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Studies on the Fungistatic Action of Hormones on Dermatophytes

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Spontaneous cure of tinea capitis with the onset of puberty has been demonstrated by several authors (Livingood and Pillsbury, 1941, Schully et al., 1948, Laur, 1951, Kligman, 1951 and 1952 and Whittle, 1953). Moreover, Seale and Richardson (1960) and Mackenzie (1961) reported that tinea capitis was more commonly encountered in children of school age up to puberty. In Egypt, Abdel-Fattah et al., (1967) achieved similar findings.

Kligman and Ginsberg (1950) and Kligman (1952) induced experimental infection of the scalp by Microsporum canis and M. audouinii in human subjects of different age groups. They noted that the infection was not terminated by puberty and tended to undergo spontaneous resolution before, or some years after the onset of puberty. The curative effect which was presumably ascribed to puberty could then be well explained by the normally selflimiting course of tinea capitis along with a greatly reduced susceptibility to primary infection after puberty.

The effect of some hormones on the growth of Candida albicans, Microsporum gypseum and Aspergillus niger has been preliminarly studied by Moursi and Refai (1968). Moreover, Refai and El-Sherif (1974) investigated the role of several hormones on Candida albicans and Cryptococcus neoformans both in vitro and in vivo.

The present work is dealing with the effect of hormones on the growth of dermatophytes.

Material and methods

Eleven hormonal agents were tested for their effect on the growth of 4 dermatophytes, namely Microsporum canis, M. gypseum, Trichophyton mentagrophytes and T. rubrum. The dermatophytes used in this investigation were studied at first for the average time of optimal growth and average diameter of the colonies. The hormones were:

Adrenocorticotropic hormone (ACTH), luteinising hormone (L.H.), posterior pituitary extract, thyroid gland secretion, insulin, oestradiol 17B, ethinyl oestradiol, Stilboestrol, Progesterone, testosterone and the contraceptive, norethisterone. Aqueous solutions or uniformly homogenous suspensions of the hormonal agents were included in the culture media (Sabouraud glucose Agar) at a fairly wide range of concentrations. For each concentration six plates were used. Control plates containing the hormonal solvents or vehicle as well as the suspending agent (alcohol + tween 80) were included in the work.

All plates were inoculated with equal inoculum of the fungi and were incubated at 30 °C and the colonies were measured at the time of optimal growth of each dermatophyte and compared with that of control plates. The changes in

size of the colonies were calculated in % and evaluated statistically.

Results

The average diameter of the colonies and optimum time of growth of control cultures are illustrated in **Table 1.**

These measurements were used as control for the evaluation of inhibition or stimulation of the growth.

levels (0.1×10^{-3} to 0.25×10^{-3} mcg/ml) it caused partial inhibition especially of M. gypseum and T. rubrum. At concentration of 0.1 mcg/ml all the 4 dermatophytes were completely inhibited **(Table 2).**

2. *Luteinising hormone*

Graded type of inhibitory response was recorded for M. gypseum and T. rubrum. As regard M. gypseum the degree of inhibition increased with increasing concen-

Table 1: Average diameter of fungal colonies at the time of optimal growth

	Median values (P50) for average time of optimal growth in days	Statistical parameters for size of the colonies at time of optimal growth.				
		Measures in cm		95 % Fiducial limits		
		Mean value	range	S. D.	lower	upper
M. canis	16 (7—21)	2.86 ± 0.27	1.7—4.0	0.88	2.17	3.55
M. gyppseum	15 (6—21)	4.59 ± 0.15	4.0—5.5	0.49	4.20	4.98
T. mentagrophytes	15 (7—21)	2.87 ± 0.60	1.9—4.0	2.0	1.33	4.41
T. rubrum	19 (10—21)	1.66 ± 0.08	1.3—2.0	0.27	1.48	1.84

Table 2: The effect ACTH on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
0.1×10^{-3}	— 6 %	—37 %	—13 %	—22 %
0.15×10^{-3}	—16 %	—41 %	—26 %	—46 %
0.25×10^{-3}	—34 %	—43 %	—31 %	—64 %
0.50×10^{-3}	—44 %	—43 %	—35 %	— 73 %
1.0×10^{-3}	—53 %	—46 %	—35 %	—73 %
0.05	complete inhibition			

