EFFECT OF ADRENALECTOMY ON THE TESTIS OF CADMIUM CHLORIDE TREATED RATS

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ABSTRACT

Adrenalectomy caused an increased proliferation of fibroblasts in the testis of $CdCl_2$ treated rats but the rate of formation of Leydig cells was not altered.

Following adrenalectomy there was mass deposition of fibrous tissue in the interstitial space : and an increase in alkaline phosphatase activity in the cellular debris contained in the necrosed tubules of $CdCl_2$ injected rats.

Adrenalectomy was partially conducive to the morphogenetic recovery of the interstitium from the adverse effects of $CdCl_2$. The resumption of endocrine activities, on the other hand, was not influenced by the operative procedure.

Besides causing an increase in alkaline phosphatase activity in the cellular debris, adrenalectomy had no effect on the necrosed seminiferous tubules.

INTRODUCTION

Parizek (1957) reported that Cd salts caused acute destruction of the gametogenic and interstitial elements of the testis in rats. Mild necrotic changes appeared within first 6 hours after the injection of $CdCl_2$ and 48 hours later the seminiferous epithelium was totally destroyed. In the interstitial tissue there were focal haemorrhages, vascular thromboses and a modicum of inflammatory reaction. However, after 10 days the entire organ was replaced by masses of eosinophilic debris containing scattered residue of basophilic chromatin material. It was interesting that during the subsequent days, while the central portion of the testis remained necrotic, there was proliferation of fibroblasts and blood vessels in peripheral regions underneath the tunica albuginea. Islets of Leydig cells were also seen in these areas and this was followed by a gradual return of the endocrine activities of the testis. Initially, the necrotic changes induced by Cd evoked castration phenomena but the atrophied accessory sexual organs retained their intrinsic ability to react to androgenic stimulation. Kar *et al.* (1959) noted that $CdCl_2$ compromised the stimulatory action of testosterone propionate on the accessory genital organs of castrated rats ; a direct action of Cd on these organs was also recorded in such animals.

The present investigation was designed to study the effect of adrenalectomy on the rate of regeneration of interstitial elements of the testis in $CdCl_2$ treated rats. As the glucocorticoids were known to have an inhibitory effect on the proliferation of fibroblasts (Noble, 1955), it was envisaged that the removal of adrenals at an appropriate time might provide a fillip to the regeneration of the interstitium by *mass* proliferation of these elements. The latter process in its turn might be expected to lead to the formation of Leydig cells in commensurately larger numbers, as it was satisfactorily established that the fibroblasts were the precursors of Leydig cells (Charney *et al.*, 1952 ; Sniffen, 1952 ; Fawcett and Burgos, 1956).

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Experimental Procedure

Adult albino rats of the Institute Colony were used in this investigation. Details of grouping of the animals and their body weights are indicated in Table I. All the animals were maintained under uniform laboratory conditions throughout the experimental period.

$CdCl_2$ was administered by the subcutaneous route (0.04 m. mol. $CdCl_2$/kg. body weight at the interscapular region, single injection). Adrenalectomy was performed 8 days after $CdCl_2$ treatment in order to ensure the removal of circulating corticoids a little before the commencement of regenerative processes in the interstitium. It may be recalled that the proliferation of interstitial elements started 10 days after the administration of $CdCl_2$ (Parizek, 1957). A group of normal animals was also adrenalectomized and all the operated animals together with the normal controls, were maintained on 0.9 per cent physiological saline solution.

The animals (including normal and untreated adrenalectomized) were sacrificed on the 30th day after the administration of $CdCl_2$. The testis, seminal vesicles (SV) and the ventral prostate (VP) were carefully dissected out and weighed to the nearest mg. For histological studies the testis were fixed in alcoholic Bouin's fluid and serial paraffin sections were stained with Ehrlich's hematoxylin followed by eosin. Sections from the same series were stained with Mallory's trichrome stain for the demonstration of fibrous connective tissue. Alkaline phosphatase activity was studied in paraffin sections of the testis fixed in a chilled absolute ethanol-acetic acid mixture (Wolman and Behar, 1952), by the technique of Gomori (1941) as laid down by Glick (1949). The sections were incubated in the substrate for 48 hours and were mounted without counterstaining. Total cholesterol content of the testis was estimated colorimetrically by the modification (Karkun and Das, *unpubliahed*) of a method by Zlatkis *et al.* (1953).

