).

#### Discussion

It is clear from the foregoing results that the 3 *Aspergillus* species, viz. *A. clavatus*, *A. fumigatus* and *A. niger*, varied in their response to the action of the various hormones. *A. clavatus* was the most sensitive and *A. niger* was the most resistant. *A. fumigatus* occupied an intermediate position. However, all 3 species were markedly inhibited by the thyroid extract in concentrations comparable to its normal physiological level in man and by L. H. in concentrations lower than that of the normal physiological level. Moreover, complete inhibition of all of them was achieved by ACTH in a concentration of 0.05 mcg/ml.

These variations in response are difficult to explain. The resistance of *A. niger* to the effect of hormones may be due to chemical changes of the hormones by the fungus as mentioned by FRIED et al. (1952), ZAFFARONI et al. (1955), WIX et al. (1957) and SHIRASAKA et al. (1960) who reported hydroxylation of steroidal agents at the 11 or 21 position by *A.*

Table 3: The effect of L. H. on the growth of *Aspergilli*

	Concentration in mcg/ml		
	0.5	1.0	2.0
<i>A. clavatus</i>	-13 %	-13 %	-16 %
<i>A. fumigatus</i>	-29 %	-57 %	—
<i>A. niger</i>	-15 %	-15 %	—

Table 4: The effect of posterior pituitary extract on the growth of *Aspergilli*

	Concentration in mcg/ml		
	0.5	1.0	2.0
<i>A. clavatus</i>	- 4 %	-15 %	-30 %
<i>A. fumigatus</i>	-29 %	-29 %	-57 %
<i>A. niger</i>	—	-14 %	-29 %

Table 5: The effect of testosterone on the growth of *Aspergilli*

	Concentration in mcg/ml					
	4.2	8.4	21.0	42.0	63.0	84.0
<i>A. clavatus</i>	+13 %	+13 %	+13 %	—	—	-30 %
<i>A. fumigatus</i>	-29 %	-29 %	-29 %	-43 %	-57 %	-57 %
<i>A. niger</i>	—	—	—	-29 %	-57 %	-57 %

Table 6: The effect of oestradiol 17 $\beta$  on the growth of *Aspergilli*

	Concentration in mcg/ml		
	50.0	75.0	100.00
<i>A. clavatus</i>	-15 %	-15 %	-15 %
<i>A. fumigatus</i>	—	—	—
<i>A. niger</i>	—	—	—

Table 7: The effect of ethinyl oestradiol on the growth of *Aspergilli*

	Concentration in mcg/ml		
	50.0	75.0	100.0
<i>A. clavatus</i>	—	—	—
<i>A. fumigatus</i>	-29 %	-43 %	-57 %
<i>A. niger</i>	—	—	—

*niger*. On the other hand, the growth promotion caused by insulin may be due to stimulation of anabolic processes in the fungal cells (VILLAR-PALASI and LARNER, 1960, KRABL, 1961).

The results of the thyroid extract and L. H. are of therapeutic interest because they inhibited the growth of the 3 *A.* species in very low concentrations. The use of such hormones in treatment may

Table 8: The effect of stilboestrol on the growth of *Aspergilli*

	Concentration in mcg/ml		
	5.0	10.0	12.5
<i>A. clavatus</i>	-44 %	-72 %	—
<i>A. fumigatus</i>	—	—	—
<i>A. niger</i>	—	—	—

Table 9: The effect of progesterone on the growth of *Aspergilli*

	Concentration in mcg/ml			
	10.0	25.0	50.0	75.0
<i>A. clavatus</i>	+13 %	+ 2 %	-15 %	-44 %
<i>A. fumigatus</i>	-14 %	-29 %	-29 %	-29 %
<i>A. niger</i>	—	—	—	—

Table 10: The effect of norethisterone on the growth of *Aspergilli*

	Concentration in mcg/ml				
	10.0	25.0	50.0	75.0	100.0
<i>A. clavatus</i>	+13 %	—	-9 %	-44 %	-72 %
<i>A. fumigatus</i>	—	-29 %	-57 %	-93 %	-93 %
<i>A. niger</i>	-29 %	-29 %	-57 %	-57 %	-79 %

Table 11: The effect of thyroid gland secretion on the growth of *Aspergilli*

	Concentration in mcg/ml		
	25.0	50.0	100.0
<i>A. clavatus</i>	-35 %	-52 %	-66 %
<i>A. fumigatus</i>	-29 %	-29 %	-71 %
<i>A. niger</i>	-29 %	-57 %	-71 %

Table 12: The effect of insulin on the growth of *Aspergilli*

	Concentration in mcg/ml				
	0.2	0.4	0.8	1.6	3.2
<i>A. clavatus</i>	+41 %	+41 %	+41 %	+41 %	-44 %
<i>A. fumigatus</i>	—	-14 %	-14 %	-57 %	-57 %
<i>A. niger</i>	—	—	—	—	—

be particularly useful especially in cases of systemic mycoses and lung infection e.g. aspergillosis, where a potent and non-toxic antifungal drug is lacking. The inhibitory effect of the thyroid extract

may be due to its damage to the mitochondria (WOLFF and WOLFF, 1964). SUTHERLAND et al. (1965) observed lipolysis in presence of inhibitory levels of L. H.

### Summary

Eleven hormones (adrenocorticotrophic hormone, luteinising hormone, thyroid extract, posterior pituitary extract, insulin, oestradiol 17 $\beta$ , ethinyl oestradiol, stilboestrol, progesterone, testosterone and norethisterone) were tested for their effects on the growth of *Aspergillus clavatus*, *A. fumigatus* and *A. niger*. The luteinising hormone and the thyroid extract inhibited the growth of all 3 *Aspergillus* species in very low concentrations (1.0 and 5 mcg/ml respectively). On the other hand, insulin, progesterone and norethisterone promoted the growth of these moulds in lower concentrations and inhibited it in higher concentrations. The other hormones exerted a graded type of inhibitory effect. *A. clavatus* was the most sensitive, *A. niger* was the most resistant and *A. fumigatus* occupied an intermediate position.

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