1. *Adrenocorticotropic hormone*

This hormone exerted an inhibitory effect on the growth of all the 4 dermatophytes. Even in concentrations equivalent to physiological or therapeutic

tration of L.H. and the reserve occurred in case of T. rubrum. M. canis and T. mentagrophytes showed a uniform degree of inhibition with the exception of the concentration 1.0 mcg/ml at which more inhibition was observed **(Table 3).**

Table 3: The effect of L. H. on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
0.25	—50 %	—	—37 %	—28 %
0.50	—50 %	—	—37 %	—40 %
1.0	—65 %	—	—41 %	—40 %
2.0	—50 %	—13 %	—37 %	—28 %
5.0	—50 %	—24 %	—37 %	—13 %

Table 4: The effect of the posterior pituitary hormone on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
1.0×10^{-3}	—34 %	— 4%	—15 %	— 8 %
2.0×10^{-3}	—34 %	— 4 %	— 19 %	—16 %
0.06	—34 %	— 13 %	—23 %	—16 %
0.13	—34 %	—24 %	—23 %	—16 %
0.25	—34 %	—24 %	—30 %	—28 %
0.50	—41 %	—26 %	—40 %	—28 %
1.00	—44 %	—26 %	—44 %	—28 %
2.00	—48 %	—28 %	—48 %	—40 %

3. *Posterior pituitary extract*

This hormone exerted graded inhibitory effect on the growth of the 4 dermatophytes (**Table 4**).

4. *Thyroid gland secretion*

In concentrations lower than that equivalent to the normal physiological or therapeutic levels in man (5—10 mcg/ml) the thyroid gland secretion inhibited the growth of the 4 dermatophytes completely (**Table 5**).

5. *Insulin*

In the lower range of concentrations comparable to the physiological level, insulin exerted a graded type of inhibition

Table 5: The effect of thyroid extract on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
0.01	—30 %	—36 %	—32 %	— 24 %
0.025	—34 %	—37 %	—37 %	— 28 %
0.050	—47 %	—59 %	—47 %	—100 %
0.075	complete inhibition			

Table 6: The effect of insulin on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
4×10^{-6}	—19 %	—	— 6 %	— 60 %
8×10^{-6}	—16 %	—	— 6 %	— 55 %
16×10^{-6}	—13 %	—	—	— 48 %
32×10^{-6}	— 8 %	+ 3 %	—	— 34 %
64×10^{-6}	— 8 %	+ 3 %	—	— 34 %
0.2	—20 %	+20 %	—13 %	— 70 %
0.4	—27 %	+18 %	—16 %	— 70 %
0.8	—30 %	+18 %	—23 %	— 70 %
1.6	—44 %	+15 %	—41 %	—100 %
6.4	—44 %	+15 %	—48 %	—100 %

inversely proportional to its quantity. Higher concentrations of the hormones caused partial inhibition of M. canis and T. mentagrophytes and complete inhibition of T. rubrum. Surprisingly, promotion of growth was observed in case of M. gypseum (**Table 6**).

6. *Oestradiol 17 β*

This hormone behaved like insulin. In concentrations equivalent to the normal physiological levels in man the inhibitory effect was inversely proportional to the increasing concentration so as at a certain concentration slight stimulation of growth was observed. In higher concentration there was an increasing degree of inhibition parallel to the amount of the hormone with the exception of T. rubrum which showed a complete inhibition suddenly at a concentration 10 mcg/ml (**Table 7**).

