mykosen **11** (10) : 747—754 (1968) 30 Eingegangen am 23. Juli 1968

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The effect of hormones on some fungi in vitro

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Introduction

In early medical studies, considerable attention was devoted to variations of the individual in his response to a constant environment. It is well known that pregnancy and other sexual stages affect the progress of several diseases, such effects may result from some intrinsic physiological changes, probably to the endocrine hormones existing during these stages.

Nowadays, fungus infections occupied an important part in human and veterinary medicine all over the world and particularly in our country. Candida albicans is the commonest organism causing fungal vaginitis. The incidence of vaginitis is higher in gravid than non gravid women (PICKHARDT and BREEN, 1957). Moreover, HESSELTINE (1959) found that the infection with C. species in the non pregnant women was self-limiting spontaneous disappearance of the fungi from the vagina, while in pregnant women, infection was persistant. In Egypt REFAI (unpublished data) noticed that there is an increased number of Candida infections of the vagina in pregnant women. Moreover, TASCHDJIAN *et al.* (1960) reported that both mice, SCHOLER (1960) and rats are susceptible to vaginal C. albicans infections but only during oestrus. SCHOLER induced a prolonged persistent infection in rats by castrating the animals and injecting oestradiol, thus maintaining permanent oestrus. REFAI (1963) isolated C. albicans from milk in cases of mastitis. It was also observed that C. albicans was incriminated as a cause of abortion in cattle (BISPING *et al.* 1964).

Microsporum gypseum infections occur both in man and animals. It is one of the causative agent of tinea capitis in man and ringworm in animals. KLIGMAN (1952, 1955) postulated that dermatophytes infection was greater in children than in adults in experimental infection. SEALE and RICHARDSON (1960) and MACKENZIE (1961) mentioned that ringworm was more frequently in children of school age up to puberty, boys are more frequently affected than girls. ABDEL FATTAH *et al.* (1967) observed that ringworm is common in young children and scarcely seen in adults.

Aspergillus niger was frequently isolated from foods and animal food-stuffs. It causes diseases and toxicity both in man and animals. JUNGHEER (1935) recorded 4 cases of abortion associated with mold, 3 from one herd in which bacteria could not be incriminated and a mold was definitely identified, JUNGHEER cites numerous other reports and several other species have been identified. WILLIAMS (1950) mentioned that molds are associated with interference with reproduction in animals concerning the diseases of genital organs. Cortisone complicated the mycotic infections on animal experiments CHAPPAZ *et al.* (1956) and specially Candida and Aspergillus infection (LEVY and COHEN, 1955). Cortical hormones may have played a role in the nature of infection with Aspergillus organism (FINEGOLD *et al.*, 1959; SIDRANSKY and PEARL, 1961).

It is evident from the above review that a relationship exists between fungus infection and hormones. The importance of investigations into the role played by hormones in human and animal infections suggest the studies of the action of hormones on fungi in vitro.

The aim of the present work is to demonstrate the effect of *sex hormones, pituitary hormones, thyroid hormone* and *corticosteroid* on 3 different species of fungi representing yeasts, dermatophytes and molds.

Experimental

The hormonal compounds used were:

1.	Stilboestrol B. P.	tablets	(Boots — England)
2.	Oestrone	tablets	(ovex Leo — Leo Denmark)
3.	Ethinyl oestradiol	tablets	(dyloform B. D. H. London)
4.	Progesterone	powder	(U. S. P. Batch C. L. S. 185)
5.	Testosterone propionate	powder	(Thomas, Tyrer, England)
6.	Pituitary anterior lobe	(total hormone)	(Antifisan, Richter, Italy)
7.	Pituitary posterior lobe extract		(Hypophysin Hoechst, Germany)
8.	Thyroid	tablets	(Evans — England)
9.	Cortisone (17-α-hydroxy-11-dehydro-corticosterone acetate)	suspension	(Ciba — Suisse)

Sabouraud agar (Difco) was poured in flasks of 90 ml quantities, 10 ml of distilled water containing the different hormone concentrations are added to the agar. The final and hormones were then autoclaved for 15 *min* at 120° C and each was distributed equally in 3 plates, each plate was inoculated with one of the following strains.

