Histological testicular parameters
in bilateral cryptorchid adult rams

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Summary. Three male lambs were rendered bilaterally cryptorchid at 15 weeks of age.
They were killed when adult during the sexual season in autumn and compared with 4 nor-
mal adult rams of the same age, slaughtered at the same time.
     In the cryptorchid testes, there was a drastic decrease in the total number of Leydig
cells per testis, in the length and diameter of seminiferous tubules, and in the numbers
of stem spermatogonia. Sertoli cell numbers were normal.

The cryptorchid testis has been investigated mainly for the morphological appea-
rance of the Leydig and Sertoli cells, the absence of differentiated germ cells, and the
biochemical parameters of both intertubular and tubular tissues (see review of
Van Demark and Free, 1970). No numerical analysis of Leydig and Sertoli cells has
been reported. The aim of our work was to present these numerical data in the cryptor-
chid testis of the ram.

Material and methods.

Three lambs were rendered bilaterally cryptorchid when they were 15 weeks
old, just after puberty. They were killed one year later during the sexual season
(autumn) and compared with 4 normal adult rams killed during the same season.
After slaughter the testes were weighed and fragments of each testis were fixed in
Bouin-Hollande solution and treated as previously described (Hochereau de Reviers
and Courot, 1978). The relative volumes of intertubular tissue (figs. 2, 3) and semini-
ferous tubules were determined with a 25 point ocular integrator (Hennig, 1957) on
20 fields for each testis. The relative proportion of Leydig cells in the intertubular
tissue was determined by the same method on 20 fields of intertubular tissue for each

testis. The total volumes of intertubular tissue, Leydig cells and tubular tissue were then calculated from the testis volume and the relative volume of each element respectively. The diameter of the seminiferous tubules was measured with an ocular micrometer on 20 cross sections of tubules per testis. The cross sectional areas of the cytoplasm and nuclei of Leydig cells (figs 4, 5) and that of the nuclei of Sertoli cells were estimated from camera lucida drawings of 20 cells per animal. From the estimation of Leydig cell volume, the total number of Leydig cells per testis was calculated. The Sertoli cells and type A_0 and A_1 spermatogonia of each animal (Hochereau-de Reviers, Ortavant and Courot, 1976) were counted in 10 μm-thick cross sections at stage 8 of Ortavant's classification (1958). The true numbers of these cells per cross section were calculated by the formula of Abercrombie (1946) as modified by Ortavant (1958). The total numbers of Sertoli cells and A_0 and A_1 spermatogonia per testis were determined as described by Attal and Courot (1963).

Results.

Cryptorchidism resulted in drastic decreases in testis weight ($\times$ 0.12, fig. 1a), total volume of intertubular tissue per testis ($\times$ 0.18, fig. 1b), and total volume of Leydig cells per testis ($\times$ 0.16, fig. 1c). However, the cytoplasmic and nuclear cross sectional
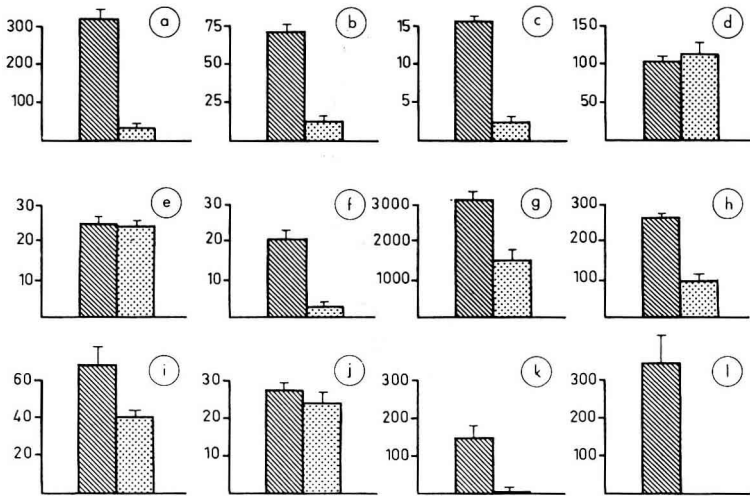
FIG. 1. — *Comparisons of histological parameters in normal* ▨▨ *and cryptorchid* ▨▨ *testes of adult rams (m* $\pm$ *s. e. m.). a) Testis weight (g)   b) Total volume of intertubular tissue per testis (cm³) ;*

c) Total volume of Leydig cells per testis (cm³) ; d) Leydig cells : individual cytoplasmic cross sectional area (μm²) ; e) Leydig cells : individual nuclear cross sectional area (μm²) ; f) Total number of Leydig cells per testis ($\times$ 10⁸) ; g) Total length of seminiferous tubules per testis (m) ; h) Mean tubular diameter of seminiferous tubules (μm) ; i) Sertoli cells : individual nuclear area (μm²) ; j) Total corrected number of Sertoli cells per testis ($\times$ 10⁸) ; k) Total corrected number of A_0 reserve spermatogonia per testis ($\times$ 10⁶) ; l) Total corrected number of A_1 renewing spermatogonia per testis ($\times$ 10⁶).

areas of the Leydig cells were not modified by cryptorchidism (figs 1d, e, 4 and 5), and from these last data we deduced that the total number of Leydig cells per testis was greatly diminished by cryptorchidism ($\times$ 0.14, fig. 1f).

