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Effects of the duration of therapy with the LHRH agonist D-ser (BUT)^6 Azgly^{10}-LHRH (ICI 118-630) on the steroid hormone content and the morphology of human testicular tissue in the treatment of patients with advanced prostate cancer

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Summary. In 20 patients with locally advanced or metastatic prostate cancer, testicular tissue obtained by bilateral subcapsular orchidectomy was examined for steroid hormone content and morphological changes. Eight patients (group I) had not received previous treatment. Twelve patients had been treated with monthly subcutaneous doses of the depot luteinizing hormone-releasing hormone (LHRH) agonist D-ser (BUT)^6 Azgly^{10}-LHRH (ICI 118-630). Six patients (group II) had been treated for less than 6 months and 6 patients (group III) for more than 6 months. The longest duration of treatment with depot LHRH was 36 months. After 2 months of treatment (group II), maximum hormone suppression was achieved and remained unchanged even if treatment was continued for 3 years. The mean serum testosterone levels were decreased in group II ($\bar{x} = 0.586$ mg/ml) and in group III ($\bar{x} = 0.575$ mg/ml) and were found to be in the range of castration; a statistically significant reduction in luteinizing hormone ($P < 0.000001$) and follicle-stimulating hormone ($P < 0.05$) was observed in the treated patient groups. The content of the steroid hormones dihydroepiandrosterone sulfate (DHEA)-S, testosterone, androstenedione, oestradiol, progesterone and 17-alpha-hydroxyprogesterone/g testicular tissue was significantly lower in patients on LHRH agonists. The differences in concentration were particularly pronounced for DHEA-S, T and A. As in the case of serum concentrations, the testicular tissues showed no differences between groups II and III. In the treated groups a significant reduction in weight was seen, depending on the duration of therapy. Similarly, the structural changes visible by the aid of light and electron microscopes increased with the duration of therapy. In contrast, the functional endocrine changes achieved in the serum and testicular tissue after 2 months of treatment remained stable even if treatment with the depot LHRH agonist ICI 118-630 was continued for 3 years.

Key words: LHRH agonist – Duration of treatment – Human testicular tissue – Steroid hormones – Morphology

Supraphysiological doses of luteinizing hormone-releasing hormone (LHRH) agonists lead to a reliable suppression of testicular androgen production [11, 12, 22]. This may result from an evacuation of luteinizing hormone (LH) stores, a desensitisation of and reduction in LHRH receptors in the pituitary gland after initial pituitary overstimulation [6, 19]. In addition, LHRH therapy was found to produce a loss of LH and human chorionic gonadotrophin HCG receptors in the gonadal tissue [5]. Moreover, direct testicular mechanisms of action have been discussed [7, 16]. The application of LHRH agonists in the management of prostate cancer is based on the fact that pharmacological castration is achieved by this therapeutic approach. With the introduction of depot preparations, a clinically practicable alternative to bilateral orchidectomy has become available [1, 17, 18, 24]. While the suppressing effect of LHRH agonist on androgen production in general has been demonstrated in several studies, there are hardly any data on the mechanisms involving the testes. Similarly, the influence of the duration of therapy has not been sufficiently described. In this study, testicular tissue obtained by subcapsular bilateral orchidectomy from patients with advanced carcinoma of the prostate was examined biochemically for steroid hormone content, as well as for morphological changes, using light and electron microscopy. The aim of this study was to investigate the effects of treatment with the depot LHRH agonist ICI 118-630 on hormone concentrations in the serum and testicular tissue, in order to give a more detailed description of the effects on hormone biosynthesis. Another purpose of the study was to determine the effects on testicular morphology in relation to steroid hormone content in the testicular tissue, depending on the duration of therapy.

Patients and methods

In 20 patients with locally advanced or metastatic prostate cancer, testicular tissue obtained by bilateral subcapsular orchidectomy was examined for steroid hormone content and morphological changes.