Results

Testis. It will be evident from Table I that adrenalectomy caused a significant decrease in absolute weight of the testis ($P < .001$) but the relative weight remained virtually unaltered. It was to be noted that the body weight gain was considerably impeded in the operated animals (as compared to the controls) and this was probably responsible for such apparent loss in absolute weight of the testis. $CdCl_2$ treatment, on the other hand, evoked a significant reduction in absolute and relative weights of the organ ($P < .001$). However, adrenalectomy in $CdCl_2$ injected animals tended to elevate the testis weight (absolute and relative) so that the difference from that of the intact $CdCl_2$ treated group was statistically significant ($P < .05$). The relative testis weight of the $CdCl_2$ injected adrenalectomized animals did not reveal a statistically significant increase as compared to the group (intact) treated with $CdCl_2$. Nevertheless, it was interesting that the testis weight (absolute and relative) of the operated plus $CdCl_2$ injected group was significantly lower than that of the normal or adrenalectomized controls ($P < .001$).

Histologically, the testis of the normal controls showed full spermatogenesis ; the tubules exhibited vigorous activity with successive stages of transformation of the seminiferous epithelium into mature spermatozoa. The latter were present in large numbers and their disposition was typical (Fig. 1). There were numerous Leydig cells in the interstitium and the vascularity of the entire organ was normal.

In Mallory preparations the thin basement membrane of the tubules stained blue (Fig. 1a). The other elements which showed similar tinctorial reaction were the tunica albuginea and the serosa of the interstitial blood vessels.

Adrenalectomy was without any effect on spermatogenesis (Fig. 2). In general, the Leydig cells appeared normal with no obvious symptoms of atrophy. However, in some locations atrophic Leydig cells with pycnotic nuclei were encountered.

TABLE I

Testis weight, testis cholesterol and weight of the accessories of adrenalectomized $CdCl_2$ treated rats.

Treatment	Mean testis weight with S.E.		Mean testis cholesterol with S.E. (Mg./gm. testis)	Mean seminal vesicle weight with S.E.		Mean ventral prostate weight with S.E.		Mean body weight (G.M.) with S.E.	
	Absolute (Mg.)	Relative (Mg./100 gm. body weight)		Absolute (Mg.)	Relative (Mg./100 gm. body weight)	Absolute (Mg.)	Relative (Mg./100 gm. body weight)	Initial	Final
Normal Controls	1022.9±30.67 (6)*	736.4±25.59 (6)	8.38±0.24 (6)	64.52±9.52 (6)	45.52±5.52 (6)	98.42±8.17 (6)	70.85±6.54 (6)	115.3±3.13 (6)	139.3±4.43 (6)
Adrenal-ectomized	830.9±50.56 (7)	748.5±24.61 (7)	8.45±0.14 (7)	29.34±4.04 (7)	27.16±2.81 (7)	54.53±9.95 (7)	48.63±8.63 (7)	106.4±3.38 (8)	109.5±4.94 (8)
$CdCl_2$	146.4±13.77 (7)	129.04±15.86 (7)	4.12±0.19 (7)	7.81±0.65 (8)	6.60±0.45 (8)	14.00±0.67 (8)	12.28±0.97 (8)	95.4±2.41 (8)	107.4±2.35 (8)
Adrenal-ectomized + $CdCl_2$	210.7±26.72 (6)	148.9±17.05 (6)	4.90±0.37 (6)	12.10±1.95 (6)	11.60±3.42 (6)	16.07±1.79 (6)	11.40±1.05 (6)	89.8±6.35 (6)	140.7±7.55 (6)

*Figure in parenthesis indicates the number of animals.

Nevertheless, considering the preponderance of normal cells such atrophic ones formed only a fraction of the total Leydig cell population.

In sections stained with Mallory's the histological features of the testis of adrenalectomized animals were similar to those of the normal controls. The thin basement membrane of the tubules, the serosa of the interstitial blood vessels and the tunica albuginea were the only elements which stained blue (Fig. 2a).