The total length of seminiferous tubules per testis and their mean diameter were reduced after cryptorchidism (respectively $\times$ 0.5, fig. 1g and $\times$ 0.4, fig. 1h), The nuclear cross sectional area of Sertoli cells was decreased after cryptorchidism ($\times$ 0.6, fig. 1i), while their total number per testis was not significantly affected (fig. 1j).

A_0 reserve stem cells were scarcely observed in the tubules of adult cryptorchid rams (fig. 1k), while A_1 spermatogonia were never observed (fig. 1l).

Discussion and conclusion.

The decrease in testis weight of the ram after cryptorchidism resulted from depletion of both intertubular and tubular tissues.

The total volume of Leydig cells per testis was drastically reduced by cryptorchidism. A similar depletion was observed in unilateral cryptorchid pigs (Van Straaten and Wensing, 1977). Despite this, neither the area of Leydig cell cytoplasm nor that of the nuclei was affected, although changes were observed in the unilateral cryptorchid (Van Straaten and Wensing, 1977 ; Hochereau-de Reviers et al., unpublished data). Cellular size of Leydig cells in normal and cryptorchid ram testes showed skewed distributions. Contrary to the bimodal distribution of these cells in the boar (Van Straaten and Wensing, 1977), the populations of Leydig cells in the ram were homogeneous. However, we formerly numbered and analysed only the morphologically active looking Leydig cells and possibly underestimated the values, as the precursors or the degenerated Leydig cells could not be identified. But to obtain an identical total volume of Leydig cells in normal and cryptorchid testes, it would be necessary for the totality of the cryptorchid intertubular tissue to be occupied by Leydig cells : in fact, there were also blood vessels, fibroblasts and peritubular cells. As a consequence of the decreased total volume and constant individual cellular volumes of Leydig cells, the total number of Leydig cells per testis was greatly reduced by cryptorchidism. This decrease in Leydig cell numbers could be due to blockage of Leydig cell mitoses by elevated temperature, to a non-differentiation of Leydig cells from their precursors, or to degeneration of Leydig cells formed before the animals were rendered cryptorchid. Despite these decreased numbers, the plasma levels of testosterone in the peripheral blood (Cahoreau et al., 1979) were equivalent to that of normal rams at the same season (Garnier et al., 1978). This may be related to increased steroid synthesis by these Leydig cells, since androgen concentrations in the interstitial fluid of the cryptorchid testis were higher than those in normal testes (Hagenäs et al., 1978a) due to the enhancement of steroid biosynthesis by elevated temperature (Gospodarowicz and Legault-Démare, 1962).

The depletion of the total volume of seminiferous tubules per testis was due to a decrease in both the length and the diameter of the seminiferous tubules. This resulted in a diminution in the surface of the basal lamina of the seminiferous tubules, possibly correlated with qualitative changes in it. Hadziselimovic and Seguchi (1973) observed an increase in collagen in the peritubular cells and a thickening of the basal membrane

in cryptorchid testes of the human infant. The total number of Sertoli cells per testis was not affected by cryptorchidism in the present experiment which was performed at an age (15 weeks) when supporting cell division has definitively stopped (Courot, 1971). In another group of animals where unilateral cryptorchidism has been induced earlier in life, when the lambs were 6 weeks old, the total number of Sertoli cells per testis was diminished (Blanc et al., 1977). The cross sectional nuclear area of Sertoli cells was decreased by cryptorchidism. In such animals the mean plasma levels of FSH were significantly elevated, compared with that of normal animals (Blanc et al., 1978), as were the interstitial fluid FSH levels (Hagenäs et al., 1978b) ; despite this, FSH receptors in the cryptorchid Sertoli cells were greatly reduced as compared to those of the normal rat testis (Hagenäs et al., 1978a). The elevation of plasma FSH levels would result from an absence of secretion of inhibin in the presence of normal testosterone plasma levels (Blanc et al., 1978). Secretion of œstrogens was reported to be normal or decreased in cryptorchid animals (Liptrap and Raeside, 1970 ; Abney et al., 1977).

In the adult cryptorchid ram few A_0 and A_1 spermatogonia were present in the abdominal testis, while gonocytes have been shown to divide in the abdominal fœtal testis at the same temperature (Courot, 1971). This could be due to the high sensitivity of differentiated germ cells to elevated temperatures in the ram (Waites and Ortavant, 1968).

In conclusion, the total number of Leydig cells per testis is drastically reduced while their androgen secretion is enhanced. The total number of Sertoli cells per testis is not modified. Although cryptorchidism occurred after cessation of supporting cell mitoses, secretion of inhibin is drastically depressed (Blanc, et al., 1978). In adult rams, most of the stem cells had degenerated after cryptochidism, despite the fact it occurred late during the life.

