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A Study of the Capacity for Regeneration of Rat and Human Leydig Cells

Th. Senge[1], B. Schenck[2], U. Tunn[1], Th. Hülshoff[1], and F. Neumann[2]

[1] Urologische Klinik des Josefs-Hospitals, 4690 Herne 1, FRG
[2] Department of Endocrinology, Schering AG, Berlin-Bergkamen, FRG

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Summary. The capacity of Leydig cells for regeneration was investigated in 12 patients with prostatic carcinoma, who underwent subcapsular orchidectomy, and in rats after testicular necrosis produced by cadmium chloride.

In rats, reappearance of Leydig cells originating from the tunica albuginea could be demonstrated by histology. Testosterone concentrations increased parallel to regeneration of Leydig cells, while LH concentrations declined.

In contrast to these findings, no rise of testosterone concentrations could be observed in patients up to 8 months after subcapsular orchidectomy. Human Leydig cells seem to have no capacity for regeneration, or endocrine function, despite the fact that some of these cells, which are present morphologically in the tunica albuginea or spermatic cord, remained.

Key words: Leydig cells - Testosterone - Subcapsular orchidectomy - Cadmium chloride.

In rats, regeneration of Leydig cells after testicular necrosis due to ischaemia caused by ligation of spermatic vessels or cadmium intoxication has been observed (7, 11). Formation of interstitial cell tumours occurs occasionally following both forms of injury (5, 12). Cadmium induced tumours are usually found 18 months after injection.

Regeneration of Leydig cells is accompanied by recovery of growth and function of the accessory sex glands (3, 7, 10). Favino et al. (2) were able to demonstrate surviving Leydig cells histochemically lying beneath the tunica albuginea of rat testes ten days after administration of cadmium chloride. Studies in vitro on testicular tissue revealed progressive recovery in the capacity of the surviving Leydig cells to synthesise testosterone as a result of hyperplasia and hypertrophy.

As in the rat testis, the tunica albuginea of the human testis contains Leydig cells (1, 8, 13). Ectopic Leydig cells have also been found in the spermatic cord (1, 15). If a potential site of Leydig cell activity exists then, following subcapsular orchidectomy, it would be theoretically possible for such cells to produce androgens which would be important in the management of a patient with prostatic carcinoma.

In an attempt to asess whether or not regeneration of testosterone producing cells occurs, peripheral testosterone concentrations have been measured in patients following subcapsular orchidectomy and in rats following cadmium administration.

MATERIAL AND METHODS

Animal Experiments

One hundred and twenty-six male Sprague-Dawley rats with an average initial body weight of 200 g were used. The rats were housed in plastic cages and fed a standard diet and water ad libitum. Seventy-two rats re-

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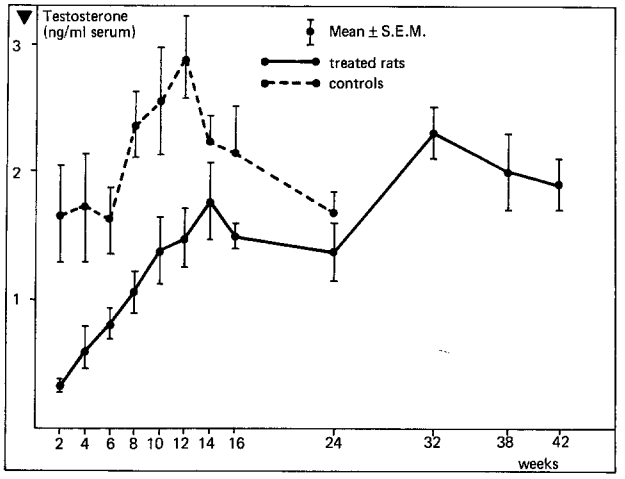

Fig. 1. Serum testosterone concentrations in male rats after administration of cadmium chloride compared with normal controls