Eight patients had not received previous treatment; the youngest patient in this group was 65 years old, the oldest 86 years. The average age was 77. Of the 12 patients treated with a depot LHRH agonist, the youngest patient was 67 and the oldest 84 years old. The average age was 77.6 years. The following three treatment groups were formed for statistical comparision:
Group I: No LHRH pre-treatment, orchidectomy.
Group II: LHRH pre-treatment until month 6 (4 patients treated for 2 months; 1 patient treated for 4 months; 1 patient treated for 5 months).
Group III: Duration of treatment with the depot LHRH agonist for more than 6 months (2 patients treated for 10 months; 2 patients treated for 13 months and 2 patients treated for 36 months). Median duration of treatment was 13.08 months.

Treatment was performed using the depot LHRH agonist D-ser (BUT)^6 Azgly^{10}-LHRH (ICI 118-630). This special biodegradable depot preparation consists of lactide glycolide copolymer (50:50) that dissolves in the tissue, releases the substance and degrades to lactate and glycolic acids. Each depot preparation containing 3.6 mg of LHRH agonist (ICI 118-630) was subcutaneously injected into the abdominal skin using a needle from the trocar system. It was possible to administer the medication without local anesthesia like a normal subcutaneous injection. The average daily amount released from the depot was 120 µg.

Plasma concentrations of dihydroepiandrosterone sulfate (DHEA), 17-alpha-hydroxyprogesterone (17-OHP), oestradiol (E2), testosterone (T), androstenedione (A), LH, follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) were measured prior to therapy, during therapy, as well as 2 days before and on the day of orchidectomy. Blood samples were collected between 7 and 9 o'clock in the morning. A radioimmunoassay method (CIS) was used to determine LH. Progesterone levels (P, E2, A and DHEA) were measured by means of a coated tube/solid-phase radioimmunoassay (DPC). T and SHBG concentrations were determined by Sorin and Serono radioimmunoassay respectively. The Serono double-antibody assay was used to measure the follicle-stimulating hormone FSH. The intra-assay of variation was 12% for all hormone determinations.

Immediately after operative removal, the testicular tissue was weighed and stored in liquid nitrogen until being processed. One milliliter of homogenization buffer (50 mM phosphate buffer, 250 mM saccharose, 7 mM dithiothreitol, 0.5 mM EDTA, pH 7.4) was added to 1 g of tissue, which was then homogenized at 1,000 rpm in a glass Teflon mixer (Potter). For this and all following steps refrigeration was required (0–4°C). The homogenate was centrifuged at 100,000 g for 1 h. The resulting supernatant was stored at −20°C for quantification of steroids. The steroids 17-OHP, A, T, E2 and DHEA were analyzed using the same radioimmunoassay methods as for serum hormone determination. Statistical evaluation of the data was carried out by one-way analysis of variance.

For morphological evaluation small tissue specimens approximately 1 mm in diameter were fixed in 3% glutaraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.4) for 2.5 h, rinsed in the same buffer and postfixed in Palade-buffered 1% osmic acid for 1 h. After dehydration in a grade series of ethanol, they were embedded in epon 812. Semithin sections were stained with toluidine blue, ultrathin sections with uranyl acetate and lead citrate.

Results

Endocrinology

Serum hormone profiles. Figure 1 contains the mean values and standard deviations of the endocrine parameter concentrations measured in the serum. In comparision to the untreated patient group (group I), patients treated with depot LHRH (group II) showed a statistically significant decrease in LH and FSH serum concentrations. While FSH dropped to approximately 50% ($P<0.05$) compared to the untreated patient group, a suppression of LH levels by 80% was achieved ($P<0.000001$). Similarly, a significant decrease in serum levels of 17-OHP ($P<0.0005$), T ($P<0.00005$) and E2 ($P<0.001$) was observed. However, no differences were found between groups II and III. After only 2 months of treatment, maximum hormone suppression was achieved and remained stable even if patients were treated for 3 years. The mean testosterone levels measured in group II had decreased ($\bar{x}=586$ ng/ml and $\bar{x}=575$ ng/ml) and were found to be in the range of castration (<0.6). During treatment with ICI 118-630 there was no change in the serum concentration of SHBG, which is physiologically and clinically the most important binding protein for testosterone.

