TINCTORIAL DIFFERENTIATION OF THE CELL TYPES IN THE PARS ANTERIOR OF THE SHEEP

By Robin Tassell* and J. P. Kennedy*

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Abstract

Histological methods have been used to study the pars anterior of the sheep in a number of physiological states with the object of identifying the cellular sources of the hormones produced by this gland.

It was possible to identify definitely four types of cell. Two of these were acidophils and stained red or orange after Herlant's tetrachrome. The orange cell showed little variation in different physiological states and probably produces growth hormone while the red was active about the time of parturition and appears to be the source of prolactin. Two classes of basophil, staining dark or light blue after tetrachrome, were identified. The former, which also were stained by complex basic dyes, were prominent after castration. The light blue cells were faintly PAS-positive when the PAS procedure was used and this reaction was slightly stronger after gonadectomy. Both types of basophil may be involved in gonadotrophin production but thyrotrophic and corticotrophic cells may also be included in these classes. Evidence supporting this theory is discussed.

I. Introduction

Gonadotrophic hormone content of the anterior pituitary is known to vary according to physiological factors such as age, sex, and reproductive state. Nutrition, temperature, and other environmental factors may also influence levels of these hormones.

The anterior pituitaries of a number of mammalian species have been examined microscopically to determine whether fluctuations in hormone levels are reflected by changes in cell numbers, structure, degree of granulation, or staining intensities. In some species, notably the rat (Purves and Griesbach 1954, 1955) and the bat (Herlant 1956), the cells which appear to produce gonadotrophin show distinctive characteristics according to the reproductive state of the animal, but in other species the changes are less marked.

The following experiments were carried out with the object of identifying the cellular sources of the gonadotrophic hormones in sheep and describing visible changes associated with different reproductive states. Preliminary studies were made to determine the most suitable fixatives and staining techniques.

* School of Wool and Pastoral Sciences, University of New South Wales, Kensington, N.S.W. 2033.

II. Materials and Methods

Pituitary glands were removed at slaughter from the following groups of animals:

(*a*) 11 adult Merino wethers, 4 of which had been implanted with testosterone propionate (four 23·5-mg pellets) 25–60 days prior to slaughter;

(*b*) 10 ovariectomized aged Merino ewes, killed 1 month (5) or 7 months (5) after surgery;

(*c*) 13 aged Merino ewes in early to mid-pregnancy;

(*d*) 15 similar ewes, in the immediate prepartum period, and their foetuses (14 females, 8 males);

(*e*) 13 lactating ewes, up to 2 weeks postpartum, and their lambs (10 females and 3 males);

(*f*) 2 non-pregnant ewes similar in age and breeding to those described above;

(*g*) 4 ewes from the same flock, which had received 0·5 g methyl thiouracil (MTU) daily as a drench for 1 month;

(*h*) 2 male foetuses at 71–72 and 88 days gestation;

(*i*) 5 crossbred ewe lambs aged approximately 7 months. Three of these animals had ovulated without showing oestrus and two had not ovulated.

Most glands were fixed in Bouin–Holland sublimate (Racadot 1963*a*) but a few were preserved in Elftman's (1959*a*) chrome–alum fixative, Zenker's fluid, or formol sublimate. In most cases the glands were split longitudinally before fixation.

The pituitaries were washed, dehydrated, and embedded according to standard histological procedure, then sectioned at 5 and 7 μm, chiefly in the sagittal plane. A few were cut in the horizontal or coronal plane.

A large number of staining techniques were evaluated. Detailed observations were made on sections stained by the following techniques:

(1) tetrachrome (Herlant 1960, as modified by Racadot 1962);

(2) Alcian blue–periodic acid Schiff–haematoxylin–orange G (OxAB–PAS–H–Or; Herlant 1960);

(3) aldehyde fuchsin (OxAF; Cameron and Steele 1959) followed by trichrome (Slidders 1961) or light green;

(4) PAS–H–Or (Jubb and McEntee 1955);

(5) resorcin–fuchsin (Krutsay 1960) alone or combined with Alcian blue.

