# The Interstitial Cell in the Testis of the Foetal Sheep

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**With one plate (fig. 1)**

## Summary

The testes of 18 foetal sheep, of crown–rump length from 2·8 to 40 cm, have been studied by the PAS, Sudan black, and plasmal tests. Typical interstitial cells differentiate from mesenchymal precursors; the nucleus alters first, becoming ovoid and vesicular; then the cytoplasm increases in amount, its processes become fewer and smaller, sudanophil lipid droplets appear, and the result is a lipid-laden epithelioid cell. Two atypical forms of interstitial cell have been noted: the first has groups of eosinophil granules in its cytoplasm, the second is shrunken and has a pycnotic nucleus. The interstitial cell of foetal sheep, unlike that of poikilotherms, contains no glycogen. The PAS-positive polysaccharide / protein granules found in the interstitial cells of adult homiotherms are absent from the interstitial cells of this foetal homiotherm. While sudanophil lipids appear at an early stage, Schiff-positive lipids (plasmalogens, acetal phosphatides, and possibly steroids) are entirely absent from the interstitial cells of the foetal sheep.

## Introduction

UNTIL comparatively recently there has been considerable controversy regarding the precise foetal origin of the testicular interstitium; embryonic sources suggested by various writers for the interstitial cell include leucocytes, lymphocytes, plasma cells, capillary endothelial cells, the sex cords, Sertoli cells, and the coelomic epithelium (Gillman, 1948). Now, however, it is widely accepted, in accordance with Gillman (1948), that the interstitial cell develops from small spindle-shaped elements lying between the seminiferous tubules. This intertubular tissue is derived in the first instance from the general mesenchyme of the primordial testis, but it also receives a contribution of mesonephric mesenchyme accompanying the blood-vessels. It thus appears that the interstitial cell arises from a stellate precursor which is morphologically indistinguishable from the cells giving rise to the cellular elements of ordinary connective tissue.

Although much time and attention have been devoted to the histogenesis of the foetal interstitial cell, there is little information available at present regarding its histochemical characteristics. This work is a study of some of the histochemical features of the foetal interstitial cell; it also seeks to furnish histochemical evidence in support of a mesenchymal origin of the interstitial cell.

## Material and Methods

In all, 18 male sheep embryos were studied; the crown–rump length varied from 2·8 to 40 cm. The details are tabulated as follows:

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| *Crown–rump length* | *No. of embryos* | *Position of testes* |
|---|---|---|
| (cm) | | |
| 2·8 | 1 | medial to kidney |
| 8·1 | 1 | abdominal |
| 9·7 | 3 | ,, |
| 17·5 | 3 | inguinal canal |
| 21·3 | 2 | ,, |
| 24·3 | 1 | ,, |
| 31·0 | 2 | scrotal |
| 33·0 | 2 | ,, |
| 36·0 | 1 | ,, |
| 40·0 | 2 | ,, |

One testis from each animal was fixed intact in a mixture of 90 ml water, 10 ml formalin, and 5 g mercuric chloride, dehydrated in cellosolve, embedded in ester wax, and sectioned at 5 $\mu$. The stains used were haematoxylin and eosin, and also the McManus/Hotchkiss periodic acid / Schiff (PAS) technique (Carleton and Drury, 1957). It has been shown (Vallance-Owen, 1948) that glycogen is as well fixed by formaldehyde fixatives as by alcohol or alcohol/picric mixtures; the former were found to give vastly superior histological results in the testis, shrinkage of interstitial tissue being minimal by this method.

With the exception of the smallest embryo, the other testis was in each case fixed in formaldehyde-calcium solution (90 ml water, 10 ml formalin, 1 g anhydrous calcium chloride), embedded in gelatin, and sectioned at 10 $\mu$ on the freezing microtome. Some frozen sections were coloured in Sudan black to demonstrate total lipids, while others were subjected to Hayes's modification of Feulgen and to Voit's plasmal reaction (Lillie, 1954) to show acetal phosphatides and possibly steroids (Dempsey, 1948).