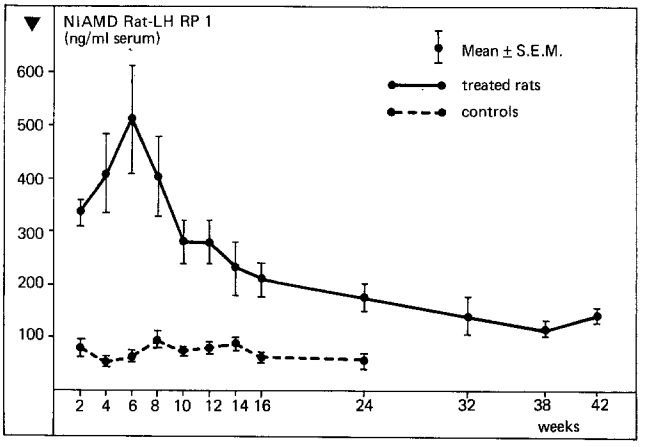

Fig. 2. Serum LH concentrations in male rats after administration of cadmium chloride

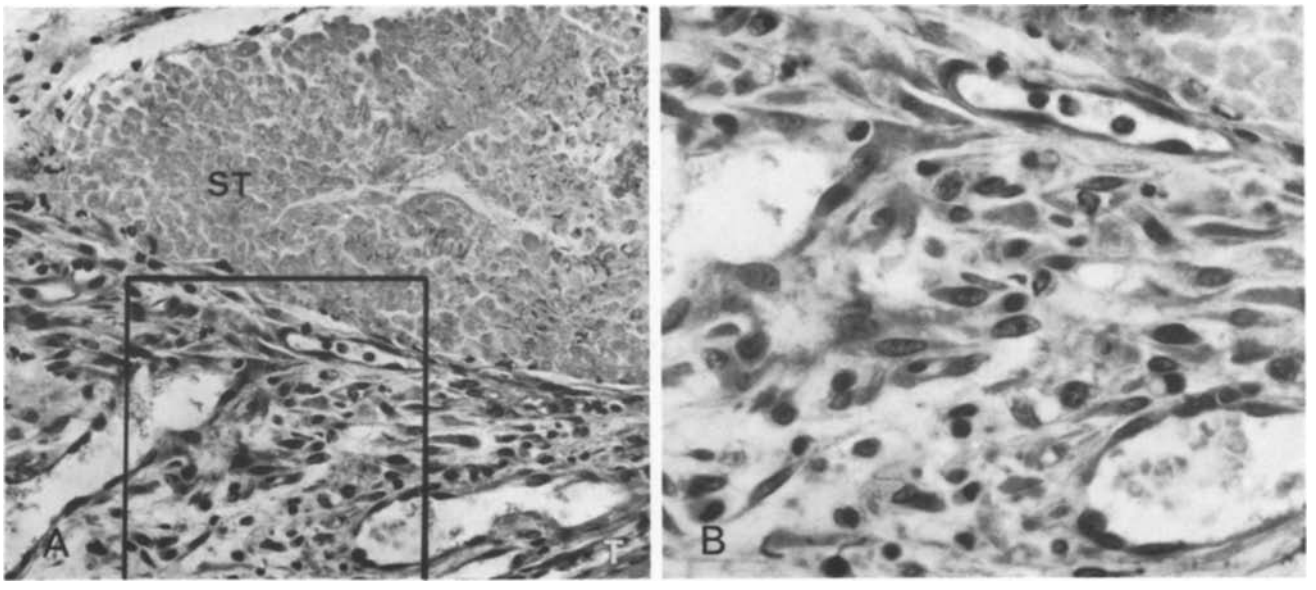

Fig. 3. Histology of rat testis 2 weeks after administration of cadmium chloride. Note the proliferation of fibroblasts and blood vessels on the inner surface of the tunica albuginea.
ST, seminiferous tubule; T, tunica albuginea.
A Magnification: 400 x; B Magnification: 800 x

ceived a single subcutaneous injection of 0.02 mmol cadmium chloride/kg body weight, 54 untreated rats served as controls. Groups of 6 rats were killed, 2, 4, 6, 8, 10, 12, 14, 16, 24, 32, 38 and 42 weeks, respectively, after the injection of cadmium chloride. Control groups of 6 untreated rats were killed at the same intervals up to the 24th week. Sera were taken for hormone determinations and the testes were removed for histological examination.

