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 present[3].

As judged by the uterine weights and the absence of vaginal estrus it seems that under HCG, the normal ovarian steroidgenesis was hardly in progress, if at all. Finally, in confirmation of early workers[4] 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 maturation[5].

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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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' rats[3]. 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 rats[5], 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 potencies[6] 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 rats[7]. This was also true for the 'control'-testes which were removed before treatment commenced, then weighing 252 $\pm$ 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 250 μ 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).

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Discussion. The atrophied testes reacted immediately after being exposed to the gonadotrophic preparations. Hereby, it became clear that the FSH principle played a key role in this experiment. It was given within the PMS preparation which has additionally inherent ICSH activities. Under these combined hormonal actions, the male gonads gradually redeveloped within 24 days all visible structures so far that they were practically indistinguishable from histological pictures of normal testes, with the exception that the Leydig cells were somewhat hypertrophied. Obviously, the HCG, with its single ICSH potency, was restricted to stimulate selectively the interstitial cells (= Leydig cells) only, since there was no spermiogenesis and practically no substantial weight increase; findings which corroborate similar studies[1,2]. The weights of the accessory sexual organs were more stimulated under PMS, probably because of its higher amount

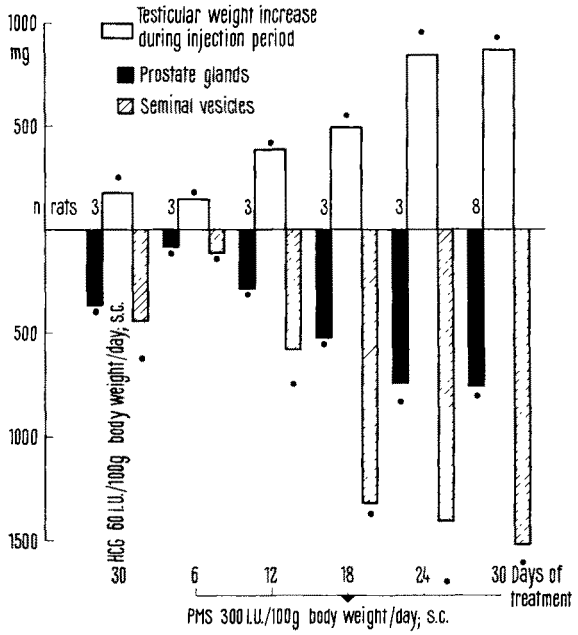

Fig. 1. Effect of a 1 year post-hypophysectomy regression period and subsequent replacement therapy with gonadotrophic hormones on testicular weight development, and on the weights of prostate gland and seminal vesicles.

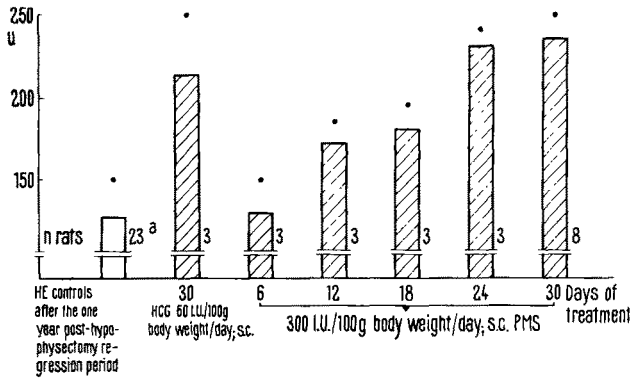

Fig. 2. Effects of a 1 year post-hypophysectomy regression period and subsequent replacement therapies with gonadotrophic hormones on the mean diameter of the seminiferous tubules. [a] Left testes, removed before treatment.