## Results

To avoid repetition, descriptions of the specimens stained with haematoxylin and eosin are presented conjointly with descriptions of the corresponding PAS slides.

*Results with haematoxylin and eosin and with PAS.* The gonad of the 2·8-cm embryo is a mesenchymal mass, covered with cuboidal coelomic epithelium, located in the dorso-medial angle of the coelom: it is coextensive with the developing kidney. The primordium consists solely of stellate mesenchymal cells (fig. 1, A), whose membranes are well defined by the PAS procedure. Occasional red, refractile, PAS-positive, diastase-resistant granules may be seen in some cells. The nuclei are usually large and oval. A few large hexagonal cells with vesicular nuclei are visible at the periphery of the primordium; these may be the primitive sex cells. No PAS-positive ground substance is visible.

The gonads of the 8·1-cm and 9.7-cm embryos resemble one another closely. The spaces between the differentiating seminiferous tubules are populated with stellate mesenchymal cells resembling those described in the

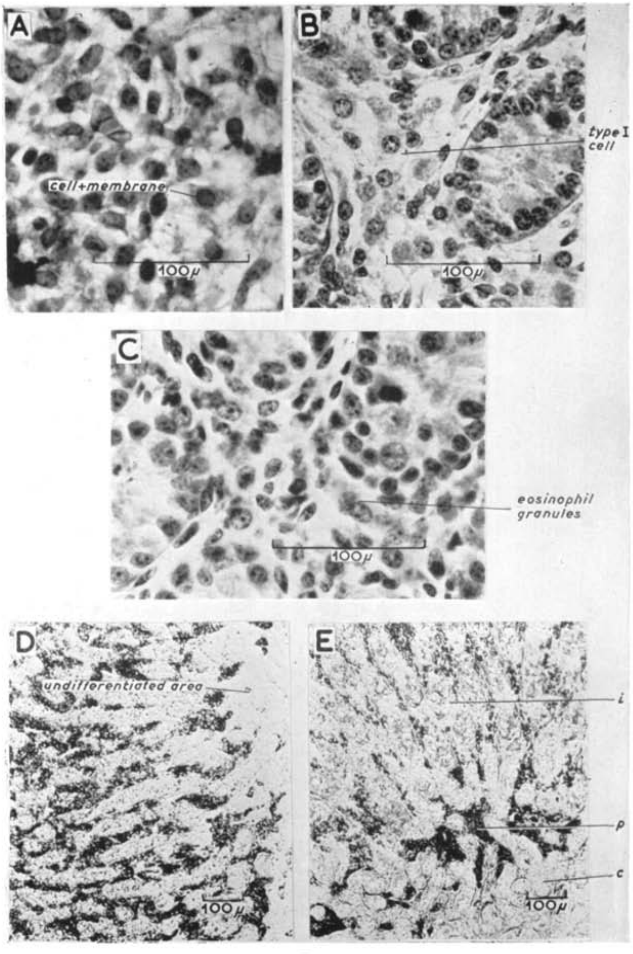


FIG. 1

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2·8-cm embryo; these cells are connected by transition forms with peritubular fibroblasts on the one hand and with interstitial cells on the other. The latter have abundant hexagonal or rounded cytoplasm, which stains pale pink with eosin; there are no PAS-positive cytoplasmic inclusions. Their nuclei are ovoid and vesicular and possess a characteristic chromatin arrangement.

The first visible event in the differentiation of the typical interstitial cell from its mesenchymal precursor is alteration of the nucleus. The nucleus, originally oval and containing dust-like chromatin, becomes vesicular and round, while the chromatin becomes localized just inside the nuclear membrane: one or more nucleoli become visible. Cytoplasmic differentiation is a slower process than nuclear differentiation. Initially the cytoplasm increases in amount, its processes diminish in size and number before finally disappearing, and the cell-body assumes a polygonal or epithelioid appearance. At a variable stage during this phase (see below), sudanophil droplets make their appearance.

