gar nicht reduziert werden[9]. Für Cortisol fanden MUROTA und TAMAOKI keine Veränderung des Substituenten am C-Atom. Der Hauptmetabolit des Cortisols im Knorpelgewebe wurde als 3α, 11β, 17α, 21-Tetrahydroxy-5β-pregnan-20-on identifiziert[7]. Er hat keine Wirkung auf den Wassergehalt der Knorpel in vitro.

Tabelle II. Wirkung verschiedener Δ^4-3-Keto-11β-hydroxy-C_{21}-Steroide auf den Hydratationsgrad in Abhängigkeit von der Konzentration

		0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	1.0 µg/ml
11β-Hydroxyprogesteron		96 ± 3.1	77 ± 2.3	79 ± 3.2	79 ± 2.4
	P	–	< 0.001	< 0.01	< 0.001
11β,17α-Dihydroxy-progesteron		88 ± 2.4	66 ± 4.6	68 ± 2.2	67 ± 1.7
	P	< 0.05	< 0.01	< 0.001	< 0.001
Corticosteron			86 ± 3.2	77 ± 2.1	71 ± 1.5
	P		< 0.02	< 0.001	< 0.001
Cortisol			81 ± 2.9	76 ± 2.9	71 ± 2.0
	P		< 0.05	< 0.001	< 0.001
21-Dehydrocortisol		99 ± 2.0	79 ± 1.9	70 ± 2.5	65 ± 4.1
	P		< 0.02	< 0.001	< 0.001

Ausmass der Projektionsbilder in Prozentzahlen der entsprechenden Kontrollen. Mittelwerte aus mindestens 4 Versuchen ± Standardabweichung des Mittelwerts. *P*, Irrtumswahrscheinlichkeit beim Vergleich Versuch–Kontrolle.

Aufgrund der vorliegenden Versuche ist die Annahme naheliegend, dass die Knorpelzelle an Steroiden, welche in die Wasserregulation eingreifen, lediglich das im Ring A lokalisierte Strukturmerkmal verändert. So wird beispielsweise das hydratisierungshemmende Cortisol in seinen diesbezüglich inaktiven Metaboliten umgewandelt. Ob die mit dem Eintritt des Wasserstoffs in 5β-Stellung verbundene konfigurative Änderung der gegenseitigen Lage der A- und B-Ringe auch bei den 21-Desoxy- und 21-Dehydroverbindungen zu inaktiven Metaboliten führt, müssten weitere Untersuchungen ergeben.

Summary. Embryonic chick cartilages were cultivated in a synthetic nutrient medium. In this experimental procedure cortisol is known to reduce the water uptake of the cartilages. It was found that some C_{21}-steroids exert a similar activity. Those of the steroids investigated, which inhibit water uptake, have in common a Δ^4-3-keto group, an 11β-hydroxy group, and a keto group in position 20; oxygen functions in positions 21 and 17α seem to be of minor importance for this activity. The possibility of these steroids being inactivated by cellular enzymes is discussed.

B. SCHÄR

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Morphological Artefacts in Biopsy Specimens of the Testis

The present study was undertaken in order to describe the histological and ultrastructural changes produced by biopsy technique in the testis tubules of the rat.

Material and methods. Ten adult albino rats weighing 200 g were used for this study. Following ether anaesthesia, a longitudinal incision of the tunica was made, the small portion of hernial parenchyma was removed and fixed in Bouin's solution. The testis on the other side was entirely removed and fixed. 3 µ thick sections were stained with haematoxylin-eosin, Hopa and Hotchkiss-McManus. Some biopsy fragments were fixed in cold 2% osmium tetroxide buffered according to MILLONIG[1] and, after dehydration, were embebbed in Araldite (Durcopan ACH). Thin sections were mounted on Formvar coated grids and examined in a Siemens Elmiskop I electron microscope, after staining with lead hydroxide according to KARNOVSKY[2].