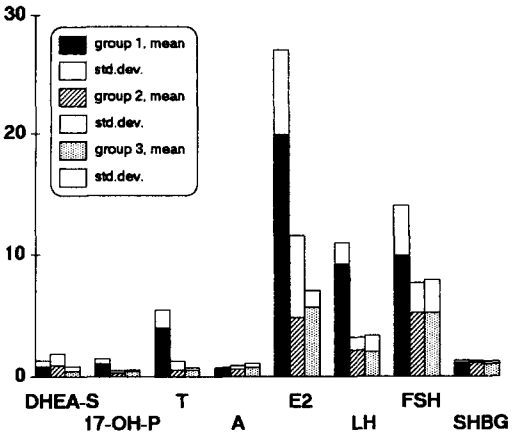

Fig. 1. Serum hormone levels before and after treatment with the LHRH agonist ICI 118-630 (DHEA-S: µg/ml, 17-OHP: ng/ml, T: ng/ml, A: ng/ml, E2: pg/ml, LH: mIE/ml, FSH: mIE/ml, SHBG: µg%)

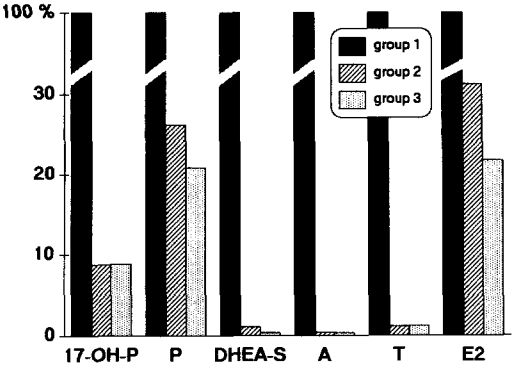

Fig. 2. Change in the content of steroids in the testicular tissue (%) before and after therapy with the LHRH agonist ICI 118-630

Steroid hormone profiles in the testicular tissue. Therapy resulted in a significant decrease in DHEA-S ($P<0.00005$), T ($P<0.00005$), A ($P<0.000005$), E2 ($P<0.005$), P ($P<0.0005$), and 17-OHP ($P<0.000001$) in the testicular tissue (group II). There were not differences between groups II and III (Fig. 2).

Concentrations of DHEA-S, A and T dropped to values around or below 1% compared to group I. Frequently the amounts of DHEA-S were even below the detection limit of the assay system applied (20 ng/ml). For 17-OHP a decrease in concentration to 7.5% was achieved, whereas P and E2 levels were depleted to one-fifth of those measured in group I. Figure 3 shows that therapy with the LHRH agonist did not only lead to pronounced inhibition of steroid synthesis in general, but also to a significant change in the quantitative ratios between the steroids. In comparison to P, the decrase in 17-OHP levels was significantly stronger ($P < 0.00005$). A parallel change was seen in the quantitative ratios between A and 17-OHP (see Fig. 4). As a result of therapy, the suppression of A was significantly more pronounced than that of 17-OHP ($P < 0.00005$). This change in the ratios of concentrations was to the same degree as observed in groups II and III.

Morphology

Macroscopy. Clinical examination showed a pronounced decrease in testicular volume after long-term treatment with depot LHRH. Within 6 months of therapy (patients in group II), the wet weight of testicular parenchyma (obtained by subcapsular orchidectomy) was reduced by 30% compared to specimens from patients without treatment (group I). Prolonged treatment with the LHRH agonist before orchidectomy (group III) resulted in wet weight reductions of even 45%. Mean values and standard deviations of the testis parenchyma weights are summarized in Table 1.