The testes of the 17·5-cm, 21·3-cm, and 24-cm embryos present a uniform histological appearance. There are three recognizable types of interstitial cell:

(1) the most common form has abundant cytoplasm which colours pink with eosin; the cell-membrane stains clearly with PAS (fig. 1, B); the nucleus is vesicular and ovoid.

(2) the second form differs from the first in that it has variable numbers of prominent eosinophil granules in its cytoplasm (fig. 1, C). The granules occur in groups and may fill a cell process or even the whole cell-body: they are not PAS-positive.

(3) The third form has scanty eosinophil cytoplasm and a shrunken pycnotic nucleus. The cytoplasm is PAS-negative.

The testicular histology of the last four embryo sizes is similar: central differentiation of the tubules and intertubular tissue is beginning to catch up on peripheral differentiation. Only typical interstitial cells persist; the pycnotic forms and those with eosinophil granules have largely disappeared. As in earlier testes, staining with haematoxylin and eosin or with PAS reveals transition forms between stellate mesenchymal cells and typical interstitial cells.

*Results with Sudan black.* The testes of the 8·1-cm and 9·7-cm embryos are alike (fig. 1, D). There are no sudanophil elements in the centre of the testis, where differentiation of the various constituents is incomplete. Elsewhere

FIG. 1 (plate). A, testis of 2·8-cm embryo. 5 $\mu$, PAS. The gonad consists entirely of stellate mesenchymal cells.

B, testis of 24-cm embryo. 5 $\mu$, PAS. Note the absence of Schiff-positive material in the cytoplasm of the interstitial cells.

C, testis of 17·5-cm embryo. 5 $\mu$, haematoxylin and cosin. Some epithelioid interstitial cells have eosinophil cytoplasmic granules.

D, Testis of 9·7-cm embryo. 10 $\mu$, Sudan black. The centre of the gonad is poorly differentiated: the radial arrangement of the tubules in the remainder is conspicuous.

E, testis of 24-cm embryo. 10 $\mu$, Sudan black. Note the lack of interstitial lipids in the centre (*c*) of the gonad, the intense sudanophilia of the paracentral (*p*) interstitial cells, and the radial arrangement of interstitial cells in the intermediate (*i*) zone.

sudanophil interstitial cells are arranged radially in columns of 2 to 8 cells between the seminiferous tubules. The abundant cytoplasm of the individual cells contains numerous minute sudanophil particles: the ground cytoplasm and nuclei are not coloured. Nondescript cells, intermediate in size and shape between typical interstitial cells and mesenchymal cells, are also visible: their cytoplasm contains a variable number of sudanophil inclusions.

The testes of the 17·5-cm, 21·3-cm, 24-cm, 31-cm, and 33-cm embryos resemble one another. The tubules are well defined in the centre of the organ and the interstitial cells may be divided into 4 groups or zones. In the first or peripheral zone, which lies just below the tunica albuginea, the cells are arranged in clumps and have sudanophil cytoplasmic granules. The cells of the second or intermediate zone (fig. 1, E) are histologically similar but are arranged in radial columns. In the third or paracentral zone, which circumscribes the area of newly-defined seminiferous tubules, the cells are coloured very deeply indeed by Sudan black and their cytoplasm is packed with dust-like black particles. In the centre of the testis the last or fourth group of interstitial cells is imperfectly differentiated; these 'immature' cells have no sudanophil cytoplasmic inclusions. At this stage, however, it will be noted that 'typical' interstitial cells are visible in the centre of the testis in the corresponding material stained with haematoxylin and eosin.

The testes of the 36-cm and 40-cm embryos only differ from the above description in so far as the cells of the central zone have acquired sudanophil inclusions and are not distinguishable from those of the paracentral zone.

*Results with the plasmal test.* At no time do the interstitial cells contain Schiff-positive lipids (plasmalogens, acetal phosphatides, and possibly steroids); nor is there any evidence of plasmalogens in the Sertoli cells.