Patients

Twelve patients aged between 56 and 76 years with stage III or IV prostatic carcinoma underwent subcapsular orchidectomy. Blood samples were taken before, 4 and 24 hours after orchidectomy and at monthly intervals up to 8 months post-operatively. Except for the 4-hour post-operative samples all samples were taken between 9 and 10.30 a.m.

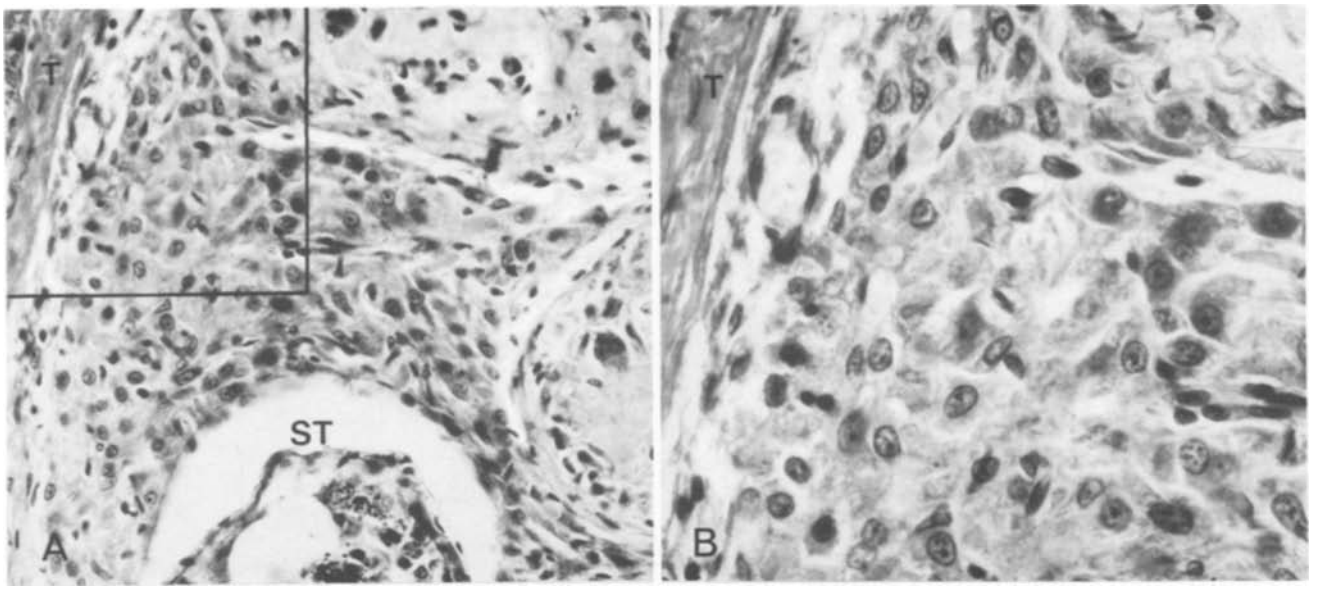

Fig. 4 A and B. Histology of rat testis 6 weeks after administration of cadmium chloride. Note the proliferation of cells with the appearance of Leydig cells in the peripheral parts of the testis. A) Magnification: 400 x; B) Magnification: 800 x

Hormone Determinations

Serum testosterone concentrations were measured by radioimmunoassay using the method of Nieschlag and Loriaux (9). Rat LH concentrations were determined by the double antibody method using NIAMD reagents.

RESULTS

Animal Experiments

Two weeks after a single injection of cadmium chloride, testosterone concentrations were significantly ($p > 0.01$) reduced in comparison with untreated controls. A continuous increase in testosterone levels then occurred up to 14 weeks after treatment. At this time testosterone concentrations were within the range of normal controls. Thereafter no significant changes were observed (Fig. 1).

The LH concentrations were significantly increased two weeks after cadmium treatment. After six weeks LH levels declined throughout the observation period and reached levels slightly above control levels (Fig. 2).

