Immunodepressive activity of Daunomycin on skin allografts in mice

Drug	Treatment route	Schedules at days[a]	Doses (mg/kg/day)	Mean survival time	Probability level (P) (comparison with control group)
–	–		–	12.4	
Daunomycin	i.p.	−3, −2, −1, 0	1.25	13.1	$\leqslant 0.05$
Daunomycin	i.p.	−1, 0, +1, +2	1.25	14.1	$\leqslant 0.05$
Daunomycin	i.p.	−8, −7, −6, −5, −4, −3, −2, −1	1.25	15.0	$\leqslant 0.05$
Daunomycin	i.p.	−4, −3, −2, −1, +1, +2, +3, +4	1.25	13.7	$\leqslant 0.05$
Daunomycin	i.p.	+1, +2, +3, +4, +5, +6, +7, +8	1.25	14.1	$\leqslant 0.05$
–	–		–	13.2	
Daunomycin	i.v.	−3, −2, −1	5	15.4	$\leqslant 0.05$
Daunomycin	i.v.	−3, −2, −1	3.3	14.3	$\leqslant 0.05$
Daunomycin	i.p.	−8, −7, −6, −5, −4, −3, −2, −1	1.25	17[b]	

[a] 0 = day of grafting. [b] Some animals are dead before the end of the experiment.

delayed death of some animals in a group of younger mice. In fact it is well known that the lethal dose of Daunomycin is higher when this drug is administered i.v. as compared to the i.p. route.

Discussion and conclusions. According to our data Daunomycin determines an increase of skin allografts survival time in mice. This increase correlates well with the cumulative doses used, since the 5 mg/kg/day i.v. treatment gave the longest survival time, without any toxic side-effects, which points to the importance of the administration route. In fact the i.v. route allowed us to reach much higher cumulative doses of Daunomycin, moreover this route is currently used in human practice. Also, Daunomycin i.p. causes a marked local inflammatory reaction.

The mechanism of action of Daunomycin as immunodepressant is not completely understood, this drug is an antimitotic agent, thus it destroys mainly immature and actively proliferating cells i.e. bone marrow cells, gastrointestinal epithelium and neoplastic cells. Unpublished observations[14] from this laboratory have shown that it has no activity upon the reticulo-endothelial system and upon macrophages. The effect displayed against delayed hypersensitivity reactions is definite and well documented[3, 6, 7, 10, 11].

On the other hand, clinical studies by KOULINSKY et al.[15] have shown that delayed hypersensitivity reactions to 4 different antigens in a group of 21 patients with acute leukemia were completely abolished by Daunomycin in 6 subjects, who presented severe, irreversible bone marrow aplasia. It would thus seem that immunodepressive activity of Daunomycin is non-specific, probably related both to its antimitotic properties and to the destruction of immature cells (stem cells).

Riassunto. L'effetto immunodepressivo della Daunomicina è stato studiato sulla sopravvivenza del trapianto cutaneo nel topo. La Daunomicina determina un aumento del tempo di sopravvivenza del trapianto cutaneo, statisticamente significativo. Gli AA. discutono l'importanza che possono assumere le dosi, la via di somministrazione e lo schema di trattamento impiegato.

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Long Term Replacement Therapies with Testosterone Propionate and Human Chorionic Gonadotrophin in Hypophysectomized Adult Male Rats[1]

Daily testosterone propionate (TP) injections commencing immediately after hypophysectomy obviously prevented substantial testicular weight losses, sustained spermatogenesis, as judged by histological criteria, during a three weeks period in adult rats[2]. Longer replacement therapies revealed that testosterone did not maintain the germ cell numbers at the normal level[3]. Questions arose for how long a period testosterone, HCG could give replacement for the rat's testicle when started with the injections immediately after hypophysectomy.