7. *Ethinyl oestradiol*

At the lower range of concentrations the results of M. canis was controversing

Table 7: The effect of oestradiol 17 β on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
0.5×10^{-4}	—	—12 %	—19 %	— 11 %
1.0×10^{-4}	—	—10 %	—16 %	— 8 %
2.5×10^{-4}	—	— 5 %	—10 %	— 7 %
5.0×10^{-4}	—	— 2 %	— 9 %	— 4 %
1.0×10^{-3}	+ 5 %	+ 2 %	— 8 %	— 6 %
5.0	+ 5 %	—13 %	—30 %	— 10 %
10.0	—23 %	—17 %	—30 %	— 10 %
25.0	—23 %	—24 %	—30 %	—100 %
50.0	—23 %	—24 %	—40 %	—100 %

Table 8: The effect of ethinyl oestradiol on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
10×10^{-4}	— 1 %	—24 %	—36 %	— 9 %
5×10^{-3}	— 5 %	—24 %	—36 %	—22 %
10×10^{-3}	+30 %	—24 %	—53 %	—31 %
2.5×10^{-2}	+27 %	—26 %	—53 %	—34 %
5.0	+12 %	— 2 %	—37 %	—88 %
10.0	+12 %	— 2 %	—37 %	—88 %
25.0	—76 %	— 2 %	—55 %	—88 %
50.0	—76 %	— 6 %	—55 %	—88 %
100.0	—76 %	—13 %	—72 %	—88 %

and statistically insignificant. However, a highly significant inhibition of growth was achieved at a concentration of 25.0 mcg/ml. Also, M. gypseum showed variable response. T. mentagrophytes and T. rubrum showed gradually progressing inhibition at lower range of concentrations and highly significant reduction in the size of the colonies at higher concentrations (**Table 8**).

at higher concentrations. T. mentagrophytes and T. rubrum were highly sensitive to this congener and complete inhibition was achieved at a concentration of 1.25 mcg/ml (**Table 9**).

9. *Progesterone*

Lower concentrations of progesterone caused slight inhibition or apparent increase in the size of the colonies, however,

Table 9: The effect of stilboestrol on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
5.0×10^{-2}	—47 %	—22 %	—47 %	—22 %
10×10^{-2}	—47 %	—24 %	—52 %	—23 %
15×10^{-2}	—44 %	—25 %	—49 %	—23 %
25×10^{-2}	—44 %	—25 %	—51 %	—23 %
1.25	+12 %	— 2 %		
2.50	+12 %	—13 %	complete inhibition	
5.0	+12 %	—13 %		
12.5	— 2 %	—17 %		
25.0	—48 %	—17 %		

8. *Stilboestrol*

The growth of M. canis and M. gypseum was significantly inhibited at lower concentrations and to a much lesser extent

all these changes were statistically insignificant. Distinct inhibition was observed at higher concentrations of the hormone especially in the case of T. rubrum (**Table 10**).

Table 10: The effect of progesterone on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
10×10^{-3}	+ 5 %	− 2 %	− 7 %	−31 %
25×10^{-3}	+ 5 %	+ 3 %	− 7 %	−16 %
5.0	+26 %	+ 7 %	+12 %	−40 %
10.0	+15 %	+ 2 %	+ 5 %	−70 %
25.0	+15 %	− 2 %	−30 %	−70 %
50.0	− 6 %	− 6 %	−48 %	−70 %
75.0	−23 %	−24 %	−65 %	−70 %
100.0	−37 %	−46 %	−65 %	−70 %

10. *Testosterone*

M. canis responded to the effect of the hormone in a triphasic manner. The initial concentrations caused slight inhibition followed by stimulation and lastly, at higher concentrations, complete inhibition of growth was achieved. The other 3 dermatophytes showed gradual increase of inhibition, especially T. rubrum was inhibited completely at a concentration of 42.0 mcg/ml (**Table 11**).

Table 11: The effect of testosterone on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
$4.2 - 84 \times 10^{-4}$	− 6 %	−12 %	− 8 %	− 7 %
4.2	+ 40 %	−30 %	−30 %	− 10 %
8.4	+ 40 %	−35 %	−30 %	− 40 %
21.0	− 30 %	−46 %	−30 %	− 40 %
42.0	− 65 %	−46 %	−30 %	−100 %
63.0	−100 %	−67 %	−65 %	−100 %