Results. Light microscopy: The tubules of the biopsy specimens show desquamated spermatocytes and spermatids in the lumen (Figure 2). Fissures are visible in the epithelium of the seminiferous tubules and they are perpendicular (Figure 2) or parallel (Figure 3) to the basement membrane. The interstitium may be edematous and sometimes filled by seminal cells (Figure 3).

Electron microscopy: In the electron microscope desquamated cells in the seminiferous lumen of the tubules observed in the light microscope appear to be spermatocytes and spermatids (Figure 4). Large clumps of the apical portions of Sertoli cells, fragments of plasma membrane, mitochondria with partly disorganized cristae and several swollen vesicles of the smooth endoplasmic reticulum are present in the tubular lumen (Figure 4). The basal portion of the Sertoli cells, the spermatogonia of type A and B nearly always maintain their normal position near the basement membrane (Figures 5 and 6). The cellular components of the spermatogoons are well preserved; however the plasma membrane often appears to be interrupted.

The appearance of the cellular (smooth muscle cells, fibroblasts) and acellular components of the boundary tissue (tunica propria) is normal.

Discussion. These findings show that the biopsy technique of testis produces morphological artefacts in the seminiferous tubules. The most evident artefact is the presence of desquamated cells in the tubular lumen.

Electron microscopic studies[3] have shown that intercellular connections are present in the basal portion of

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the Sertoli cell. These connections have not been demonstrated between cells of the germinal epithelium nor between Sertoli cells and germinal cells. The spermatocytes and spermatids are contained in plasma membrane invaginations of the Sertoli cells[3-6]. The invaginations surround the seminal cells and keep them in place by a wedging system. Therefore it may be presumed that the mechanical injury due to the biopsy technique disorganizes the seminal cells which do not have intercellular connection systems, and produces a cellular desquamation. Besides the bioptic trauma could be considered a stimulus for the contraction of the smooth cells of the boundary tissue (tunica propria). This contraction may enhance the cellular desquamation.

The presence of germinal cells in the interstitium is probably due to the lesion in the tubular wall.

Our findings in the bioptic specimens of the rat suggest that analogous artefacts may be present in the histological observations in human testis obtained by the same technique. These findings seem also to indicate that some histological aspects present in the human testis (desquamation of germinal cells) and considered to be indicative of pathological conditions such as oligospermia and infertility[7-9], may be artefacts due to the biopsy technique[10,11].

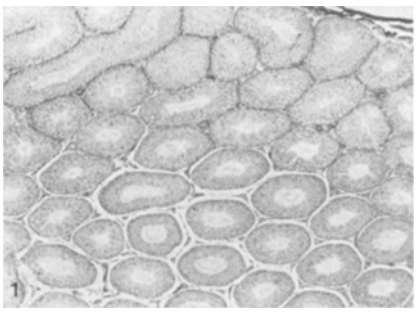

Fig. 1. Normal rat testis fixed in toto. The epithelium of the seminiferous tubules appears compact and there are no desquamated cells in the tubular lumen. Hopa staining. × 140.

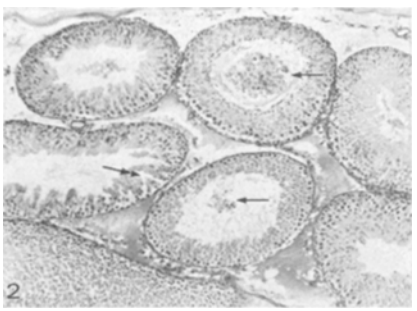

Fig. 2. Rat testis obtained at biopsy. Clumps of germinal cells are visible in the tubular lumen (single arrow). In the other seminiferous tubules, lesions affecting the entire depth of the epithelium can be seen and are found perpendicular to the tunica propria (double arrow). Hopa staining. × 350.