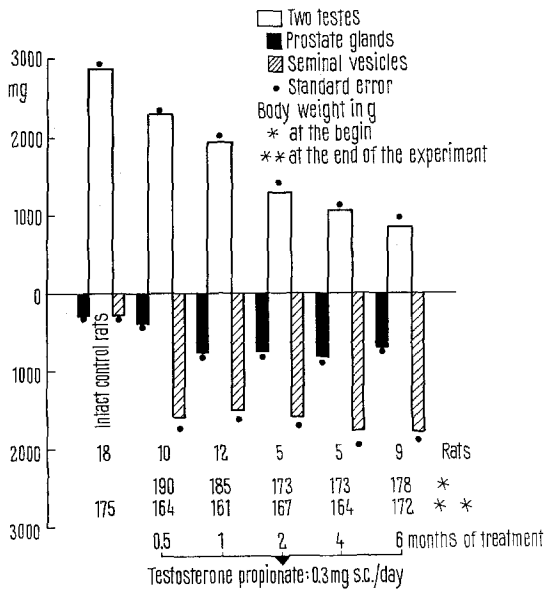

Fig. 1. Effects of long term TP replacement therapy on the weights of the testes, prostate glands, and seminal vesicles of hypophysectomized rats.

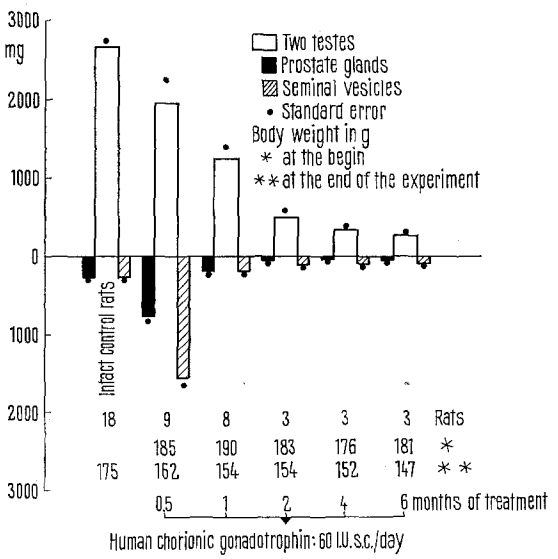

Fig. 2. Effects of long term HCG replacement therapy on the weights of the testes, prostate glands, and seminal vesicles of hypophysectomized rats.