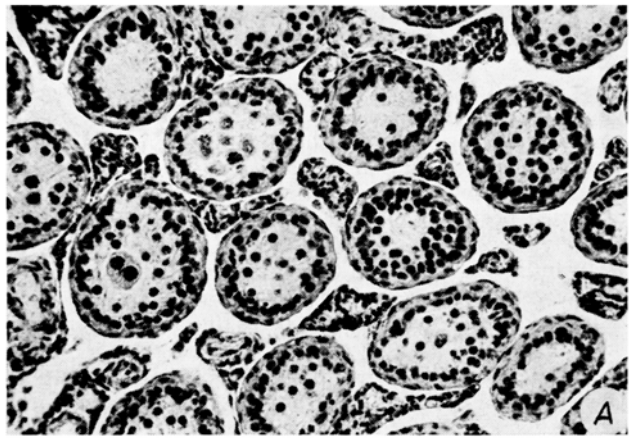

Fig. 3 A. 'Control'-testes before treatment: tubules and Leydig cells atrophic. The germinal epithelium has practically nothing but spermatogonia and spermatocytes. × 235.

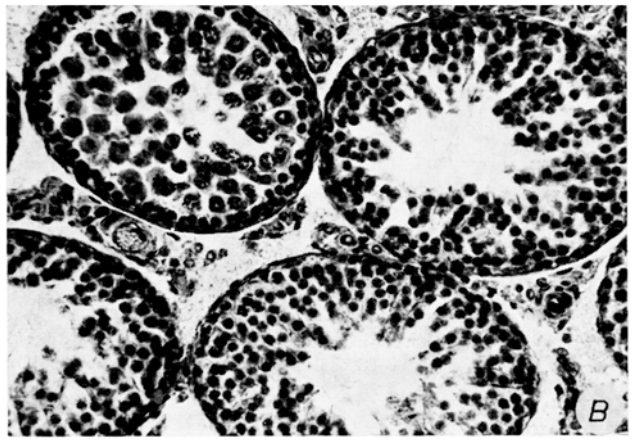

Fig. 3 B. Subcutaneous injections of 60 I.U. HCG/100 g body weight/day for 30 consecutive days: tubuli medium sized. The germinal epithelium is generally no further advanced than the early types of spermatids. More mature types of spermatids are not frequently found. The Leydig cells are hypertrophied, but not as far as under the PMS dosage. Notice that, in spite of the enlarged tubules, no spermiogenesis is detectable. × 235.

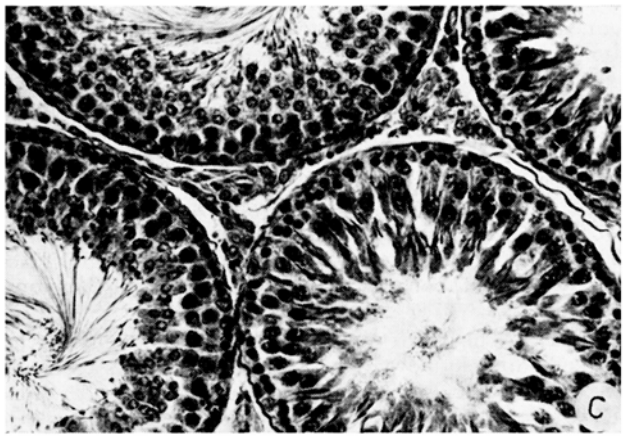

Fig. 3 C. Subcutaneous injections of 300 I.U. PMS/100 g body weight for 24 or 30 consecutive days: the tubules are in most cases comparable with those of normal intact rats. Complete regeneration of the germinal line and a rather pronounced spermiogenesis takes place everywhere. The hypertrophied Leydig cells are no longer too impressive (as they used to be after 6 days of replacement therapy) because of the normal diameter of the tubules. × 235.

of ICSH potency, as compared with the HCG preparation[8]. It remains to explain why, after a post-hypophysectomy regression period, the testicular events are FSH plus testosterone dependent, whereas testosterone or HCG, if given immediately after the extirpation of the pituitary, maintains a rather normal spermatogenesis[1,2,7]. Long term experiments are in progress in order to find out if this androgen replacement therapy can go on indefinitely with full success, or if spermatogenesis is restricted by a time factor when it is generated by androgen therapies only. In other words, it must be studied if testosterone becomes ineffective without the FSH synergism after about 48 days. This period of time is supposed to cover four 12-day cycles of the seminiferous epithelium which is believed to be necessary to complete once all events of spermatogenesis in the rat[9].