Other methods evaluated included modified Heidenhain's azan (Dawson and Friedgood 1938), Crossman's (1937) trichrome, aldehyde fuchsin as used by Halmi (1952), PAS–methyl blue (Wilson and Ezrin 1954; Rennels 1957), colloidal iron–PAS (Mowry 1958), aldehyde thionine–PAS (Ezrin and Murray 1963), OxAF–PAS (Elftman 1959*b*), and fluorescent Schiff's reagent (Culling 1963).

The sections were examined to determine the type and distribution of cells. In a limited number of sections, AF-positive cells were counted.

To define reproductive state more precisely, the ovaries of the ewes were collected and examined histologically to determine the degree of follicular activity and the condition of any corpora lutea. The results obtained from these examinations are reported by Tassell (1971). Uteri, seminal vesicles or testes, thyroids, and adrenals from some animals also were studied.

III. Results

The general structure of the pituitary is shown in Figure 1. The pars intermedia, pars tuberalis, and zona tuberalis were devoid of acidophilic cell types, as were some central areas close to the main blood vessels.

(a) Pars anterior of Wethers and Ovariectomized Ewes

Using the techniques described above, four types of cell were identified clearly in the pars anterior of gonadectomized animals.

After tetrachrome, OxAB–PAS–H–Or, or PAS–H–Or, finely granular cells were stained with orange G (Fig. 2). These aurantiphil cells were distributed singly or in small groups, their numbers being greater dorsally and to a lesser degree laterally. The numbers and degree of granulation did not vary in any of the groups of adult sheep studied.

Another type of acidophil was stained with erythrosine after the tetrachrome procedure (Fig. 2). After OxAB–PAS–H–Or, this type was orange-pink, its slight affinity for Schiff's reagent being enhanced by oxidation in acid permanganate. These cells were rectangular or irregular in shape, were coarsely granulated, and occurred in groups or in palisade-like blocks in which the nuclei were frequently oriented towards the blood vessels. When heavily granulated, a Golgi image often was visible, while strongly degranulated cells appeared bluish. Erythrosinophils were the commonest cells of the pars anterior.

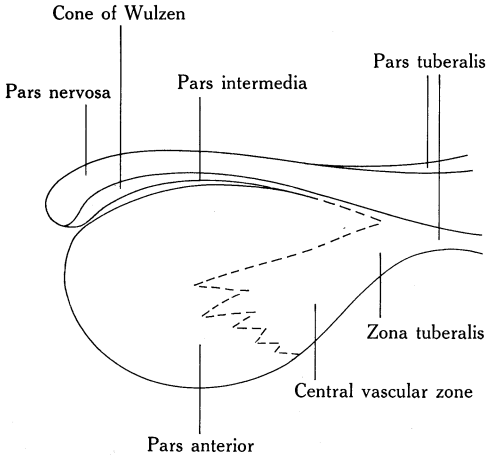

Fig. 1.—General structure of the ovine pituitary gland—mid-sagittal section. The division between the zona tuberalis and the pars tuberalis, and between the pars intermedia and the pars anterior is not sharply defined. The border between the cone of Wulzen and the pars intermedia is clear only if cell types are examined.

Tetrachrome staining revealed rounded, dark blue basophils which were distributed singly throughout the pars anterior and especially the zona tuberalis (Fig. 2). The cells appeared to be similar to those in adjacent sections which reacted with Alcian blue or aldehyde fuchsin. After use of the latter stains, differentiation was clearer and the granulation could be seen to be coarse. Aldehyde fuchsin-positive cells were numerous and strongly stained in the partes anteriores of most wethers and ovariectomized ewes. There was no significant difference in AF cell numbers between normal and testosterone-treated wethers although at low magnifications the treated animals appeared to have greater numbers. The glands from short-term ovariectomized ewes showed few of these dark basophils but their fixation may have been at fault. Some, but not all of the dark basophils were strongly PAS-positive after PAS–H–Or, those near the blood vessels being darkest.

In addition to the dark basophils, groups of light blue cells, chiefly in the zona tuberalis and the central vascular areas, were stained after the tetrachrome procedure.