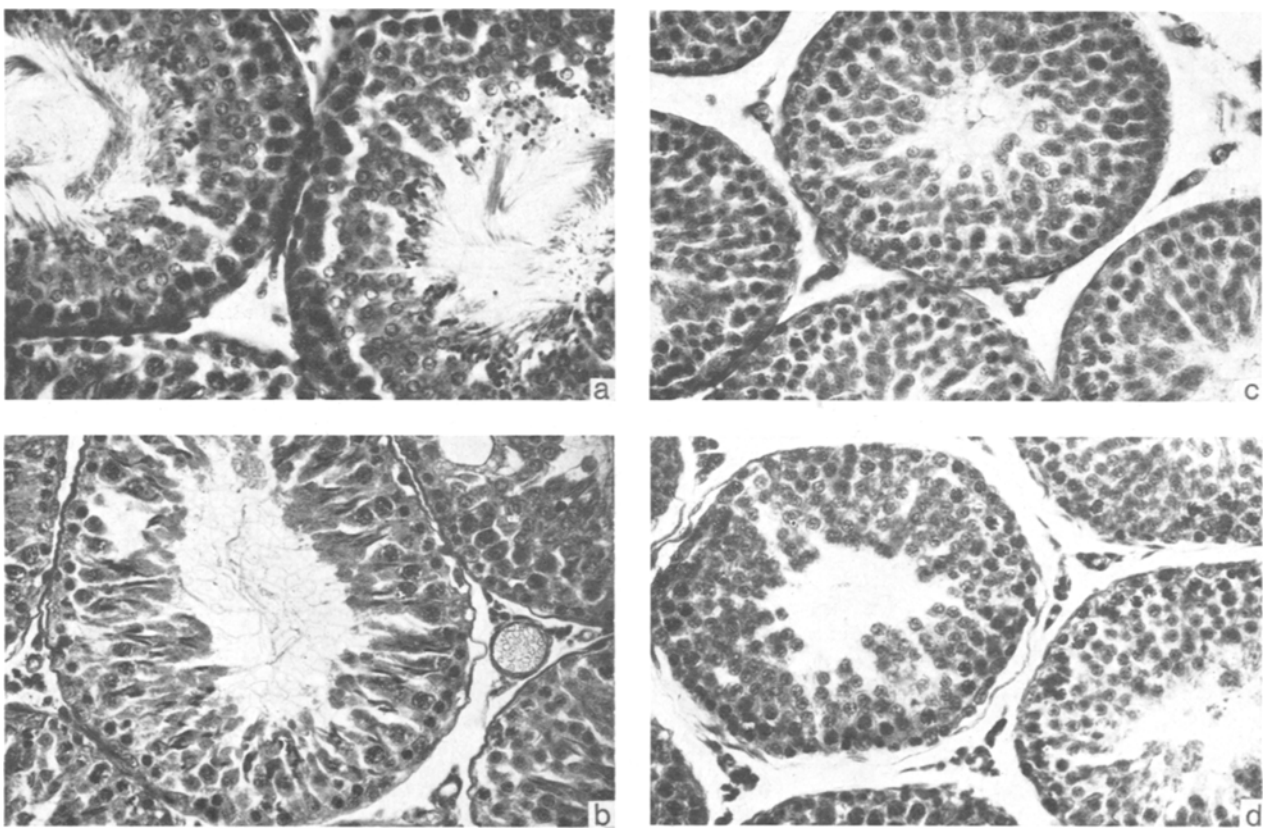

Fig. 3. Long term testosterone propionate replacement therapy in hypophysectomized rats: 0.3 mg s.c./day (× 400). Note that the sequence for the signs of degeneration under this steroid as given in this plate advanced in a smooth and quasi 'organized' way. Even after 6 months of continuous treatment there were still numerous 'young' spermatids visible. a) 21 days. Note the absence of interstitial cells. b) 2 months. Spermatogenesis no further advanced than to the spermatid level, occasionally spermatozoa. c) 4 months. Widths of tubules slightly smaller; reduction of cell population within the germinal epithelium comes into prominence. Slight intraepithelial edema observable. d) 6 months. Beginning of the disorganisation of the cellular elements.

Methods. Mature male rats[4] weighing 180–200 g were hypophysectomized. 18 intact animals served as controls. Daily injections commenced immediately after hypophysectomy as indicated in the figures. Testes, prostate glands, seminal vesicles, adrenals were removed at autopsy and weighed. Testes were inspected histologically. The HCG preparation was extensively tested for its FSH potencies[5,6] and found to be devoid of detectable amounts of FSH.

Results. Under the influence of testosterone propionate, there was a gradual decrease of testicular weight over the period of 6 months. Prostate gland and seminal vesicles were stimulated by far beyond normal (Figure 1).

Under the influence of HCG, however, an abrupt decline of testicular weight took place. After 2 months the testicles reached a level, rather typical for HE control rats. Excessively stimulated prostate glands and seminal vesicles were found after 2 weeks time only. Later on, these glands were in an atrophic condition (Figure 2).

As judged by histological inspections, first deviations from normal were found to take place after 21 days of TP treatment (Figure 3, a). Those testes which had been under TP influence for 2 months exhibited first signs of an intraepithelial edema; simultaneously spermiogenesis was arrested (Figure 3, b).

The seminiferrous tubules retrogressed gradually throughout the experiment (Figure 3, b–d). In correlation with this parameter, there was a continuous diminution of the cellular elements too. After 6 months of TP treatment, the overall impression of the testicular histology turned out to give a picture of disorganisation with a more important intraepithelial edema, as compared with the transitory situation found after a 4-months period (Figure 3, c–d).

Finally, as a rule, there were positively no spermatozoa observed later than 2 months after hypophysectomy (Figure 3, c–d).