Table 12: The effect of norethisterone on the growth of dermatophytes in vitro

Concentration in mcg/ml	M. canis	M. gypseum	T. mentagrophytes	T. rubrum
0.25	− 35 %	− 42 %	− 2 %	−10 %
0.50	− 35 %	− 41 %	− 6 %	−15 %
0.75	− 33 %	− 41 %	− 6 %	−16 %
1.00	− 33 %	− 43 %	− 8 %	−16 %
5.0	+ 40 %	+ 9 %	+ 5 %	−10 %
10.0	+ 5 %	− 2 %	+ 5 %	−40 %
25.0	− 3 %	− 50 %	+ 5 %	−40 %
50.0	−100 %	− 78 %	− 30 %	−40 %
75.0	−100 %	−100 %	−100 %	−40 %

11. *Norethisterone*

This hormone exerted a triphasic action on M. canis, M. gypseum and T. mentagrophytes comprising initial inhibition then stimulation at a certain concentration and lastly a powerful inhibitory effect at higher concentrations. T. rubrum was slightly inhibited at the lower range of the concentrations and significant and uniform inhibition throughout the whole higher range (Table 12).

Discussion

The results of this investigation revealed that most of the hormones caused inhibition of the growth of dermatophytes. This inhibition was either quantal (abrupt) or gradual with the rise of the concentration. The quantal type of inhibition was manifested by ACTH, stilboestrol and norethisterone as regard T. mentagrophytes and oestradiol 17 β in case of T. rubrum. The pure graded type of inhibitory activity was observed in case of ACTH on T. rubrum, L. H. on M. canis, thyroid extract on T. mentagrophytes and ethinyl oestradiol as well as stilboestrol on M. gypseum. In such cases inhibition of growth was proportional to concentration of the test compound with the exception of ethinyl oestradiol and stilboestrol on M. gypseum, where the degree of inhibition decreased with increased concentration of the hormone.

These results may be of great therapeutic interest because such hormones exerted a potent fungistatic effect at concentrations comparable to their therapeutic levels.

The inhibition of fungal growth by hormones may be attributed to their inhibitory effects on protein synthesis (Halkerston et al., 1964, Farese, 1966). Wolff and Wolff (1964) were of the opinion that the multiple effects of the thyroid hormone together with its structural damage to the mitochondria may suppress the fungal growth. Sutherland et al. (1965) observed increased lipolysis in presence of inhibitory levels of L.H. Similarly. Jennings (1970) described the lipolytic effect of ACTH.

Some hormones caused stimulation of growth at lower concentrations then followed by inhibition at a certain concentration and this phenomenon is known as the biphasic action. This was observed in case of progesterone and ethinyl oestradiol on M. canis and insulin on M. gypseum. A triphasic action i. e. alternative inhibition and stimulation at different concentrations, was observed in case of norethisterone, testosterone and oestradiol 17 β on M. canis as well as progesterone and norethisterone on T. mentagrophytes. These bi- and tri-phasic effects of some hormones were explained by Monod et al., (1963, 1965), Monod (1966) and Munk (1968) on the basis of allosteric interactions at the cellular level. On the other hand, Williams-Ashman (1965), Means and Hamilton (1966) and Clegg (1966) explained the effects of such hormonal agents as due to disturbance in the gene transcription or translation leading to the formation of new protein molecules.

Moreover, the multiple effects of insulin, at the cellular level, involving alterations in the cell membranes (Hechter et al., 1964), stimulation of anabolic processes (Krable, 1961) and alterations in intracellular enzymes might also account for its heterogenous effect on the size of the fungal colonies with variation of its concentration.

Summary

Eleven hormonal agents: adrenocorticotropic hormone, luteinising hormone, thyroid gland secretion, posterior pituitary extract, insulin, oestradiol 17 β, ethinyl oestradiol, stilboestrol, progesterone, testosterone and norethisterone, were tested in vitro for their effects on the growth of 4 dermatophytes: Microsporum canis, M. gypseum, Trichophyton mentagrophytes and T. rubrum. Most of the hormones caused inhibition of the fungal growth either of quantal or graded type. Several hormones exerted their potent inhibitory effect at concentrations comparable to their normal physiological levels in man. Some hormones caused stimulation of growth at lower concentration followed by inhibition and other exerted a triphasic action i. e. inhibition followed by stimulation then again inhibition of the growth.

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