1. *Candida albicans (C. albicans)* represents yeasts.
2. *Microsporum gypseum (M. gypseum)* represents dermatophytes.
3. *Aspergillus niger (A. niger)* represents molds.

The plates were inoculated on the surface with one platinum lope (diameter 2 mm). Sabouraud agar plates without hormones were also inoculated as control.

All plates were incubated for one week at room-temperature and the results were recorded as follows.

Results

A. *The effect of sex hormones on fungi in vitro:*

Table 1: The effect of stilboestrol on the growth of fungi

Fungi	Hormone doses mg/ml									
	Control	0.001	0.0025	0.01	0.03	0.05	0.25	0.5	3	6
Candida albicans	++++	+++++	++++	++++	++++	++++	+	+	(+)	0
Microsporum gypseum	++++	+++++	++++	+++	+	+	+	+	(+)	0
Aspergillus niger	++++	++++	++++	+++	++	+++	+	(+)	(+)	(+)

+++++++	= more strong stimulation	+++	= slight inhibition
++++++	= strong stimulation	++	= inhibition
+++++	= stimulation	+	= strong inhibition
++++	= normal growth	(+)	= more strong inhibition
		0	= total inhibition

Table 1 shows that stilboestrol in small dose (0.001 mg/ml) stimulates the growth of C. albicans and M. gypseum more than in the control. No effect was seen in conc. of 0.0025 to 0.05 mg/ml on C. albicans, while an inhibitory action began from 0.01 mg on M. gypseum and A. niger. A strong inhibition on the growth of alle the strains was produced from 0.25 to 3 mg/ml and a total inhibition of the growth of C. albicans and M. gypseum at concentration of 6 mg/ml, however, it was a strong inhibition in the growth of A. niger at 6 mg/ml.

Table 2: The effect of oestrone on the growth of fungi

Fungi	Hormone doses mg/ml			
	Control	0.001	0.0025	0.01
Candida albicans	++++	++++	++++	++++
Microsporum gypseum	++++	++++	++++	++++
Aspergillus niger	++++	++++++	+++++	++++++

Table 2 shows that oestrone has no effect on C. albicans and M. gypseum while it has a strong stimulatory action on A. niger in the used concentrations in comparison with the control.

Table 3: The effekt of ethinyl oestradiol on the growth of fungi

Fungi	Hormone doses mg/ml			
	Control	0.001	0.0025	0.01
Candida albicans	++++	++++	++++	++++
Microsporum gypseum	++++	+++++	+++++	+++++
Aspergillus niger	++++	+++++	++++++	++++++

Ethinyl oestradiol has no effect on C. albicans as shown in **table 3**, while it stimulates the growth of M. gypseum. The stimulation was strong on the growth of A. niger as compared with the control.

Table 4: The effect of progesterone on the fungi

Fungi	Hormone doses mg/ml						
	Control	0.05	0.25	0.5	1	3	6
Candida albicans	++++	++++	++++	++++	++++	++++	++++
Microsporum gypseum	++++	++++++	++++++	++++++	+++	+	+++
Aspergillus niger	++++	++++++	++++++	++++++	++++++	+	+++

Table 4 indicates that progesterone has no effect on the growth of C. albicans, while it stimulates the growth of M. gypseum in doses from 0.05 to 0.5 mg/ml and of A. niger in doses from 0.05 to 1 mg/ml. An inhibitory action on the growth of M. gypseum began at 1 mg/ml and at 3 mg/ml in case of A. niger.

Table 5: The effect of testosterone propionate on the fungi

Fungi	Hormone doses mg/ml						
	Control	0.05	0,25	0.5	1	3	6
Candida albicans	++++	+++++	+++++	+++++	+++++	+++++	+++++
Microsporum gypseum	++++	++++	++++	++++	++++	+++++	+++++
Aspergillus niger	++++	++++	++++	++++	+++++	+++++	+++++

Table 5 shows that testosterone propionate exerted various degrees of stimulation on the growth of the 3 strains. The hormone favoured the growth of C. albicans more than the other 2 strains in doses from 0.05 to 6 mg/ml, on M. gypseum from 1 to 6 mg/ml.