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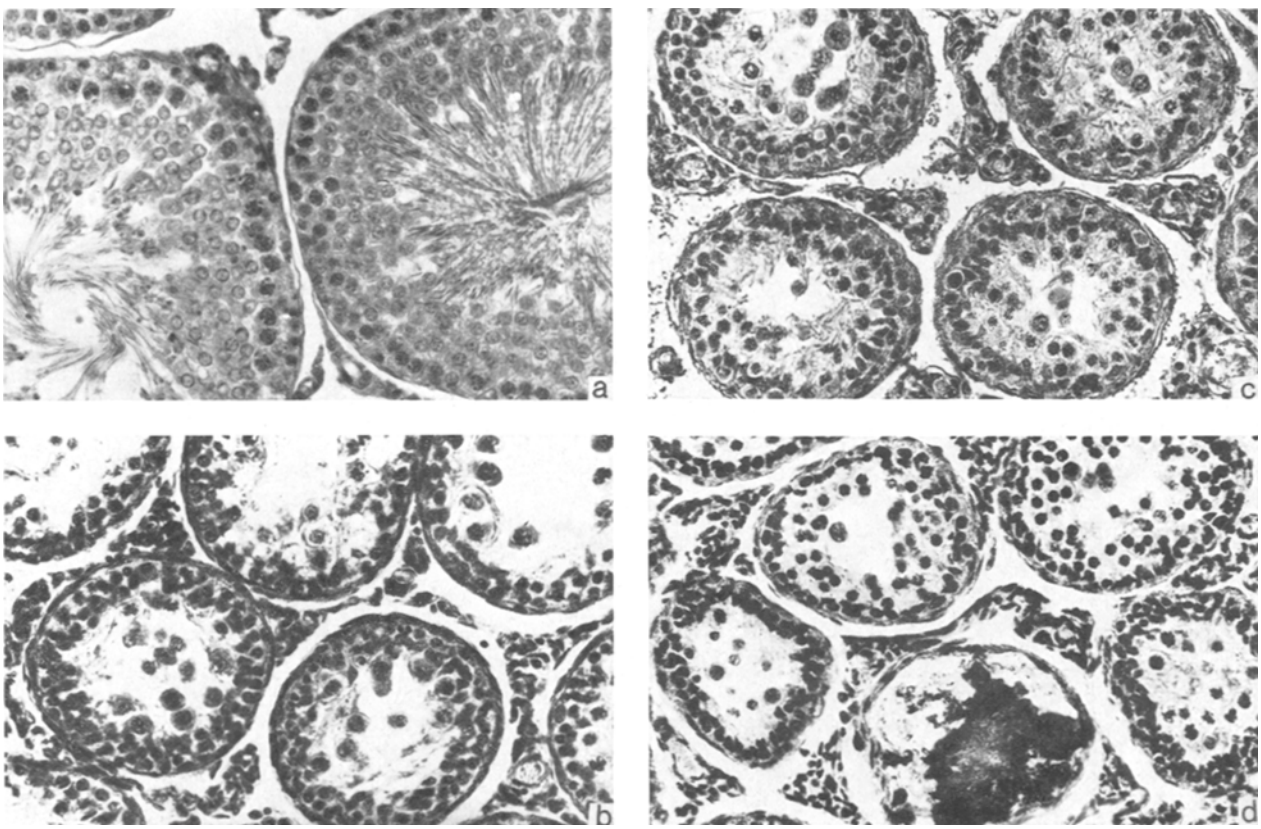

Fig. 4. Long term HCG-replacement therapy in hypophysectomized rats: 60 I.U. s.c./day (× 400). The quintessence of the illustrations as given in this plate seems to be the abrupt change from rather normal histology to an advanced disorder between the pictures a) and b). This demonstrates the complete failure of HCG injections to give successful replacement at the testicular level in hypophysectomized rats, over a period commencing somewhere between 3 weeks and 2 months. a) 21 days. The interstitial cells in a stage of beginning retrogression. b) 2 months. Note the appearance of far reaching degenerative changes: A striking intraepithelial edema within the interstitial sites. Furthermore, in addition, there were cellular elements, probably activated mesenchyme of vessels. Very occasionally only, a few spermatids within some tubules. The same was true for single giant cells. c) 4 months. Predominance of disorganization rules this scene: a still more advanced decrease of the tubular diameters, with a depopulation of the germinal line, touching those levels which are typical for rat testes after long post-hypophysectomy regression periods. d) 6 months. Note the ultimate retrogression of testicular atrophy within the limits of this experiment.

Concerning the testes of the HCG-treated rats, first signs of histologically visible events, slightly deviating from normal, were observed after a 3 weeks period (Figure 4, a). In spite of 2 months of HCG influence normal-looking interstitium was visible no longer (Figure 4, b).

Along with considerably reduced tubular diameters, the germinal epithelium was in a stage of heavy depopulation. Practically no spermiogenesis and elements no further advanced than primary spermatocytes (Figure 4, b).

