

XI SCIENTIFIC MEETING OF THE GERMAN SPEAKING MYCOLOGICAL SOCIETY IN BASEL, SEPTEMBER, 26-28, 1974

Department of Microbiology,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Studies on the effect of some hormones on *Candida albicans* and *Cryptococcus neoformans* in vitro and in vivo

M. REFAT and SAFINAZ EL SHERIF

The widespread use of certain hormonal agents in the treatment of various infectious diseases has been the subject of interest to many researchers. RUSS and COLLINS (1940) proved the successful treatment of candidal vaginitis by diethylstilboestrol. Also DOBES (1955) found that oestrone was most effective in curing tinea capitis caused by *Microsporum audouinii*. On the other hand, some of the naturally occurring hormones may adversely affect the course and severity of fungal infections.

Illustrative examples may be found in the case of cortisone, which was shown to increase the severity of experimental candidosis in rats and mice (ROTH et al., 1952; SCHERR, 1955). Likewise, oestradiol aggravated the mortality of mice caused by *Candida albicans* as indicated by marked reduction in the rate of their survival following infection. Moreover, SCHOLER (1960) induced a prolonged and persistent vaginal infection in ovariectomized rats injected with oestradiol.

The present work has been conducted for testing the in vitro effect of several hormones on the growth of *Candida albicans* and *Cryptococcus neoformans*, and the in vivo influence of oestradiol 17 β , progesterone and adrenocorticotrophic hormone (ACTH) on the course of experimental infection in rats and mice.

Material and methods

I. In vitro studies

The hormones used in this work were: Anterior pituitary extracts

adrenocorticotrophic hormone (ACTH) and luteinising hormone (L. H.), posterior pituitary extract, thyroid extract, insulin, follicular hormones and synthetic oestrogenic congeners (oestradiol 17 β , ethinyl oestradiol and stilboestrol).

Progesterone, the contraceptive norethisterone and the male testicular hormone testosterone.

In order to elucidate the effect of such hormones on the growth of *Candida albicans* and *Cryptococcus neoformans*, the hormones were incorporated in Sabouraud's dextrose agar in

concentrations comparable to and considerably exceeding their normal levels in human body (Table 1 and 2). The agar was poured in plates which were inoculated with the yeasts using standardized loop. In order to obtain statistically reliable results, six plates were used for each concentration. In addition, control plates were used in which the hormonal solvents were incorporated.

II. In vivo studies

a) Experimental candidosis of the vagina in rats

In this study the following groups of animals, each of 18 rats, were experimentally infected in the vagina with a swab soaked in *Candida albicans*-suspension: non-pregnant, ovariectomized and pregnant rats. From all rats used in the experiments vaginal swabs were examined for any possible infection. Some of the rats showed spontaneous vaginal candidosis, these were collected in groups, each of 18 rats, and were examined in parallel with the other groups. Three groups of ovariectomized

Table 1: Effect of hormones on the growth of *C. albicans* and *Cr. neoformans* in lower concentration

| Hormones | mcg/ml | <i>C. albicans</i> | <i>Cr. neoformans</i> |
|-----------------------|----------|--------------------|-----------------------|
| ACTH | 0.0001 | -11 % | no effect |
| | 0.00025 | -25 % | no effect |
| L. H. | 0.25-5 | - 4 % | -18 % |
| Post. pituitary | 0.002 | no effect | no effect |
| Thyroid extract | 0.125 | no effect | no effect |
| Insulin | 0.000064 | no effect | no effect |
| Oestradiol 17 β | 0.001 | no effect | no effect |
| Ethinyl oestradiol | 0.0005 | no effect | no effect |
| | 0.015 | -19 % | -22 % |
| | 0.10 | -13 % | - 8 % |
| Stilboestrol | 0.25 | no effect | no effect |
| | 0.001 | +26 % | +38 % |
| | 0.005 | +26 % | +22 % |
| Progesterone | 0.10 | +26 % | +11 % |
| | 0.0084 | no effect | no effect |
| Testosterone | 1.0 | no effect | no effect |
| Norethisterone | | no effect | no effect |