Résumé. Trois agneaux Préalpes âgés de 15 semaines ont été rendus bilatéralement cryptorchides. Ils ont été abattus à l'âge adulte durant la saison sexuelle (automne) et comparés à 4 béliers adultes normaux castrés au même âge et à la même saison.
Dans les testicules des béliers cryptorchides, on a observé une diminution très importante du nombre total de cellules de Leydig par testicule, du diamètre et de la longueur des tubes séminifères et des spermatogonies souches alors que le nombre de cellules de Sertoli est maintenu normal.

FIGS. 2, 3. — Microscopic appearance ($\times$ 200) of cryptorchid (2) and normal (3) testes : ☐ intertubular tissue ; ▨ seminiferous tubules.

FIGS. 4, 5. — Microscopic appearance ($\times$ 1 000) of intertubular tissue in cryptorchid (4) and normal (5) testes : The drawing shows the nuclear and cytoplasmic limits of a cross-sectioned Leydig cell ▨
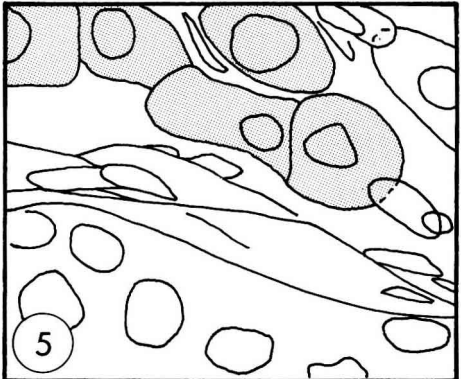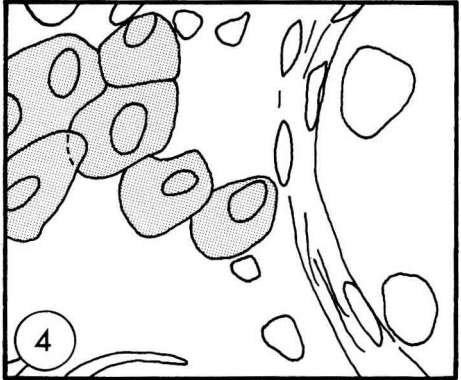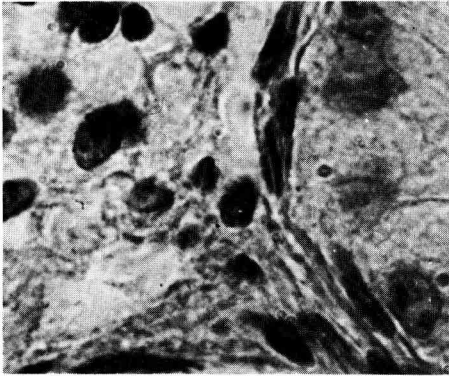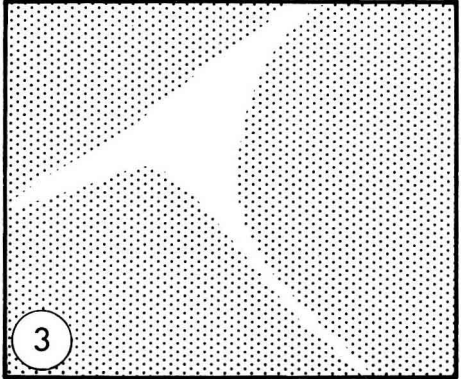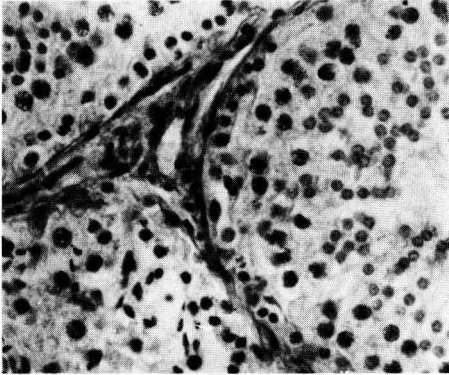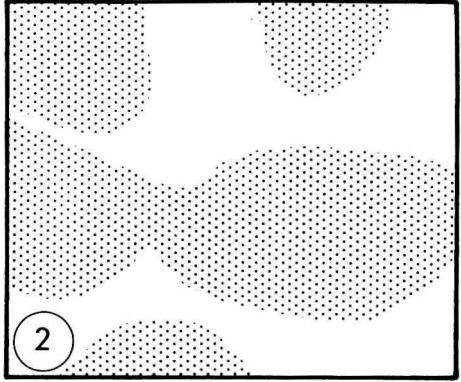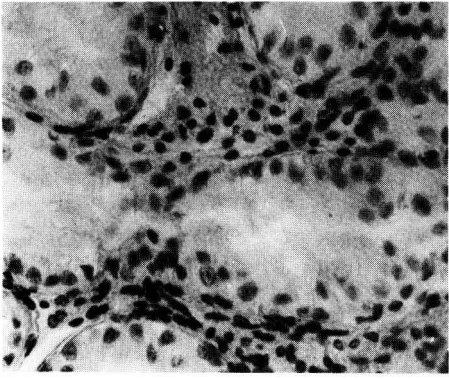
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