Almost identical histological impressions gave slides of the testes which had been 4 and 6 months under the HCG therapy respectively. In fact, there were still areas left containing a few spermatocytes. On the other hand, however, quite a few tubules were completely devoid of normally occurring cellular structures. Instead of this, they were occupied with an abundance of necrotic materials (Figure 4, c). It might be left open to discussion whether the severity of degeneration and necrosis was even further advanced after 6 months as compared to the 4 months pictures (Figure 4, c–d).

Discussion. Although a high dose of TP was given daily up to 6 months, the testicular events retrogressed in the hypophysectomized rat continuously. The longer the replacement therapy, the more a phenomenon became transparent, which is not seen earlier under such an experimental condition[2]. The androgen alone could not sustain spermiogenesis, testicular weight in the adult rat in the long run: almost identical with what was observed after the ablation of the pituitary gland in control rats[7], however, extended over a much longer period of time the well-known sequence of degeneration within the germinal line took place under TP too. However, it must remain open to discussion whether the ultimate stages of atrophy as described earlier in HE rats[8] could also be reached although this steroid is injected.

HCG was much less effective than testosterone, probably, first of all, because of its inability to stimulate androgenic secretion(s) of the interstitial cells beyond a given term. The comparatively high mortality of these HCG-treated rats during the second month of the experiment points to the likelihood of anti-body formation in consequence of the rather high and chronic dose of foreign protein. Under testosterone, such a lack of androgenicity was ruled out: the established parameters for the male hormone, prostate glands and seminal vesicles had been stimulated excessively even after 6 months. These findings support the theory that the dynamics of the seminiferous epithelium and its morphology depend not only on steroidal androgenicity but also on the synergistic action of the FSH[9,10]. At first sight there might arise the somewhat remote objection that the stage of hypophysectomy per se, in other words the absence of the pituitary with its various hormones, could cause this failure of the androgen therapy. However it has been shown extensively that FSH plus endogenous or exogenous androgen gave full replacement for the rat's testis, even after ultimate atrophic conditions such as are seen after extended post-hypophysectomy regression periods[8]. Although the dynamics of spermatogenesis in man and rat are not necessarily comparable, this fact may be of importance in clinical practice.

Zusammenfassung. Langzeitversuche mit hypophysektomierten Ratten ergaben, dass Testosteron oder HCG allein die Spermiogenese, Hodengewicht, nicht aufrechterhalten konnten.

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RNA Synthesis in the Sex Chromosomes of the Opossum, *Didelphis virginiana*. I. Female

The single active X (Lyon) hypothesis[1] is supported by the following observations: a) female mammalian somatic cells frequently demonstrate sex chromatin bodies[2]; b) these cells contain a late replicating X chromosome[3–7]; c) late replicating chromosomes are heterochromatic in interphase nuclei[8,9]; d) heterochromatin synthesizes RNA at a lower rate than does euchromatin[10–13]; and e) when more than two X chromosomes are present, the number of sex chromatin bodies is one less than the total number of X chromosomes present, and all but one are late replicating[14].

Whereas the majority of evidence favors this hypothesis, certain other findings suggest that both X chromosomes may be genetically active during brief portions of the cell cycle. For example, sex chromatin cannot be identified in all female somatic cells in interphase[15]. Similarly, during periods of rapid growth, both female X chromosomes may fail to undergo heterochromatization in cells with short cycles[16,17]. In addition, all prophase chromosomes of female human lymphocytes incubated briefly with H^3-uridine were found to synthesize RNA immediately prior to mitosis[18,19].

The opossum possesses many unusual cytogenetic characteristics which made it an ideal animal in which to test the single active X hypothesis. Quantitation of RNA synthesis was made possible since the X chromosomes are easily identifiable as the smallest of the complement[20]. In addition, the following observations have been made: a) cultured opossum lymphocytes demonstrate a short cell cycle[21]; b) sex chromatin is present in both sexes[22,23]; c) the amount of heterochromatin appears to vary little between the X chromosomes of lymphocytes from female opossums[24]; and d) there is no typical late replicating X chromosome[25].

Materials and methods. Lymphocytes obtained from cardiac blood of female opossums were cultured according to a previously described method[20]. Following 42–48 h of incubation, the cultures were labeled terminally for