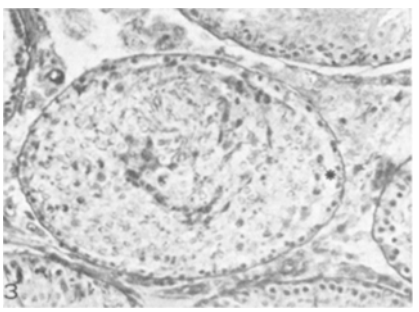

Fig. 3. Rat testis obtained at biopsy. The structure of the seminiferous tubule is significantly modified. The tubular lumen is filled with desquamated cells of the germinal epithelium. In the lower portion of the seminiferous tubule, a lesion is clearly visible running almost parallel to the tunica propria (asterisk). Hopa staining. × 560.

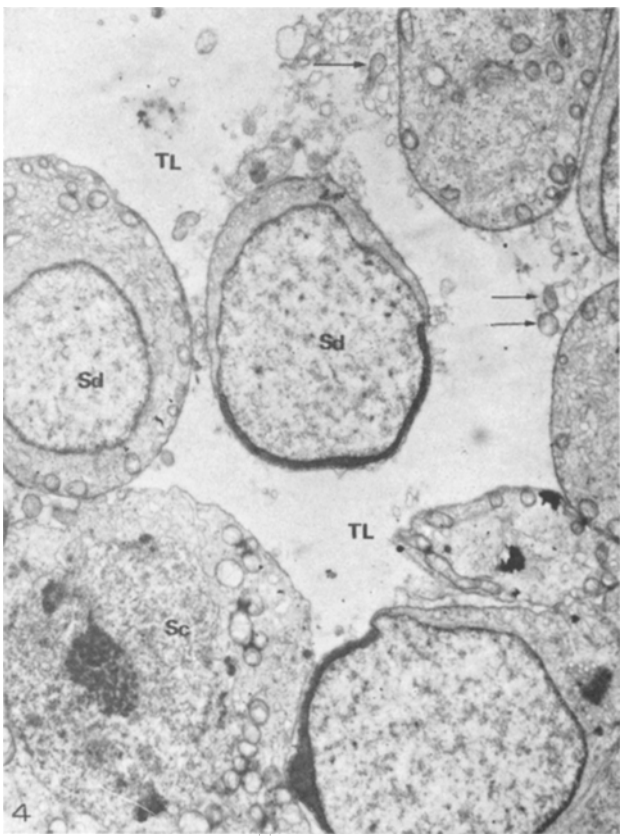

Fig. 4. Apical portion of the rat seminiferous tubule obtained at biopsy. Free spermatids (Sd), in addition to numerous free cellular organelles such as mitochondria (arrow) and vesicles of the smooth endoplasmic reticulum are clearly visible in the tubular lumen. TL, tubular lumen; Sc, spermatocyte. × 10,000.

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[10] We wish to thank Prof. A. Fabbrini and Prof. C. De Martino for their helpful criticism and advice.
[11] This paper is supported by grant No. 1247 of Consiglio Nazionale delle Ricerche, Roma, Italia.