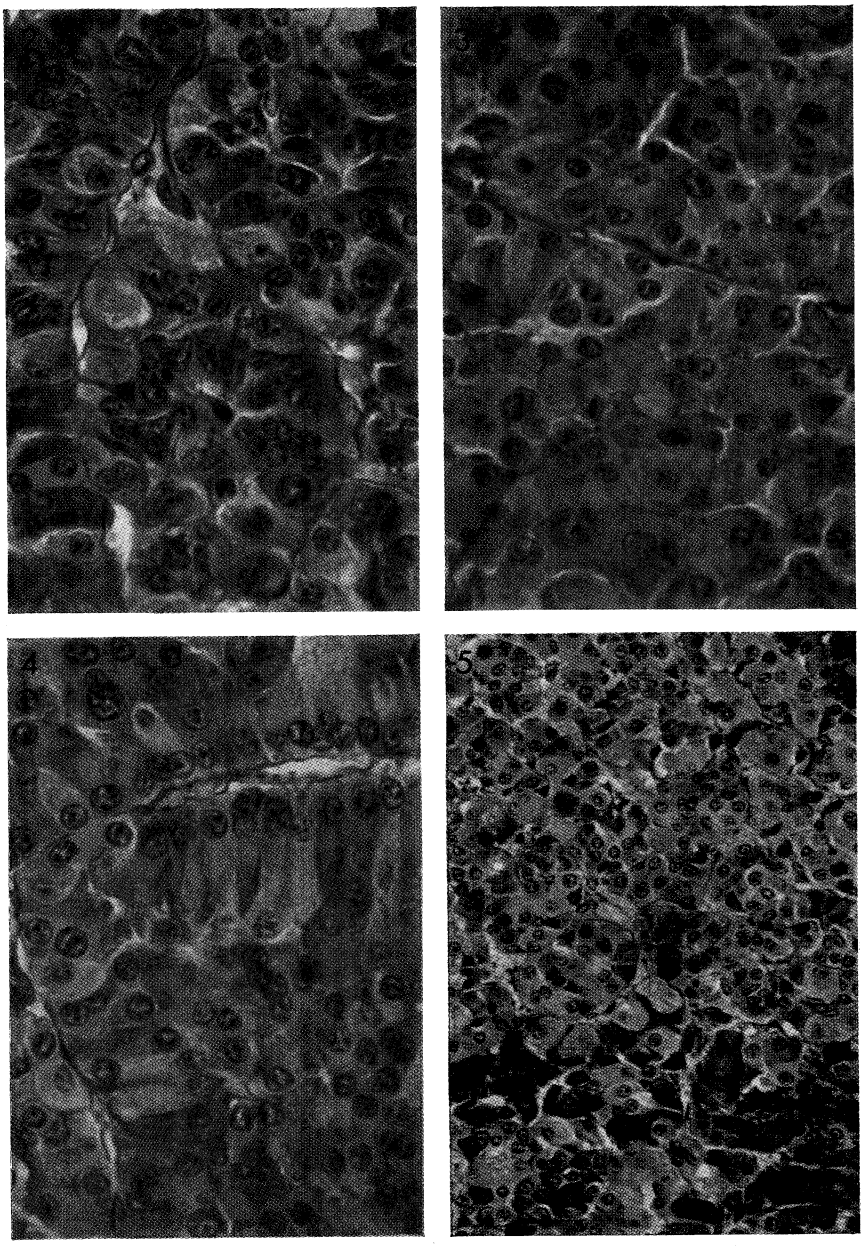

Fig. 2.—Pars anterior of an ovariectomized ewe after tetrachrome staining. Numerous dark blue cells are present, some showing degranulation or Golgi images. Aurantiphil (orange) and erythrosinophil (red) cells are also visible. ×500.

Fig. 3.—Pars anterior of a prepartum ewe, showing well-developed erythrosinophil cells. Some of these have giant nuclei and Golgi images. Tetrachrome. ×500.

Fig. 4.—Pars anterior of a postpartum ewe. Erythrosinophil cells are partly degranulated. Tetrachrome. ×500.

Fig. 5.—Pars anterior of a ewe treated with methyl thiouracil. Numerous large pale blue cells are present in this central part of the gland. Tetrachrome. ×400.

These cells, which were rounded and sometimes large and well defined, differed from the dark blue type in distribution and in that their granules appeared finer. Although they occurred mainly near the blood vessels, in some individuals the cells were also seen elsewhere. After OxAB–PAS–H–Or or PAS–H–Or, these cells were faintly pink. It was difficult to determine differences in numbers between individuals because of the importance of plane of section.