Table 2: Effect of hormones on the growth of *C. albicans* and *Cr. neoformans* in higher concentration

| Hormones | mcg/ml | <i>C. albicans</i> | <i>Cr. neoformans</i> |
|-----------------------|---------|--------------------|-----------------------|
| ACTH | 0.05 | — 29 % | —31 % |
| | 0.10 | —100 % | —72 % |
| L. H. | 7.50 | — 7 % | —21 % |
| Post. pituitary | 0.6—2.0 | no effect | no effect |
| Thyroid extract | 25—50 | — 29 % | —31 % |
| | 100.0 | — 71 % | —72 % |
| Insulin | 0.4 | — 29 % | —31 % |
| | 1.6 | — 29 % | —72 % |
| | 3.2 | — 57 % | —72 % |
| Oestradiol 17 β | 10.0 | — 14 % | — 3 % |
| | 50.0 | — 57 % | —31 % |
| | 100.0 | — 71 % | —44 % |
| Ethynyl oestradiol | 10.0 | no effect | —10 % |
| | 50.0 | — 7 % | —24 % |
| | 100.0 | — 21 % | —31 % |
| Stilboestrol | 5.0 | no effect | —31 % |
| | 12.5 | — 10 % | —31 % |
| | 25.0 | — 21 % | —31 % |
| Progesterone | 5.0 | + 29 % | +40 % |
| | 25.0 | + 29 % | +25 % |
| | 50.0 | + 43 % | +11 % |
| Testosterone | 20.0 | no effect | +11 % |
| | 60.0 | no effect | +25 % |
| Norethisterone | 5.0 | + 29 % | +25 % |
| | 50.0 | — 7 % | +11 % |

Table 3: The influence of ovariectomy, pregnancy and hormonal treatment on the course of spontaneous or experimentally induced candidosis in rats

| Rats | No. of intervals + cultures on 3 days | | | | | | | Total | |
|--|---------------------------------------|----|----|----|----|----|----|-------|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Non-ovariectomized spontaneous induced | 14 | 14 | 14 | 13 | 13 | 13 | 11 | 92 | |
| | 15 | 12 | 12 | 11 | 10 | 10 | 10 | 80 | |
| Ovariectomized spontaneous induced | 14 | 13 | 13 | 10 | 9 | 8 | 6 | 72 | |
| | 17 | 14 | 13 | 12 | 12 | 11 | 10 | 89 | |
| Pregnant spontaneous induced | 18 | 18 | 18 | 18 | 18 | 11 | 2 | 103 | |
| | 15 | 18 | 18 | 18 | 18 | 9 | 1 | 97 | |
| Hormonal treatment | | | | | | | | | |
| | Oestradiol 17 β | 12 | 8 | 8 | 6 | 3 | 2 | 2 | 41 |
| | Progesterone | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 126 |
| | Sesam oil | 12 | 9 | 8 | 5 | 5 | 5 | 3 | 47 |

infected rats were treated with sesame oil which is used as a vehicle for the hormones, oestradiol and progesterone. Each of the two later hormones was injected in 3 groups of rats intramuscularly in doses of 5, 10 and 20 mcg/animal for 7 days. Cultural examination of vaginal swabs from all animals was carried out every 3 days for a period of 3 weeks.

b) Experimental intraperitoneal infection in mice by *Candida albicans*.

Ten groups of animals, each of 6 white mice, were injected i. p. with gradually increasing 5 doses of yeast suspension in saline (starting from 1×10^5 up to 10×10^8 cells/animal). Each 2 groups received the same dose. One day prior to the infection the animals were injected i. m. with ACTH in 2 dose-levels of 7.6×10^2 and 15.2×10^2 mcg/animal. Another 5 groups of mice infected with *C. albicans* received no hormones and 2 groups were treated with the two doses of hormones used in the experiment without being infected with *C. albicans*. The dose mortality data were evaluated by the procedure of LITCHFIELD and WILCOXON (1949). The statistical significance of the results was determined by application of chisquare (X^2) test.