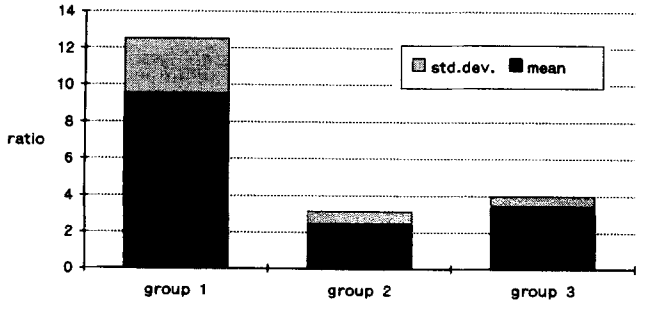

Fig. 3. Change in the ratio of 17-OHP to P contained in the testicular tissue

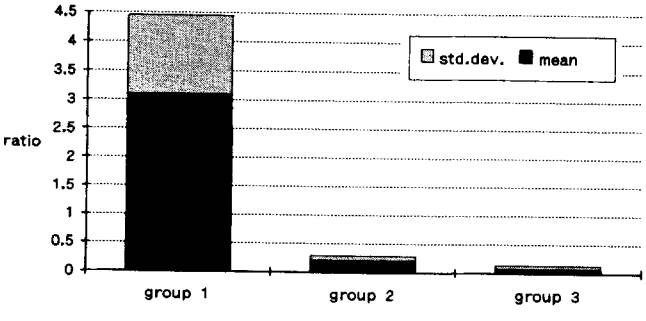

Fig. 4. Change in the ratio of A to 17-OHP contained in the testicular tissue

Table 1. Mean values and standard deviations of testicular tissue weights

	$\bar{x}$
Group I ($n = 16$ untreated)	13.16 g ± 3.25
Group II ($n = 12$ LHRH therapy < 6 months)	9.04 g ± 4.19
Group III ($n = 12$ LHRH therapy > 6 months)	7.29 g ± 1.22

Microscopy. Four stages of tubular degeneration, which might be responsible for the reduction in testicular volumes and weights, were observed after long-term therapy.

1. Complete atrophy of the testis tubules with hyalinization of the basement membranes, complete or nearly complete loss of the lumina, and peritubular fibrosis (Fig. 5).
2. Complete germ-cell loss with reduction of the lumina and, as above, hyalinization of the basement membranes and peritubular fibrosis (Fig. 6).
3. Cessation of spermatogenesis of the spermatogonium stage without alterations in the basement membranes and surrounding connective tissue (Fig. 7).
4. Development of spermatogenesis up to early spermatid stages (Fig. 8).

This stage occurred very rarely in patient group III. Late spermatids or even ripe spermatozoa were never found in our specimens.

In the interstitium, the Leydig cells exhibited numerous vesicles and dense granules after 36 months of treatment (Fig. 9), which were identified as lipid droplets and large lysosomes on electron microscopical level (Fig. 10). Furthermore, an increase in filaments and a decrease in smooth endoplasmic reticulum, possibly a sign of inactivation of steroid production, was seen.

Discussion

The aim of the present study was to contribute to the solution of the question as to how much the duration of therapy with a depot LHRH agonist can affect androgen biosynthesis and morphology in the human testicular tissue. This investigation was based on the comparative data collected within this study involving serum and tissue hormone levels and morphology. In contrast to morphology, where even in the long run the effects of treatment with the LHRH agonist were directly dependent on the duration of therapy, the suppression of testicular steroid synthesis was achieved after only a short period of treatment (group II) and remained unchanged thereafter (group III). All preliminary stages of T, i.e., P, 17-OHP, DHEA-S and A, as well as E2, were present in a