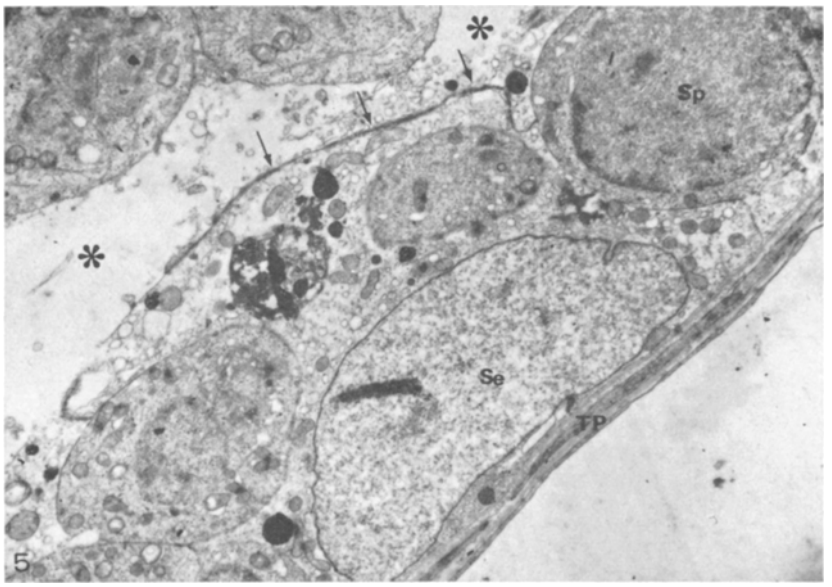

Fig. 5. Lower portion of the rat seminiferous tubule obtained at biopsy. A deep lesion can be seen in the seminiferous epithelium separating the basement regions from the more proximal regions (asterisk). The lower portion of the cytoplasm of the Sertoli cell (Se) remains attached to the tunica propria (TP) of the seminiferous tubule with no morphological modification, whereas the apical portions are detached presumably desquamated in the tubular lumen. Clearly visible is a joint between the plasma membrane of 2 Sertoli cells (arrow). Sp, spermatogonium. × 10,000.

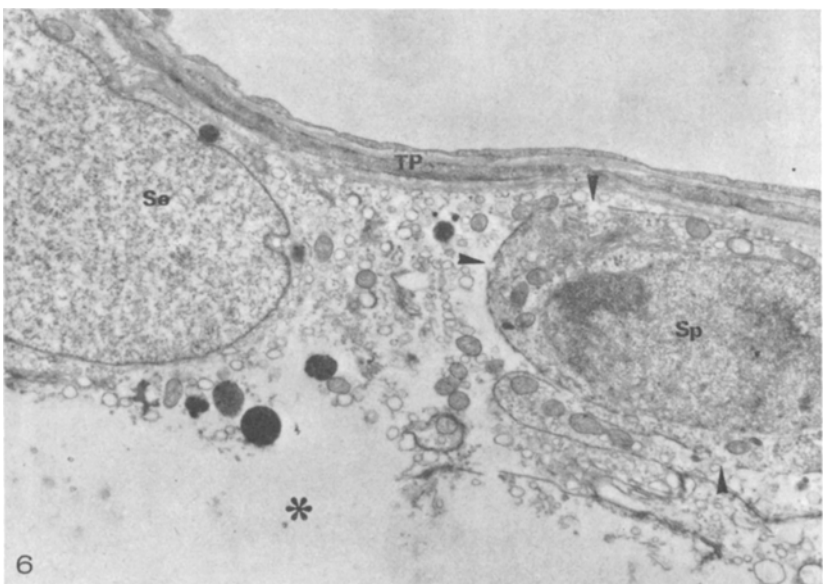

Fig. 6. Lower portion of the rat seminiferous tubule obtained at biopsy. Part of the cytoplasm of the Sertoli cell (Se) has remained attached to the basement membrane of the tunica propria (TP) of the seminiferous tubule. Biopsy trauma has caused a fracture (asterisk) between the basal cytoplasm and the apical cytoplasm of the Sertoli cell. Also visible is a spermatogonium (Sp) still in its normal position, but presenting numerous lesions in the peripheral plasma membrane (arrow). × 14,000.

Riassunto. Gli Autori hanno effettuato uno studio sperimentale sulle alterazioni strutturali provocate dalla manovra bioptica nel tubulo seminifero di ratto. Il principale artefatto è la desquamazione di cellule germinali nel lume tubulare. Analoghi reperti presenti nel testicolo umano e considerati indicativi di condizioni patologiche possono pertanto essere artefatti secondari alla manovra bioptica.

M. RE, M. BELLOCCI and G. SPERA

Istituto di Medicina Costituzionale ed Endocrinologia dell'Università, Roma (Italy), 30 October 1968.