$CdCl_2$ treatment caused complete destruction of the seminiferous tubules. The epithelial elements were totally necrosed and the tubular lumen was filled with an eosinophilic debris containing scattered residue of basophilic chromatin material (Fig. 3). Proliferation of fibroblast was clearly seen under the tunica albuginea and the deposition of peritubular fibrous tissue was also not uncommon. In some necrosed tubules the fibroblasts even invaded the eosinophilic debris. There was proliferation of blood vessels under the tunica albuginea and clumps of Leydig cells were also seen in this area. In contrast to the more central portions of the testis, the peripheral areas contained more fibroblasts and greater amounts of peritubular fibrous tissue (Fig. 3). Such histological difference between the two areas was more prominent in Mallory preparations (Fig. 3a). It was also noteworthy that the tunica albuginea was considerably thickened and stained a deep blue with Mallory's.

In $CdCl_2$ treated adrenalectomized animals the necrosis of the tubules persisted throughout the testis. However, in most of the animals there were more fibroblasts and greater amounts of fibrous tissue than in the unoperated group injected with $CdCl_2$. This could be gauged from the fact that the fibroblasts invaded even the central portions of the testis and the amount of fibrous tissue was almost equal throughout the organ (Fig. 4). It may be recalled that in the intact $CdCl_2$ treated group the fibroblastic proliferation and deposition of fibrous tissue were virtually confined to the peripheral areas underneath the tunica albuginea. It was also interesting that in the latter group the disposition of fibrous tissue was predominentally peritubular whereas, in the adrenalectomized animals (given $CdCl_2$) this tissue was seen in varying amounts thoughout the interstitial space with characteristic peritubular concentration, particularly underneath the tunica albuginea (Fig. 4). Leydig cells were present mostly in the peripheral areas but their number did not increase after adrenalectomy. Vascular proliferation was also seen in these areas. Mallory preparations demonstrated this equal distribution of fibroblasts and fibrous tissue in the two regions (central and peripheral) of the testis even more clearly (Fig. 4a). Further, the characteristic blue staining reaction confirmed the presence of fibrous tissue *throughout* the interstitial space.

In agreement with the findings of Dempsey *et al.* (1949) and Kar *et al.* (1950) it was seen that in the control animals the basement membrane of the tubules and the spermatogonia showed intense phosphatase activity (Fig. 5). The other elements of the seminiferous epithelium contained only moderate amounts of the enzyme. The Leydig cells and the endothelium of the interstitial vessels were strongly reactive.

Adrenalectomy caused an overall reduction in alkaline phosphatase activity in the testis. The seminiferous epithelium was virtually devoid of enzyme activity except in the nucleus of the spermatogonia (Fig. 6). The Leydig cells and the endothelium of the interstitial vessels contained very little alkaline phosphatase activity.

Testicular phosphatase activity was markedly inhibited after $CdCl_2$ treatment. However, some of the necrosed tubules underneath the tunica albuginea retained considerable amounts of the enzyme ; the cellular debris in these tubules was the specific reactive material (Fig. 7). The centrally located tubules were negative for alkaline phosphatase activity except a few which showed only a faint reaction in the cellular debris. Other elements of the testis were invariably devoid of enzyme activity.

The pattern of distribution of alkaline phosphatase in the testis of $CdCl_2$ treated adrenalectomized animals was the same as in the intact group injected with $CdCl_2$. Thus the enzyme activity was confined to the debris of the necrosed tubules albeit in more intense manner than in the previous group (Fig. 8). Further, phosphatase activity was seen in greater number of tubules both in the central and peripheral areas. Other elements of the testis, however, continued to give negative reactions for enzyme activity.

Adrenalectomy did not evoke any change in total cholesterol content of the testis (Table I). However, $CdCl_2$ treatment (intact or adrenalectomized animals) caused a significant reduction in testicular cholesterol concentration as compared to either normal or adrenalectomized controls ($P < .001$). It was to be noted that there was no significant difference between the two $CdCl_2$ treated groups as regards any aberration in cholesterol content of the testis.

Seminal vesicles. Adrenalectomy caused a significant reduction in absolute and relative weights of the SV ($P < .02$). $CdCl_2$ treatment (adrenalectomized or intact animals) was associated with a more drastic reduction in weight (absolute and relative) of this organ as compared to either normal or adrenalectomized controls ($P < .001$). The SV weight (absolute and relative) of the two $CdCl_2$ treated groups did not differ significantly (Table I)

Ventral prostate. The absolute weight of the VP was significantly lowered after adrenalectomy ($P < .01$) ; but the relative weight of the organ did not reveal a statistically significant difference inspite of the fact that it was appreciably less than that of the normal controls. $CdCl_2$ treatment (normal and adrenalectomized animals) was responsible for a significant decrease in VP weight (absolute and relative) as compared to either normal or adrenalectomized controls ($P < .001$). The two $CdCl_2$ treated groups did not differ significantly with respect to their VP weights (Table I).