Zusammenfassung. Nach einer einjährigen Involutionsperiode im Anschluss an die Hypophysektomie gelang es mit Hilfe eines PMS-Präparates bereits nach 24tägiger Applikationsdauer, die völlig atrophierten Testes zu annähernd normalen Gewichten mit vollständiger Spermatogenese zu restaurieren. Im Gegensatz hierzu war ein HCG-Präparat, das keine messbaren FSH-Potenzen aufwies, praktisch nur in der Lage, die Leydigschen Zwischenzellen selektiv zu stimulieren. Diese Ergebnisse deuten an, dass bei Ratten die Hodenfunktion trotz maximaler Atrophie wieder völlig hergestellt werden kann, sofern ein Präparat eingesetzt wird, das sowohl ausreichende ICSH- als auch FSH-Potenzen enthält.

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[8] Measurements of relative potency (with juvenile hypophysectomized rats, ventral prostate test[6], 3 dose factorial assay, including the covariance procedure) revealed that the ICSH potencies of PMS and HCG were identical when I.U. were compared.

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Development of Black Down Feathering in Hybrid Chick Embryos (Cross New Hampshire ♂ × Light Sussex ♀) after Pituitary Implantation

The male embryos and newly hatched chicks of the cross New Hampshire ♂ × Light Sussex ♀ are white, i.e. have no down pigmentation; the females develop a reddish-brown down pigmentation from the eleventh day of incubation onwards.

In previous communications (GROENENDIJK-HUIJBERS[1,2]) evidence was presented in favour of the concept that the formation of the red pigment in the female embryos is bound to an adequate level of gonadal hormones, either ovarian or testicular. While studying the influence of endocrine glands on the formation of the red pigment, an unexpected phenomenon was observed after pituitary implantation. The preliminary results of this study are reported here.

Material and methods. The hybrid eggs of the above cross were 'windowed' on the fourth day of incubation according to a method already described (GROENENDIJK-HUIJBERS[3]). On the fifth day, 1–3 pituitaries from chick donors of various ages were implanted into the coelomic cavity of the host embryos according to DOSSEL's method[4]. The age of the donors varied from 16 days of incubation to 6 weeks after hatching. As sham implants, fragments of embryonic heart, liver, thyroid and adrenal gland were used. Moreover, as a sham treatment, some embryos received on the fifth day of incubation a single dose of a synthetic hormone, such as testosterone-propionate (Orchisterone, Frosst; or Neohombreol, Organon), oestradiol-benzoate (Dimenformon, Organon), thyrotrophic hormone (Ambinon, Organon; or TSH, Sigma) or ACTH (Cortrophine and Cortrophine-Z, Organon). Finally, synthetic α-MSH (melanocyte-stimulating hormone) was administered either in 3 doses on the fifth, eighth and tenth day, or in 5 doses on the seventh to eleventh day of incubation. The drugs were administered by instilling the chorio-allantois through the shell window.

Autopsy was done at 12–15 days of incubation. The down on head, back, tail and wings was carefully examined with the aid of a dissecting microscope and photographed. The host's gonads and thyroids as well as the implants were removed and prepared for histological examination.

Results. The results are summarized in the Table and demonstrated in the Figures. When a sufficient amount of pituitary tissue has developed in the implant, a peculiar black-feathered area is found on the occiput of the host embryos of either sex. When only a few black feathers are present (which is arbitrarily designated as Degree 1: the number of black feathers ranging from 3–20), they are found along the median line (Figures 1 and 1a) just cranially and caudally to the pineal body, that in the 14-day-old chick embryo lies in direct contact with the scalp (Figures 2 and 2a).

When a larger amount of pituitary tissue has developed in the implant, some 20–40 black feathers embrace the pineal body in the form of a diamond and extend along the median line proximally and distally (Degree 2, Figures 2 and 2a). When the blackening is still more advanced, tiny black feathers are found in line with the lateral angle of the eyes (Degree 3, Figures 3 and 3a). Proximally the feathers extend as far as the vertex, close to the root of the comb.

As a rule the 'black feathers' are only partially black: the black pigment is arranged in longitudinal stripes along one side of the feather. In the females the fine-dotted brown pigment can be clearly distinguished lying side by side with the coarse black pigment (magnification × 40).

The degree of blackening is related to the amount of tissue developed in the implant rather than to the age of

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