Results

I. In vitro

The posterior pituitary extract, the thyroid extract, the insulin, the oestradiol 17 β , the testosterone and the norethisterone had no influence on the growth of both *C. albicans* and *Cr. neoformans* when they were used in concentrations comparable to the physiological or therapeutic levels in man. Even in higher concentrations the posterior pituitary extract failed to influence the growth of both yeasts and testosterone had no effect on *C. albicans*.

On the other hand, ACTH and L. H. exerted a distinct inhibitory effect on *C. albicans* and *Cr. neoformans* respectively, and stilboestrol on both yeasts

Progesterone was the only hormone which promoted the growth of yeasts significantly (Table 1).

In concentrations exceeding the normal physiological or therapeutic levels of the hormones in man, ACTH, L. H., thyroid extract, insulin, oestradiol 17 β , ethinyl oestradiol and stilboestrol inhibited the growth of both yeasts significantly, while progesterone and norethisterone stimulated their growth, testosterone promoted the growth of *Cr. neoformans* only (Table 2).

II. In vivo

a) Influence of bilateral ovariectomy and pregnancy on vaginal candidosis in rats.

The bilateral ovariectomy caused a highly significant reduction in the incidence of candidosis among rats spontaneously infected, but not in experimentally infected animals. On the other hand, pregnancy tended to promote the infection. A very highly significant difference was observed between the groups of ovariectomized and pregnant rats suffering from spontaneous vaginal candidosis. However, the corresponding difference between the analogous groups of rats with induced vaginal candidosis proved to be non-significant (Table 3).

The daily treatment of bilaterally ovariectomized rats for one week with 0.4 ml sesame oil which is used as a vehicle for the hormone resulted in a very highly significant decrease in the rate of infection. Similar effect was recorded in groups of animals treated with oestradiol 17 β in daily doses of 10–20 mcg.

On the other hand progesterone, in daily doses of 10–20 mcg, considerably increased the persistence of the infection so that all the animals gave positive cultures during the whole period of the experiment (Table 3).

b) Mortality of adult mice as a result of i. p. injection of *Candida albicans* and

Table 4: Mortality in mice caused by *C. albicans* with and without treatment with ACTH

| % of mortality | No. of <i>C. albicans</i> cells/ml | | | | |
|-----------------------------|------------------------------------|--------|--------|--------|--------|
| | 100 t | 250 t | 500 t | 750 t | 1 mill |
| observed | 33.3 % | 33.3 % | 50 % | 66.7 % | 91.2 % |
| expected | 29 % | 44 % | 55 % | 62 % | 67 % |
| treatment with ACTH | | | | | |
| 7.6 $\times 10^2$ mcg/mice | | | | | |
| observed | 33.3 % | 33.3 % | 33.3 % | 50 % | 83.3 % |
| expected | 14 % | 36 % | 61 % | 69 % | 81 % |
| 15.2 $\times 10^2$ mcg/mice | | | | | |
| observed | 16.7 % | 16.7 % | 33.3 % | 50 % | 50 % |
| expected | 14 % | 25 % | 36 % | 42 % | 48 % |

t = thousand mill = million

the influence of ACTH on the infection.

The mortality among mice injected intraperitoneally with *C. albicans* displayed a direct proportionality to the number of cells injected. The values for the LD₁₆, LD₅₀ and LD₈₄ were 3.5×10^4 , 4.0×10^5 and 4.0×10^6 cells respectively.

