Studies of the skin follicle development in Border disease of sheep.

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GENERAL CONTENTS

SUMMARY

Qualitative and quantitative techniques have been used to compare the follicle populations of control and Border disease affected foetuses and lambs of the medium - fine fleeced Cheviot x Dorset Horn cross, and the coarse fleeced Scottish Blackface breeds.

In the Cheviot cross, the main effect of the disease is to cause primary follicle hypertrophy, which is first apparent at 115 days' gestation and is accompanied by an increased fibre size and an increased frequency of medullation. These changes are not known to occur in any other infectious ovine disease. The normal suppression of follicle activity which occurs peri-natally and at about the time of birthcoat shedding, and also during the winter months is present in Border disease affected animals. Thus, the differences observed between the follicle populations of control and affected animals at these times are less obvious. A reduction in the number of secondary follicles which develop is also apparent in Border disease, but this may be an indirect effect of the disease process caused by increased primary follicle size or possibly by impaired foetal nutrition resulting from the placentitis which is also a feature of the disease.

The developing primary follicles are susceptible to the effects of Border disease following maternal inoculation at up to 80 days' gestation. The reason for the loss of susceptibility thereafter is not clear, although it may be related to the development of a foetal immunological response to the agent.

Studies of the literature on epidermal-dermal interaction and studies of the disease process in the Cheviot cross suggest that the follicle papilla determines the morphology of the follicle and fibre. Thus modification of the cells of the papillae of primary follicles by the disease process probably causes follicle hypertrophy. Post-natal studies indicate that this effect is durable if not permanent, and thus a new concept is introduced, namely that of a developmental anomaly leading to ordered hyperplasia rather than the more usual disordered hypoplasia.

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The development of the follicle population of the control Scottish Blackface resembled that of the Border disease affected medium - fine fleeced Cheviot cross. Border disease in the Blackface did not cause further primary follicle hypertrophy. As a working hypothesis to explain these findings, it is proposed that Border disease prevents the function of an inhibitor substance which normally controls primary follicle growth in medium and fine fleeced breeds. Interference with the functioning of the inhibitor contributes to the development of a coarse fleece.

An analogy exists between the effects of Border disease and the effects of the mutant N and nr genes in the N-type Romney, which also cause primary follicle hypertrophy, and the analogy underlines the hypothesis that Border disease mimics a genetic effect in the skin.

Baselines can be established to determine abnormal medullation in fine and medium fleeced breeds, and it is suggested that the frequency of medullae or large medullae in central primary follicles should be used depending on the breed studied. Peri-natal sampling and sampling during the birthcoat shedding phase, during the winter months and during terminal illness should be avoided if useful results are to be obtained. With these provisos, histological examination of the skin could be a useful tool in medium and fine fleeced breeds to detect adult carriers and affected lambs.

CHAPTER I

Literature review of Border disease

1. History, distribution and clinical symptoms of Border disease in the field.

In the first account of Border disease in the field, Hughes, Kershaw and Shaw (1959) reported that the disease had been endemic for at least ten years among the mainly Kerry Hill and Clun Forest flocks of the Border country between England and Wales, where it was the cause of considerable economic loss to the sheep farmer. It was a disease affecting lambs, which were born with an abnormally hairy birthcoat, with or without locomotor dysfunction and abnormal conformation. They were generally smaller than their normal fellows, throve badly and had a high mortality rate in the first few months of life. Affected lambs were usually born to ewe hoggs, i.e. sheep mated in the first autumn of their lives, although a few older ewes gave birth to affected lambs. This observation was also noted by Dry (1959, 1960 unpublished observations), and later supported by data published by Shaw (1962).

Affected lambs often had disproportionately short long bones and prominent foreheads (Hughes, Kershaw and Shaw, 1959), so that they appeared "stunted".

The locomotor dysfunction of affected lambs was described as ranging from a slight swaying movement when walking to a definite overall tremor, which could be so severe that the lambs were unable to stand unaided. In later work, Shaw (1962) showed that myelin formation in the central nervous system of affected lambs was defective. It is relevant to discussion later in the text that in laboratory tests carried out on normal and affected lambs, Shaw found that the blood copper and liver copper levels of affected lambs were within the normal range.

Locomotor dysfunction of newborn lambs associated with defective myelin formation characterised a very similar condition, called "hypomyelinogenesis congenita" by Markson, Terlecki, Shand, Sellers and Woods (1959). The breeds involved were Leicester cross, Oxford, Clun, Radnor cross and Kerry, so that although no mention was made of abnormal birthcoats in affected lambs, the variety of birthcoat types among these breeds may have made these difficult to detect.

Barr (1964) described outbreaks of "hypomyelinogenesis congenita" in Swaledale, Swaledale cross, Rough Fell cross, Border Leicester cross and Suffolk cross lambs, but only in the Suffolk cross lambs were the birthcoats described as abnormally "hairy".

It appears that there may have been confusion between the conditions of "hypomyelinogenesis congenita" and that of Border disease arising from inadequate knowledge of normal fleece types and their alteration by Border disease. The situation was clarified by Barlow and Dickinson (1965) who put forward diagnostic criteria for Border disease in the flock. These were:

a. an abnormally pigmented hairy birthcoat or fleece in normally smooth-coated breeds,

b. a flock history of poor growth and viability of the lambs, with no overt cause and/or a proportion of lambs showing rhythmic clonic spasms, and

c. the occurrence in lambs less than six months of age of myelin defects and clusters of swollen interstitial glia. Over six months of age, the presence of many abnormal glial cells could also be of some value in diagnosis in the absence of myelin defects provided that other disease processes could be excluded.

These criteria suggest that Border disease and "hypomyelinogenesis congenita" could be the same condition. However, the conflicting evidence of the significance of the fleece changes in these field reports illustrates the need for a particular study of the skin lesion.

Recently, Border disease and Border disease-like conditions, have been reported from many countries - from New Zealand (Hartley and Kater, 1962; Manktelow, Porter and Lewis, 1969), from California (Bell, 1967b; Osburn, Crenshaw and Jackson, 1972), and from Ireland (Hamilton and Donnelly, 1970).

2. Experimental transmission and aetiology

Dickinson and Barlow (1967) first showed that a transmissable agent was involved in the causation of Border disease, and that the agent reached the embryo from its infected dam. Pregnant Dorset Horn ewes were inoculated subcutaneously and intraperitoneally at various ages of gestation with suspensions of brain and spleen tissue from Border disease affected lambs. Several ewes aborted, and several lambs were stillborn, but the majority of lambs produced at term were "hairy" and several were "shakers".

Shaw, Win kler and Terlecki (1967) also reproduced "hypomyelinogenesis congenita" experimentally, in lambs born to Hampshire and Welsh Mountain cross ewes, by subcutaneous and intraperitoneal injection of nervous tissue, spleen, liver and kidney suspensions from affected lambs, and although the majority of ewes produced lambs with clinical and neurological lesions consistent with those of Border disease, the situation with regard to fleece type could not be resolved, perhaps due to the normally coarse fleece of the breed used.

The similarity between Border disease and Hairy Shaker Lamb Disease became more marked when Manketelow, Porter and Lewis (1969) showed that the latter condition could be transmitted experimentally by inoculating pregnant Romney ewes between 16 and 50 days' gestation with an inoculum prepared from affected lamb tissues. As with experimental Border disease, there was a high occurrence of abortions. Live lambs were all "hairy" and unthrifty, and most were "shakers". The variability of symptoms in experimental and natural cases was illustrated by Terlecki, Hebert and Done (1973), who showed that no one clinical feature of the disease occurred in all offspring of Border disease injected ewes. The abnormal birthcoat and "nervous signs" were the most frequent abnormalities observed, occurring in 13 and 12 lambs respectively in a group of 17.

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On inoculating ewes by various routes, Gardiner and Barlow (1972) found that although intraperitoneal, subcutaneous and intratracheal injection produced Border disease, administration per os and per vagina did not. Semen proved to be non-infective. Lewis, Porter and Manktelow (1970) on the other hand, found that oral and conjunctival inoculation did produce Hairy Shaker Lamb Disease, although less consistently than intramuscular and intraperitoneal injection. They suggested that the former might be the natural route of infection following contamination of the pasture by aborting ewes.

The Border disease type condition reported by Jackson, Osburn and Crenshaw (1972) from California was also transmitted experimentally by foetal injection through the uterine wall between 57 and 70 days' gestation.

In all these reports, the timing of inoculation has been important in the reproduction of the disease. Inoculation before 16 days' gestation resulted in the birth of apparently normal lambs (Manktelow , Porter and Lewis, 1969), as did inoculation after 118 days' gestation (Gardiner and Barlow, 1972; Jackson, Osburn and Crenshaw, 1972).

The nature of the disease agent was investigated by Gardiner, Barlow, Rennie and Keir (1972), who showed that it was inactivitated by heating to 60°C for 90 minutes, and by ether treatment, and that its size was approximately 27 nm (270Å) in diameter. They suggested that it might be a small RNA virus with an ether-soluble envelope.

3. Pathology and biochemistry

Barlow and Dickinson (1965) based the third of their diagnostic criteria on the results of histological examination of the central nervous system of sheep up to nine months of age, which were considered on clinical grounds, to be affected by Border disease. The lesions were generally more pronounced in animals less than six months of age, especially in "Shakers", but their severity /

severity was not related to the degree of locomotor disturbance. The lesion was basically one of hypomyelinogenesis, the myelin sheaths being absent in some cases and sparse in others, and showing irregular swellings which were best appreciated among the smaller nerve fibres of the dorsal funiculi of the cord. Abnormal lipid droplets, probably composed of triglycerides, cholesterol esters and hydrophobic phospholipids, were also seen lying within the dilated sheaths or in the interstitium and perivascular spaces of lambs up to six weeks of age. "Hairy non-shaker" lambs showed similar but milder changes. Interstitial glial cells were often abnormally numerous, especially in the occipital white matter, cerebellum and spinal cord.

In the first of a series of papers on Border disease, these lesions were again described as being pathognomonic of Border disease by Barlow and Gardiner (1969), and Barlow, Gardiner, Storey and Slater (1970) showed that following maternal inoculation at 54 days' gestation, the lesions could be recognised as early as 16 days later. Barlow et al suggested that the disease might inhibit myelination directly or cause concurrent demyelination of newly myelinated fibres.

Hypomyelinogenesis was the outstanding neurological lesion of the Border disease type condition reported by Manktelow, Porter and Lewis (1969) from New Zealand and by Osburn, Crenshaw and Jackson (1972) from California.

The ultrastructure of the spinal cord in normal and Border disease affected lambs was described by Cancilla and Barlow (1968). This was followed by an ultrastructural study of the resolution of these lesions in older animals (Cancilla and Barlow, 1971).

Early reports of the effect of inoculation on the ewe indicated that ewes showed no evidence of malaise, even before abortion (Barlow and Gardiner, 1969). Barlow (1972a) showed that following inoculation between /

between 25 and 54 days' gestation, lesions were restricted to the uterus, its drainage lymph nodes and the conceptus, and appeared about ten days post inoculation. In the uterus there was necrosis of placental septae, which either extended to diffuse necrosis of the crypt zone with local atrophy of the trophoblast or remained small and focal. Extensive necrosis was associated with changes in the foetus suggesting imminent abortion, and restricted lesions were, it was suggested, likely to heal with restoration of tissue, since abnormalities were not consistently observed in full term placentae.

The severity and extent of the placental lesions varied according to the gestational age at inoculation, and Barlow showed that these became greater as the age of inoculation increased from 25 to 54 days' gestation. He suggested that lesions developed only in caruncles very closely associated with the trophoblast, and that this might account for the failure to transmit the disease in early gestation (Manketelow, Porter and Lewis, 1969).

Studies of lipid metabolism in the central nervous system of Border disease affected animals were begun by Davison and Oxberry (1966), who observed that both Border disease and "Swayback" were ataxic disorders of lambs in which lesions of myelin sheath had been shown to occur; and that Swayback was associated also with low tissue levels of copper and reduced amounts of myelin lipids. These authors showed that Border disease shaker lambs, like lambs affected by Swayback, did indeed have less of the characteristic myelin lipids - cerebroside, cholesterol and plasmalogen. However, the cholesterol esters known to accumulate in nervous tissue as a result of active demyelination, and found in some cases of Swayback, were absent from the white matter samples from Border disease. The authors suggested that active demyelination was not a feature /

feature of Border disease. The lesions of Swayback were known to result from copper and cytochrome oxidase deficiency leading to impaired lipid metabolism and myelin aplasia (Howell and Davison, 1959). Davison and Oxberry (1966) concluded that although the final biochemical findings in Border disease resembled those of Swayback, the lack of evidence of copper and cytochrome oxidase deficiency in their brains suggested that the primary causes of the lesions were different.

Later, speculation on the role of copper metabolism in Border disease was again aroused by Patterson and Sweasey (1973), who demonstrated a "relative hypocupraemia" in Border disease affected lambs which, they speculated, might have been secondary to the placentitis described by Barlow (1972a), and which might have been implicated in the development of abnormal birthcoat fibres and in abnormal myelin development. However, examination of the data reveals that the relative hypocupraemia is not outwith the normal range of copper values, and casts doubt on the validity of such speculation.

Patterson, Terlecki, Done, Sweasey and Hebert (1971) attempte d to relate the lipid composition of the spinal cord to the clinical symptoms of Border disease affected lambs. They found that a general deficiency of myelin lipid tended to be associated with the birthcoat abnormalities. Unlike Davison and Oxberry (1966), they found that an increased amount of esterified cholesterol could be a feature of the disease, and particularly of "shaker" lambs, which did not consistently have a reduced amount of myelin lipid. Patterson et al suggested that the "hairiness" and abnormal lipid biosynthesis might be connected through the metabolism of the amino acid methionine which is used in the synthesis of fibre keratin, and in the synthesis of phosphatidyl choline in the spinal cord.

Davison and Oxberry (1966) and Patterson et al (1971) did not describe the occurrence of lipid droplets in the spinal cord. These

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were observed first by Barlow and Dickinson (1965), whose suggestion that the lipid droplets were composed of triglycerides, cholesterol esters and hydrophobic phospholipids, was later confirmed by Storey and Barlow (1972). These authors suggested that the droplets could be either the accumulation of premyelin lipids or phagocytosed products of myelin degeneration, and the new observation that similar lipid accumulated in the liver and kidneys supported the second hypothesis, since lipid could have been transported from the central nervous system for excretion.

4. Immunology.

It had been observed that ewes which had produced a Border disease affected lamb produced normal lambs on subsequent challenge (Gardiner and Barlow, 1972), suggesting that exposure to the disease could lead to the development of resistance to it. Shaw, Winkler, Gibbons, Terlecki, Hebert, Patterson and Done (1969) attempted to induce acquired immunity in susceptible ewes by "vaccinating" them one month before mating, with a suspension of tissue from a Border disease affected lamb, and repeating the injection 20 days after mating. There was a significant reduction in the occurrence of the disease in the lambs born to these ewes.

That immunity could be acquired by contact was demonstrated by Gardiner and Barlow (1972). Susceptible pregnant ewes were penned with Border disease injected ewes, and produced normal lambs, both that season and in subsequent lambing seasons when they had been challenged during pregnancy with Border disease inoculum. In newborn affected lambs, too, Gardiner (1967) suggested that an immunological response might occur, since in one experiment approximately 35 per cent of affected lambs had globulin (IgG) in precolostral blood. Later this was supported by data from further experiments in which 14 out of 20 lambs had 7S λ globulin (Barlow and Gardiner, 1969). The globulin was not found in any of the controls. Although placental lesions might allow leakage of maternal serum /

serum proteins to the foetus, the maternal and foetal immuno-electrophoretic patterns were dissimilar (Gardiner, 1967) and it was suggested that the 7S λ globulin present in the precolostral blood was foetal in origin (Barlow and Gardiner, 1969). However, Patterson and Sweasey (1969) published conflicting data in which they showed that their Border disease inoculum failed to produce serum IgG in affected lambs.

A remarkable and unexpected discovery in the investigation of the immunology of Border disease was that it is antigenically similar to Bovine Viral Diarrhoea/Mucosal Disease (BVD/MD). Acland, Gard and Plant (1972) showed that examination of sera from Border disease injected lambs and pregnant ewes for the presence of BVD/MD antibodies, using the agar gel precipitation test, gave strong positive results. Acland et al suggested that the BVD/MD virus might be involved in the aetiology of Border disease. These findings were supported by the work of Hamilton and Timoney (1972).

Later, a third virus, that of Swine Fever, was also identified as having immunological similarities to Border disease and BVD/MD by Plant, Littlejohn, Gardiner, Vantsis and Huck (1973), and these authors had little doubt that the three diseases were caused by very closely related viruses.

5. Fleece changes in Border disease

An abnormal birthcoat is one of the most consistent of the signs of Border disease, both in the field (Hughes, Kershaw and Shaw, 1959; Dry, 1959, 1960, unpublished observations; Hartley and Kater, 1962; Shaw, 1962; Hamilton and Donnelly, 1970; Manktelow, Porter and Lewis, 1969) and under experimental conditions (Dickinson and Barlow, 1967; Patterson, Terlecki, Done, Sweasey and Hebert, 1971; Terlecki, Hebert and Done, 1973).

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Of these, only Shaw's (1962) account of the disease in Clun and Kerry Hill lambs gave any details of the birthcoat changes. Affected Clun lambs had an unusually long and hairy birthcoat, which was sandy-coloured, brown or black, with colour in patchy areas over the sides and back. As the lambs aged, the coloured patches often disappeared. In affected Kerries, the birthcoat seemed hairier and whiter than normal, and patches of colour sometimes occurred, usually at the back of the neck. A similar description was applied to affected lambs in California (Bell, 1967b; Osburn, Crenshaw and Jackson, 1972).

In their report of a Border disease-like condition from Ireland, Hamilton and Donnelly (1970) described affected Galway cross lambs as "hairy" with a prominent frontal "tuft" of hair, a feature which has not been noted by other workers.

Barr (1964) described the birthcoats of affected lambs of several breeds - Swaledale and Swaledale cross, Rough Fell cross, Border Leicester cross and Suffolk cross. In only the Suffolk cross lambs was the birthcoat said to be abnormally "hairy". In this report, as in others in which birthcoat changes have not been mentioned or have been "difficult to resolve", it may be that the wide range of normal birthcoat types made any abnormal hairiness difficult to detect.

The first study of the fibres of the birthcoat in Border disease was made by Nott and Shaw (1967). In natural outbreaks of the disease in Kerry flocks, affected lambs were recognised by the density and coverage of long coarse birthcoat kemps or halo hairs (Chapter II, p. 32).

Fibre-type arrays (Chapter II, p. 33) showed that a much greater proportion of the total fibre population was medullated than in normal lambs. Thus this report related the abnormal Border disease "hairiness" to an increased frequency of medullated fibres in the birthcoat.