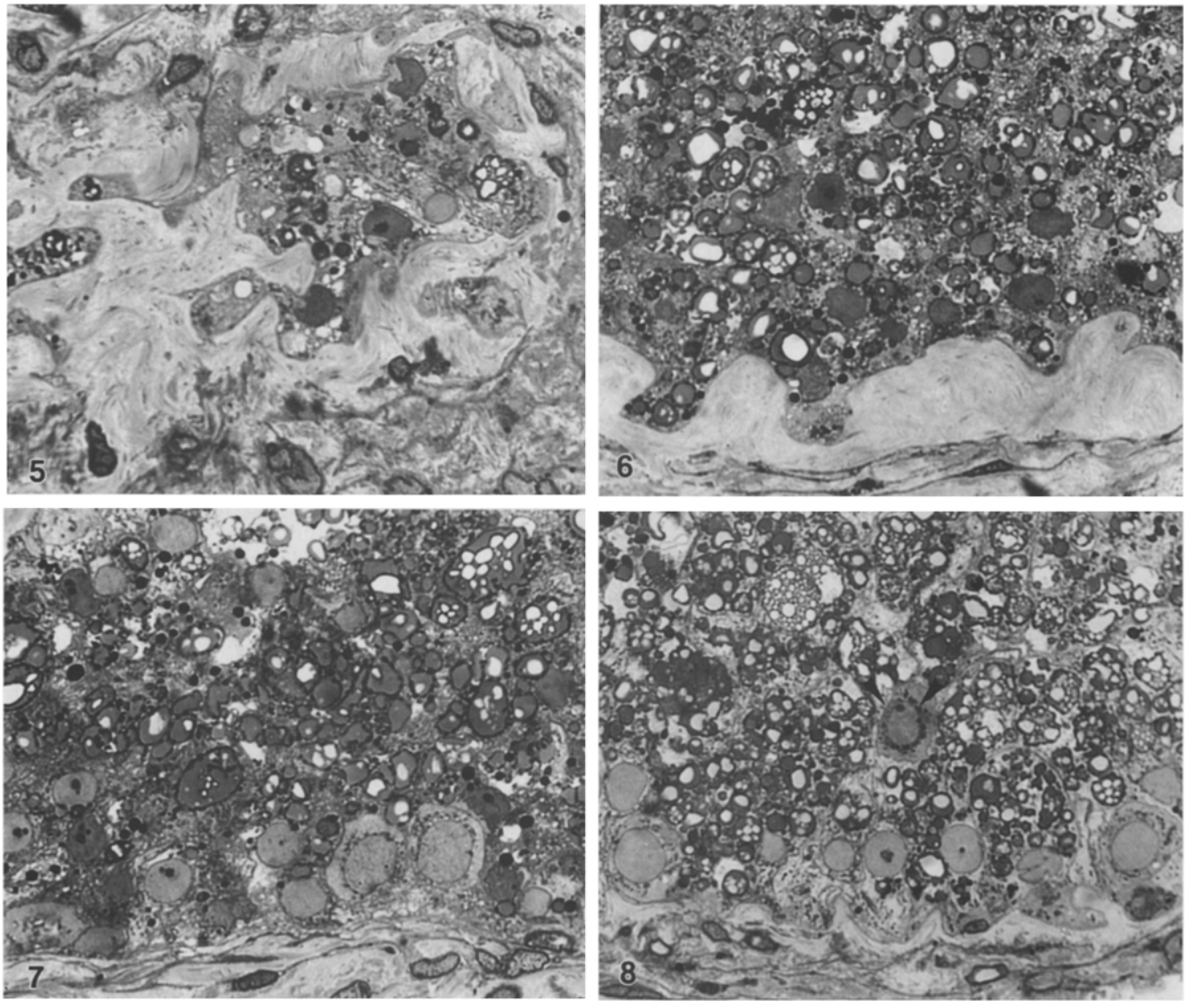

Fig. 5–8. Tubuli seminiferi 36 months after the onset of treatment. Semithin sections, toluidine blue staining. ×2,700

Fig. 5. Tubular atrophy with hyalinization of the basement membrane and peritubular fibrosis

Fig. 6. Testis tubule with only Sertoli cells, hyalinization of the basement membrane and peritubular fibrosis