Discussion

The data presented in this report indicated that adrenalectomy did influence certain morphogenetic events in the interstitium during its recovery from the adverse effects of $CdCl_2$. On the other hand, the seminiferous tubules were irreversibly destroyed and adrenalectomy failed to exert any recuperative effect on the defunct epithelial remnants.

Parizek (1957) noted that during progressive necrosis of the testis after administration of $CdCl_2$, a stage was reached when the gametogenic and the interstitial portions of the testis appeared to be equally affected. But subsequently while the spermatogenic elements continued to persist merely as a morass of dead tissue, focal regenerative changes commenced in the interstitium. There was no doubt that these changes heralded both morphogenetic and functional recovery of the interstitium ; as clumps of active Leydig cells, presumably formed from the proliferated fibroblasts were consistently encountered. It was precisely this process of fibroblastic proliferation which lent itself to experimental modification for a prompt and more complete resumption of endocrine activities of the interstitium. However, the reason why such modification was attempted specifically through the adrenocortical pathway was already indicated (*vide supra*).

Notwithstanding such provocative syllogism, the removal of adrenals did not prove conducive to a more complete functional recovery of the interstitium. There was no doubt that the fibroblasts were proliferated in larger numbers than in the unoperated $CdCl_2$ treated animals but the Leydig cell population failed to show the expected increase, at least during the experimental period employed in this study. Further, the weight of the accessories in the two $CdCl_2$ treated groups (intact and adrenalectomized) was virtually similar which indicated that adrenalectomy did not improve the endocrine status of the regenerating interstitium

through an enhanced rate of formation of Leydig cells from their precursors. The similar cholesterol concentration of the testis in the two $CdCl_2$ treated groups also pointed towards such a conclusion.

Changes in alkaline phosphatase activity after $CdCl_2$ treatment merited a comment. It was noteworthy that the enzyme activity was markedly reduced in the testis after adrenalectomy. However, the administration of $CdCl_2$ was also associated with a similar overall decrease in phosphatase activity although the dead cellular debris in some necrosed tubules gave positive reactions for the enzyme. Curiously, adrenalectomy accelerated phosphatase activity in the cellular debris of such tubules. Whether the positive histochemical reaction in the cellular debris of defunct tubules was indicative of a true mobilization of the enzyme could not be determined from the present data.

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Explanation of plate I

(All figures are photomicrographs. Figures 1, 1a, 2, 2a, 5 and 6 are magnified × 130)

Fig. 1. Testis of a normal rat. H and E.