However, the identification of fibre types and their inter-relationship is only possible by examination of skin samples, and a report on the /

the effect of Border disease on the main characters of the skin follicle population of Dorset Horn lambs was later presented by Carter, Terlecki and Shaw (1972). The advantage of Dorset Horn sheep for such work was that their fleece normally has no medullated fibres. The lambs used were those involved in the "vaccination" experiment of Shaw, Winkler, Gibbons, Terlecki, Hebert, Patterson and Done (1969), described above. In affected newborn lambs, the primary follicles produced fibres which were 27 per cent wider and approximately 36 per cent of which were medullated, and 9 of the 13 affected lambs showed definite primary fibre medullation in skin samples from the midside. The authors concluded that this was a specific effect on primary follicles, and that other characters of the follicle population were not affected to the same degree. It is interesting that in affected lambs from "vaccinated" ewes, the primary fibre medullation was present, but in fewer fibres. The authors, referring to the work of Patterson, Terlecki, Done, Sweasey and Hebert (1971) on the same lambs, suggested that there was a relationship between the aberrant birthcoat structure and a defect in lipid biosynthesis. The infective agent might attack ectodermal tissue at a time when the biochemical systems of both lipid and protein synthesis were vulnerable, although it was acknowledged that such a general effect might be expected to interfere with the development of other ectodermal tissues. The fascinating suggestion was made that the follicle abnormalities in Border disease could be considered to be "a phenocopy of certain fleece variations whose genetic basis is well established". This stimulating and often provocative paper thus indicated the value of further study of the effect of Border disease on the skin follicle population in investigation of the pathogenesis of the disease.

Patterson, Terlecki, Done, Sweasey and Hebert (1971) had suggested that the amino acid methionine might be implicated in the disease processes underlying /

underlying the skin and spinal cord abnormalities, since it is used in keratin synthesis and in the synthesis of a myelin precursor, phosphatidyl choline. This line of thought was followed up by Patterson and Sweasey (1973), who proposed that an imbalance of sulphur containing amino acids might occur in the wool of lambs affected with Border disease. This possibility was investigated by hydrolysing wool keratin and estimating the total cysteine content and total sulphydryl group content by semi-quantitative thin-layer ion exchange chromatography (Patterson, Brush, Foulkes and Sweasey, 1974). No difference in amino acid content was found between the wool keratin of experimentally produced and naturally occurring cases. The effect on such estimations of the presence of an increased number of low-sulphur medullae (Barritt and King, 1926) in the primary fibres of the experimental animals was not considered however, and so the validity of these results is in doubt.

Attempts have been made to relate the birthcoat changes to abnormal copper metabolism in affected lambs (Patterson and Sweasey, 1973). However, although experimental affected lambs were said to show a "relative hypocupraemia", and copper deficiency is known to alter the metabolism of sulphur containing amino acids (Marston, 1955), the abnormally weak wool fibres and reduced wool growth characteristic of copper deficiency in the skin are not features of the Border disease fleece.

The involvement of skin follicles in the Border disease syndrome and the accessibility of skin and wool for sampling give them considerable diagnostic potential in both live and dead lambs.

The increased occurrence of medullated fibres in the birthcoat of affected lambs, also the apparent increase in the density of the inter-fascicular glia in the central nervous system were recognised by Barlow (1972b) as being two fairly consistent features of the disease which could be quantitated. Affected Dorset Horn lambs were found to have more than

36 per cent medullated fibres and more than 266 glial nuclei per field (0.0575 sq.mm.), values which together were not reached by any of the other lambs in the experiment. These base-lines could be used diagnostically in the Dorset Horn, but it was pointed out that for general application, base-lines would have to be established for each breed, and the laborious techniques necessary would tend to make such an undertaking impractical. Furthermore, the use of wool samples rather than skin samples in Border disease diagnosis could be misleading, even in fine fleeced breeds in which the abnormal "hairiness" is very marked clinically, because although Carter, Terlecki and Shaw (1972) showed that Border disease caused an increase in primary fibre medullation which they identified in histological sections of skin, it is known that in fine fleeced breeds such as the Dorset Horn, the secondary fibres can be larger than the primary fibres and may be medullated (Ryder and Stephenson, 1968). Since primary and secondary fibres cannot be accurately distinguished in wool samples, it would be wrong to attribute the presence of medullated fibres in a wool sample to the effects of Border disease.

Confusion between the Border disease birthcoat and that of coarse fleeced breeds is evident in the literature. This confusion can only be resolved by definition of the lesion at skin level in breeds of different fleece types, thereby providing a possible basis for a diagnostic test. As has been suggested in the literature, such a study could also contribute to an understanding of the factors involved in the differentiation of foetal tissues, in particular the dermal-epidermal interactions in the skin.

There are, however, more fundamental aspects of the skin lesion in Border disease which have been neglected, including its pathogenesis, its permanency, and the effect of infection at different stages of gestation. Their investigation is fundamental to an understanding of the Border disease process as a whole.

CHAPTER II.

Comprehensive literature review of skin follicles of sheep and wool growth

A Sheep skin, wool follicles and fibres

1. Sheep skin

Kozlowski and Calhoun (1969) made a comprehensive review of the structure of sheep skin. They showed that the epidermis on the fleece-bearing surface was thin, ranging from 27 μm in a Shropshire ewe to 42 μm in a Merino ram, and the thickness of the skin, (epidermis and dermis) was approximately 2.7 mm. The rete pegs of the epidermis were very shallow. Of the two dermal layers, the more superficial papillary layer, which was found by Dempsey (1948) to be relatively thicker in the sheep than in other mammals, contained a fine meshwork of elastic fibres. In the deeper reticular layer there were fewer elastic fibres. The mesh of elastic fibres was closer and denser beneath the epidermis and around the follicles. The dermal collagen fibre distribution described by Mikhailova (1958) was quoted by Kozlowski and Calhoun (1969). The horizontal undulating bundles of collagen fibres in the reticular layer of the dermis of the young lamb were replaced by inter-twining horizontal and diagonal bundles running in different directions after about five months of age.

The cell population of the dermis consists largely of fibrocytes, Selye (1965) and Kozlowski and Calhoun (1969) also noted the presence of mast cells between follicle groups and along the walls of small dermal blood vessels, and Margolena (1963) was quoted by Kozlowski and Calhoun (1969) as counting 1 to 35 mast cells per sq.mm. of histological section of dermis in her study of a Columbia-Southdale lamb. Apart from fibrocytes and mast cells, other fully differentiated cell types which have been observed in the normal dermis are histocytes or fixed macrophages, plasma cells and small numbers of lymphocytes and polymorph neutrophils (neutrophils) (Kelsall and Crabb, 1959; Draper and Chalmers, 1968).

Networks /

Networks of blood vessels in the skin were shown by Ryder, (1956a) to occur at three levels, and he named these the inner or dermal, middle or mid-dermal and outer or sub-epidermal networks.

The innervation of the skin of the sheep was described by McEwan Jenkinson and Blackburn (1967). Large nerves in the reticular layer of the dermis branched to supply the cutaneous blood vessels, the epidermis, the sebaceous glands and erector muscles and formed nerve palisade endings around primary and secondary hair follicles. The hair palisade endings, it was suggested, might have a neuro-secretory as well as a sensory function.

2. Wool follicles

Wildman and Carter (1939) recognised primary follicles, formed first in the foetus, and secondary follicles. Primary follicles are characterised by the possession of a sweat gland, a bi-lobed sebaceous gland and an erector muscle (Diagram 1). The secondary follicles usually have a single lobed sebaceous gland but neither a sweat gland nor erector muscle (Diagram 2).

The sweat gland of primary follicles was described by Ryder and Stephenson (1968) as being of the apocrine type, lying on that side of the follicle towards which the follicle sloped (the ental side) at about the level of the follicle bulb, the duct opening into the follicle neck. 'Ectal' and 'ental' refer to directions away from and towards the slope of the follicle respectively.

The sebaceous glands are of the holocrine type. Their twin lobes lie on the ental side of the primary follicle, on either side of the sweat duct, and they open into the follicle neck beneath the sweat gland orifice.

The primary follicle erector muscle lies obliquely in the dermis, on /

on the ental side of the follicle and extends from its origin beneath the epidermis to its insertion in the follicle wall above the follicle bulb. Dempsey (1948) described its ramifications with elastic fibres at its origin and insertion.

The fine structure of the wool follicle was studied in detail by Auber (1952), and in more recent years, electron microscopy has contributed to our knowledge of the ultrastructure of the follicle of the guinea pig (Parakkal and Matoltsy, 1964), mouse (Parakkal, 1967), man (Birbeck and Mercer, 1957; Ballin and Happey, 1965) and sheep (Chapman and Gemmell, 1971).

Each follicle has an inner root sheath invested by an outer root sheath, which merge at the base of the follicle in the follicle bulb. The follicle bulb contains the follicle papilla, and from the bulb, the fibre grows up into the hair canal (Diagram 1).

Auber (1952) adopted the terms 'proximal' and 'distal' to refer to directions towards and away from the skin surface, and 'axial' and 'peripheral' to refer to directions towards and away from the follicle axis. These terms will be used below.

Auber (1952) described the <u>follicle bulb</u> as being usually deflected from the main axis of the follicle. The cells overlying the papilla and in the proximal bulb are undifferentiated, and have characteristically little cytoplasm with a large round nucleus generally containing several nucleoli. Mitoses occur frequently and in pigmented fibres, ramified melanocytes containing small, dark-brown granules are interspersed between the basal cells. Ultrastructurally, the basal cells have been shown to be columnar, and attached at one end to the basement membrane of the papilla (Birbeck and Mercer, 1957). The undifferentiated cells of the bulb have very many ribosomes and a moderate /

moderate number of mitochondria. Profiles of rough surfaced endoplasmic reticulum occur rarely, but a Golgi complex (consisting of tubules and agranular vesicles) is present (Parakkal and Matoltsy, 1964). At scattered points, the cell membranes are specialised into desmosomes, with short filaments extending into the cytoplasm (Parakkal, 1967). In the most central portion of the undifferentiated matrix of large bulbs, lie the presumptive medullary cells (Parakkal and Matoltsy, 1964). These develop relatively more cytoplasm, and in this, amorphous granules appear.

As the proliferating cells move outwards, they begin to differentiate, and form five or six concentric cylinders (Auber, 1952). In peripheroaxial order, these are cells continuous with the outer root sheath, presumptive cells of the Henle, Huxley and cuticle layers of the inner root sheath and cells of the cuticle and cortex of the fibre. In large bulbs there is a central core of presumptive medullary cells. At the boundary between the bulb and papilla is a well-defined basement membrane (Parakkal, 1967).

The shape of the follicle papilla varies with bulb size. Auber (1952) noted that small follicle bulbs, producing fine non-medullated fibres have slender papillae, whereas larger follicle bulbs, producing coarser fibres, have broad, oval or pear-shaped papillae.

Auber (1952) observed that the papillary fibrocytes like those of dermal connective tissue, are small and compact with an irregular outline. Ultrastructurally, Parakkal (1967) described the fibrocytes as having a well developed rough surfaced endoplasmic reticulum, with a large prominent Golgi complex situated near the nucleus. Reports of papillary cells other than fibrocytes are few, but mast cells have been observed in the papillae of active hair follicles in man, (Selye, 1965).

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The outer root sheath extends up around the more central layers of the follicle to become continuous with the epidermis of the skin surface (Diagram 1). In the mid-third of the follicle, Auber (1952) described the outer sheath as being usually thicker on the ental side of the follicle. The cells are irregular in shape, and the cytoplasm of the most peripheral cells often contains numerous small vacuoles. The nuclei of these cells are dislocated axially. Ryder (1958) in the sheep, and later Parakkal (1967) in the mouse, noted that these cells contain large accumulations of glycogen particles. More distally, the nuclei of the basal layer again become central (Auber, 1952). Having observed mitoses at all levels, although infrequently, (Auber, 1952) proposed that upward movement of the outer root sheath was slight. Straile (1962) also suggested that cells of the outer root sheath migrated very slowly towards the skin surface in the Merino, to be sloughed off to the follicle neck from the zone of corrugations below the sebaceous gland duct orifice.

The inner root sheath is a highly differentiated part of the follicle wall, comprising, in peripheral-axial order, Henle's layer, Huxley's layer and cuticle (Diagram 1). These layers differentiate in the same sequence of phases, however the levels at which the changes occur differ in each layer. Auber (1952) described the characteristics of inner root sheath differentiation as follows. As the cells move distally from the bulb, small droplets of trichoyalin appear, which gradually increase in size and number as the cells move distally, but disappear as the cells became homogenous in the prekeratinisation zone, Finally, the nuclei become flattened and smaller as the cells keratinise, show greater refringency and fuse, continuing distally as corrugations in the neck of the follicle.

In Henle's layer, the cells beoome keratinised just above the bulb. (Diagram 1) whereas in Huxley's layer and the inner root sheath cuticle, keratinisation /

keratinisation occurs relatively higher in the follicle. Straile (1965) suggested that the inner root sheath disappearance at its distal limit was caused by enzymatic action, perhaps from the outer root sheath. He considered that the inner root sheath and the hair moved distally together, with Henle's layer moving over the almost static outer root sheath, and suggested that the function of the inner root sheath was to provide support and attachment for the keratinising hair fibre.

3. Fibres

The nature of this thesis demands a definition of fibre types. In the adult fleece there may be kemps, hairs and wool fibres, and these have been described by Ryder and Stephenson (1968). Kemps, the coarsest fibres with diameters of 100 μm or over, are short, since they grow for a few months only before being shed, and they often become flattened or ribbon-shaped. They have very wide medullae, usually latticed containing air-filled spaces.

Hair fibres (also known as coarse wool fibres or hetero-types) grow for longer than kemps, and are usually medullated in summer, non-medullated in winter. The medullae can be latticed but are usually narrower than those of kemps. Their diameter varies from 50 μm to 100 μm approximately.

Wool fibres are usually tightly crimped or waved, and non-medullated. They are usually approximately 15 μm to 40 μm wide. Some of the coarser wool fibres have a small medulla, but this is narrow and non-latticed and often interrupted. The frequency of the fibre crimp increases as fibre diameter decreases.

Auber (1952) described the histology of these fibres during keratinisation. The cuticle cells undergo changes in shape reciprocal to those /

those of the root sheath cuticle cells, so that a projection from each cell extends periphero-distally from the cell (Diagram 1).

As the differentiating cells of the cortex move distally from the undifferentiated region of the bulb, fine fibrillae orientated vertically appear. The cells become elongated and the fibrillae form small bundles which eventually become rod-like refringent structures.

In heavily medullated fibres with large papillae from a Herdwick sheep, Auber (1952) noted that the cellular elongation occurred at a lower level in the bulb than in non-medullated fibres from the same sheep, and extended distally from approximately one-fifth or one-sixth of the follicle's length compared with one-third of the follicle's length in the non-medullated fibres. However, the level at which keratinisation was complete was found by Auber to be constant at just over one-third of the follicle's length from its base. Thus the length of the pre-keratinisation region was relatively greater in heavily medullated than in non-medullated fibres. Since few fibres from each breed were examined, these results may not be significant.

To Auber, the fully keratinised cortex appeared homogenous, but ultrastructural studies later showed that it comprised filaments embedded in an amorphous matrix (Birbeck and Mercer, 1957; Parakkal, 1967).

In follicles producing fine fibres, keratinisation began nearest to the thinnest point of the follicle wall, the ectal side, before spreading across the fibre. This Auber called 'segmental' keratinisation. In the larger follicles with a central fibre, keratinisation spread from the periphery of the cortex towards the central axis - 'periphero-axial' keratinisation. Horio and Kondo (1953) first described the relation between segmental keratinisation and the formation of two types of cortex in crimped fibres - ortho and para- cortex. From this and subsequent work /

work, Ryder and Stelphenson (1968) concluded that two main factors could lead to crimp formation. The centre of cell division in the bulb could be deflected, causing the stream of cells which was first ready for keratinisation to be on one side. Secondly, the rotation of these segments around the fibre could occur, and the differential keratinisation between ortho- and para- cortex which resulted could lead to crimp formation. These observations supersede Auber's postulate that keratinisation always begins first on the ectal side of the fibre.

The structure of cortical keratin was uncertain until Mercer (1953) and later Birbeck and Mercer (1957) found, in chemical and ultrastructural studies respectively that keratin was composed of a fibrillar low sulphur fraction (α keratin) and an amorphous high sulphur fraction (γ keratin) which formed a cementing matrix in which the fibrils were embedded. Mercer (1953) noted that the ortho-cortex was more chemically reactive and contained less sulphur than the para-cortex. However, Ryder (1958a) could not demonstrate any difference in sulphur content between the ortho- and para - cortex and later (Ryder, 1963) suggested that any difference must be small.

Under some circumstances, copper deficiency can be associated with the production of abnormal wool. Such wool is straight and fragile, and is known colloquially as "steely wool" (Marston, 1955). Marston found that the keratogenous zone of the fibres of such sheep was abnormally long, and this he attributed to a deficiency of copper ions or a copper-containing enzyme involved in the oxidation of free sulphydryl groups to disulphide bonds during keratinisation. Later, however, Ryder (personal communication) using the Mercury Orange method for the demonstration of free sulphydryl groups, was unable to confirm Marston's observations. Thus although it seems to be generally accepted that the keratinisation process in the development of "steely wool" is abnormal, the nature of the abnormality is not known.

Energy /

Energy for the chemical processes involved in keratinisation was said to be supplied by glucose in the blood stream, or by the glycogen of the outer root sheath cells of active hair follicles (Montagna, Chase and Lobitz, 1952; Ryder, 1958).

Little research has been undertaken to investigate the role of lipids in keratinisation of hair, but Montagna (1950) and Montagna, Chase and Hamilton (1951) found small sudanophilic granules arranged peri-nuclearly or at the poles of the nucleus of the outer root sheath cells, but their nature and function were unknown.