Fig. 7. Testis tubule with spermatogonia and Sertoli cells

Fig. 8. Testis tubule with Sertoli cells and germ cells up to early spermatids (*arrowheads*)

significantly reduced concentration in the testicular tissue. This indicates a general inhibition of testicular steroid synthesis. The concentrations of T measured in the serum ($P < 0.6$ ng/ml) show that the aim of chemical castration had been achieved. With a decrease in concentrations to 15% of those measured in the control group, the result was identical with that achieved by surgical castration [10]. This confirms that in the male, T is synthesized primarily in the testicular interstitial cells of Leydig. On the other hand, the adrenal cortex secretes a considerable percentage of A as well as the major part of DHEA-S, which is, however, likely to be of only secondary biological importance [14]. The serum levels of the DHEA-S and A were unchanged during treatment with ICI 118-630. This suggests that there is neither a direct nor indirect effect of these agonists on steroid synthesis in the adrenal cortex. The suppressing effect of long-term treatment with LHRH agonists on testicular steroid production [8, 9, 15, 23] is the consequence of concurrent gonadal and pituitary inhibition [16]. A down-regulation of LHRH receptors and a related decrease in gonadotropin secretion has been reported in the literature [20, 21, 23]. In the present study, a decrease in LH and FSH concentrations by 80% and 50% were observed, respectively.

In earlier studies, a direct effect of LHRH agonists on testicular function has been described [2, 13], and LHRH receptors have been observed in the interstitial cells of Leydig [16]. Furthermore, a down-regulation of gonadal LH receptors has been suggested [3, 4]. In addition, an inhibition of testicular enzymes, such as 17-alpha-hydro-