B. *The effect of the pituitary hormones:*

It is seen from **table 6** that the anterior pituitary lobe extract exerted an inhibitory action on the growth of the fungi as compared with the control. A. niger was the most sensitive to the effect of the hormone, the total inhibition of the growth was obtained at contentration of 0.3 U/ml.

Table 6: The effect of the anterior pituitary lobe extract on the growth of fungi

Fungi	Hormone doses U/ml			
	Control	0.05	0.15	0.3
Candida albicans	++++	++++	+++	+
Microsporum gypseum	++++	+++	++	+
Aspergillus niger	++++	+	(+)	0

It is shown from **table 7** that posterior pituitary lobe extract has no effect on the growth of the 3 strains by using the above concentrations.

Table 7: The effect of the posterior pituitary lobe extract on the growth of fungi

Fungi	Hormone doses U/ml					
	Control	0.05	0.15	0.30	0.50	1.00
Candida albicans	++++	++++	++++	++++	++++	++++
Microsporum gypseum	++++	++++	++++	++++	++++	++++
Aspergillus niger	++++	++++	++++	++++	++++	++++

C. *The effect of thyroid hormone on fungi:*

Table 8: The effect of the thyroid hormone on fungi

Fungi	Hormone doses mg/ml			
	Control	0.6	6	12
Candida albicans	++++	++++	+++++	++++++
Microsporum gypseum	++++	++++	+++++	++++++
Aspergillus niger	++++	++++	+++++	++++++

Table 8 indicates that the thyroid hormone had no effect on the growth of C. albicans, M. gypseum and A. niger in dose of 0.6 mg/ml, while it had a stimulatory effect in dose of 6 mg/ml. This increase was strong at 12 mg/ml as compared with the control.

D. *The effect of corticosteroid on fungi:*

Table 9: The effect of cortisone® on the growth of fungi

Fungi	Hormone doses mg/ml								
	Control	0.005	0.04	0.08	0.2	0.5	1	2.5	1
Candida albicans	++++	++++	++++	++++	++	++	++	++	++
Microsporum gypseum	++++	++++	++++	+++	++	++	+	+	0
Aspergillus niger	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++

As shown in **table 9,** Cortisone® (17-α-hydroxy-11-dehydro-corticosterone acetate) had no effect on C. albicans from doses of 0.005 to 0.08 mg/ml and on M. gypseum in

doses from 0.005 to 0.04 mg/ml. Inhibition effect was produced at conc. of 0.2 to 5 mg/ml on C. albicans and from 0.08 to 2.5 mg/ml on M. gypseum and a total inhibition of the growth of M. gypseum at 5 mg/ml. However, the growth of A. niger was increased more than in the control from 0.005 to 5 mg/ml.

Discussion

It was noticed that mycotic diseases of the scalp in children were spontaneously healed at the onset of puberty. This fact leads to think that the sexual hormones should play an important role in the healing process: Appropriate treatment trials showed no direct support for such connection (RIETH, 1962). DOBES (1950, 1955) postulated that after parenteral administration of sexual hormones quick healing takes place.

The present in vitro study shows that sex hormones has different effect on the growth of fungi and the responsiveness of each hormone differs according to the dose.

Stilboestrol, a synthetic oestrogen, stimulated the growth of C. albicans and M. gypseum in small dose (0.001 mg/ml). An inhibitory effect was produced in doses of 0.25 to 3 mg/ml; 0.01 to 3 mg/ml, and 0.01 to 6 mg/ml on C. albicans, M. gypseum and A. niger, respectively. Moreover, stilboestrol showed a fungicidal action at concentration of 6 mg/ml on both C. albicans and M. gypseum. These findings correlate with those reported by HARDER (1956) who found that diethylstilboestrol is effective as fungistatic in the high dilution, and total inhibition was produced in dilution of 1 : 20.000 on M. gypseum; good results were obtained with local applications of diethylstilboestrol on Microsporum (MIRANDE and BARBERO, 1958).