Medullae are not found in fibres of less than about 40 µm diameter in Herdwick sheep (Auber, 1952). In the largest fibres, of diameter 206 µm, the medullae width was 193 µm. In Romney sheep, fibres were smaller but medullation tended to appear at the same fibre diameter as in the Herdwick. Ryder and Stephenson (1968) showed that this minimum fibre diameter for medullation varied between breeds, although the medulla width consistently became relatively greater with increasing fibre diameter. The largest medullae contained many air-filled spaces, and were named latticed medullae by Auber (1952). In the differentiation of medullae, Auber observed that trichohyalin droplets appeared in the cytoplasm of medullary cells a short distance above the bulb. Further distally, small vacuoles also appeared in the cell cytoplasm. These enlarged as the cells moved distally. The trichohyalin granules disappeared below the level of cortical keratinisation and filaments appeared around the vacuoles. The vacuoles enlarged and the filaments formed strands across the medullary width, extending vertically from one nucleus to another. The smallest medullae tended to be interrupted with narrow fusiform cells. Slightly larger medullae of 15 µm diameter or more had wedge-shaped cells in an alternating arrangement. As the medullary width increased, the orientation of the strands /

strands became more horizontal and the medullae acquired their latticed appearance as cytoplasmic spaces developed and formed a system of interconnected air-filled spaces. The most heavily medullated fibres were often very bilaterally compressed or ribbon-shaped. The medullary strands fused so that this shape was retained, but in the proximal third of the follicle, the follicle and fibre had a round cross-section. Ultrastructurally, Parakkal (1967) noted that the first sign of differentiation of the medulla in the mouse was an increase of rough-surfaced endoplasmic reticulum and increased prominence of the Golgi elements. Although Auber (1952) described the medullary granules as trichohyalin, Parakkal (1967), observing that they consisted entirely of an amorphous substance, unlike the filament-matrix structure of the inner root sheath trichohyalin, preferred to call them "medullary granules".

Auber (1952) observed melanocytes between the medullary cells in the basal cell layer of the bulb of pigmented fibres, and observed that the cytoplasm of medullary cells could contain melanin granules. Possible causes of medullation were considered by King and Nichols (1933) who suggested that lack of "sulphur" in the blood supply to the papilla tip could cause medulla formation. Auber (1952) also thought that the genetically determined capillary arrangement in large follicle papillae by providing an inadequate supply of "sulphur" to the papilla tip could lead to medulla formation. However Ryder (1956b), by the injection of labelled cystine into a lamb, deduced that there was as much sulphur in the medulla as in the cortex. In his studies on blood supply to the wool follicle Ryder (1956a) found that large papillae were supplied by a straight vertical vessel rising from the base of the papilla to its tip where it divided into several branches. These followed the surface of the papilla down to its base, where they united into a single large drainage vessel. This observation makes the earlier theories of medulla formation less plausible, but the processes underlying medulla formation remain obscure.

B The follicle group and breed differences.

The earliest studies of hair follicles made by German scientists in the last half of the nineteenth century failed to reveal any particular arrangement of the follicles in the skin. J.C.H. de Meijere (1894) first noted the arrangement of the skin follicles of sheep in threes or multiples of three. Duerden and Ritchie (1924), Wildman (1932) and Duerden (1939) later confirmed the observation that primary follicles of sheep were usually arranged in groups of three. Carter (1943) described in full the arrangement of the secondary follicles around the primary follicle trio, and showed that the trios tended to show the same orientation in any region of the body. In all groups the primary follicles were arranged along one side, sloping away from a variable number of secondary follicles arranged behind them (Figure 2). Ryder and Stephenson (1968) described the usual arrangement of these groups in rows in the skin, and pointed out that some groups comprised two or even one primary follicle with related secondary follicles. They also discussed the marked variation between and within breeds of sheep in the relative number of primary and secondary follicles, in their relative sizes and in their arrangement within the group.

In the primitive wild sheep, the central primary and its associated lateral primaries are very large (Ryder, 1960), and grow coarse heavily medullated kemps which form the outer coat and are shed in spring and, sometimes, autumn. The associated secondary follicles are small and lie between the primaries. They grow fine, non-medullated wool fibres, which are shed only in spring during the general seasonal moult of the whole coat, and which form the fine, dense inner coat of these breeds. The double-coated domestic breeds show a follicle group pattern and fibre types similar to the wild sheep. Such double-coated breeds are the British Mountain breeds (Scottish Blackface /

Blackface, Herdwick, Swaledale and some Welsh Mountain strains), the N type variant of the New Zealand Romney, and the double-coated breeds of India, North Africa and Middle East (Ryder and Stephenson, 1968). In these domestic sheep, however, many of the fibres are long heterotypes which grow for longer periods than the kemps of wild sheep. The primary follicles of the carpet-fleeced British Mountain breeds grow long coarse hairy fibres, and the secondary follicles produce shorter, finer fibres which are sometimes medullated. In the New Zealand Romney N-type birthcoat there appeared to be a tendency for coarse medullation in the primary follicle fibres to be associated with a great degree of medullation in the secondary follicle fibres (Ross, 1951) and a similar association was found in comparisons between the different Indian breeds (Narayan, 1960). The follicle group in the double-coated breeds also differs from that of primitive breeds in that the secondary follicles tend to move from their place of initiation between the primaries towards the ectal margin of the follicle group (Ryder and Stephenson, 1968).

In the long wool and short wool breeds, the main difference from double-coated breeds is a further reduction in the relative coarseness and hairiness of the primary fibres, so that the primaries and secondaries both grow fibres of an approximately similar length and diameter. The $\frac{S}{P}$ fibre ratio (the ratio of secondary to primary fibres in the skin) is higher for the long and short wool breeds than for double-coated breeds, and this Fraser and Hamada (1952) suggested could be due to a decrease in competition from the primary follicles. The tendency of the early formed secondaries to move to the ectal margin was more pronounced than in the double-coated breeds.

The main difference between a long wool breed and a short wool breed is that the former shows an increase in the growth and diameter of fibres /

fibres from all follicles compared with the latter. Thus the short-wools produce a shorter, finer fleece of lighter weight than the long-wools. However, the relative diameters of the primary and secondary follicles, and the $\frac{S}{P}$ fibre ratios are similar in the two groups.

C Birthcoat fibres and shedding

Since many follicles in sheep begin to grow fibres before the lamb is born, newborn lambs have a well-developed birthcoat. In some breeds the lambs have a coarse hairy coat while others have a fine woolly coat formed into tight coils. Between these extremes there is a gradation of hairiness which was described by Fraser (1951), using a twelve grade scale. The anterior parts of the body, especially the side of the neck and behind the elbow, tend to have finer wool, and there is a gradient of increasing coarseness from neck, shoulders and side to the quarters and dorsum. Duerden and Seale (1927) first described different fibre types in the birthcoat. These authors observed in the South African Merino birthcoat the presence of fibres with medullated sickle-shaped tips which became finer for the remaining part grown before birth. After birth, these fibres again became coarser. Later, Duerden (1932) found this fibre type in many breeds of sheep. Dry (1933, 1934), in his work on the New Zealand Romney, first described the birthcoat fibre types. Dry's description of these was later illustrated by Stephenson (1956). The birthcoat fibres are divided into three broad categories according to the shape of their fibre tip, i.e. (a) pre-curly tips,

(b) curly tips, and

(c) histerotrichs (Diagram 3)

a) The pre-curly tips have three separate regions along the fibre. First, a pre-natal coarse tip region; second, a pre-natal curly region ("neck") which is finer than the tip, and strongly crimped; and third, a post-natal coarser region. The relatively coarse and straight tip region terminates in a fine point. This tip may be short and represented by a coarse, straight sickle tip at the distal end of the fibre (as in the super sickle and sickle series of fibres) or it may be long, curved and /

and strongly medullated as in halo-hairs. The post-natal portion of the fibre is coarser and has wider crimps than the pre-natal curly region and, is more frequently medullated. Fibres of this group, beginning with the earliest formed, are termed halo-hairs, super sickle and sickle fibres.

b) The curly tip fibres are thought to be the second type of fibres to be formed, and these lack a coarse tip, having only two regions - a fine pre-natal curly tip resembling the middle region of the pre-curly tip fibres and a coarser, less curly post-natal region frequently medullated. They therefore resemble the pre-curly tip fibres except that they lack the coarse sickle-shaped tip. Fibres of this group, beginning with the earliest formed are hairy-tip curly tip, checked curly tip, medullated curly tip and non-medullated curly tip fibres.

c) The histerotrichs are the shortest and finest fibres of the birth-coat, and usually lack a medulla, but they can be medullated in some hairy double-coated sheep (Ross, 1951). They appear above the skin about the time of birth and later (Goot, 1945).

Dry (1933) arranged the fibres from a lock of wool from the birth-coat in what he considered to be their order of appearance on the foetus, to form a "fibre type array". To do so, he assumed that within the pre-curly tip type the first formed fibres were those with the longest pre-curly tip region and that the first formed fibres within the curly tip type were those with the greatest number of curls in the tip. Dry divided fibre type arrays into five main sections, according to the presence or absence of, and the relative numbers of different fibre types within the pre-curly tip and early curly tip groups. He called these arrays Plain, Valley, Ravine, Saddle and Plateau, so that through this series there was a progressive increase in the medullation of pre-curly tip and early developing curly tip fibres. /

fibres. The fibre type array was considered by Burns (1954a) to be a useful indication of the adult fleece type, since the coarser birthcoats (Plateau, Saddle) developed into adult fleeces in which the primary fibre diameter: secondary fibre diameter ratio was high, and the finer birthcoat types (Plain, Valley) developed into adult fleeces in which the fibre diameters were more uniform.

It has long been known that hair and wool growth occur in cycles in which periods of active growth alternate with periods of rest. The active phase (anagen) in which the fibre is grown, is followed by a retrogressive phase (catagen) lasting for approximately two days, in which fibre production stops and the follicle shrinks in size. The proximal end of the fibre keratinises to form a "brush end" lying within the follicle. Telogen is the phase of follicle inactivity, and the fibre is shed at the end of this phase, when the new fibre produced at the onset of the next anagen grows (Ryder and Stephenson, 1968). The anatomy of the active follicle (in anagen) has been described above. With the onset of catagen, the morphology of the fibre changes, it becomes narrower with loss of the medulla and any pigmentation. The living root of the fibre keratinises and the follicle shortens to less than half its normal length, so that in telegen the brush end lies at the level of attachment of the erector muscle. Ryder (1964) comments that the erector muscle seems to play no part in the shortening process, and that it is not instigated by poor nutrition. The nuclei of the basal layer of the outer root sheath become orientated with their long axes towards the fibre during catagen and telogen. A distinctive feature beneath the shortened follicle is an epithelial "stalk", the remains of the lower part of the outer root sheath, which lies beneath the brush. The papilla and bulb are reduced in size, and the /

the inner root sheath appears to be lost towards the end of catagen. Auber and Burns (1947) stated that, in some domestic breeds there was often a time lag before shed fibres were replaced, but Ryder (1960) showed that in wild sheep, replacement of the old fibre before it shed was normal. Moulting, a mechanism for providing different coats for summer and winter, seems to be the primitive basis for seasonal variation in wool. In wild sheep, such as the Mouflon, Ryder (1960) showed that the whole fleece was cast each spring. In contrast, the highly evolved Merino sheep has almost continuous wool growth. Although most domestic breeds do not have a true moult, many show considerable shedding.

The first shedding is that of the birthcoat, which is lost between one and five months of age (Slee, 1963). Dry (1933) thought that the tendency towards continuous growth in domestic sheep with fine fleeces was a result of the pre-natal check so that they did not shed, and that their birthcoats, comprising mostly curly tips and histerotrichs, continued to grow into the adult fleece as wool fibres. In breeds with a coarser birthcoat, the halo hairs and super-sickles were usually replaced by kemps and heterotypes while the sickles and curly tip fibres became narrower. Slee (1963) showed that the hairiness of the birthcoat did not affect shedding, nor did follicle density or $\frac{S}{P}$ ratio. However, he suggested that there could be some genetic variation, since twins behaved more alike than half-siblings. Nutritional factors seemed to be of importance in the timing of shedding. Well-fed lambs, gaining weight, shed earlier. There was also a synchronisation in the timing of shedding in lambs born at different times, except for very early or very late lambs, which usually shed earlier. Slee suggested that an environmental factor, possibly day length, and age interacted in the control of shedding. Later, Slee (1965) found

that /

that lambs kept indoors under constant dim artificial lighting showed retarded moulting. Doney and Smith (1964) showed that poor nutrition in Scottish Blackface lambs delayed birthcoat shedding.

The control of fibre shedding was discussed by Slee and Carter (1962), who postulated three systems of control: 1) innate systemic control; 2) innate local control by the follicle; and 3) environmental control. Kemp fibres, they suggested, were under a system of local control in which the fibres grew for fixed periods of time (approximately three months) whereas hair and wool were under systemic and environmental control, and these factors are discussed below.

D. Pre-natal follicle development

Various features in the development of wool follicles in pre-natal life have been described by Spottel and Tänzer (1923), Duerden and Ritchie (1924), Tänzer (1926), Wildman (1932) and Carter (1943).

Hardy (1949) described how the development of the mammalian hair follicle could be divided into eight stages, and these are illustrated in Diagram 4, and defined as follows:

Stage F1: A localised thickening of the basal layer of the epidermis or periderm occurs above an aggregation of dermal cells, the prepapilla. From the epidermal thickening, a plug of cells grows down into the dermis or primitive mesoderm.

Stage F2: The base of the epidermal plug flattens.

Stage F3: The base of the plug becomes invaginated over the dermal papilla.

Stage F4: The elongated cells of Henle's layer of the inner root sheath form a cone directed along the axis of the follicle, and lying in the proximal part of the follicle.

Stage F5: The tip of the hair cone reaches the level of the sebaceous glands.

Stage F6: The tip of a keratinised hair fibre appears within the hair cone.

Stage F7: The tip of the keratinised hair reaches beyond the base of the epidermis and lies in the hair canal.

Stage F8: The hair fibre penetrates the skin.

These developmental changes apply to primary and secondary follicles, and they were extended by Hardy and Lyne (1956) for primary follicles.

Stages F2 and F3 were sub-divided as follows:

Stage /

Stage F2a: The base of the epidermal plug flattens when it is relatively short and the sweat gland plug appears on the ental side of the follicle.

Stage F2b: The sebaceous bud glands appear, one on each side of the sweat gland duct.

Stage F3a: The length of the dermal papilla is less than its width.

Stage F3b: The length of the dermal papilla is more than its width. The erector muscle appears.

Hardy and Lyne also noted an ental swelling of the outer root sheath, also observed by Duerden and Ritchie (1924) and Auber (1952), in primary follicles at about Stage F4.

Secondary follicle development is known to differ from that of primary follicles in several minor details (Spottel and Tänzer, 1923), and these were described fully by Hardy and Lyne (1956). The secondary follicles were usually longer and narrower than primary follicles before reaching Stage F2, and since no sweat gland developed this stage was not sub-divided. From Stage F3b new secondary follicles sometimes arose by branching from the neck region of established secondary follicles. This secondary follicle branching was also observed by Tänzer (1926) in the Karakkul, and Orwin (1961) suggested that secondary follicle branching occasionally occurred in the New Zealand Romney. Auber and Ryder's (1956) description of compound follicles and Ryder's (1959) description of some unusual outgrowths from the secondary follicles of Soay sheep suggest that budding may also occur in these breeds. Branching could occur by bifurcation or by lateral branching and occurred from the neck region of the established follicle at or just below sebaceous gland level (Hardy and Lyne, 1956). In other respects, the development of derived and established secondary follicles was similar.

The /

The ultrastructure of differentiating mammalian follicles is not well documented. However, Bell (1967) described some of the ultrastructural features of differentiating hair follicles in the Rhesus monkey. At a stage corresponding to Hardy and Lyne's (1956) F3, the bulb cells were fairly homogenous, although some adjacent to the basal lamina were particularly electron-dense. Epithelial products, such as filaments, were sparse. With further differentiation, the cells of the presumptive outer sheath developed clumps of amorphous material and rosettes of glycogen could be seen. By the stage at which growing hairs had reached the skin surface, the basal cells of the bulb were more electron dense, with few filaments but with the desmosomes characteristic of epithelial cells.

Ryder (1956) showed that capillary networks began to form around primary follicles by approximately 100 days' gestation, and capillaries invaded the papilla of the larger follicles of advanced foetuses when fibres were being produced.

Studies of the development of the follicle group began in 1924, when Duerden and Ritchie (1924) first suggested that the follicles might be grouped around primary follicle trios in the early stages of follicle development. In 1932, Wildman confirmed that this arrangement existed during follicle initiation. During the thirties a number of scientists studied the follicle group, and Galpin (1935) found a trio stage of follicle development followed by a "nine" stage. This may have been due to a confusion of developing secondaries with primaries, since it has not been observed by other workers. However, Galpin and later Carter (1943) and Ruttle and Sorensen (1965) gave the ages at which the different follicle types appeared in the fleece-bearing skin of the foetal sheep. Isolated central primary follicles appeared over most of the body at about /

about 56 to 60 days' gestation, and trio formation was complete at about 72 days' gestation.

The pattern of development of follicle groups was described in detail by Hardy and Lyne (1956) in their work in the Merino and its variation over different parts of the body was described by Stephenson (1957) in his work on the New Zealand Romney. The following account is based on these two works.

Follicle development begins with the appearance of central primary follicle plugs, fairly equally spaced. They appear first on the head and limbs, followed by the neck, shoulder, flank, dorsum, and finally over the rest of the body, between 54 and 63 days of foetal age in the New Zealand Romney. This "wave" of development occurs with each succeeding phase of development (Stephenson, 1957). Hardy and Lyne (1956) identified two types of primary follicle in the early stages of development: the primary X and primary Y follicles. These were distinguished by the earlier appearance and larger size of the former compared with the latter. By about 76 days, the development of trios and hence the initiation of primary follicles was complete (Hardy and Lyne, 1956). Stephenson (1957) found that primary follicle initiation had ceased by 90 to 99 days in the New Zealand Romney.

Secondary follicle plugs first appear between central and lateral primary follicles on their ectal side between 80 and 90 days in the New Zealand Romney (Stephenson, 1957) and at 86 days in the Merino (Hardy and Lyne, 1956). The first formed secondary follicles slowly move towards the ectal margin of the group defining that margin, and later-developing secondary follicles develop between the secondary and primary follicles already present. In the Merino, most of the later-developing secondary follicles develop by branching from established secondary follicles as described /

described above. There is much secondary follicle branching by 102 days' gestation, and secondary follicles continue to be initiated in this way almost to term.

Concensus of opinion suggests that in most breeds, secondary follicles are initiated by 135 days' gestation, and the remainder before birth (Stephenson, 1957; Carter and Hardy, 1947; Schinckel, 1955 a andb; Short, 1955), although Burns (1953) considered that, in the Scottish Blackface, secondary follicles could be initiated up to several weeks after birth. In the Merino, the earliest-developing secondary follicles were producing fibres above the skin surface by 133 days' gestation (Hardy and Lyne, 1956), and all secondary follicles were producing emergent fibres by 4 or 5 months of age (Fraser, 1954; Schinckel, 1955), although in the Romney all secondary follicles were at this stage by 30 days of age (Fraser, 1954).

In studies of secondary follicle development, the terms $\frac{S}{P}$ follicle and $\frac{S}{P}$ fibre ratio have been used to refer to the ratios of the number of secondary follicles to the number of primary follicles in a given area and the number of fibre-producing secondary follicles to the number of primary follicles in a given area respectively. This terminology will be used in the text below.

