

Extrahypophyseal Gonadotrophins in Adult Female Rats after a Long Term Post-Hypophysectomy Regression Period

It was of interest to investigate if the female reproductive tract could be restored in rats to normal size and function after a very long deprivation of endogenous hypophyseal secretions.

Fifteen mature female rats were used under the identical experimental conditions as described in the accompanying paper¹. The ovaries were removed in analogy to the testes and the uteri were weighed after dissection. Body-, adrenal- and kidney-weights were reduced to levels which are normal for hypophysectomized rats. The 'control' ovaries were taken out at the beginning of the replacement therapy, 1 year after hypophysectomy. These gonads were in a stage of considerable atrophy. The contralateral ovary was removed at autopsy. The average weight of the 'control' ovaries was 5.8 ± 0.5 mg. Thirty days of HCG administration practically failed to increase this weight substantially. In contrast, the PMS preparation provoked a fourfold weight increase already after 6 days. This weight level became stationary until after 18 days of PMS replacement therapy. Then, after 24 and 30 days, the ovaries increased enormously. The uterine weights remained low under the HCG, whereas, under PMS, this organ was stimulated significantly (Figure 1).

Histologically, there were no signs pointing to processes of follicular development in the atrophied 'control' ovaries. Only small primordial follicles and many corpora lutea with relatively small diameters were visible (Figure 2 A). After 30 days of HCG treatment the follicular growth was never found to be further advanced than to the smaller types of secondary follicles (Figure 2 B). In contrast, after a replacement therapy with PMS for 6 days only, within the enlarged ovaries, there were already cavity formations and transformations into tertiary follicles. After 12 days of PMS treatment, there were no

longer corpora lutea visible and the follicular development took place everywhere. Eighteen days of PMS injections gave rise to the appearance of a great number of large follicles, the cystic type, causing the extreme weight increase of the ovaries (Figure 2 D).

This trend became predominant after 24 days of PMS application, and it was probably favoured by the hypothyroid condition of the hypophysectomized animals². It must be assumed that no ovulations had taken place. This was expected since it is well known that a particular

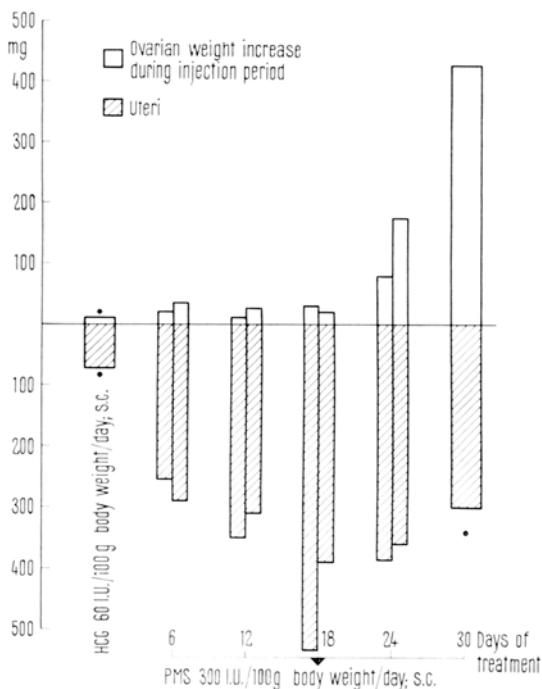


Fig. 1. Effects of a 1 year post-hypophysectomy regression period and subsequent replacement therapy with gonadotrophic hormones on ovarian weight development and on the uterine weight.