On the other hand oestrone and ethinyl oestradiol stimulated the growth of A. niger more than in the control in doses from 0.001 to 0.01 mg/ml. Moreover, ethinyl oestradiol stimulates the growth of M. gypseum in the same doses. Whether natural oestrogens possess an inhibitory or fungicidal effect, more doses should be done; due to the lack of these hormones at present it was unable to carry on more concentrations.

The growth of C. albicans was normal as in the control by using progesterone in concentrations of 0.05 to 6 mg/ml. However, progesterone stimulated the growth of M. gypseum and A. niger at doses of 0.05 to 0.5 mg/ml and 0.05 to 1 mg/ml respectively. Moreover when the doses increased, an inhibitory action was produced on M. gypseum at doses of 1 to 6 mg/ml and on A. niger at 3 to 6 mg/ml. Testosterone propionate exerted various degrees of stimulation on the growth of the 3 strains in doses ranging between 0.05 to 6 mg/ml. The hormone favoured the growth of C. albicans more than the other 2 strains that its increase in growth was in doses ranging between 0.05 to 6 mg/ml. These results were in agreement with (TARBET *et al.*, 1953) that there was stimulation of M. gypseum in vitro by using testosterone and testosterone diethyl-amino-ethyl carbonate. However, our findings are not in agreement with PANA (1930) who studied the action of endocrine glands on the development of some fungi and mentioned that development of fungi culture was inhibited to a lesser degree by the testiculer substance.

Our findings indicated that the total anterior pituitary lobe extract gave an inhibitory effect on the growth of the 3 strains from 0.15 to 0.3 U/ml on C. albicans; from 0.05 to 0.3 U/ml on M. gypseum and from 0.05 to 0.15 U/ml on A. niger. The inhibitory effect on A. niger was very strong. At 0.3 U/ml a fungicidal effect was produced. These results should be more confirmed whether this antifungal effect was due to the total anterior lobe extract or to any of the hormones of the anterior lobe of pituitary as the lactogenic, thyrotropic, adrenocorticotropic, growth and gonadotropic hormones. This will be continued in our further work. However the posterior pituitary lobe extract possess no effect on the growth of C. albicans, M. gypseum and A. niger in doses ranging between

0.05 to 1.00 U/ml as compared with the control. These findings are not in agreement with PANA (1930) who found that the culture of Aspergilli was favoured by the addition of pituitary substance. The thyroid hormone stimulated the growth of C. albicans, M. gypseum and A. niger at 6 mg/ml the stimulation was heavy at 12 mg/ml. Our results are not in agreement with PANA, 1930, who found that the culture of Aspergilli was inhibited by the thyroid substances.

17-α-hydroxy-11-dehydro-corticosterone acetate exerted an inhibitory effect on the growth of C. albicans and M. gypseum in doses ranging between 0.2 to 5 mg/ml and 0.08 to 2.5 mg/ml respectively. Moreover a fungicidal effect was obtained by 5 mg/ml on M. gypseum. These findings confirm the observations of LEDIG (1958) and SCHUPPLI (1957) that local application of hydrocortisone in 0.1—1% in combination with antimycotica are proved to be useful and the cure get well. It was observed also that this compound favoured the growth of A. niger from 0.005 to 5 mg/ml more than in the control. This confirms the reports that aspergillosis is common infection associated with the use of the adrenal cortical hormones (FINEGOLD *et al.*, 1959; SIDRANSKY and PEARL, 1961).

This present in vitro study shows that the responsiveness of the fungi differs according to the hormonal substance used. The variations in the action could be attributed either to the degree of variation in the metabolic process of the fungi, or may be either to the decrease on the oxygen uptake of the fungi after being treated with such hormonal substances.