Dry (1933) postulated that a pre-natal check to fibre growth caused the narrowing of the pre-curly-tips and curly-tips in the region grown immediately before birth (Diagram 3). However, he did not discuss the possible mechanisms by which the pre-natal check could operate. Galpin (1935) suggested that variations of skin expansion rate might affect follicle density during the pre-natal differentiation of follicles, and so permanently affect the vigour of growth of their fibres. Stephenson (1957), in his work on the N-type Romney, found that although there was

no /

no difference in follicle density between normal and N-type Romney foetuses, the former had fine birthcoats with a strong pre-natal check, and the latter had coarse birthcoats, with a weak pre-natal check. Fraser (1952) suggested that the pre-natal check was caused by competition for substrate from neighbouring secondary follicles when the primary follicles started to produce fibres. He suggested that the shape of the birthcoat fibres could best be explained by different intensities of competition at the time of follicle formation and maturation of the early secondaries and second-wave secondary follicles. In an extension of this idea, Fraser and Short (1960) proposed that the cause of the pre-natal check should be sought in the differentiation of primary follicles.

Side and Rudall (1964) made some quantitative studies of fibre growth rates in relation to the pre-natal check. They found that there were two periods during intra-uterine life when fibre growth rates were checked. The first check occurred at the time when the neck regions of the pre-curly-tips were being formed, and it was mostly associated with this type of fibre. The second check found mainly in halo-hairs and the coarser curly-tip fibres from the britch, occurred later after about 130 days' gestation. The authors considered that this second check might not be significant since a reduction in fibre growth rate prior to birth was not unexpected, and evidence of peri-natal depression had been found by Duerden and Boyd (1930) in the Blackhead Persian sheep. A pronounced thinning occurred in the coarsest birthcoat fibres of this breed near the time of birth, and this appeared to have no lasting effects. The first pre-natal check appeared to correspond to that described by Dry (1933), and it varied in expression from one part of the body to another. Of the three areas studied, the "shoulder patch" showed greatest effect of the check, the britch area showed very little effect

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of the check, the back being intermediate (Side and Rudall, 1964). Dry's (1933) idea that the degree to which a fibre was affected was determined by its "age" at the time of onset of the check, was not supported by Side and Rudall (1964), since they found that the various pre-curly-tips appeared to start growth at the same time on any one position, but that halo-hairs were less affected than fibres of the sickle group. These authors grouped the theories of the cause of the pre-natal check into two classes: (1) those implicating a systemic, possibly endocrine, change, which occurred at the relevant phase of gestation, and (2) those which argued that factors localised in the skin gave rise to the "check". The latter theories included those of Galpin (1935) and later Fraser (1951b, 1952). Side and Rudall's (1964) own results, in which the pre-natal check occurred earlier in the anterior parts of the body, also suggested that systemic effects did not have an important role in its causation. In addition, Rudall and Wickham (1965) suggested that systemic factors were unlikely causes of "check" when they took some fleece-bearing skin from a foetal lamb and replaced it as an autograft after birth. In the graft, initiation of new follicles occurred and sickle fibres were found when the birthcoat began to grow, showing that a check to fibre growth had occurred, but after birth. The possibility that this check was related to impaired follicle productivity following transplantation was not discussed, however.

Burns' (1966) described the pre-natal check as a reduction in the potential of primary fibres to produce medullated fibres which occurred some time before birth, the intensity and duration of its effect varying from one breed to another. In her study of the follicular origin of Merino birthcoat fibres, Burns stated that the pre-natal check caused a reduction in follicle volume which was the result most often of a greater reduction in follicle bulb diameter than follicle length, although /

although both were affected. Diomidova (1961) also found that the follicle bulb diameter increased in the Merino foetus, and at about 105 days' gestation. She noted that in follicles that were about to shed, papilla and bulb size were also reduced. The possibility that, in foetuses, the reduction in follicle size observed by Diomidova (1961) and the smaller size of follicles containing checked fibres, were due to the approach of the shedding (telogen) phase was considered by Burns (1966). However, since the formation of brush ends did not occur in the checked follicles, the approach of telogen did not seem to be the cause of the reduction in follicle size.

Dry's (1933, 1934) early descriptions of the effect of the pre-natal check were simplified by Burns (1966), who explained the different birth-coat structures of different breeds in terms of an interaction between an inherent potential for medullation or "base" of medullation and the pre-natal check. In coarse fleeced breeds, the "base" was strong and the check weak or perhaps late acting, so that a coarse birthcoat developed. In fine fleeced breeds the "base" was weak and the check acted earlier, so that a fine birthcoat developed. However, the mode of action of the pre-natal check remains to be resolved in physiological and biochemical terms.

Histochemical studies of foetal sheep skin have been few. Montagna (1956) showed that there was considerable storage of glycogen in the skin of man and other animals during the first half of foetal life. The epidermis, he claimed, stored glycogen until it differentiated to stratified squamous epithelium when glycogen storage was taken up by the liver. Pinkus (1972), in work on sheep foetuses, confirmed that, in foetuses of "intermediate" age, glycogen packed the epidermal basal cells and /

and that this was not present in "advanced" foetuses. He also showed that the cells of the early "hair germ" were packed with glycogen, which was lost and later re-acquired by the cells of the outer root sheath in "intermediate" and "advanced" foetuses. Pinkus also stated that collagen fibrils were demonstrable around the follicles of advanced foetuses, but elastic fibres were seen only in late gestation.

E Factors affecting follicle development.

Schinckel (1953) suggested that poor maternal nutrition during pre-natal life could prevent initiation of some secondary follicles, since he found a close correlation between birth weight and $\frac{S}{P}$ fibre ratio of the adult. Claxton (1963) suggested that, in the Merino, secondary follicle initiation was more protracted than in other breeds, and that it was thus more susceptible to the effects of poor nutrition. However, Short (1955a) and later Hugo (1958) using Merinos, and also Ryder (1955) using Cheviot sheep, found that poor maternal nutrition had no effect on the number of secondary follicles initiated before birth. The question of whether maternal nutrition could affect secondary follicle initiation has produced conflicting answers, therefore.

There was more agreement on the effects of pre-natal and early post-natal nutrition on the maturation of secondary follicles, i.e. their development to the fibre producing stage. Short (1955b) and Schinckel (1955b) found that birth weight and $\frac{S}{P}$ fibre ratio at birth were correlated.

Schinckel and Short (1961) collaborated in an attempt to resolve the effect of pre-natal and early post-natal maternal nutrition of secondary follicle development. They found, using Merino sheep, that low pre-natal maternal nutrition restricted foetal size and the total number of secondary follicles, while early post-natal restriction of nutrient intake suppressed secondary follicle maturation. However, these workers pointed out that reduced foetal growth rate might affect the adult fleece type independently and the separation of this effect from that of pre-natal maternal nutrition was not possible.

Turner (1961) found that 87 per cent of the reduction in clean

wool /

wool weight of sheep which had been twins or lambs of young ewes compared with sheep which had been single lambs, was due to a reduced number of secondary follicles.

That the Merino breed was unusual in its response to poor pre-natal nutrition was again suggested by Doney and Smith (1964). Using the Scottish Blackface, they found that the difference between the $\frac{S}{P}$ follicle ratio of single lambs and the lower ratio of twin lambs at birth, disappeared by six weeks when post-natal nutrition was adequate. Poor post-natal nutrition also delayed secondary follicle maturation and birthcoat shedding but the differences had again disappeared by one year of age. These results were later supported by Wildman (1965), using the Romney breed.

Thyroidectomy at birth prevented secondary follicle maturation, and thyroxine administration restored their normal development (Ferguson, Schinckel, Carter and Clarke, 1956). Labban (1957) showed that Growth Hormone treatment of Suffolk lambs increased the $\frac{S}{P}$ fibre ratio significantly, although the mechanisms of this effect were not discussed.

The factors involved in the initiation of primary follicles are not known. However, Cohen (1967) suggested that the original pattern for follicle groups ante-dated any actual follicle origins and that it was determined by the vascular network supplying the embryonic skin. The reaction between dermis and epidermis, at points related to blood flow, would lead to the formation of dermal pre-papillae which would differentiate to form primary follicles.

An interesting observation of Sengal (1964) was that neural extract was a necessary ingredient in the culture medium of avian skin tissue cultured before the appearance of skin feather primordia if these structures /

structures were to develop. The tissue interactions between skin ectoderm and dermal mesoderm which result in differentiation of skin appendages have only recently been studied, and have been focussed on the domestic fowl. The relative importance of the dermal and epidermal components of the presumptive feather or scale have been assessed by their separation and exchange using skin from feathered and scaled regions at different developmental stages (Rawles, 1965). The recombined tissues were cultured on chorio-allantoic membrane and the resultant appendages examined. The results showed that both dermis and epidermis participated actively in the formation of the feather and scale, but that there were significant differences in the regional properties of both dermis and epidermis that conditioned the development of the final product. The dermis from different regions of the body differed in the developmental age at which it became capable of eliciting a specific response from the overlying epidermis. For instance, scale-forming dermis from the foot could not evoke scale formation from epidermis overlying it until late in incubation (approximately the thirteenth day). Epidermis overlying dermal tissue "younger" than twelve days tended to produce feathers. The strength of the dermal influence was therefore related to its age and region of origin.

Epidermal tissue or prospective epidermis from any part of the body of the embryo could be induced to form any of the epidermal derivatives under the influence of an appropriate dermal stimulus. This ability of the epidermis to react to a specific stimulus from the dermis persists for a relatively long time in ontogeny, and epidermis at relatively late stages in the formation of its derivatives is potentially capable of changing its course of differentiation under strong dermal influence.

The nature of these dermal-epidermal interactions is not known, but //

but Rawles (1965) stated that a dependent relationship existed between dermis and epidermis. Close contact of epidermis and dermis was necessary for this interaction and a necessary condition for normal differentiation of normal dermis and the formation of dermal follicle papillae was an ectodermal covering. Billingham and Silvers (1965) in a review of work such as that of Rawles (1965) concluded that there seemed to be no predetermined regional specificity in the embryonic epidermis; its morphological differentiation depended on stimuli from the prospective dermis.

The follicle papilla of hairs becomes rich in acidic mucopolysaccharides during hair growth (Montagna, Chase and Melaragno, 1951) and probably induces the hair to form (Cohen, 1961). The maintaining role of connective tissue mucopolysaccharides was discussed by McLoughlin (1963), who also raised the interesting point that these substances might specifically affect epidermal differentiation.

F Environmental and genetic aspects of wool growth

The relationship between the level of nutrition and wool production has received considerable attention. Marston (1955) stated that pastured sheep rarely reached their potential for wool production, and Ryder (1972) showed that abundant food stimulated wool growth rate and fibre diameter. Poor nutrition resulted in reduction of growth rate and fibre diameter (Daly and Carter, 1956), so that any factor which caused a reduction in food intake could depress wool growth without necessarily having a specific effect on the follicle (Ryder, 1972). Wool growth in fact continued, but at a much reduced rate even when body tissues were being depleted by under-nutrition (Ryder and Stephenson, 1968).

Copper is a trace element with a specific part to play in keratinisation and, as described above, deficiency can lead to the development of "steely wool". Such sheep respond rapidly to oral doses of copper and even to topical application of copper sulphate solution, by growing a normal crimpy fleece (Marston, 1955).

In their description of the effects of hormones on wool growth, Ryder and Stephenson (1968) stated that removal of the pituitary gland suppressed wool growth and pituitary extract injections restored wool growth. Of the nine pituitary hormones, those with an influence on wool growth were not known, however. Several pituitary hormones were injected intradermally by Downes and Wallace (1965) who observed no marked change in wool growth rate with growth hormones, and a slight increase in growth rate in some fibres with prolactin. However, other workers showed that thyroxine and adrenocorticotrophic hormone had a more potent effect on wool growth rate. Ferguson, Schinckel, Carter and Clarke (1956) observed that thyroxine treatment of adult sheep had the effect of increasing wool growth, although the /

the increase in food consumption necessary to maintain body weight invalidated any practical application of this finding. Lambourne (1964) considered that the mechanism of action of thyroxine was non-specific and a result of the increased metabolic rate, which led to increased mitotic rate in the follicle bulb, and appeared as increased wool length. The observation that thyroxine often failed to increase wool growth in spring and summer (Coop and Clarke, 1958) could be explained by the fact that the natural secretion of thyroxine was greater during this time due to increasing day length (Ryder and Stephenson, 1968), but Ryder (1973) later noted that thyroxine failed to restore wool growth to inactive follicles in winter. Rougeot (1965) postulated that thyroxine acted by increasing cell size, not mitotic rate, and that this contributed largely to the growth rate increases.

Adrenocorticotrophic hormone (ACTH) was said by Ferguson (1956) to suppress wool growth by stimulating the release of hormones from the adrenal cortex. In contrast to thyroxine, ACTH injected daily suppressed wool growth by reducing fibre length and diameter, so that the fibres broke easily and simulated the fleece "break" that often occurred in severe stress (Lindner and Ferguson, 1956). This effect could also be produced by parenteral or topical administration of cortisone, a substance chemically similar to the adrenal cortex hormone hydrocortisone.

Disease, cold and malnutrition are among the stressful conditions known to stimulate release of hormones from the adrenal cortex, and Lindner and Ferguson (1956) suggested that these factors might lead to suppression of wool growth in winter. Morris (1961) suggested that adrenocortical activity was induced by daylight length changes and /

and that these were the main cause of the seasonal wool growth pattern. Downes and Wallace (1965) demonstrated that the effect of cortisone varied with its dose since small doses (0.6 and 3.0 µg per day) increased wool growth, although larger doses (320 and 1600 µg per day) depressed wool growth.

Mohn (1972) showed that in rats adrenaline from the adrenal cortex was important in the regulation of epidermal mitotic rate, the rate being higher when blood adrenaline levels were low and vice versa.

The sex hormones, too, have been shown to affect hair follicle activity in some mammals, but little work has been done with sheep. However, Slen and Connell (1958) showed that the synthetic oestrogens, oestradiol and diethylstilboestrol, reduced wool production by reducing the length but not diameter of the wool fibres, and testosterone increased wool growth. Draper, Haynes and Lamming (1966) found that with oestrogen treatment, wool growth was reduced only when the food was restricted, but increased under conditions of ad lib feeding. These effects could reflect a disturbance of hormone balance involving the pituitary gland.

The mechanism of action of these hormones and their interactions are not fully understood. However, it has long been known that hormonal changes are important in the induction of seasonal wool growth patterns. Concensus of opinion suggests that these hormonal changes are basically mediated through the eyes, hypothalamus and anterior pituitary gland, so that increasing day length leads to a stimulation of thyroxine secretion from the thyroid gland, and decreasing day length to an increase in adrenal cortex activity.

The birthcoat and fleece type of sheep are a result of interaction between their genetic and environmental factors. Fraser and Short (1960) pointed /

pointed out that study of the effect of mutant genes on wool growth was an almost unexplored field. Ryder and Stephenson (1968) mentioned that as well as being caused by single major genes, genetic control of wool growth could arise from a continuous range of effects on all traits of wool which were of economic importance. This continuous inherited variation was exploited in breeding for improvement of wool production. Of the single major genes affecting fleece type, the "felting lustre" mutant of the Merino has been studied by Short (1958). This gene caused a great reduction in the number of skin folds, absence of fibre crimping, slight reduction in fibre diameter and a reduction of the amount of wool grown by a unit area of skin, but it had no effect on fibre length growth rate. The "Low Ratio" gene and the "gog" gene of the Merino were mentioned by Fraser and Short (1960), who described the effect of the "Low Ratio" gene as a reduction in the $\frac{S}{P}$ fibre ratio and therefore wool production, since the primary follicle density and fibre length growth rate were unaltered. The "gog" gene had a similar effect but also eliminated sweat glands.

The "carpet" genes of the New Zealand Romney caused the development of a hairy birthcoat and fleece but did not affect follicle density or wool growth rate. These genes are of particular interest in the present study since their clinical effects resemble those of Border disease. The discovery of two major non-allelic mimic genes, N and nr, as causes of this condition was made by Dry (1955 a and b), who confined his studies to the birthcoat. The hairiness of the birthcoat of N-type lambs was due to the presence of a great number of long coarse hairy fibres which Dry called "halo-hairs". Dry (1955 a and b, 1958) described N as being an incomplete dominant gene, and nr as a recessive gene. The birthcoat of N-type lambs showed a quantitative gradation from /

from apparently normal to completely hairy, depending on the degree of gene dosage. Stephenson (1956) has made an analysis of the effects of different genotypes on the birthcoat.

The development of the follicle population in the N-type foetus has been studied by Ross (1954) and Stephenson (1957, 1958, 1959a, b, and c) and in N-type lambs by Carter and Tibbits (1959). Cockrem and Rae (1959) and Cockrem (1959a, b and c) described their post-natal studies of the effects of the N gene, so that a fairly comprehensive study of its effects has been made. Fraser and Short (1960) suggested that the nr gene effects were very similar to those of the N gene.

The gene N was found to have no effect on the ages of initiation of primary or secondary follicles or on their rate of maturation (Stephenson, 1957). Primary follicle density and the $\frac{S}{P}$ follicle ratio were unaffected at least up to 126 days' gestation (Stephenson, 1958, 1959c) as was the size of secondary follicles and fibres. The first noticeable effect of the N gene was an increase in diameter and medullation of primary follicle fibres, followed by an increase in primary follicle size (Stephenson, 1959a). Although Ross found no difference in the $\frac{S}{P}$ fibre ratio at birth, Stephenson (1957, 1958) suggested that there were fewer mature secondary follicles in N-type animals in late gestation, an observation also made by Carter and Tibbits (1959) in newborn lambs. The pre-natal growth rate and birth weight of N-type animals was apparently normal (Stephenson, 1959b) but Cockrem and Rae (1959) suggested that post-natal growth of N-type lambs could be retarded.

The pleiotropic effects of the N gene which have been reported are increased growth of horns, sometimes a brown patch at the base of the dorsum of the neck (Dry, 1955a) and occasionally a lengthening of the spines of the thoracic vertebrae (Cockrem and Rae, 1959).

CHAPTER III.

METHODS.

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METHODS

Introduction

In the examination of skin samples from foetal sheep, lambs and adult sheep, sections were prepared for histological, histochemical and ultrastructural examination. The methods used were either established methods adapted to suit the facilities available at Moredun Institute, or devised by the author for the special requirements of the study. In this Chapter, in addition to details of the technique, reasons are given for the selection of the methods used.