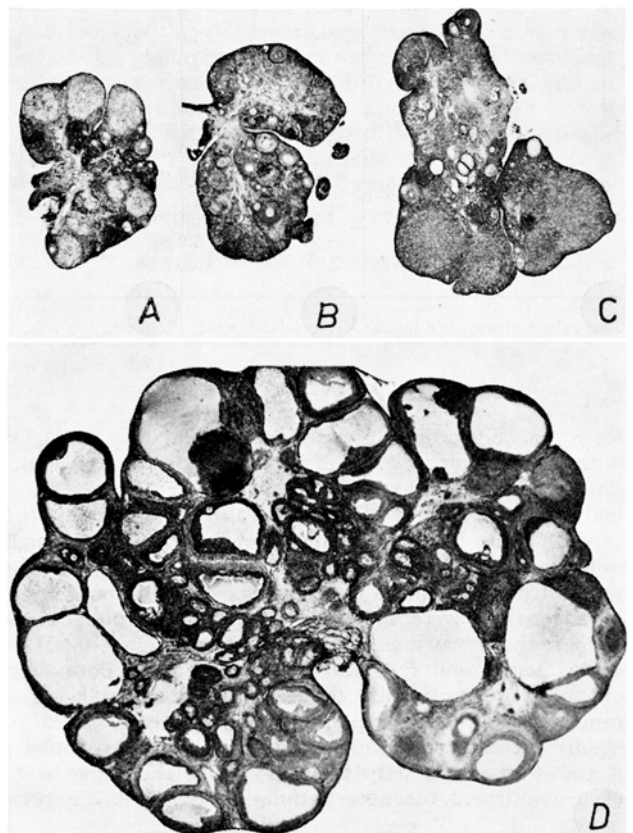


Fig. 2 A. 'Control' ovary, 1 year after hypophysectomy. There are primary follicles only, and no maturation processes are detectable. All ovaries had a great number of small persisting corpora lutea. $\times 15$.

Fig. 2 B. Subcutaneous injections of 60 I.U. HCG/100 g body weight/day for 30 consecutive days: as compared with the 'control' ovaries, there are almost no alterations. Sometimes, there is a rather doubtful follicular development resulting in very small secondary follicles, but nowhere, tertiary follicles were found. $\times 15$.

Fig. 2 C. Identical treatment as described under Figure 2 B. For unknown reasons there were found persisting corpora lutea in several ovaries. These organites were possibly slightly stimulated. $\times 15$.

Fig. 2 D. Subcutaneous injections of 300 I.U. PMS/100 g body weight for 24 consecutive days: extremely enlarged cystic ovaries. In all cases there were no signs detectable indicating that ovulations had taken place. $\times 15$.

¹ R. VON BERSWORDT-WALLRABE and F. NEUMANN, *Experientia* 24, 499 (1968).

² G. V. CALLARD and J. H. LEATHEM, *Proc. Soc. exp. Biol. med.* 118, 996 (1965).

single injection of either FSH or ICSH at a critical period of time is required to induce ovulations in hypophysectomized rats when mature follicles are present³.

As judged by the uterine weights and the absence of vaginal estrus it seems that under HCG, the normal ovarian steroidogenesis was hardly in progress, if at all. Finally, in confirmation of early workers⁴ all 'control' ovaries contained persisting corpora lutea even after this extended post-hypophysectomy regression period. Interestingly, these organites disappeared rapidly under the PMS influence, whereas, under the HCG, there were in some ovaries still persisting corpora lutea after 30 days of injections, however, in other cases they had disappeared (Figure 2 C).

This long term experiment supports 2 facts: in spite of this extended deprivation of hypophyseal secretions, the female gonad was not impaired irreversibly. It conserved in all cases its sensitivity for gonadotrophic hormones. Again, in analogy to the male gonad, in this experiment with female rats too, the FSH played a key role in synergism with the ICSH within the normal sequence of reproductive events. This underlines the paramount importance of the FSH within the phenomena of follicular growth and maturation⁵.

Zusammenfassung. Fünfzehn erwachsene weibliche Ratten wurden ein Jahr nach erfolgter Hypophysektomie mit HCG und PMS behandelt. Trotz der extrem langen Involutionsperiode reagierten die völlig atrophierten Ovarien unmittelbar auf die parenteral applizierten Hormonpräparate. Jedoch nur das FSH- plus ICSH-Aktivitäten enthaltende PMS (Serumgonadotropin) induzierte ein deutliches Follikelwachstum. Ovulationen konnten nicht nachgewiesen werden, wohl infolge der acyclischen Applikationsform der Substanzen.

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³ A. J. LOHSTROH and R. E. JOHNSON, *Endocrinology* 79, 991 (1967).