As well as the four cell types described above, a number of cells stained medium blue after tetrachrome. They usually were small but varied in number and resembled the "undifferentiated" cells of foetuses and lambs.

(*b*) *Pars anterior of the Ewe in Early to Mid-gestation*

The dark blue or AF/AB cells were smaller and inconspicuous compared to those in long-term ovariectomized ewes. In other respects the appearances of the glands were similar to those seen in the latter group.

(*c*) *Pars anterior of the Prepartum Ewe*

The overall picture of dark and light blue basophils after tetrachrome staining in this group was similar to that seen in early pregnancy, although there appeared to be fewer dark basophils in most prepartum animals. The erythrosinophil cells were generally fully granulated and numerous, while giant nuclei and Golgi images were visible in some sections (Fig. 3).

(*d*) *Pars anterior of the Postpartum Ewe*

After tetrachrome, the dark and light blue cells did not appear different from those seen in the previous group. Overall numbers of AF-positive cells were slightly greater than in prepartum ewes. In some postpartum ewes the erythrosinophils were strongly degranulated while partial degranulation was seen in some others (Fig. 4). However, in one group of five ewes, these cells were moderately and fairly evenly granulated.

(*e*) *Pars anterior of Ewes Treated with Methyl Thiouracil*

The dark blue cells were similar to those seen in early pregnancy. The light blue cells were quite strongly granulated and were present in the scattered form. Extensive vascular areas containing these cells were seen in two ewes and in another the cells were very large (Fig. 5). Some of the latter showed Golgi images. The acidophils did not appear to be affected by the treatment.

The main differences observed between the partes anteriores of the adult sheep were the greater prominence of the dark basophils in the glands of castrated animals and the apparently greater development of the vascular, light blue or faintly PAS-positive cells in three out of four of the MTU-treated ewes. Strongly PAS-positive cells were numerous only in glands from castrates.

(*f*) *Lambs and Foetuses*

In both lambs and foetuses, the whole pars anterior was highly vascular and the zona tuberalis was difficult to delineate. There were no apparent differences between male and female glands.

After tetrachrome staining, some cell differentiation was evident even in the youngest foetuses (71–88 days). The orange cells were first to become recognizable, all other cells at this stage appearing undifferentiated mid-blue. A few individuals appeared to have relatively more orange cells than adults. In the term foetuses, the erythrosinophilic cells had differentiated but they were less plentiful than in adults. Dark blue cells were not seen in foetuses and rarely in lambs. However, a few AB-positive cells were seen in the partes anteriores of both lambs and term foetuses except where these had been fixed in formol sublimate. Alcian blue-positive granules were visible in a few cells of the glands from the 88-day-old foetus.

Aldehyde fuchsin stained more cells than Alcian blue and positive cells were present in the youngest foetuses examined. Numbers were rather variable and were often higher near blood vessels.

The undifferentiated cells decreased in number as other types became identifiable. Light blue cells became distinguishable about the time of birth and were unusually prominent in some lambs.

IV. Discussion

Four cell types have been identified positively by using the procedures described above. These were stained orange, red, light blue, and dark blue in the tetrachrome procedure.

The two acidophil types (orange and red) correspond well with those observed by other workers (Clarke and Purves 1960; van Blom 1965; Mikami and Daimon 1968). The aurantiphil (orange) type is similar to the cell identified as a somatotroph in the bovine by Dubois and Herlant (1968). It has been shown that acidophil granules from sheep pituitaries contain somatotrophin and it seems likely that the aurantiphil cells are the source of this hormone.

Erythrosinophils (red) differ from aurantiphils in both staining affinity and the manner in which they occur. Apparent activity about the time of parturition and in early lactation supports the designation of erythrosinophils as the source of prolactin, suggested by Racadot (1963*b*). Mikami and Daimon (1968) described a similar cell in sheep.

The light blue cells found in the vascular areas contained very little stainable material. They were AF- and AB-negative, and probably correspond to the delta 2 cells of Mikami and Daimon (1968). In wethers some of these cells were stained with acid fuchsin after Slidder's trichrome and in most gonadectomized animals a weak PAS-positive reaction was seen. Since pituitary FSH content is higher in wethers than ewes (Bindon and Tassell 1969), these cells may be involved in production of this hormone as postulated by Mikami and Daimon (1968). However, such designation must be considered tentative. It is possible that more than one cell type was included in this class as there were signs of activity in MTU-treated ewes and newborn lambs. A second type could be responsible for corticotrophin production since corticosteroid concentrations in foetal plasma are elevated about the time of parturition (Bassett and Thorburn 1969).