Technical Methods:

A. Sampling procedure

1. Hysterectomy and perfusion

The ewes were placed in a position of dorsal recumbency while under pentobarbitone anaesthesia. A midline approach was made, and the uterus exteriorised. The uterine horn was opened over the foetus. 5 mls. of a 10^{-4} solution of heparin in saline was injected into the larger of the umbilical arteries, in order to prevent intravascular clotting prior to perfusion. In foetuses aged 105 days' gestation and over, $\frac{1}{2}$ - 1 ml. pentobarbitone was injected with the heparin. The umbilical cord was then clamped, and the foetus was removed from the placenta and weighed. Each foetus was then placed in lateral recumbency and perfused via the umbilical artery with 1 per cent glutaraldehyde, buffered to pH 7.4 with 0.24M phosphate buffer. The perfusion rate was approximately 20 mls. per minute. The second of each pair of twins was not perfused.

2. Skin sampling

Three sites were selected as standard sampling sites. These were:

a) over the shoulder, at a point half-way along the spine of the scapula.

b) over the distal half of the second last rib and

c) over the hip, at a point one-third of the distance from the hip to the stifle (Diagram 5). The sites are referred to in the text as the shoulder, side and hip sites, respectively. The side site was selected for sampling when only one sample was required.

One of two basic sampling methods was employed, depending on /

on whether or not the animal was alive, and on its age. These methods were as follows:

i) For foetuses and dead lambs

In those animals in which fibres were growing above the skin surface, the sites were prepared by close clipping with curved scissors. At each sampling site a metal trephine 6 or 10 mm. in diameter was used to define an area of skin. The smaller trephine was used for foetuses aged 85 days' gestation or less, and for lambs; the 10 mm. trephine was used for other foetuses. The excision of each sample was performed by applying the trephine gently to the relaxed skin so that the whole circumference of the cutting edge rested gently on the skin surface. With firm pressure, a half twist to the left and right cut through the whole skin thickness to the dermal fascia above the cutaneous muscle. The excision was completed with curved scissors. In most cases a strip of skin approximately 15 mm. x 5 mm. was removed from a site immediately adjacent to the side site, the long side of the strip lying parallel to the "lie" or expected "lie" of the fleece. In some cases, two squares of skin, approximately 8 x 8 mm. were also removed from sites adjacent to the side site for special stains and lipid histochemistry, and a sample of similar size was removed from the hip site, adjacent to the sampling area, for ultrastructural studies.

ii) For live lambs:

As a rule, local anaesthesia was used in the sampling of live lambs. The wool over the sampling site was first clipped closely with curved scissors. Approximately 0.3 ml. of "Lignocaine" (Macfarlan Smith Ltd.) was injected subcutaneously at /

at each of three places anterodorsal to the sampling site. Approximately 2-3 minutes after administration of the local anaesthetic, the 6 mm. diameter trephine was used to define a circular sample of skin, as described above. The skin around the defined area was lifted in a fold between the finger and thumb of the left hand, and a snip of skin containing the area was removed with one cut of the curved scissors. From this snip of skin the trephined area was dissected out, and from the remaining skin a small strip was cut, so that the long edges of the strip lay parallel to the "lie" of the fleece. This strip was usually approximately 5 mm. x 2.5 mm.

Wound bleeding rarely occurred and was very slight. No aseptic precautions were taken during the operation, but the wound was sprayed with "Chloromycetin" tincture aerosol (Parke-Davis), or dusted with sulphonamide powder after sampling.

3. Fleece score

After skin sampling at birth or within a few weeks of birth, lambs were given a fleece score based on the extent and degree of any "hairiness" or coarseness of the birthcoat. Since this hairiness tends to appear on the rump and extend antero-ventrally over the body, Fraser (1951) devised a 12-grade system to describe the extent of coarseness. Since consecutive grades were often difficult to distinguish, in the present work, this scale was simplified to 6 grades as illustrated in Diagram 6. Each grade was made more descriptive by combining it with an estimate of the degree of the "hairiness", based on a 6-grade system of Roman numerals. These grades were defined as follows:

I	II	III	IV	V	VI
no coarse hairs	few coarse hairs	some coarse hairs	many coarse hairs	very many coarse hairs	dense mat of coarse hairs

After /

After a few weeks of age the birthcoat coarseness was difficult to assess, and subjective appraisal of the fleece type was therefore not related to a scoring system.

B. Preparation of histological sections

1. Preparation of paraffin sections:

Immediately after sampling, the skin samples were placed, epidermal side up, on to small rectangular pieces of white card. After being air-dried for approximately 1 minute, the samples were adherent to the pieces of card, which were then immersed in formol phosphate fixative* for at least 10 days. Using an "Elliott Tissue Processor", the following programme was used for tissue dehydration:

50% alcohol	2 baths of 1 hour
70% alcohol	2 baths of 1 hour
90% alcohol	2 baths of 2 hours
Absolute alcohol	2 baths of ½ hour
carbolic xylol*	2 baths of 4 hours
xylol	2 baths of ½ hour

For some of the early samples a dehydrating machine was not available. As an alternative a Henley Relay machine, which kept one basket rotating, was used, and changes of alcohols were made by hand. The following programme was found to be suitable for this machine:

50% alcohol	2 baths of 40 minutes
70% alcohol	2 baths of 40 minutes
90% alcohol	2 baths of 1 hour 20 minutes
97% alcohol	overnight
absolute alcohol	2 baths of 20 minutes
carbolic xylol	2 baths of 2 hours 40 minutes
xylol	2 baths of ½ hour

Following /

* See Appendix

Following dehydration, each sample was placed in a small warmed glass beaker, covered with molten hard wax (melting point 56°C) and placed in a vacuum oven at 60°C. The vacuum was drawn slowly to a negative pressure of 24" mercury. There followed one change of wax at 1½ hours and the samples were removed from the oven after 3 hours. The samples were then transferred, using warmed forceps, to the base of a glass mould made from three microscope slides and filled with molten hard wax (melting point 56°C) on a glycerinised tile.

The samples for horizontal sectioning (i.e. the trephine samples and skin squares for special stains), were orientated with the epidermal surface downwards. Using a warmed brass plunger 1 cm. square, each sample was flattened gently against the base of the mould. As the wax began to harden, the skin sample was held in place by the wax and the plunger was removed. Each skin strip was held with a long side against the base of the mould, and the skin surface at right angles to the base until the wax became hard enough for support to be withdrawn.

The wax moulds were placed in cold water until they had hardened. The excess wax was trimmed from around each sample, and the resultant blocks were mounted on to wooden chucks.

To facilitate sectioning, the blocks were placed on ice for a few minutes before sectioning began. A Jung microtome or a smaller Beck rotary microtome was used. Both had an adjustable head so that the plane of sectioning of the block could be altered. For horizontal sections the blocks prepared from the trephine samples were secured in the head of the microtome and the position of the head was adjusted so that the plane of sectioning wss parallel to the epidermal surface of the skin. These samples were completely sectioned, at 9 µm, and the ribbon /

ribbon of sections transferred to numbered albuminised slides, so that the order of sectioning was maintained. The most convenient method of doing this was to lay the sections in rows running from top to bottom of each slide, with the first cut sections in two rows on slide 1, those next in series on slide 2, and so on. Usually 6 or 7 slides were used, but as many as 10 or 11 were necessary with long ribbons of sections. The sections were spread on the slides by adding 6 - 8 drops of cold water to each slide, which was placed on a hot plate, at a temperature warm enough to melt the wax around the sections in 10 - 15 seconds. As the wax melted, dissecting needles or scalpel blade tips were used to tease out wrinkles and to maintain the position of the sections on the slide. The excess water was then drained from the slides by carefully tipping them on edge. The slides were stored overnight in a warm oven ($38^{\circ}C$).

For vertical sections of skin, blocks containing skin strips were fixed in the head of the microtome, and the position of the head adjusted so that the skin surface of the sample lay at right angles to the plane of sectioning. Since the strip of skin had been embedded in such a way that a long side lay along the base of the mould (now facing the microtome knife) and the skin surface lay at right angles to the base, little adjustment was necessary in most cases. Sectioning at 9µm. was commenced and continued until the strip was being fully sectioned. 6 or 7 adjacent sections were then placed on each of 8 to 10 albuminised glass slides. The sections were spread on the slides with warmed water, as described above, and placed in a warm oven ($38^{\circ}C$) overnight.

2. Preparation of frozen sections:

Immediately after sampling, the skin squares destined for lipid histochemistry were placed in Baker's formalin calcium* for at least 10 days. /

* See Appendix

days. After washing in distilled water, each sample was positioned on the head of a Sartorius carbon dioxide freezing microtome, so that the epidermal surface was uppermost. The frozen sample was sectioned completely at 12 µm., and the sections floated out in distilled water.

To minimise explanations in the text, sections cut parallel to the skin surface will henceforth be referred to as "horizontal sections", and those cut at right angles to the skin surface will be "vertical sections".

C. Staining Methods

1) Routine Sacpic stain:

A modification of Auber's (1952) Sacpic stain (M.L. Ryder, personal communication) was used for all routine histological preparations of skin (Diagram 7). The procedure was as follows:

a) Remove wax in xylol 1 (10 minutes)
xylol 2 (10 minutes)

Change xylol frequently so that dissolved wax is not taken on into the alcohols

b) Hydrate — Absolute alcohol (2 minutes)
95% alcohol (2 minutes)

c) Nuclear stain: Weigert's Haematoxylin* (15 minutes). Wash.

d) Differentiate: Acid alcohol* (until only the nuclei are stained). (10 seconds) Wash (2 minutes).

e) Rinse in 50% alcohol (2 minutes)

f) "Pre-keratin" stain: Basic fuchsin* (4 - 5 minutes). Rinse

g) Upgrade: 50% alcohol (2 minutes)
90% alcohol (2 minutes)

h) Differentiate in absolute alcohol containing 2.5 gm. picric acid in 100 ml. absolute alcohol until only the pre-keratinisation zone is stained red. (20 minutes)

i) /

* See Appendix

i) Downgrade: absolute alcohol (2 minutes)
90% alcohol (2 minutes)
50% alcohol (2 minutes)
distilled water (2 minutes)

j) Stain connective tissue: Picro-indigo-carmine* (4 - 5 minutes)

k) 50% alcohol (2 minutes)

l) Differentiate in 70% alcohol (10 seconds) until collagen fibres are blue-green

m) Upgrade quickly through 90% and absolute alcohol, to avoid further differentiation of the picro-indigo-carmine

n) Clear in carbolic xylol (to complete dehydration) (2 minutes)

o) Then xylol (15 minutes)

Results:		
	Collagen fibres	blue-green
	Smooth muscle fibres	green
	Undifferentiated epithelium	pale green
	Keratinised inner root sheath	red
	Pre-keratinised cortex of fibre	red
	Keratinised cortex	yellow
	Medulla of fibre	pale green
	Trichohyalin granules	dark blue
	Nuclei	dark blue
	Erythrocytes	yellowish green
	Mast cell granules	dark red

2. Special staining methods:

Five special staining methods were used:

a. Barlow's (1957) tribasic stain for mast cell granules

b. Pinkus' (1944) acid-orcein Giemsa stain for mast cell granules

c. Gordon and Sweet's stain (1936) for reticulin fibres

d. PAS reaction for glycogen (Wachstein, 1949), with diastase control

and e. Mercury Orange stain for sulphydryl groups (Bennett, 1951).

* See Appendix

Two types of section were used for these techniques.

For the tri-basic stain and Pinkus' Giemsa, horizontal paraffin sections, 9µm. thick, were prepared from side skin samples. From the ribbon of sections obtained by the complete sectioning of each sample two or three sections from three different levels in the skin were selected for each stain, i.e. sections from just beneath the skin surface, from mid-follicle level and from follicle bulb level. It was found that sections of the dermis below follicle bulb level had a characteristic "frayed" appearance which aided selection of appropriate, more superficial sections.

For Gordon and Sweet's stain, the PAS reaction and the Mercury Orange stain, vertical paraffin sections of skin were used. Localisation of glycogen in the PAS-stained sections was aided by use of diastase-treated control slides. By pre-treatment of some sections with human saliva (containing diastase, a salivary enzyme which specifically destroys glycogen) at 22°C for 1 hour, glycogen was removed. On PAS-staining these sections, only PAS positive non-glycogen substances reacted, so that by comparison with non-treated sections from corresponding sites, the sites of glycogen deposition could be located.

3. Lipid histochemistry

For the OTAN (osmium tetroxide alpha naphthylene) and Sudan IV stains, Baker's formalin-Ca fixed frozen sections were prepared as described above. Several sections from 3 or 4 different levels in the skin were selected and stained by Adams' (1959) OTAN, and Brönte Gatenby and Beams' (1950) Sudan IV for unsaturated lipid.

D. Preparation of sections of follicle bulbs for electron microscopy:

For electron microscopy, the skin samples from the hip are were used.

From /

From these samples small blocks of skin approximately 1 to 1.5 mm. square were prepared. Each block was cut in two transversely, at mid-follicle level, with a scalpel blade. The half of the block containing the epidermis and top (distal) half of the follicles was discarded. The other half of the block was placed in 1 per cent glutaraldehyde for 4 - 5 hours, before being processed as follows:

1) Place in phosphate buffer overnight
2) Transfer to 1% osmium tetroxide in buffer for 1 - 2 hours.
3) Rinse in buffer.
4) Place in 10% alcohol 30 minutes
   followed by 60% alcohol 30 minutes
   absolute alcohol 30 minutes
   absolute alcohol 30 minutes
   ethanol 15 minutes
5) Move through two baths of epoxypropane 15 minutes each
6) Place in araldite mixture* overnight.
7) Embed in araldite mixture and place in oven at 60°C for 3-4 days.

At stages 2), 4) and 5), the sample containers were attached to a small rotating machine, which rotated approximately 12 times per minute. The skin samples were embedded (Stage 7) so that the cut ends of the follicles lay against the base of the container, i.e. they were presented first for sectioning. Several 1µm. thick sections were prepared using a Sorvall "Porter-Blum" Ultramicrotome (MTI). These were mounted on slides in a drop of distilled water, and dried at 60°C in an oven. The dried sections were stained with a $\frac{1}{10}$ dilution of Giemsa for 3 - 5 minutes, in a Coplin jar at 60°C. The stained sections were examined with a light microscope to ascertain the level of sectioning. Sectioning and staining were continued until appropriate sections, i.e. those in which most of the primary follicle bulbs were transected, were /

* See Appendix

were obtained. Thin sections (500 - 600 Å) were then prepared and stained with lead citrate. The prepared grids were examined with a Siemens 51 electron microscope at 50 KV. Electron micrographs were taken on Ilford EM6.

Methods for microscopic examination of skin sections

A. Adaptation of established methods.

1) Stages of follicle development.

To evaluate the stages of development of the primary and secondary follicles in each sample, the scale of Hardy and Lyne (1956) was used. This has been described in Chapter II and is illustrated in Diagram 4.

From the series of horizontal Sacpic stained sections of each sample, several were selected in which the follicles were represented at several levels of sectioning. The rows of follicle groups were slowly scanned at magnification x 50, or in some cases x 125. The most advanced and least advanced stages of development of the central and lateral primaries and secondary follicles were recorded.

2) Frequency of secondary follicle branching

Vertically-sectioned, Sacpic Stained skin sections were examined for the presence of branching secondary follicles. A subjective estimate of the frequency of branching secondary follicles was made, and recorded using the following scales:

\+ few, ++ some, +++ many branching secondary follicles.

3) Follicle density

The method of estimating primary follicle density in the present work was based on that of Carter and Dowling (1954) for cattle skin. These authors described the biopsy punch used in their sampling, and similar punches, or trephines as they will be called in this text, were made for use in the present work. These trephines were made /

made with known diameters (6 mm. and 10 mm.), so that the area of skin removed could be calculated. Sections in which the trephine sample had been sectioned at the level of the sebaceous glands were used (Diagram 7), and in most cases this was a complete horizontal section of the skin sample.

The counting procedure was aided by using a Reichert Neopan microscope with a drawing arm. Before the count, a micrometer slide was placed under the microscope and, at magnification x 50, an area representing 1 sq. mm. was drawn under the drawing arm on to a piece of white card. When the micrometer slide was replaced by the selected slide and the field to be counted brought into view, the image of the outline on the card was superimposed on that of the field to be counted. Thus the primary follicles within 1 sq.mm. of the section were delineated and these were counted. As stated above, this process was repeated for each of three fields, and the average count recorded. However, this count represented the number of primary follicles per square mm. of section. To convert this to the original density of primary follicles in the skin, a correction factor was applied. This was calculated as follows:

Let $\underline{x}$ be the number of follicles counted in 1 sq. mm. of section; let $\underline{a_1}$ be the area of the section and $\underline{a_2}$ the area of the original sample:

$\therefore$ The number of follicles in the section is

$x \times$ area of section $= x \times a_1$

$\therefore$ The number of follicles in the original sample is $x \cdot a_1$

$\therefore$ The follicle density in the original sample

$$= \frac{x \cdot a_1}{a_2}$$

Therefore $\frac{a_1}{a_2}$ is the correction factor.

In /

In practice, the factor was reduced to $\frac{d_1^2}{d_2^2}$ where d_1 was the diameter of the section used and d_2 was the diameter of the original sample. The diameter of the section was measured with a hand textile counter, which is a small hand held lens fixed above a graduated scale, allowing measurements to be made to within 0.05 mm. Four diameters were measured and the average value (d_1) used in the estimation.

4. $\frac{S}{P}$ follicle and fibre ratio:

Burns (1949) described a method for the estimation of $\frac{S}{P}$ follicle ratio which was adapted for use in the present study. She projected a magnified image of the selected skin section at mid-sebaceous gland level on to a piece of white paper, and traced the follicle outlines in approximately 7 complete follicle groups in 4 areas of 10 sections from each sample. However, in the present study, only one section in which most follicles were transected at sebaceous gland level was used. The follicle rows were slowly scanned at magnification x 125, and the number of follicles of each follicle type, their fibre type, and in the case of immature follicles, an approximate estimate of their stage of development were recorded. Hardy and Lyne's (1956) 8-stage scale was used, and the follicles classed as plugs if they were at stage F1 - F3, keratinising follicles if at stage F4 or F5, and keratinised follicles if they were at stage F6 or more. This procedure was continued until 100 primary follicles and their related secondary follicles had been included. The data was recorded on sheets such as that illustrated in Diagram 8. From the data, the number of secondary follicles per primary follicle ($\frac{S}{P}$ follicle ratio) and the number of secondary follicles producing keratinising or keratinised fibres per primary follicle ($\frac{S}{P}$ fibre ratio) were calculated.

5. Frequencies of medullated fibres and of "brush end" and "shed empty" follicles.

An extension of method 4. enabled a measure to be made of the frequency and degree of medullation. Non-medullated fibres were classed K, those whose medulla diameter was equal to or less than half of their fibre diameter were classified as "small medullae" (Km) and the remainder were "large medullae" (KM). From the data sheets obtained as described above, and illustrated in Diagram 8, the frequencies of medullation and of large medullae were calculated for primary and for secondary fibres. These figures therefore represented the frequency of medullation (Km and KM), and the frequency of heavily medullated (KM) fibres, at sebaceous gland level.