Two weeks after administration of cadmium chloride the testicular tissue was necrotic. The seminiferous tubules were filled with detritus. No cells either in the seminiferous tubules or in the interstitial tissue were visible. Underneath the tunica albuginea, fibroblasts and new blood vessels could be seen. After 4 weeks fibroblasts and blood vessels had proliferated on the inner surface of the tunica albuginea. During the next few weeks thickening of the tunica albuginea and seminiferous tubules occurred. Underneath the tunica proliferation of connective tissue occurs and fibroblasts grow from the peripheral to the central parts of the testes. Six weeks after cadmium administration, relatively large polyhedral cells appeared deep to the tunica. The nuclei were spherical or ovoid and contained vesicles and two or three nucleoli. These are typical characteristics of Leydig cells. In the following weeks an increase in the number of these cells occured, and they formed clusters on the inner surface of the tunica albuginea. At twenty-four weeks after cadmium treatment, 2 of 6 rats had developed the Leydig cell tumours in the peripheral parts of the testes, displacing the necrotic and shrunk tubules. At 32 weeks, interstitial cell tumours were found in 4 of 6 rats, at 38 weeks in 3 of 6 rats, and in 3 of 5 rats after 42 weeks (Figs. 3, 4, 5).

Clinical Observations

Testosterone concentrations of the 12 patients declined from a mean value of 2.4 ng/ml

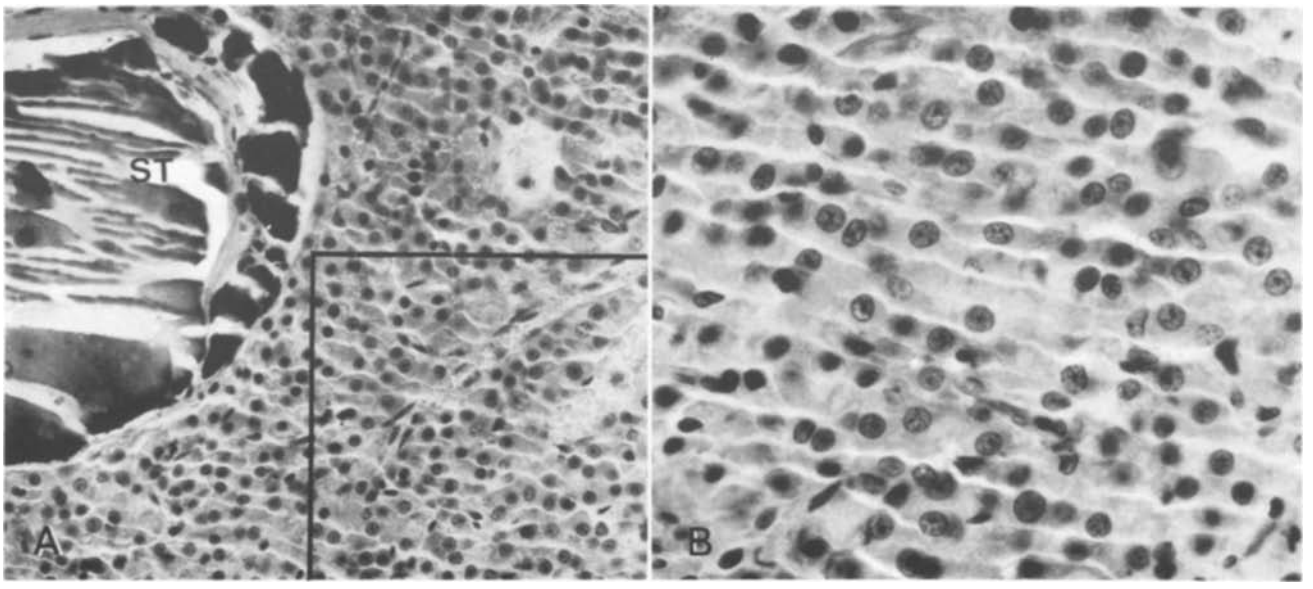

Fig. 5. Histology of rat testis 24 weeks after administration of cadmium chloride. This section shows a Leydig cell tumour in proximity to a necrotic seminiferous tubule A) Magnification: 400 x; B) Magnification: 800 x