Summary

1. The effect of sex hormones (stilboestrol, oestrone, ethinyl oestradiol, progesterone, testosterone propionate), pituitary hormones, thyroid hormone and corticosteroid on the growth of C. albicans, M. gypseum and A. niger were studied in vitro.
2. Stilboestrol in small doses (0.001 mg/ml) stimulated the growth of C. albicans and M. gypseum, while in higher doses at (6 mg/ml) possessed fungicidal effect. Inhibitory effect was seen at 0.25 to 3 mg/ml on C. albicans, from 0.01 to 3 mg/ml on M. gypseum and from 0.01 to 6 mg/ml on A. niger. In small doses (0.001 to 0.01 mg/ml) oestrone favoured the growth of A. niger as well as ethinyl oestradiol on M. gypseum and A. niger. Progesterone had no effect on the growth of C. albicans from 0.05 to 6 mg/ml, but it stimulated the growth of M. gypseum and A. niger in doses of 0.05 to 0.5 mg/ml and 0.05 to 1 mg/ml. An inhibitory effect on M. gypseum was seen in large doses from 1 to 6 mg/ml and on A. niger from 3 to 6 mg/ml.

   Testosterone propionate favoured the growth of C. albicans, M. gypseum and A. niger in doses of 0.05 to 6 mg/ml; from 3 to 6 mg/ml and from 1 to 6 mg/ml respectively.
3. Anterior pituitary lobe extract exerted an inhibitory action on the growth of the used fungi in doses ranging between 0.05 to 0.3 U/ml. A fungicidal effect was obtained at 0.3 U/ml on A. niger.
4. Thyroid hormone favoured the growth of the used fungi in doses of 6 and 12 mg/ml.
5. 17-α-hydroxy-11-dehydro-corticosterone acetate favoured the growth of A. niger at doses of 0.005 to 5 mg/ml, inhibited the growth of C. albicans at 0.2 to 5 mg/ml. A fungicidal effect on M. gypseum was demonstrated at 5 mg/ml.

Zusammenfassung

1. Die Wirkung von Sexualhormonen (Stilböstrol, Östron, Äthinylöstradiol, Progesteron, Testosteronpropionat), Epiphysenhormonen, Schilddrüsenhormonen und Nebennierenrindenhormonen auf das Wachstum von Candida albicans, Mikrosporum gypseum und Aspergillus niger wurde in vitro untersucht.

2. Stilböstrol stimulierte in kleinen Dosen (0,001 mg/ml) das Wachstum von Candida albicans und Mikrosporum gypseum, während es in höheren Dosen (6 mg/ml) eine fungizide Wirkung aufwies. Eine Hemmwirkung wurde beobachtet bei 0,25 bis 3 mg/ml auf Candida albicans, von 0,01 bis 3 mg/ml auf Mikrosporum gypseum und von 0,01 bis 6 mg/ml auf Aspergillus niger.

   In kleinen Dosen (0,001 bis 0,01 mg/ml) förderte Östron das Wachstum von A. niger, ebenso wie Äthinylöstradiol das Wachstum von M. gypseum und A. niger. Progesteron hatte keine Wirkung auf das Wachstum von C. albicans von 0,05 bis 6 mg/ml, aber es förderte das Wachstum von M. gypseum und A. niger in Dosen von 0,05 bis 0,5 mg/ml und 0,5 bis 1 mg/ml. Eine Hemmwirkung auf M. gypseum wurde bei hohen Dosen von 1 bis 6 mg/ml beobachtet, auf A. niger in Dosen von 3 bis 6 mg/ml.

   Testosteronpropionat förderte das Wachstum von C. albicans in Dosen von 0,05 bis 6 mg/ml, M. gypseum in Dosen von 3 bis 6 mg/ml, A. niger in Dosen von 1 bis 6 mg/ml.
3. Epiphysenvorderlappenextrakt hemmte die Testpilze in Dosen zwischen 0,05 bis 0,3 E/ml. Fungizid wirkten 0,3 E/ml auf A. niger.
4. Schilddrüsenhormon förderte das Wachstum der Testpilze in Dosen von 6 und 12 mg/ml.
5. 17α-hydroxy-11-dehydro-corticosteronacetat förderte das Wachstum von A. niger in Dosen von 0,005 bis 5 mg/ml und hemmte das Wachstum von C. albicans in Dosen von 0,2 bis 5 mg/ml. 5 mg/ml zeigten eine fungizide Wirkung auf M. gypseum.

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