Similarly, the presence of follicles in telogen was noted on the data sheet. These were recognised by their keratinised root with its characteristic "frayed" appearance. At higher levels of sectioning, the fibres were non-medullated and the thickened outer root sheath with the orientation of its cells at right angles to the axis of the follicle, denoted the fact that the follicle contained a "brush end". Those follicles from which the fibre had been lost were classed as "shed empty" follicles (Chapter II, pp. 34-35). The percentages of "brush end" and "shed empty" primary and secondary follicles were calculated.

B. Personally devised methods.

1) Length and bulb diameter of the largest follicles.

The longest follicles seen in the vertical Sacpic stained sections from each sample were outlined under the drawing arm of the Neopan microscope. A magnification of x 50 was used, but for the shorter follicles of the skin of younger foetuses (i.e. less than 85 days' gestation), a magnification of x 125 was necessary. To find their absolute length they were compared with the outline of the scale on a micrometer slide viewed under the microscope at the same magnification.

To /

To measure the diameter of the largest bulbs, a horizontal Sacpic stained section of skin was selected in which most of the follicles were transected at bulb level. The largest bulbs seen in this section were outlined at magnification x 125 under the drawing arm of the Neopan microscope. The smallest diameter of the largest of these was found by using a micrometer slide, as described above.

2) Diameter of the largest central primary fibres.

That section from each sample which was used in follicle density measurements, was again used to determine fibre diameter. The rows of follicles were slowly scanned at magnification x 125 and the outlines of the largest central primary fibres were drawn under the drawing arm of the Neopan microscope. The smallest diameter of the largest of these was found with the use of a micrometer slide as described above. This method, however, gave a misleadingly low value for those fibres with very large latticed medullae, which were oval, dumb-bell shaped or ribbon-shaped in cross section. These fibres were distinguished from obliquely sectioned fibres by the uniform thickness of their cortex and by the orientation of the medullary strands across the widest part of the fibre. In obliquely sectioned cylindrical fibres, the cortex at its widest diameter was thicker than at its narrowest diameter, and the strands of the medullae usually did not lie parallel to its largest diameter. In flattened fibres only, the widest diameter, that which lay parallel to the medullary strands, was measured as described above.

3) Estimate of the frequency of mast cells, lymphocytes and neutrophils in the deep dermis.

A method was devised to quantitate the numbers of lymphocytes, mast cells and neutrophils in the reticular layer of the dermis.

From each of the series of Sacpic stained horizontal sections from each sample, one was selected in which the dermis was transected through the /

the reticular layer from the level of the follicle bulbs to the level of the subcutaneous muscle, or as deep as possible. In some cases, two sections had to be selected to give this range of depth of sectioning.

These sections were slowly scanned at magnification x 125 and an estimate of the density of each of these cell types was made. This assessment varied with cell type, as follows:

Mast cells:

These were usually found lying along the dermal blood vessels, but were sometimes found free in the dermis. The apparent density of these thus depended on how much of the dermal blood vessel net was present on the section examined, and this the subjective estimate of density took into consideration. The scale used was as follows:

+ < 6 mast cells per section

++ 6-20 mast cells per section

+++ > 20 mast cells per section

These values were reduced by approximately half for the smaller sections of the foetuses aged 85 days gestation or less.

Lymphocytes:

Large and small lymphocytes were present in the dermis, clustered in small (up to 20 cells) or large (up to 100 cells) groups, or scattered throughout the dermis. The lymphocyte groups were usually found around the bulbs of the follicles or deep in the dermis near the dermal blood vessel network. Their density was recorded using the following scale:

+ < 10 scattered lymphocytes or one small group per section

++ 10-40 scattered lymphocytes or one large group or 2-3 small groups per section.

+++ >40 scattered lymphocytes or more than 2 large or 3 small groups per section.

These values were halved for the smaller sections of the foetuses aged 85 days gestation or less.

Neutrophils: /

Neutrophils:

Both juvenile and mature neutrophils were seen lying in the dermis, usually scattered at bulb level or around the dermal blood vessel network. In some cases, they formed clusters around this network. Only the mature neutrophils were recorded, since juvenile forms often could not readily be distinguished from other cell types such as immature macrophages and young lymphocytes. Their density was recorded on the same scale as that described above for mast cells.

DISCUSSION:

The foetuses from which skin samples were taken were first subjected to whole body perfusion with 1 per cent glutaraldehyde. Although perfusion is not necessary for skin preservation, it was attempted to give good preservation of the brain and spinal cord, since these tissues autolyse rapidly after death, and their histological examination was an important part of collateral work on Border disease. The quality of the perfusion, that is, thoroughness of the flushing of the foetal vascular system with perfusate varied, so all skin samples were fixed and stored by immersion in formol phosphate after perfusion.

The sampling sites described in previous studies of wool growth have varied in number and distribution, from approximately 38 at different sites (Carter and Hardy, 1947) to the single mid-side sites of Carter (1943) and Ryder (1974). The mid-side site described by Carter lay on the heart-girth line, one-third of the distance between the dorsal and ventral median lines. The mid-side site of Ryder was just behind the last rib, half-way down the body. These single sites were selected to give values representing the average values of the follicle population as a whole, since fleece type tends to be coarser over the dorsal and posterior parts of the body. Follicle development spreads from the neck and shoulders postero-ventrally (Stephenson, 1957). Thus for the present study sites from the shoulder and hip areas as well as a mid-side site were selected to indicate variations in the follicle population over the fleece bearing surface of the body during prenatal life. In post-natal studies, a standard mid-side site was used. This was slightly different to those of Carter (1943) and Ryder (1974), but it was selected as being intermediate between the shoulder and hip sites and by being related to specific topographical features it could be accurately located.

The /

The sampling of live animals described in the literature has been performed without anaesthesia, since small snip samples or samples taken with a biopsy punch (Carter, 1939; Carter and Clarke, 1957a and b; Burns, 1949, 1953, 1954a and b) could be taken quickly. In the present study, lambs were given a local anaesthetic prior to sampling, to minimise their distress on regular sampling at 3 to 4 week intervals. The anaesthetic was of the grid type described above, so that little anaesthetic would diffuse into the sampling area.

Skin can be a difficult tissue to process, since it tends to harden and curl during processing by conventional methods (Rudall, 1955). Spottel and Tänzer (1923), Wildman (1932), Carter (1939), Carter and Dowling (1954) and Carter (1955) have described the developments which led to the histological technique described by Carter and Clarke (1957a, b) in their sheep breed studies. Modificationsof this technique have been devised (M.L. Ryder, personal communication) and were used throughout the present study for the reasons discussed below.

Hardening of the tissue occurred after fixation in 10 per cent formaldehyde (Rudall, 1955). Carter and Clarke (1957a) first described the use of 5 per cent neutral formalin as a more suitable fixative for skin, since, like the 4 per cent neutral formalin used in the present study, it caused less hardening.

To prevent curling and distortion of skin samples during fixation, pins have been used to peg the samples on to a cork board (Carter, 1939; Carter and Hardy, 1947) or on to pieces of india-rubber (Burns, 1953). This method was not suitable for small trephine samples, because the pins would have damaged much of the tissue, fairly large containers and volumes of fixative were required, and it was a time-consuming procedure. The simpler method of air drying samples on white card

was /

was found to be satisfactory. During fixation, the samples were stored in small 1 oz. plastic containers, each containing approximately 10 ml. of 4 per cent formol phosphate. During processing of the skin samples the dehydration through alcohols was followed by clearing in carbolic xylol, as well as the xylene used by Carter and Clarke (1957a), since these solutions effectively cleared the tissue and helped to keep the samples soft.

To flatten skin samples during embedding, a warmed brass plunger was used, since this was found to be much quicker and more effective than the use of small pieces of glass as described in the literature (Carter, 1939, Carter and Hardy, 1947). This method of embedding, and the use of the adjustable microtome head to hold the paraffin block, helped to ensure that the plane of sectioning lay parallel to the skin surface of the embedded sample. To ensure that the sections were unscored, care had to be taken to keep the microtome knife very sharp. Sectioning of keratinised fibres quickly blunted the knife edge, and so a fresh part of the knife edge was used for each block.

The Sacpic stain devised by Auber (1952) was selected for routine staining of paraffin sections of skin, rather than the triple stain (Mayer's haemalum, eosin and picric acid) used by Carter and Clarke (1957). Although the Sacpic stain was more laborious, it revealed more details of keratinisation and distinguished between its different stages. In addition, it was found to be a very useful method for staining mast cell granules.

Two other stains for the demonstration of mast cells, Pinkus' Giemsa (Pinkus, 1944) and the tribasic stain (Barlow, 1957), were tried, using paraffin sections, but these stained the mast cells weakly and inconsistently, and the resultant sections were therefore difficult to interpret, and will not be included in the text.

Barlow /

Barlow (1957) stated that with his tribasic stain, mast cell granules of different species frequently showed shade differences. This might account for the weak staining of mast cells in the sheep skin examined.

The papilla and bulb of primary follicles were selected for ultrastructural study in the present work. Previous ultrastructural studies of sheep hair follicles have used plucked hairs (Birbeck and Mercer, 1957; Ballin and Happey, 1965) most of which had lost their papillae. The method of preparing, embedding and sectioning skin samples described above was designed to enable primary follicle bulbs and their papillae to be examined "in situ". By commencing sectioning at mid-follicle and gradually working down to the level of the deepest follicle bulbs, these could be identified with confidence as primary follicles - an identification not possible with plucked hairs.

The microscopic examination of the skin follicle population in the present study involved the measurement of features which were selected for their importance in fleece type determination. The methods for the measurement of some of these parameters have been described in the literature, and methods were devised for the evaluation of the remainder. Among the established methods are those for the assessment of the changes of follicle development during intra-uterine life. Although various features of wool follicle development in foetal sheep have been described by Spöttel and Tänzer (1923), Duerden and Ritchie (1924), Tänzer (1926). Wildman (1932), Carter (1943) and Carter and Hardy (1947), none of these descriptions is sufficiently detailed to permit an assessment of the stage of development of individual follicles. Probably the only detailed description of the stages of wool follicle development was provided by Hardy and Lyne (1956) (Chapter II, pp. 37-38). Using the Merino, Hardy and Lyne (1956) recorded the most advanced stage of each of the follicle types (central and lateral primary follicles and secondary follicles) for each foetus. They also described 18 stages in the development of the follicle group, based on the most advanced stages of development /

development of the central primary, lateral primary and secondary follicles present. In the present study, this 18-stage description of the follicle group was not used, since in preliminary work it was apparent that secondary follicle development occurred relatively earlier in the Merino than in the Cheviot x Dorset Horn cross or Scottish Blackface foetuses used in the present work. Thus this system of group classification was not used . However, the method of assessment of the stage of development was adopted, and extended to cover the range of follicle stages for each follicle type. This gave a more accurate assessment of the stage of development of the follicle population as a whole.

To examine skin sections for the presence of branching secondary follicles, Hardy and Lyne (1956) examined 7μm.or 8μm. thick horizontal and vertical sections stained with haemalum, eosin and picric acid. Individual follicles and groups were studied in each of a series of sections and reconstructions made from camera lucida drawings. However, in the present study it was found that if vertical Sacpic stained sections, 9μm. thick were examined, the branching secondary follicles could be identified without recourse to montage construction. Although it was sometimes difficult to distinguish between developing sweat glands and branching secondary follicle plugs, the latter could usually be identified by the cluster of presumptive papilla cells beneath their bulbs.

Perhaps the first worker to attempt counts of follicle density was Carter (1939). His skin samples were of undetermined area, but from the stained, horizontal sections prepared from these, he counted primary and secondary follicles at sebaceous gland level in 4 contiguous fields of 1 sq. mm, using an ocular graticule. The average value

was used to represent the number of primary and the total number of follicles per sq. mm. Thus in this early work, no correction was made for tissue shrinkage. Later, Carter and Hardy (1947) realised that compression of the sections would occur during sectioning, and so they multiplied their observed count by a correction factor:

$$\text{correction factor} = \frac{\text{area of section from which counts were made}}{\text{area of skin surface in paraffin block}}$$

They conceded that skin distortion during sampling and fixation could make their estimates inaccurate, but no allowance was made for this. Burns (1949) first recognised the importance of sampling a known area of skin in density estimations. By sampling between marks tattooed on the skin of the sheep at the required site, she obtained samples of known size. Burns then estimated the amount of shrinkage that occurred during each stage of the processing of the sample. She found that no appreciable shrinkage occurred during fixation, but shrinkage during dehydration and embedding was considerable. During sectioning, compression led to further shrinkage, but sometimes, after expansion during mounting, the area of the section was greater than that of the block. Burns concluded that, for an accurate assessment of follicle density, the follicles in the whole of the tattooed area should be counted and related to the original sample area.

Fraser and Short (1952), in their measurements of the follicle population, made use of skin samples collected with a 10 mm. diameter trephine. After sectioning each sample, its area (a_1) was measured and stated as a proportion of its original area (a_2). This proportion was used as a correction factor $\frac{a_1}{a_2}$, and in the three preparations studied, the correction factors were 0.56, 0.72 and 0.69. Details of their counting methods are not given, but the method is the first to relate measurements on the prepared section to those on the living animal /

animal by using a correction factor which takes account of shrinkage at all stages of processing.

Carter and Dowling (1954) describe in detail the biopsy punch used in their work on cattle skin. These authors selected one section from the series of stained sections of each biopsy in which most of the follicles were transected at sebaceous gland level. Counting was done at a magnification of x 115 on a random sample of 10 fields, each being 1 sq. mm. of the section. A microprojector was used. A correction factor ($\frac{a_1}{a_2}$), as used by Fraser and Short (1952), was applied to the result, to give the original follicle density in the skin. The correction factor was found to be approximately 0.45 for adult cattle skin.

Burns' (1953, 1954) skin samples from Blackface and Suffolk sheep were, as in her earlier work (Burns 1949), taken from a tattooed known area. No correction factor was needed, since all the follicles in the tattooed area were counted. Ruttle and Sorensen (1965), in their work on Rambouillet sheep, counted primary follicle density at magnification x 100, on each of three 1 sq. mm. fields on each selected section. A micrometer disc in the eye-piece was used as a guide for counting. To account for tissue shrinkage, they applied a tissue correction factor ($\frac{a_1}{a_2}$), as did Fraser and Short (1952) and Carter and Dowling (1954). They found that the average shrinkage for all samples was 45.9 per cent, and therefore a correction factor of 0.541 was used in all cases.

For the present study, trials were made using some of the techniques described. Tattooing skin samples proved unsatisfactory because the tattooed areas were difficult to find on histological sections. In addition, it was often not possible to count all the follicles within the tattooed area. As Burns (1953) has noted, the sections were often obliquely cut, or slightly torn or wrinkled, making complete counting difficult. /

difficult. Thus, the method adopted was based on that of Carter and Dowling (1954), and samples were taken using a trephine. As described above, precautions were taken during fixation, embedding and sectioning to ensure that the skin samples remained flat and the skin follicles were sectioned in an even place. As Burns (1949, 1953) found, the sections, having been shrunk during dehydration and compressed during sectioning, often expanded noticeably when floated on warm water prior to mounting. The methods of estimating follicle density were chosen as being fairly quick and accurate. To the average follicle density in three 1 sq. mm. areas of the selected section, a correction factor, as used by Fraser and Short (1952) was applied. It was found that this correction factor varied greatly from sample to sample, within the range 0.5 to 1.0, a variation which might be accounted for by the greater intracellular fluid volume during prenatal life. Thus, unlike Ruttle and Sorensen (1965) the correction factor was calculated anew for each density estimation made.

Because of the usefulness of the $\frac{S}{P}$ follicle and fibre ratios in evaluating the follicle population of sheep, many workers have made a study of $\frac{S}{P}$ ratios. Burns (1949) described the basic method used. She projected a magnified image of the selected skin section on to a piece of paper, on which the outlines of the follicles were traced, and marked with a symbol to indicate whether they were central or lateral primary or secondary follicles. Only complete follicle groups were included. The section selected for the count was one in which the primary follicles were sectioned at mid-sebaceous gland level. Burns' preliminary tests indicated that an area containing approximately 21 primary follicles with their related secondaries would be the minimum area likely to give /

give a satisfactory estimate of follicle $\frac{S}{P}$ ratio. Four such counts were made on each of ten sections from each sample. Burns found that although this technique gave satisfactory results, an accurate assessment of $\frac{S}{P}$ ratio (as with follicle density) can only be obtained by counting all the secondaries and primaries in the sample area. This approach was made in the present study, which was based on the technique of Burns (1949). However, by including all the follicles in rows of groups, and not, as Burns did, only groups containing three primary follicles, a more accurate value was obtained. Burns' studies did not cover the prenatal phase of follicle development, being confined to study of the adult skin, and so a method was devised to include immature follicles in the count. As described above, these were described as follicle plugs, keratinising follicles or keratinised follicles. Thus estimates of the relative proportions of follicle fibre types and their stages of development could be calculated.

The methods described so far have been adapted from those described in the literature. However, for some of the parameters which were to be measured, there were no descriptions of suitable methods. Among this second group were measurements of fibre medullation in the skin, "brush end" and "shed empty" follicles, follicle and fibre dimensions and the frequency of lymphocytes, mast cells and neutrophils in the dermis. Measurement of fibre medullation was an important part of this study, and the method described above for the measurement of $\frac{S}{P}$ follicle and fibre ratios was used to provide data with which to assess this. Previous attempts to classify medullated fibres were based on the structure of the medullae (Burns, 1953, Ryder, 1955, 1956), which were described as latticed or non-latticed. In the present work, no clear-cut distinction was observed between these types, and by

referring /

referring to Auber's (1952) text, it can be seen that the transition from latticed to non-latticed is gradual. A more definitive method was therefore devised based on the relative width of the medulla to fibre width. Auber (1952) showed that the diameter of the medulla relative to fibre diameter increased as fibre diameter increased. Thus the larger fibres have relatively larger medullae than smaller fibres. Medulla types were accordingly divided into two groups - large and small. In the fibres with large medullae (KM) the medulla was usually latticed. Small medullae were non-latticed.

A method of measuring the dimensions of individual follicles was described by Burns and Clarkson (1949). However, the method involved the construction of a montage of each follicle by tracing it through a series of horizontal sections of skin for its entire length, and this was obviously impractical in the present study. The length and bulb diameter of the larger follicles were selected as the most useful indicators of follicle size. These follicles could be identified with confidence as central primary follicles, on vertical sections, because it was found from observations on deep horizontal sections that except in some Cheviot x Dorset Horn cross lambs, the longest central primary follicles were always longer than any lateral primary follicles, and secondary follicles were invariably smaller than primary follicles. The exceptions to this general rule are discussed in relevant parts of the text.