## Discussion

Most recent workers agree with Gillman's (1948) statement that the testicular interstitial cell arises from a spindle-shaped mesenchymal precursor. In the foetal sheep testis, while many of the peripheral interstitial cells make their appearance during a period not covered by the present available material (i.e. between the 2·8-cm and the 8·1-cm stage), the more centrally placed cells can be seen to arise by transformation of mesenchymal elements derived from the undifferentiated central portion of the gonad. A similar, centrally placed portion which differentiates more slowly than the remainder of the gonad has been described in the foetal rat testis (Roosen-Runge and Anderson, 1959). On the basis of the PAS and Sudan tests, one can easily recognize that histochemical and histological metamorphosis of the interstitial cell from a mesenchymal precursor does occur.

In the intertubular spaces of testes traversing the inguinal canal (crown–rump length 17·5 cm, 21·3 cm, and 24 cm), there are three types of interstitial cell. While the occurrence of the typical hexagonal epithelioid cell is to be expected, the significance of the shrunken pycnotic forms and of the clumps of eosinophil granules in otherwise cytologically typical cells is problemati-

cal. Abnormal shrunken cells are also to be seen in the postnatal mouse testis (Baillie, 1958); they may represent atrophic or regressive forms of interstitial cell.

In man and the horse (Gillman, 1948) foetal interstitial cells reach a developmental maximum at or about mid-term. Thereafter the interstitial tissue undergoes a reduction whose occurrence and timing have not been satisfactorily explained. By contrast, in the rat (Roosen-Runge and others, 1959), in the rabbit (Allen, 1903), and in cattle (Bascom, 1923) similar interstitial cell maxima do not occur until birth. The ensuing interstitial tissue regression in these animals has been ascribed to deprivation of maternal oestrogens and gonadotrophins. In the present material there is no histological or histochemical evidence, at any stage, of significant interstitial cell degeneration; the post-natal fate of these cells in the sheep has not been pursued.

The cytoplasm of the interstitial cells of the foetal sheep contains no glycogen, glycolipid, or glycoprotein masses, i.e. no PAS-positive granules. This contrasts with the cytoplasm of the interstitial cells of poikilotherms (Cavazos and Melampy, 1954), which contains glycogen, and with that of adult homiotherms, which contains diastase-resistant polysaccharide / protein complexes (Montagna and Hamilton, 1952). On the basis of these findings it appears that glycogen is entirely absent from the foetal and adult homiothermal interstitial cell; the prominent red, refractile, diastase-resistant granules to be seen in the interstitial cell of the adult ram (Cavazos and Melampy) must make their appearance some time after birth.

In common with the sparrow, chaffinch, greenfinch, mouse, and Leghorn cockerel (Lofts and Marshall, 1956), rat (Lynch and Scott, 1951), deer (Wislocki, 1949), and man (Mancini, Nolazeo, and Balze, 1952) the interstitial cells of the foetal sheep contain sudanophil material. The material occurs in the form of very fine droplets which are only a fraction of the size of the corresponding droplets in interstitial cells of mice (Baillie, 1958).

No Schiff-positive lipids occur in the interstitial cell of the foetal sheep. Similarly, these lipids are absent from the interstitial cells of neonatal mice, cold-stressed mice (Baillie, 1958), the adult rat (Albert and Leblond, 1946), and the adult deer (Wislocki, 1949). It would appear that Schiff-positive lipids do not occur in the foetal testes of species so far investigated; they are present in large amounts in the interstitium of most adolescent animals so far studied; they are usually present in reduced amounts in the corresponding adult testes, although some adult testes contain no demonstrable plasmalogens. In addition to age and species variation, climatic factors also influence the amount of interstitial cell plasmalogen (Baillie, 1958).

While the above histochemical findings suggest that interstitial cell androgens are present in small quantities, if at all, in the foetal sheep, androgen has been demonstrated in the testis of the foetal bull (Moore, 1950), and it has recently been shown (Price and Pannabecker, 1956) that the foetal rat testis *in vitro* produces demonstrable androgens. It is difficult to reconcile these observations with the classical view that the male secondary sexual organs

require merely an initial androgenic stimulus for their differentiation, and that, for a time thereafter, their development is independent of the male sex hormone (Moore, 1950).

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