Cells which were dark blue after tetrachrome also were AB- and AF-positive. In the absence of basic stains, some of these cells seemed to have affinity for PAS. Examination of AF-stained sections under high magnification ($\times 1500$) revealed that

the differences between wether groups in numbers of positive cells seen with low magnification were not real. Some cells had been overlooked because of low granule content.

In other species cells which stain with AF generally are considered to be thyrotrophs but, as pointed out by Purves (1966), one or more of the gonadotrophs may also stain under some circumstances. The cells designated as the sources of TSH and LH in cattle by Dubois and Herlant (1968) both stained with AB and AF. Treatment with MTU did not induce changes in the AF-positive cell type but as the effect of this substance on pituitary thyroid stimulating hormone concentration is unknown, this evidence does not preclude a thyrotrophic function. The treatment resulted in macroscopic and microscopic changes in the thyroid which suggested marked stimulation of the gland. It seems probable that the AF cell class in sheep includes the thyrotrophs (Mikami and Daimon 1968) but it may also include a gonadotroph since these cells were prominent in castrated sheep.

It had been hoped that changes in the pituitaries of castrated animals would permit identification of the gonadotrophic cells. In the rat such changes are marked and "castration cells" develop (Purves and Griesbach 1951). Mikami and Daimon (1968) have described castration cells in sheep but these were not seen in the present study although the pars anterior of one ovariectomized ewe contained a few cells similar to those described by these authors. The hypothesis that changes in the gonadotrophs would occur following castration was also based on the evidence that FSH, LH, and total gonadotrophin concentrations increase in ovine pituitaries at this time (Warwick 1946; McDonald and Clegg 1966; Maraček and Arendarčík 1968; Roche *et al.* 1970).

The data of Roche *et al.* (1970) suggest that some changes in cells producing LH should have been observed in the short-term ovariectomized ewes. Cells producing FSH also may be active at this time (McDonald and Clegg 1966; Maraček and Arendarčík 1968). No marked changes were observed in ewes ovariectomized for 1 month although the cells which were light blue after tetrachrome were prominent in most glands.

Partes anteriores of the testosterone-treated and control wethers did not differ in appearance. However, the treatment did not significantly alter the height of the epithelium in the seminal vesicles so perhaps the amount of hormone absorbed was insufficient to affect the pituitary.

Little is known of gonadotrophic changes in the pituitary of the sheep during pregnancy. There could be a suppression of both synthesis and release of pituitary gonadotrophin as pregnancy advances since high levels of oestrogen and progesterone would exert a feedback effect on the gland. The ovaries of ewes in the prepartum period contained only very small follicles indicating that little gonadotrophin was being released (Tassell 1971). After parturition larger (up to 4–5 mm diameter) follicles were seen; however, this does not provide information on pituitary gonadotrophin levels.

Several types of cell are likely to be active in both ewes and their offspring around the time of parturition since a number of hormones are involved in initiation and maintenance of lactation (Cowie 1966) and some, such as ACTH, probably play a role in parturition. The thyroids in lambs and foetuses appeared histologically to

be active and some ovaries from newborn lambs showed marked follicular development suggesting FSH release. The evidence of Liggins and Kennedy (1968), Mauléon and de Reviers (1969) and others indicates that the foetal pituitary is capable of producing most if not all of the hormones produced by the adult.

The number of cells staining with AF in many foetal glands was greater than the number staining with Alcian blue. This suggests either that AF is more sensitive than AB or that two cell types are involved with AF-positive cells appearing before AB-positive types. The latter type would also stain with AF. In adult glands PAS alone appears to stain some but not all of the cells usually stained by Alcian blue.

A major problem with the methods used in this study was the within-group variation in the appearance of the glands. This may have been due to small differences in the environment of individual animals. For example, some animals may have been subjected to greater stress than others.

It seems possible that the faintly PAS-positive cell type and the AF-positive cell classes might be further subdivided and some immunofluorescent studies have been undertaken in the hope of achieving this (Tassell 1971).

V. Acknowledgment

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