Estimates were made of the numbers of lymphocytes, neutrophils and mast cells in the dermis. Although groups of lymphocytes (Kelsall and Crabb, 1959), mast cells and scattered neutrophils (Draper and Chalmers, 1968) have been described, in the normal dermis, there are no data on their frequency and distribution in the skin of foetal sheep. The methods devised for the assessment of the frequency of these cell types in the dermis /

dermis were fairly crude, but were considered to be adequate to indicate any significant differences in the numbers of these cells between animals.

CHAPTER IV.

The effect of Border disease on the pre-natal skin follicle population of Cheviot x Dorset Horn cross foetuses

CHAPTER IV.

Introduction

One of the most consistent of the clinical features of Border disease in medium and fine-fleeced breeds is an abnormally "hairy" birthcoat (Plates 1 and 2) (Dry, personal communication; Hughes, Kershaw and Shaw, 1959; Shaw, 1962; Hartley and Kater, 1962; Barlow and Dickinson, 1965; Osburn, Crenshaw and Jackson, 1972). This resembles macroscopically the birthcoat of coarse fleeced breeds such as the Scottish Blackface and the N-type mutant of the New Zealand Romney (Dry, 1955a).

One previous study of the follicle population in Border disease lambs has been undertaken (Carter, Terlecki and Shaw, 1972), but the developmental stages of the lesion have not yet been examined. Studies of normal follicle development have been confined to the Merino, Rambouillet and Romney breeds (Hardy and Lyne, 1956; Ruttle and Sorensen, 1965; Ryder, 1956c), and the development of the "hairy" N-type Romney mutant has received considerable attention (Ross, 1954; Stephenson, 1957, 1958, 1959 a, b, and c).

It is important to an understanding of the pathogenesis of Border disease that the nature of the skin abnormality is established. In the present study, this was attempted by comparing histological, histochemical and ultrastructural features of the developing follicle population in Border disease affected and control foetuses.

Materials and methods:

Approximately seventy South Country Cheviot ewes from farms with no history of Border disease and in lamb to a Dorset Horn tup were used in experiments performed in the years 1971-72 and 1972-73. Several of the experimental ewes aborted after inoculation or contained mummified foetuses unfit for examination. Fifty-five ewes provided foetuses and lambs suitable for study.

Table 1. 1971 - 72 Experimental material.

Type of animal	Duration of gestation at slaughter (days)													Total number of animals
	55	60	65	70	75	85	95	105	115	125	135	145	newborn	
Control group														
No. of ewes	1	1	1	1	1	2	1	2	2	1	2	1	2	18
No. of foetuses and lambs	2	1	1	1	1	2	1	2	2	1	2	1	2	19
Border disease affected groups														
No. of ewes	2	2	1	2	1	2	2	4	1	3	2	2	-	24
No. of foetuses and lambs	2	2	1	2	1	2	3	5	1	3	2	2	-	26

Table 2. 1972 - 73 Experimental material.

Type of animal	Duration of gestation at slaughter (days)		Total number of animals.
	95	115	
Control group			
No. of ewes	2	2	4
No. of foetuses	4	3	7
Experimental group			
No. of ewes	4	5	9
No. of foetuses	6	8	14

1971-1972: Twenty-four ewes were inoculated at 54 days' gestation, part intraperitoneally and part subcutaneously with standard Border disease inoculum, i.e. 6 mls. of a $\frac{1}{200}$ suspension of brain tissue from Border disease affected newborn lambs with 100 I.U./ml. penicillin and streptomycin. Eighteen control ewes were inoculated with a similar preparation of normal newborn lamb brain. The ewes were killed at a range of gestational ages, and the numbers of ewes and their foetuses were as shown in Table 1. Two of the control ewes were allowed to lamb normally. The control ewes produced seventeen foetuses and two lambs; twenty-six foetuses (including two sets of twins) were produced from the experimental ewes.

1972-1973: Thirteen ewes were used to provide material for more specific study. Nine of these were inoculated with the standard Border disease inoculum at 54 days' gestation as described above. The remaining four were inoculated with control material. These ewes were killed at 95 days' or 115 days' gestation and the number of ewes and foetuses were as shown in Table 2.

Diagnosis of Border disease was established by histological examination of the brain, spinal cord, and uterine caruncles (Barlow, Gardiner, Storey and Slater, 1970; Barlow, 1972a). In addition to trephine samples and side site skin strips, side skin squares and hip skin samples were removed from the 1972-1973 foetuses for histochemical and ultrastructural studies (Chapter III, p. 59). The newborn lambs were sampled as described in Chapter III, pp. 59-60.

The methods described in Chapter III were used for the estimation of the range of stages of development, the frequency of secondary follicle branching, follicle density, frequency of medullation, $\frac{S}{P}$ ratios, central primary follicle bulb diameter, length and fibre diameter, and the frequencies of lymphocytes, neutrophils and mast cells in the dermis. The side site values and in most cases the average of the three sites for each foetus or lamb were calculated for each of these parameters, and plotted against foetal age.

Using the side skin squares from the 1972-73 foetuses, the special stains and lipid histochemical techniques described on pp. 65-66 of Chapter III. were applied. The hip skin squares from these foetuses were used to provide material for electron microscopy as described on pp. 66-67 of Chapter III.

For estimations of the approximate surface area of each foetus, the formula

$$S.A. = B.W.^{0.77} \times 3.543$$

was applied (Schinckel, 1955b), where S.A. = body surface area (sq.cm.) and B.W. = body weight (g.). Surface area estimations were made for all foetuses but the 1972-73 115 days' gestation group, for which body weights were not recorded.

Results

Border disease was confirmed in all the experimental foetuses by histological examination of spinal cord (Plates 3 and 4) and placenta (Chapter I , pp. 9-10).

1. Follicle development

Figure 1 a, b, c, and d shows the range of development stages of each follicle type throughout gestation in control (Figure 1a and c) and Border disease affected (Figure 1b and d) foetuses. Figure 1a and b shows the combined range of values from the three sites examined, and Figure 1c and d shows the range of values for the side site only.

Since the assessment of the developmental stages was subjective and unlikely to be completely accurate, only differences of two or more developmental stages between groups at the same age were considered to be significant. On this basis, follicle development of control and experimental groups was similar at most ages except 95 days' gestation. Follicle development began with the initiation of central primary follicle plugs between 55 and 75 days' gestation (Plate 5). Lateral primary follicle initiation occurred between 75 and 85 days' gestation, so that by 85 days the primary follicle trios were distinct and complete (Plates 6 and 7). At 95 days' gestation, most primary follicles contained developing fibres, and secondary follicle plugs had made their appearance, but at this stage it was apparent that both primary and secondary follicles of Border disease were less well developed than those of control animals (Plates 8 and 9). Between 95 and 105 days' gestation most primary follicles had produced a fibre above the skin surface, an event which coincided with a thickening of the epidermis (Plate 8) and the differentiation of the periderm to stratified squamous epithelium. Some secondary follicles had developed to stage F3. There was no difference in the stages of follicle development at 105 days' gestation (Plates 10 and 11) or subsequently, between Border disease affected and control animals. The shoulder site sometimes seemed /

Table 3. Frequency of secondary follicle branching for each foetus.

Gestational age of foetus (days)		Border disease	Control
105	1.	(+)	(+)
	2.	(+)	(+)
	3.	+	
	4.	+	
	5.	+	
115	1.	(+)	(+)
	2.	+	+
	3.	+	++
	4.	+	++
	5.	+	+++
	6.	+	
	7.	++	
	8.	++	
	9.	+++	
125	1.	(+)	+++
	2.	(+)	
	3.	+	
135	1.	(+)	+++
	2.	+	++
145	1.	(+)	+
	2.	+	+
	3.		+

seemed more advanced in its stages of follicle development than the side and hip sites, so that the side site values tended to be slightly lower than the combined values of the three sites.

Secondary follicles continued to develop throughout gestation, often by branching from established secondary follicles. This phenomenon, secondary follicle branching, was observed at all ages from 105 to 145 days' gestation, (Table 3, Plates 12 and 13), but occurred most frequently between 115 and 135 days' gestation. The "parent" secondary follicles were at all stages of development from F3b, just before the formation of a keratinising hair cone, to F8, the production of a fibre above the skin surface. The assessment of the frequency was again very subjective, but from Table 3, it can be seen that branching occurred as frequently in Border disease affected as control animals except at 125 and 135 days' gestation, when it was less frequent in Border disease.

By 115 days' gestation, all primary follicles in Border disease affected and control foetuses were producing fibres (Plates 14 and 15) which had grown above the skin surface (Plates 16 and 17), and the most advanced secondary follicles contained fibre tips. By 135 days' gestation, some secondary follicles in Border disease affected and control animals were producing fibres above the skin surface, and by 145 days' gestation, most contained a keratinised fibre (Plates 18 and 19).

2. Follicle density

Figure 2a and b shows the changes in follicle density during gestation in Border disease affected and control foetuses, recorded as the average values for the three sites and the side site values respectively.

Primary follicle density showed no significant difference between Border disease affected and control animals, although at 95 days' gestation there was a tendency for primary follicle density to be higher in Border disease than in the controls. Total follicle density was measured from the age at which secondary follicles /

follicles could be included in the counts, 105 days' gestation, and the values for the controls tended to be higher than those of the Border disease affected foetuses. There was little difference in follicle density between sites so that the side site values and the average values for the three sites were similar.

For comparison with follicle density changes during gestation, the surface area changes with foetal age are illustrated in Figure 3. Foetal surface areas increased most rapidly from 95 to 135 days' gestation, so that the greatest rate of skin expansion occurred at this time. Surface areas of control and Border disease affected lambs were similar at all ages except 105 to 125 days' gestation, when they were lower in the experimental group.

3. Fibre medullation

The frequencies of primary fibre medullation and of large medullae (defined as described in Chapter III, p. 71) in control and Border disease affected foetuses have been recorded, both as average values for the three sites used, and as side site values only (Figure 4a and b). Figure 4c and d records the same parameters for central primary fibres only.

The greatest frequencies of medullation in the control foetuses occurred at 115 days' (Plate 14) and 145 days' gestation (Plate 18), but were never more than 25 per cent, in any animal. Fibre medullation was usually greatest at the hip site, so that side site values tended to be lower than the average values for the three sites. Primary fibre medullation occurred much more frequently in Border disease affected foetuses than in control foetuses at all ages after 105 days' gestation and was more than 40 per cent from 115 days' gestation (Plate 15), reaching its maximum of almost 100 per cent at 125 to 135 days' gestation before falling to 40 to 50 per cent at term (Plate 19). Fibre medullation was usually greatest at the hip site, so that side site values tended to be lower than the average values for all sites.

The frequency of large medullae in controls was always less than 5 per cent, but parallelled that of medullation as a whole, so that it occurred only at 115 and /

and 145 days' gestation. In Border disease affected foetuses, the frequency of large medullae was much greater, reaching maximum values of 30 to 40 per cent at 125 to 135 days' gestation, before falling to approximately 20 per cent at term. Many of these heavily medullated fibres were flattened or ribbon-shaped at term (Plate 19).

Plates 14, 15, 18 and 19 and Figure 4 a, b, c and d show that more central primary fibres were medullated and that these were more heavily medullated than primary fibres as a whole, throughout gestation.

4. Follicle and fibre dimensions

Some dimensions of the largest of the central primary follicles were recorded; these were follicle bulb diameter (Figure 5a and b), follicle length (Figure 6) and fibre diameter at sebaceous gland level (Figure 7).

In control foetuses, follicle bulb diameter rose steadily to 95 days' gestation, more rapidly to 105 days' gestation, and after dropping again at 115 days' gestation, rose steadily to term. Follicle length also increased steadily to 95 days' gestation, more rapidly to 105 days' gestation, and thereafter more slowly to term. Fibre diameter remained fairly steady throughout gestation, but tended to be maximal at 115 days' gestation. In the Border disease affected foetuses, the follicle size changes throughout gestation differed. Follicle bulb diameter and length increased slowly to 85 days' gestation, and after a slight drop at 95 days' gestation, rose very rapidly to 115 days' gestation (Plate 17) and 125 days' gestation, when they were maximal. Thereafter they fell to term, when they were still greater than those of the controls (Plates 20 and 21).

Like the frequency of medullation, follicle bulb diameter tended to be greater at the hip site than at the shoulder or side sites, so that the values for the side site tended to be slightly lower than the average values for the three sites (Figure 5a and b).

5. /

5. $\frac{S}{P}$ follicle and fibre ratios

$\frac{S}{P}$ follicle and fibre ratio changes with foetal age have been shown in Figure 8a and c as the average values for the three sites, and in Figure 8b and d as the side site values only.

At all ages, the Border disease values were lower than those of the controls (Plates 14, 15, 18 and 19). The $\frac{S}{P}$ follicle ratio of control foetuses rose rapidly from 105 to 115 days' gestation and more slowly to term, when it was about 3 to 3.5. In Border disease, the $\frac{S}{P}$ follicle ratio increased steadily but more slowly to about 2 to 2.5 at term (Figure 8a and b). The $\frac{S}{P}$ fibre ratio in controls increased steadily to about 2.5 to 3 at term, but in Border disease, its increase was slower, and after 125 days' gestation increased only very slightly to its maximum of about 1.5 at term (Figure 8c and d). Thus the proportion of fibre producing secondary follicles at term was greater in the control foetuses (80 per cent) than in the Border disease affected foetuses (60 per cent).

Total follicle density was calculated using the $\frac{S}{P}$ follicle ratio and primary follicle density, as described above (Chapter III, p. 70). It has been shown that primary follicle density did not differ between groups, so that the lower values for total follicle density in the Border disease affected animals were related to a lower number of secondary follicles. The values for $\frac{S}{P}$ follicle ratios and thus for total follicle densities differed little between sites, and so, for these parameters, the side site values and average values for three sites were similar (Figures 2a and b and 8a, b, c, and d).

6. Cell types in the dermis

Figure 9a and b shows the numbers of lymphocytes, mast cells and neutrophils in the dermis in control and Border disease affected animals throughout gestation, recorded as the average values for the three sites. The numbers of cells in both groups rose gradually to 95 days' gestation, and thereafter the mast cell and neutrophil concentrations fell towards term. However, the lymphocyte concentrations remained fairly steady from 105 days' gestation to term. /

term. Although the pattern of change was the same, there were differences in dermal cell numbers between the Border disease affected and control groups. In Border disease, the concentration of all these cell types seemed to be higher at 95 days. At other ages, the concentrations of neutrophils and mast cells were similar, but lymphocyte concentrations showed a tendency to be higher in Border disease from 95 days' gestation to term.

7. Glycogen, reticulin fibres and sulphydryl groups

Special stains were used to demonstrate the presence of glycogen, reticulin fibres and sulphydryl groups in the side skin of foetuses at 95 and 115 days' gestation.

The PAS stain (Plate 22) with the diastase control from which the glycogen had been selectively removed (Plate 23) showed that there was abundant glycogen in the follicle outer root sheath and epidermis at 95 days' gestation, and at 115 days' gestation the glycogen had disappeared from the epidermis, but was still plentiful in the outer root sheath cell cytoplasm (Plates 24 and 25). There seemed to be no difference in the strength of the reaction or in its distribution between the Border disease affected and control foetuses at either age.

The distribution of reticulin fibres in the dermal connective tissue was demonstrated by Gordon and Sweets' method. At 95 days' gestation, the papillary and reticular layers of the dermis were apparent. The more superficial papillary layer contained fine strands of reticulin, in an open network, but forming a dense shallow mesh beneath the basal layer of the epidermis, which extended around the follicles. The follicle bulbs extended into the reticular layer of the dermis in which the reticulin strands were coarser and tended to be parallel to the skin surface. The fibres seemed finer and more loosely arranged towards the subcutis (Plate 26). At 115 days' gestation, the distribution of the reticulin fibres was similar, but they formed an irregular mesh in the reticular layer in which no pattern was discerned. The papillary and reticular layers occupied the same relative proportions of the dermis at both ages studied, and no difference was observed between Border disease affected and control foetuses in the characteristics of the reticulin fibres, at either age.

The Mercury Orange method was used to demonstrate the sulphydryl or keratogenous zone in primary fibres of foetuses aged 115 days' gestation. The zone extended from approximately 0.21 to 0.28 of the follicle length from the bulb in control foetuses, and from approximately 0.30 to 0.44 of the follicle length from the bulb in Border disease affected foetuses so that in this group it appeared to be shifted distally. The length of the zone, about one-fifth of the follicle's length, was not significantly different between these groups, but its position in Border disease was significantly more distal ($p < .01$) than its position in control foetuses (Plates 27 and 28).

8. Lipid histochemistry

The Sudan IV and OTAN methods were used to demonstrate lipid in the skin of foetuses at 95 and 115 days' gestation. The sebaceous glands stained strongly with both stains (Plate 29), due to the presence in the sebum of cholesterol esters (Ryder and Stephenson, 1968). Small Sudanophilic and Otanophilic droplets were often present in the cytoplasm of the cells of the outer root sheath (Plates 30 and 31). These droplets were present at both ages in both groups of animals. No difference in the distribution or intensity of the reactions was apparent between Border disease affected and control animals.

9. Electron microscopy

Ultrastructural study was confined to the follicle bulb and papilla at the hip site. At 95 days' gestation, the basal cells of the bulb were attached at their base to the basal membrane (Plate 32), and desmosomes were frequently observed between cells. The cell cytoplasm was packed with very small dense granules. Very little endoplasmic reticulum was evident but many mitochondria were observed. The dermal-epidermal junction consisted of a continuous structureless extracellular membrane. The cells of the papilla contained paler cytoplasm in which mitochondria and some endoplasmic reticulum could be discerned. In general, their nuclei seemed larger than those of the bulb cells, but in both cell types nucleoli were prominent. The nuclei of the outer root sheath cells were displaced towards the differentiating central core and the voluminous cytoplasm was packed with granules of glycogen (Plates 33 and 34) and occasionally showed lipid droplets (Plate 33).

By /

By 115 days' gestation, the basal cells were low columnar and orientated at right angles to the basement membrane, to which they were attached (Plate 35). The cytoplasm was more densely packed with small dark granules, and the occasional mitochondrion was observed. The nuclei were large, with prominent nucleoli. The basal membrane was distinct, and the cells of the papilla showed a more varied morphology than at 95 days' gestation. Most had large irregular nuclei, abundant endoplasmic reticulum and many mitochondria, and occasionally dispersed granules were observed. Blood capillaries were present in the papillae at this stage. The papillae also contained cells with large electron dense cytoplasmic granules which tended to lie towards the periphery of the cells (Plate 36). These cells were generally found near the basement membrane, and their surface showed numerous fine finger-like projections. They were not observed in the histological sections prepared for light microscopy, but were occasionally seen in the papillae of primary follicles in the "thick" Araldite sections, Giemsa stained, which were prepared prior to thin sectioning. There was no difference in their numbers or in their distribution between control and Border disease affected animals. Nor was there any apparent difference in the ultrastructure of the cells of the primary follicle bulbs and papillae between groups.