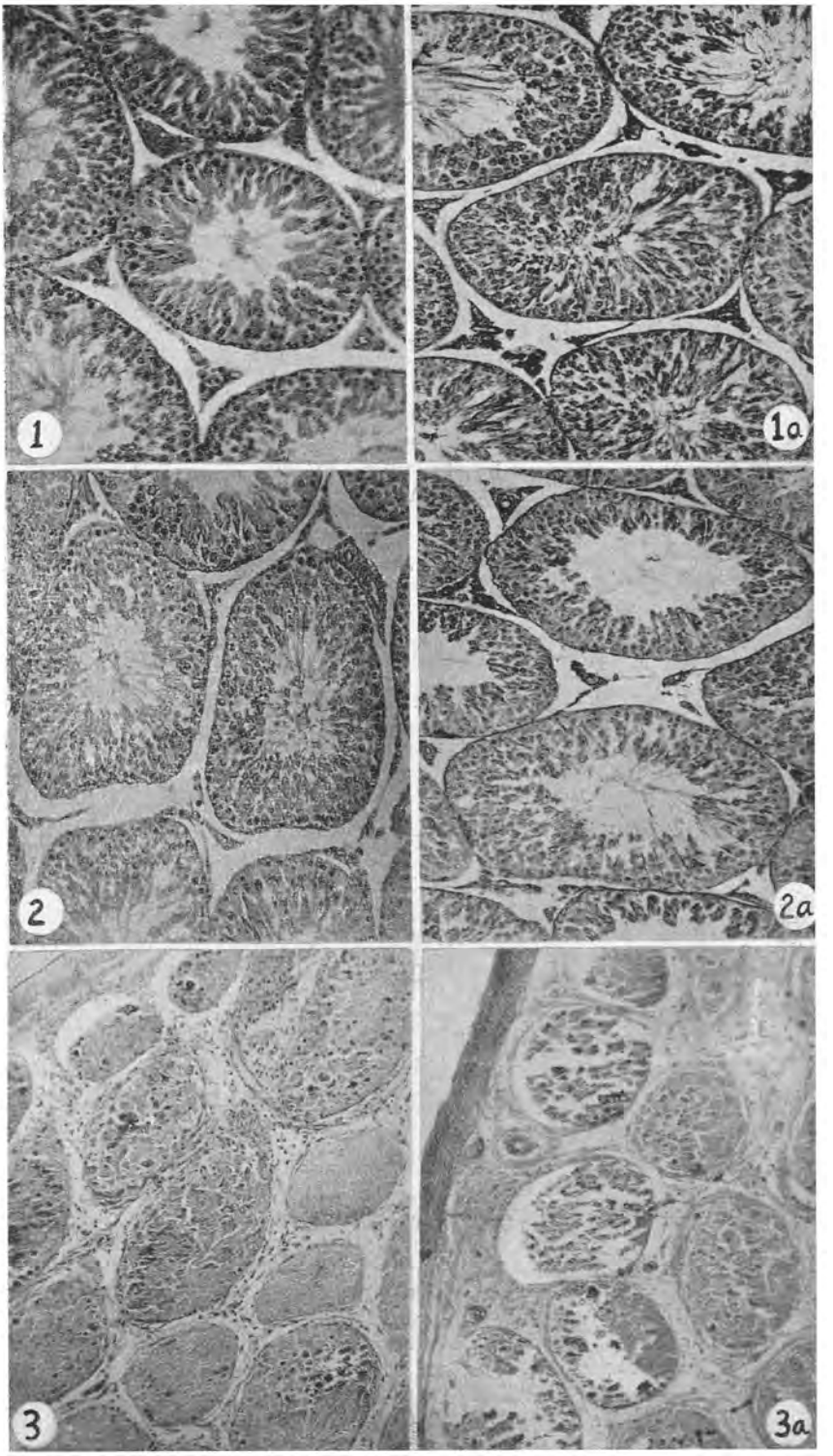
Fig. 1a. Testis of a normal rat. Mallory's trichrome stain. The thin basement membrane of the tubules stains blue.

Fig. 2. Testis of an adrenalectomized rat. H and E. Full spermatogenesis as in the controls. Compare with fig. 1.

Fig. 2a. Testis of an adrenalectomized rat. Mallory's trichrome stain. Features similar as in fig. 1a.

Fig. 3. Testis of a $CdCl_2$ treated rat. H and E. The tubules are totally necrosed and contain a dead cellular debris. Note proliferation of fibroblasts (small black dots) under the tunica albuginea and peritubular deposition of fibrous tissue. The fibroblasts and fibrous tissue are more at the periphery (upper left of the fig.) than in the central portions of the testis (lower left and lower right of the fig.).