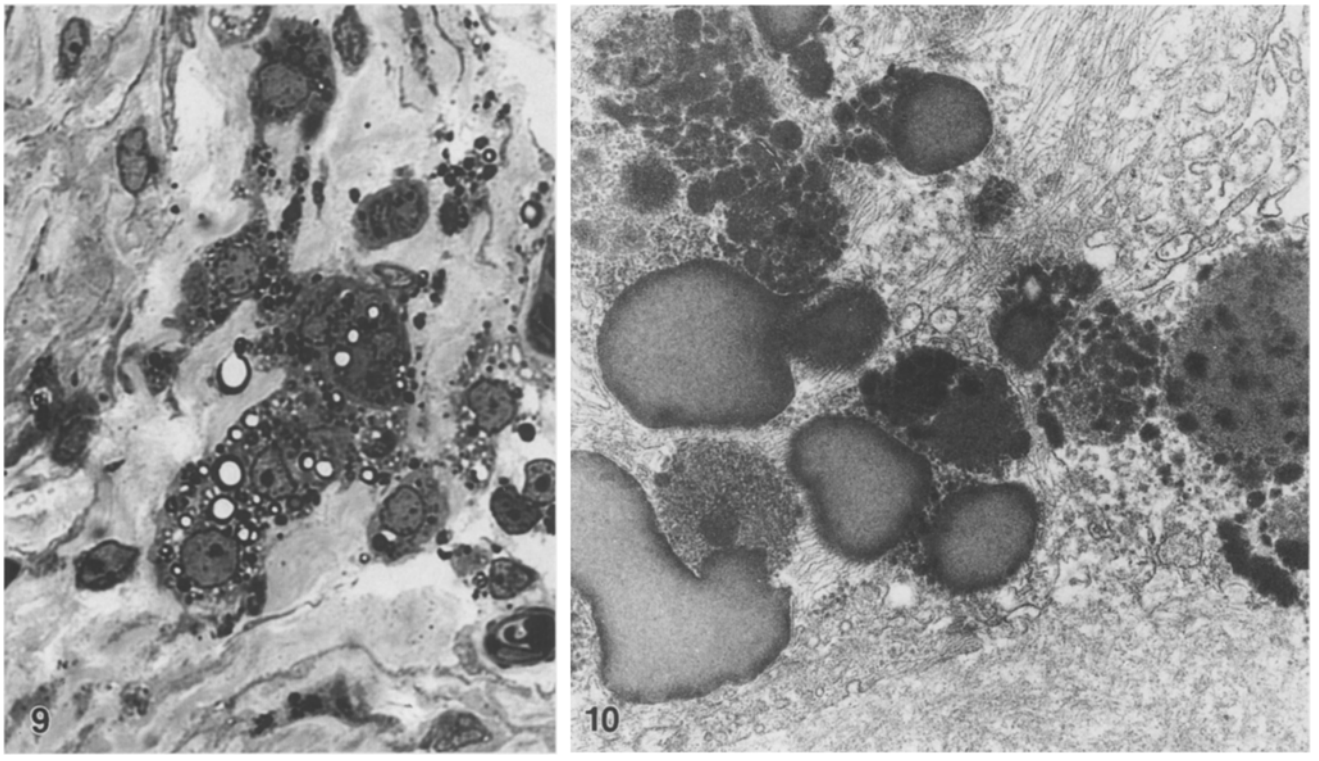

Fig. 9–10. Leydig cells 36 months after the onset of treatment

Fig. 9. Semithin section of a group of Leydig cells with numerous vesicles and dense granules. Toluidine blue staining. ×3,100

Fig. 10. Ultrathin section: part of a Leydig cell exhibiting lysosomes, lipid droplets and filaments. The smooth endoplasmic reticulum is reduced. Uranyl acetate and lead citrate staining. ×26,000

xyprogesterone aldolase (17,20-desmolase, E.C. 1.4.2.30) and steroid (17-alpha-monoxygenase (17-hydroxylase, E.C. 1.14.99.9) occurs [7, 15]. This ultimately leads to a reduction in testosterone levels. The drop in the ratio of 17-OHP to P by approximately 70% in this study indicates the inhibition of the steroid 17-alpha-monoxygenase. Similarly, the decrease in the ratio of A to 17-OHP by more than 95% is likely to result from an inhibition of 17-alpha-hydroxyprogesterone aldolase. To sum up, one can say that the synthetic LHRH agonist ICI 118-630 produced chemical castration in patients with carcinoma of the prostate even after short-term therapy. On the one hand, total suppression of testicular steroid synthesis was achieved by general inhibition of synthesis, probably the consequence of reduced pituitary LH release. On the other hand, inhibition of two important enzymes of androgen biosynthesis was observed, which is one of the possible direct effects of the agonist on testicular function. It seems to be an important observation for clinical practice that the endpoint of functional endocrine change was reached after only one or two administrations of 3.6 mg of the depot LHRH agonist ICI 118-630. The endocrine situation achieved after this short period of treatment was identical with the endocrine changes found in the serum and testicular tissue after 3 years of treatment. However, atrophy and degeneration increased considerably as treatment continued.

In the present study, testicular weights were shown to be reduced by 50% after 3 years of treatment with LHRH. On these grounds it is to be expected that regeneration of the exogenous and endocrine parts of the testicular tissue will take a very long time, provided complete recovery is possible. Based on the data and results from this study, it is to be expected that recovery of the endocrine function and morphological condition of the testicular tissue occurs after short-term transitory therapy with the depot LHRH agonist D-ser (BUT)^{6} Azgly^{10}-LHRH (ICI 118-630) in the possible clinical range of application, such as pretreatment of patients with radical prostatectomy, stopping spermatogenesis in patients with malignant germinal testicular tumor on cytostatic therapy, or use as a contraceptive in males. After several years of therapy with the depot LHRH agonist ICI 118-630, considerable changes in testicular tissue were found and the endocrine changes remained stable. Recovery after several years of therapy with the LHRH agonist is presently of no clinical relevance. As indicated by the data presented in this study, it is certain that recovery with regard to morphology is only to be expected after a longer period of regeneration.

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