The treatment of animals with the larger dose of ACTH (15.2×10^2 mcg) resulted in a uniform protection of considerable magnitude throughout the full range of i. p. test dosage of *C. albicans*-suspension. This was indicated by the statistically significant reductions in the expected values for percentage mortality as compared with the untreated groups of injected animals. Also, the LD₅₀ of *C. albicans* for the animals treated with ACTH was much higher (1.2×10^6 cells) as compared with the

corresponding LD₅₀ value of 4×10^5 *C. albicans*-cells in untreated animals (Table 4 and 5).

The ACTH itself caused death of 17 % and 33 % of mice injected with 7.6×10^2 mcg and 15.2×10^2 mcg.

Discussion

According to the aforementioned results the hormones can be classified to 3 groups with regard to their effect on the growth of yeasts. The first group constitutes the posterior pituitary hormone and testosterone. Both hormones failed to influence the growth of *C. albicans*, and the posterior pituitary hormone exerted no effect on *Cr. neoformans*. On the other hand, both hormones had an inhibitory effect on the growth of dermatophytes (EL-

Table 5: Lethal doses of *C. albicans* in mice

| | LD ₁₆ | LD ₅₀ | LD ₈₄ |
|-----------------------------|-------------------|-------------------|-------------------|
| No hormone treatment | 3.5×10^4 | 4.5×10^5 | 4.0×10^6 |
| Treatment with ACTH | | | |
| 7.6 $\times 10^2$ mcg/mice | 1.2×10^5 | 3.6×10^5 | 1.2×10^6 |
| 15.2 $\times 10^2$ mcg/mice | 1.2×10^5 | 2.1×10^6 | 1.2×10^7 |

SHERIF and REFAI, 1974), therefore, the resistance of *C. albicans* to the effect of these hormones is difficult to explain. This may be due changes in the chemical configuration of the hormones (FRIED et al., 1952; SHIRASAKA et al., 1960) or due to the poor chitin content of the cell walls of the yeasts (BRAIN, 1960) which did not allow the posterior pituitary extract to influence the permeability of the cells as claimed by LEAF (1964).

The second group of hormones, viz., progesterone, testosterone norethisterone, exerted a fungal growth promoting activity. This result of progesterone is of special interest. Its stimulating effect on *C. albicans* was also observed in rats, where the incidence of *C. albicans* was higher in pregnant than in non-pregnant rats. Moreover, ovariectomized rats experimentally infected with *C. albicans* in the vagina showed persistent infection when treated with progesterone in daily doses of 10–20 mcg. These results are similar to those reported in women experimentally infected with *C. albicans* in the vagina by DAWKINS et al. (1953), HALD and ARAGON (1956), STOUGH and BLANK (1958) and HESSELTINE et al. (1959). These authors reported a more persistent and long-lasting vaginal candidosis in the pregnant women. The infection in non-pregnant women was self-limiting and disappeared spontaneously. The third group of hormones, viz., ACTH, L. H., thyroid extract, insulin, oestradiol 17 β , ethinyl oestradiol and stilboestrol inhibited the growth of yeasts. The growth inhibitory effect of oestradiol 17 β was also verified in vivo by injecting the hormone in bilaterally ovariectomized rats infected with *C. albicans*. These results are contrary to those obtained by SCHOLER (1960) who reported a persistent infection of the vagina in ovariectomized rats injected with oestradiol.

It was also noticed that treatment of mice with ACTH afforded a partial protection against systemic candidosis. This can be explained on the basis of the direct inhibitory influence of ACTH on the growth and multiplication of *Candida* in vitro. It is interesting to note that the injection of ACTH alone (15.2 \times 10² mcg) caused death of 33 %

of the animals, while the mortality was reduced to 14 % when the same dose of the hormone was injected in animals infected with *C. albicans*. It seems that an interaction took place between the yeast and the hormone which induced a mutual antagonism resulting in the highly significant reduction in the fatality, well below half the expected percentage value of mortality attributed to the hormonal treatment. This may be explained, that the large number of yeast cells might produce excessive amounts of metabolites that rendered the hormone inactive or that because of the large number of yeast cells so that a considerable number of cells escaped the effect of the hormone.