Fig. 3a. Testis of a $CdCl_2$ treated rat. Mallory's trichrome stain. Note the distribution of fibroblasts and fibrous tissue. The tunica albuginea is greatly thickened.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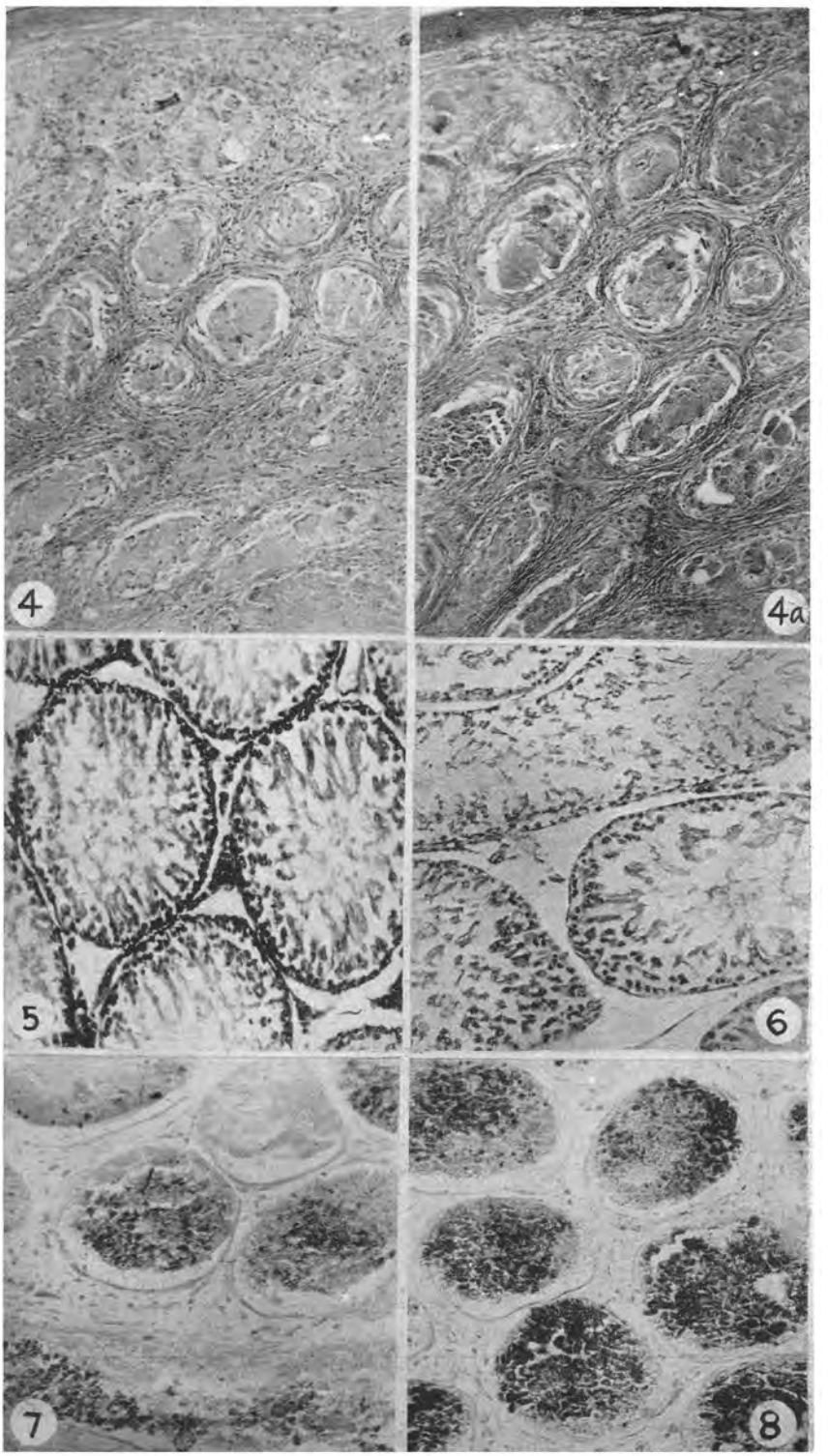
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Explanation of Plate II

(Figures 3, 3a, 4, 4a, 7 and 8 are magnified × 80).

Fig. 4. Testis of a $CdCl_2$ treated adrenalectomized rat. H and E. Note mass proliferation of fibroblasts (black dots) and deposition of fibrous tissue throughout the interstitial space with characteristic peritubular concentration. Compare with fig. 3.

Fig. 4a. Testis of a $CdCl_2$ treated adrenalectomized rat. Mallory's trichrome stain. Note the deposition of fibrous tissue. Compare with fig. 3a.

Fig. 5. Testis of a normal rat. Gomori technique. Note phosphatase activity in the basement membrane and spermatogonia. The Leydig cells are also highly reactive.

Fig. 6. Testis of an adrenalectomized rat. Note overall reduction of enzyme activity.

Fig. 7. Testis of a $CdCl_2$ treated rat. Gomori technique. The cellular debris in some tubules shows phosphatse activity.

Fig. 8. Testis of a $CdCl_2$ treated adrenalectomized rat. Gomori technique. Note intense phosphatase activity in the cellular debris of the tubules. Compare with fig. 7.