⁴ R. O. GREEP, *Endocrinology* 23, 154 (1938).

⁵ J. H. GAARENSTROOM and S. E. DE JONGH, *A Contribution to the Knowledge of the Influences of Gonadotropic and Sex Hormones on the Gonads of Rats* (Elsevier Publ. Co., New York 1946).

Successful Reinitiation and Restoration of Spermatogenesis in Hypophysectomized Rats with Pregnant Mare's Serum after a Long-Term Regression Period

After an adequate post-hypophysectomy regression period, both repair and continued development of the testis necessitates in rats the synergism of FSH and androgens, probably testosterone^{1,2}. When this synergism was abolished with an anti-androgen, the FSH principle by itself was devoid of any detectable effects within the testis of hypophysectomized 'androgen-free' rats³. On the other hand, testosterone propionate, ICSH and HCG had very limited abilities to reinitiate, restore and maintain spermatogenesis after an adequate post-hypophysectomy regression period^{1,2,4}. The purpose of this study was to investigate if the testis could be restored to normal size and function after having been deprived of hypophyseal gonadotrophic hormones for an extremely long period of time.

Methods. Twenty-three mature male rats⁵, weighing 220–280 g, were hypophysectomized and 1 'control'-testis was removed after 1 year and weighed. The contralateral gonad was removed at autopsy, weighed and inspected histologically. The diameters of the testicular tubules were determined. All doses of hormones (Figures 1 and 2) were referred to body weight at the time of injection. The HCG preparation was extensively tested for its FSH potencies⁶ and found to be devoid of detectable amounts of FSH.

Results. The body weight and average weight of the adrenals and kidneys were uniformly reduced to levels which are typical for hypophysectomized rats⁷. This was also true for the 'control'-testes which were removed before treatment commenced, then weighing 252 ± 43 mg. After 30 days of HCG treatment, there was practically no testicular weight increase. By contrast, under the PMS preparation, this parameter gradually increased during the period of replacement therapy, to reach a plateau after 24 days (Figure 1). The diameter of the tubules was considerably enlarged under both preparations. They

reached practically normal values of 250μ when pregnant mare's serum (PMS) was given for 24 or 30 days (Figure 2).

The histology of the 'control'-testes showed a male gonad in a stage of a rather pronounced atrophy (Figure 3 A). After the HCG treatment, the seminiferous epithelium was generally no further developed than the early stages of spermatids. Simultaneously, the interstitial cells were hypertrophied (Figure 3 B). However, after 6 days of PMS treatment, there were already pachytene spermatocytes. In spite of this, the germinal epithelium was still largely depopulated, whereas the Leydig cells were enlarged and hypertrophied in a distinctive way. After 12 days of PMS therapy, the tubules were enlarged, the germinal epithelium became more populated and as far advanced as the early types of spermatids. This development of germ cells was even more advanced after 18 days. Ripe sperms finally became observable in the testes of those animals that had been under the more prolonged PMS influence (Figure 3 C). The weights of the prostates and seminal vesicles showed a continued increase under the PMS (Figure 1).

¹ J. H. GAARENSTROOM and S. E. DE JONGH, *A Contribution to the Knowledge of the Influences of Gonadotropic and Sex Hormones on the Gonads of Rats* (Elsevier Publ. Co., New York 1946).

² M. C. WOODS and M. E. SIMPSON, *Endocrinology* 69, 91 (1961).

³ R. VON BERSWORDT-WALLRABE and F. NEUMANN, *Excerpta med. Int. Congr. Series* 133, 950 (1967).

⁴ A. V. BOCCABELLA, *Endocrinology* 72, 787 (1963).

⁵ H. STEINBECK and R. VON BERSWORDT-WALLRABE, *Z. Versuchstierkde* 8, 167 (1966).

⁶ F. J. A. PAESI, S. E. DE JONGH, M. J. HOOGSTRA and A. ENGELBRECHT, *Acta endocr., Copenh.* 19, 49 (1955).

⁷ F. NEUMANN and R. VON BERSWORDT-WALLRABE, *J. Endocr.* 35, 363 (1966).