Summary

The in vitro studies of the effect of hormones on the growth of *Candida albicans* and *Cryptococcus neoformans* revealed that some hormones had no influence at all e. g. the posterior pituitary extract; some hormones exerted a distinct inhibitory effect e. g. ACTH, L. H. and stilboestrol and a third group of hormones promoted the growth of yeasts e. g. progesterone, testosterone and the contraceptive, norethisterone.

The in vivo studies showed that treatment of ovariectomized rats experimentally infected with *C. albicans* in the vagina with oestradiol 17 β or sesame oil resulted in a very highly significant decrease in the rate of infection. It was also proved that treatment of mice with ACTH afforded partial protection against systemic candidosis.

Zusammenfassung

Die in-vitro-Untersuchungen über die Wirkung von Hormonen auf das Wachstum von *Candida albicans* und *Cryptococcus neoformans* führten zu der Feststellung, daß einige Hormone überhaupt keinen Einfluß auf das Wachstum dieser beiden Hefen haben, z. B. Hypophysenhinterlappenextrakt; einige Hormone übten eine geringfügige Hemmwirkung aus, z. B. ACTH, (Adrenocorticotropes Hormon), L. H. (Luteinisierendes Hormon) und Stilboestrol; eine dritte Gruppe von Hormonen schließlich för-

derte das Wachstum von Hefen, z. B. Progesteron, Testosteron und das Kontrazeptivum Norethisteron.

Die in-vivo-Untersuchungen zeigten, daß die Behandlung ovariectomierter Ratten — nach experimenteller Infektion der Vagina mit *Candida albicans* — mit 17 β -Oestradiol oder Sesamöl zu einer hochsignifikanten Abnahme der Infektionsrate führte. Es wurde außerdem nachgewiesen, daß die Behandlung von Mäusen mit ACTH einen partiellen Schutz gegen eine systemische Candidose gewährte.

References

1. BRAIN, P. W.: *Trans. Brit. Mycol. Soc.* 43, 1–13 (1960).
2. DAWKINS, S. M., J. M. B. EDWARDS and R. W. RIDDELL: *Lancet*, 2, 1230–33 (1953).
3. DOBES, W. L.: *Arch. Derm.* 72, 252–265 (1955).
4. FRIED, J., R. W. THOMA, J. R. GERKE, J. E. HERZ, M. N. DONIN and D. PERLMAN: *J. Am. Chem. Soc.* 74, 3962–63 (1952).
5. HALD, C. and G. ARAGON: *Am. J. Obstet. Gynec.* 72, 363–66 (1956).
6. HESSELTINE, H. C.: *Ann. New York Acad. Sci.* 83, 245 (1959).
7. LEAF, A.: In "The biochemical aspects of hormone action." J. & A. Churchill Ltd., London.
8. LITCHFIELD, T. and F. WILCOXON: *J. Pharmacol. & Therap.* 96, 99 (1949).
9. ROTH, F., J. J. FRIEDMAN and J. T. SYVERTON: *J. Immunol.* 78, 122–127 (1957).
10. RUSS, J. D. and C. G. COLLINS: *J. Am. Med. Ass.* 114, 2446 (1940).
11. REFAI, M. and S. EL-SHERIF: (1974) under publication.
12. SCHERR, G. H.: *Mycologia* 47, 305–310 (1955).
13. SCHOLER, H. J.: *Path. Microbiol.* 23, 62 (1960).
14. SHIRASAKA, M., A. NAITO, S. SUGAWARA and M. NAKAMURA: *Takamine Kenkyusho Nempo* 11, 52–56 (1960).
15. STOUGH, W. V. and H. BLANK: *Obstet. & Gynec.* 12, 338–40 (1958).

Address for reprints: Prof. Dr. MOHAMED REFAI, Dept. of Microbiology, Faculty of Veterinary Medicine, Giza, Egypt.