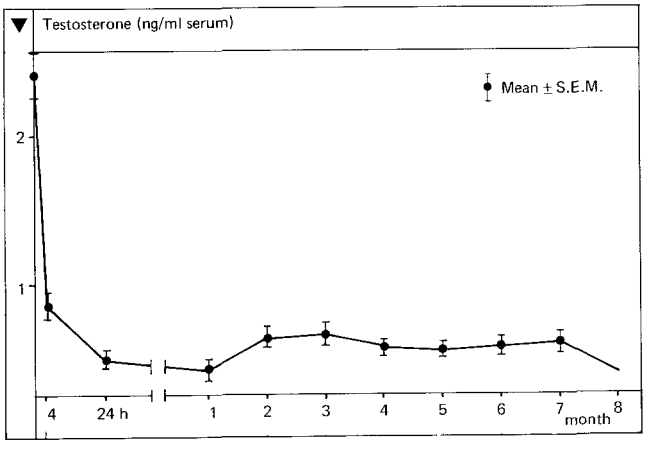

Fig. 6. Serum testosterone concentrations in 12 patients before and after subcapsular orchidectomy.

serum before subcapsular orchidectomy to 860 pg/ml 4 h after operation and 500 pg/ml 24 h after operation. The mean values of testosterone concentrations from 1 to 8 months after orchidectomy varied between 420 and 670 pg/ml serum. The differences were not statistically significant. A tendency for serum testosterone concentrations to rise with time after subcapsular orchidectomy could not be observed (Fig. 6).

DISCUSSION

The studies in rats showed that after testicular necrosis, caused by a single subcutaneous injection of cadmium chloride, regeneration of Leydig cells occured. The first step in the repair of the interstitial tissue was proliferation of fibroblasts and formation of new blood vessels originating from the tunica albuginea. This mesenchymal reaction was already visible two weeks after cadmium administration. After 6 weeks, cells with the typical appearances of Leydig cells were found on the inner surface and within the inner layers of the tunica albuginea. No conclusion can be drawn as to whether the regenerating Leydig cells were derived from mesenchymal cells such as fibroblasts or from surviving Leydig cells. The interstitial cells increased in number and, as proliferation progressed, these cells formed clusters. In the later stages, the formation of Leydig cell tumours was observed. These results are in agreement with other studies (5, 7, 11, 12).

The reappearance of Leydig cells correlated well with the increase in serum testosterone concentrations and a decrease in LH concentrations. Fourteen weeks after cadmium administration, the serum testosterone concentrations were not significantly different from the levels in intact rats. This rise in testosterone concentrations to normal levels is

proof of the endocrine function of the regenerated Leydig cells, which could be predicted on the basis of indirect evidence, such as recovery of growth and function of accessory glands 8-12 weeks after cadmium treatment (2, 7, 10, 11).

Despite the return of testosterone levels to normal the LH concentrations remained slightly above those of controls. This could indicate altered function of these interstitial cells, as has been proposed by other investigators (2, 4, 7). However, these results clearly demonstrate the high capacity of rat Leydig cells for regeneration.

In contrast to the findings in rats, there is no evidence at all for the regeneration of Leydig cells or compensatory testosterone production in patients after subcapsular orchidectomy. Throughout the observation period testosterone concentrations remained in the range which has been reported for castrates (6, 12, 16). None of the individuals showed any tendency of an increase in testosterone levels. Although Leydig cells are present in the tunica albuginea and spermatic cord (1, 8, 13, 15), which remained after subcapsular orchidectomy, these cells seem to have no capacity for regeneration or for increased compensatory testosterone production. Our observations therefore indicate that, subcapsular orchidectomy has no disadvantages in comparison with castration for therapy of prostatic carcinoma.

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Dr. Th. Senge
Urologische Klinik des
Josefs-Hospitals
D-4690 Herne 1
Federal Republic of Germany