Discussion

The birthcoats of medium and fine-fleeced breeds are short, fine and tightly coiled. In Border disease, the birthcoats of these breeds become straight and coarse (Plates 1 and 2), and Barlow (1972b) described an increased frequency of medullated fibres in these birthcoats. Although affected lambs have, in the past, been described as "hairy" or "fuzzy", these are ill-defined and colloquial descriptions. The nature of the birthcoats of lambs in the present experiment, and Barlow's (1972b) findings suggest that "straight" and "coarse" are more appropriate adjectives to describe affected birthcoats, and these terms will be used below.

For the present study of the effect of Border disease on follicle population development, the Cheviot X Dorset Horn cross was used. Ryder (1956c) has never observed large medullae in the birthcoat fibres of newborn Cheviot lambs, and found that the frequency of medullation generally was low. The Dorset Horn has a fleece with very little medullation, if any (Ryder and Stephenson, 1968). Thus, the birthcoat of the normal Cheviot X Dorset Horn cross, being likely to show little medullation, was suitable for this study of medullation increase in Border disease.

In Chapter II it was shown that fibre medullation is generally considered to be related to follicle bulb size, and so follicle dimensions might be abnormal in Border disease. The other parameters which could be affected are follicle density, the rate of follicle development, and the relative numbers of primary and secondary follicles. All these parameters were measured in the present study. In the control and Border disease affected foetuses, follicle development began at approximately 55 days' gestation, an age similar to those reported in other prenatal studies (Chapter II, pp. 39-40). All primary follicles were initiated by /

by 85 days' gestation (Figure 1, Plate 6), and the increasing follicle density throughout the initiation phase, in spite of the concurrent skin expansion (Figure 3), indicated a rapid rate of initiation.

At 95 days' gestation, the central primary follicles of most of the control foetuses were beginning to produce fibres. Simultaneously, the epidermis with its superficial layer of periderm, differentiated to stratified squamous epithelium, and thickened markedly (Plate 8). The thickening of the epidermis at this stage may be due to the parakeratosis of the first cornified epidermal layers, described and illustrated by Lyne (1957). At this age, the primary follicles in Border disease, as judged by their stages of development, density and size, seemed to be retarded compared with those of control foetuses at the same site (Figures 1, 2, 5 and 6; Plates 8 and 9). The follicle populations of the two groups were similar again at 105 days' gestation (Figure 1, Plates 10 and 11), and subsequently (Plates 14, 15, 18 and 19). Thus, the retardation of follicle development at 95 days' gestation is temporary, and since it is accompanied by an increase in the numbers of dermal leucocytes and mast cells, it is the first indication of an abnormal process in the skin. The abnormality in the skin at this stage is essentially one of defective development.

At 105 to 115 days' gestation, the controls' central primary follicles underwent a phase of rapid growth, as indicated by increases in their bulb and fibre diameters and fibre medullation (Figures 4, 5 and 7). The subsequent fall in these dimensions, together with a reduction in follicle length increase (Figure 6), almost certainly corresponds to the "prenatal check" described by Dry (1933), and discussed in Chapter II, pp. 41-44. These observations demonstrate the validity of Burns' (1966) speculation that the prenatal check to fibre growth could be caused by a reduction in follicle size affecting diameter more than length.

In /

In Border disease there was also a phase of vigorous primary follicle activity at the onset of fibre production, but it was extended and much more fully expressed than that of control foetuses. Central primary follicle and fibre size, and the frequency of primary fibre medullation were greater than those of controls from115 days' gestation, and reached maximal values at 125 to 135 days' gestation (Figures 4, 5, 6 and 7; Plates 14, 15, 16, 17, 18, 19, 20 and 21). Although a prenatal check occurred after 135 days' gestation at term the primary follicles and fibres of affected foetuses were still significantly larger with more medullation than those of control foetuses (Plates 18 and 19).

In the course of the histological work, it was observed that in control and Border disease affected foetuses, medullation normally appeared in fibres approximately 25 m. in diameter, and as described in the literature (Chapter II, pp. 24, 27), the degree of medullation tended to increase as fibre diameter increased, so that fibres approximately 40 m. in diameter usually had large medullae. The greater increase in medullation in Border disease was thus not associated with a greater expression of medullation at comparable fibre diameters.

The medullation of central primary fibres tended to be greater than that of primary fibres as a whole, and maximum medullation occurred at 125 days' and 135 days' gestation respectively (Figure 4). Thus it seemed that lateral primary fibres had a lower frequency and degree of medullation than central primary fibres, and their follicles were subject to the prenatal check later in gestation than the central primary follicles.

No control foetus had more than 30 per cent primary fibre medullation or more than 5 per cent large medullae in primary fibres at any stage so these values will be used in future studies as "ceilings" /

"ceilings" for normal medullation in foetuses of this breed. In Border disease, the frequency of medullation after 115 days' gestation was more than 40 per cent with more than 10 per cent large medullae, and these values will be used as baselines for abnormal medullation in foetuses of this breed.

In both groups, the rate of initiation of secondary follicles was rapid at first, since total follicle density increased rapidly to 105 to 115 days' gestation (Figure 2) and the $\frac{S}{P}$ follicle ratio showed a steep rise from 105 to 115 days' gestation (Figure 8). Thereafter the ratio of initiation was slower, since total follicle density fell to term. In the control foetuses, there was a second "wave" of secondary follicle initiation between 125 and 135 days' gestation, as indicated by a marked $\frac{S}{P}$ follicle ratio increase at this time. This observation was similar to that of Fraser (1951a), who found that secondary follicle initiation occurred in two "waves" in the Merino.

In Border disease, the number of secondary follicles initiated was less, as indicated by the lower total follicle density and the lower $\frac{S}{P}$ follicle ratio throughout gestation (Figures 2 and 8). The first "wave" of initiation was delayed and the second "wave" seemed to be absent, an observation possibly related to the reduced frequency of secondary follicle branching in Border disease at 125 to 135 days' gestation (Table 3).

The smaller size of the Border disease affected foetuses from 105 to 125 days' gestation (Figure 3) would tend to lead to an apparent increase in follicle density by "concentrating" the follicles in a smaller surface area. The total follicle density in Border disease, in spite of this effect, was lower than in control foetuses, suggesting a marked reduction in the number of secondary follicles which developed.

As /

As well as a reduced number of secondary follicles in Border disease, a small proportion of these had developed to a fibre-producing stage at term.

The literature suggests two possible causes of this secondary follicle suppression in Border disease. According to the "competition theory" of Fraser (1951b), large primary follicles are more effective competitors for space and substrate than the smaller, later developing secondary follicles, whose development is therefore suppressed. This theory was proposed to account for the low $\frac{S}{P}$ ratios of coarse fleeced breeds. Burns (1953) argued that during follicle development, primary follicles produced "organiser" substance, which induced the development of secondary follicles around them. It may be that, in Border disease, the enlarged primary follicles produce less "organiser" so that fewer secondary follicles develop in association with them. Alternatively, the impaired foetal maternal exchange which almost certainly occurs as a result of the placentitis of Border disease (Barlow, 1972a) could result in secondary follicle suppression, since it is known that this can be caused by poor maternal nutrition in late gestation (Schinckel, 1955a; Short, 1955b). These theories will be discussed more fully in Chapter VIII.

In the present study, the histological results discussed above have been obtained from the average of the three sites sampled, but throughout the study data were also compiled for the side site only, so /

so that an assessment could be made of the values of side site sampling in future studies. For all the parameters measured but those of medullation and bulb diameter, the comparison showed close agreement. Although follicle development tended to be more advanced at the shoulder than at side and hip sites, and subsequent changes during follicle development tended to follow this pattern, the side site values were only slightly lower than the average values for the three sites. Medullation and follicle bulb diameter tended to be relatively greatest at the hip site so that the side site value was lower for these parameters, as Carter, Terlecki and Shaw (1972) suggested would be the case for medullation. Since these discrepancies were small, it was considered that use of the side site only was justified in future studies.

The differences in the activity of primary follicles of Border disease affected and control foetuses prompted a comparison of some aspects of primary follicle metabolism. Based on the histological findings, two age groups in which critical stages of follicle development had been reached were selected. The stage immediately before primary fibre production and the stage of increasing follicle bulb size, 95 and 115 days' gestation respectively, were used for this study.

Abnormalities of lipid metabolism have been indicated in the central nervous system of affected lambs by use of such methods as the Sudan IV and OTAN stains (Barlow and Dickinson, 1965). These tests were applied to skin sections in an attempt to detect concurrent abnormalities of lipid metabolism in the skin follicles. No differences were observed between the control or experimental foetuses at the ages studied, although droplets of lipid were demonstrated in the cytoplasm of the cells of the proximal and middle thirds /

thirds of the outer root sheath in both groups (Plates 30, 31, and 33). There is very little literature on lipid metabolism from which the significance of these droplets can be assessed, but they probably correspond to the small Sudanophilic droplets in the outer root sheath described by Montagna (1950), and Montagna, Chase and Hamilton (1951) (Chapter II, p. 27).

It is known that the follicle outer root sheath cell cytoplasm contains much glycogen (Montagna, 1956; Ryder, 1958) (Chapter II, p. 27). On the premise that this "store" might be modified by any abnormality in follicle metabolism, the PAS test for glycogen was applied. No difference between the Border disease affected and control animals was apparent. It is of interest to note that in both groups glycogen was also present in the periderm at 95 days' gestation, but at 115 days' gestation, when the periderm had differentiated to become stratified squamous epithelium, it was absent (Plates 22, 23, 24 and 25). This accorded with the findings of Rothman and Schaaf (1929), who according to Rothman (1954), provided histochemical evidence that there was considerable storage of glycogen in human and animal skin in the early part of the foetal life.

In their studies of the fibre keratinisation process in copper deficiency of sheep, Marston (1952, 1955) and Gillespie (1964), by demonstrating the elongation of the keratogenous zone of these fibres (Chapter II, pp. 26,50) showed the keratinisation process was abnormal. Ryder (1958) used the Mercury Orange method to demonstrate the keratogenous zone of sheep wool fibres. It was felt that a similar technique applied to the fibres of the Border disease affected foetuses might indicate abnormality in keratinisation which could contribute to the birthcoat coarseness. The Mercury Orange method applied /

applied to 115 days' Border disease and control foetuses showed that, although in Border disease this zone was shifted towards the skin surface, it occupied the same proportion of follicle length (Plates 27 and 28). This observation may represent an abnormality in fibre keratinisation in Border disease. Alternatively, in large medullated fibres, the keratinisation zone may be situated more distally than it is in smaller, non-medullated fibres. Full discussion of fibre keratinisation in Border disease is presented in Chapter VII.

During the histological studies, it was observed that the connective tissue structure often varied from one section to another. Gordon and Sweet's method for reticulin was used to demonstrate any difference in connective tissue structure between Border disease and control animals (Plate 26). The results indicated that no difference occurred in the distribution of reticulin fibres between the control and Border disease affected groups. It may be that the apparent differences in connective tissue observed with the Sacpic stain were due to slight differences in the thickness of the sections, or inconsistencies in the staining technique.

In an attempt to detect any inflammatory or immunological responses to the Border disease process in the skin, a study of some cell types in the dermis was made. Neutrophils and lymphocytes were chosen as cell types in which abnormal numbers might indicate such responses. An estimation of mast cell numbers was included in the study since these cells have been implicated in the control of hair growth (Selye, 1965). The concentrations of these three cell types increased from 55 to 95 days' gestation before falling to term. It was not possible to estimate how the absolute numbers of these cells changed, since the surface area of the foetuses increased /

increased throughout gestation. The tendency for lymphocytes to be present in higher concentrations in Border disease affected foetuses than in control foetuses from approximately 105 to 125 days' gestation may indicate a type of immunological response to Border disease in the skin.

In general, the ultrastructure of the differentiating cells of the bulbs and papillae resembled that described by Bell (1967a) for the Rhesus monkey foetus. The many small dense granules in the basal cell cytoplasm (Plates 32 and 35) Mercer (1958) regarded as ribonucleoprotein particles. The associated small amount of endoplasmic reticulum was taken by Mercer to be a feature of differentiated cells with a great potential to synthesise protein, but with no secretory mechanism. In the foetal sheep, the presence of these granules at 95 days' gestation probably indicates protein synthesis in cells which are becoming specialised towards the production of keratin. Between 95 days' gestation, immediately before fibre production, and 115 days' gestation, at which age the follicles were producing fibres, blood vessels invaded the papillae and the morphology of the papillary cells became more diverse (Plate 35). In addition, the number of cells with large cytoplasmic electron dense granules increased. These were seldom seen at 95 days' gestation but appeared frequently at 115 days' gestation in both Border disease affected and control foetuses (Plate 36). The cells were not apparent on light microscopy using the stains described (Chapter III, pp. 64-65).

Selye (1965) observed that mast cells occurred in the papillae of actively growing human follicles. It has been established that, in the present study, at 115 days' gestation the primary follicles of sheep foetuses were active, so that there is a possibility that they /

they were mast cells. However, if so, their failure to stain with the Sacpic stain, Barlow's Tribasic stain and Pinkus' Giemsa, (Chapter III, p. 65) cannot be explained.

Melanocytes also have numerous dense cytoplasmic granules, but they possess long dendritic processes (Barnicot and Birbeck, 1972), and in the sheep, very seldom occur in the papillae, being confined to the basal layer of the cells of the follicle bulb and the outer root sheath (Auber, 1952).

Thus the identity of the cells is not known. However, they conform to the description of protein secreting cells given by Mercer (1958), and in view of their situation near the basal membrane in the follicle papilla, it is possible that they may participate in the epidermal-dermal processes involved in follicle differentiation. As such, they might be involved in the pathogenesis of the skin lesion of Border disease, but evidence against this hypothesis is provided by the observation that their numbers and their morphology did not appear to differ between control and Border disease affected animals.

Conclusions.

1. Border disease had no effect on the ages of initiation of primary follicles, on their density or on their development to 105 days' gestation.

2. The main effect of Border disease was to extend the phase of primary follicle activity prior to the prenatal check, so that the primary follicles became larger and produced fibres with a greater frequency and degree of medullation. From 115 days' gestation to term, Border disease affected foetuses had more than 40 per cent medullated primary fibres with more than 10 per cent of primary fibres containing large medullae, whereas these parameters for control foetuses were less than 30 per cent and 5 per cent respectively at all gestational ages. The keratogenous zone of primary fibres was situated more distally in the hypertrophied primary follicles of Border disease affected foetuses at 115 days' gestation.

3. Secondary follicle branching occurred frequently in the control Cheviot X Dorset Horn cross foetuses, probably contributing to the second wave of secondary follicle development in late gestation. In Border disease the first wave was delayed and the second wave was reduced. Fewer secondary follicles were initiated and a smaller proportion of these was producing fibres at the end of gestation.

4. The side site values resembled the average values for the three sites originally selected as being representative of the fleece type as a whole. In further studies, a single side site sample would therefore be justified.

5. /

5. The dermal concentrations of lymphocytes tended to be higher after 105 days' gestation in Border disease affected foetuses.

6. No other abnormalities were detected in a histological study of the cell populations and structure of the dermis or in a histochemical and ultrastructural study of primary follicles in Border disease.

CHAPTER V.

The effect of prenatal Border disease infection on the post-natal skin follicle population of Cheviot X Dorset Horn cross lambs

CHAPTER V

Introduction

In reports of Border disease in the field, it has been observed that adult affected sheep have a "scruffy" appearance (Shaw, 1962), and Barlow (personal communication) comments that the coarseness of the birthcoats of affected lambs persists into adult life. Post-natal histological studies of the fleece abnormalities in Border disease have been confined to newborn lambs (Carter, Terlecki and Shaw, 1972), although Dry (unpublished observations) and Nott and Shaw (1967) studied the birthcoat fibres of older affected lambs. No study has been made of post-natal birthcoat fibre production, the birthcoat shedding process and its replacement with the adult fleece in Border disease. Such a study has been undertaken in the present series of experiments to determine the duration of effect of pre-natal infection on the follicle population. It was thought that this would provide an important contribution to knowledge of the pathogenesis of Border disease in the skin, and also that it might elucidate the mechanism of action of the disease on the follicle population. In addition, the potential value of skin studies in Border disease diagnosis could be assessed.

Materials and methods:

The animals used in the following experiment can conveniently be divided into two groups.

Group I. Seventeen South Country Cheviot ewes from farms with no history of Border disease and in lamb to Dorset Horn tups were used. Nine were inoculated with Standard Border disease inoculum, and eight control ewes were inoculated at the same gestational age with material from normal lambs at 54 days' gestation. For the purposes of concurrent experimental work on the copper status of Border disease affected lambs, the ewes were fed a standard low copper diet, and housed in individual pens from about two weeks before inoculation until the end of the experiment. Eight experimental and five control ewes produced live lambs at term, and these were skin sampled as described in Chapter III, pp. 59-60, at birth and at three weeks of age, when they were killed. The numbers of lambs at each sampling age are shown in Table 4.

Group II. From two concurrent Border disease experiments, two clinically normal Cheviot X Dorset Horn lambs were taken as controls, and two with the body tremor and coarse straight birthcoat characteristic of Border disease were selected for further studies of the follicle population from birth to six months of age. One male control lamb and one female Border disease affected lamb were born to uninoculated Cheviot ewes in an experiment designed to simulate conditions for natural transmission of the disease. During their pregnancy, these ewes were in contact with pregnant ewes which had been "hairy shaker" lambs themselves, one of which aborted during the period /

Table 4. The numbers of lambs at each sampling age in Group I.

Disease status	Number of lambs	
	Newborn	3 weeks of age
control	6	6
Border disease affected	9	7

Table 5. The numbers of lambs at each sampling age in Group II.

Disease status	Number of lambs					
	Newborn	Age (weeks)				
		3	6	9	12	24
control	2	2	2	2	2	2
Border disease affected	2	2	2	2	2	1

period of contact, and they had been mated with the Dorset Horn ram which had previously served the "hairy shaker" ewes.

The other two lambs, a control female and an affected male, were born to Cheviot ewes injected at 54 days' gestation with standard Border disease inoculum, in an experiment designed to test the immunity of ewes with previous experience of Border disease or Bovine Viral Diarrhoea/Mucosal Disease (BVD/MD). The dams of the two lambs had been inoculated during a previous pregnancy with the Border disease inoculum and BVD/MD respectively.

The control and affected lambs were assessed at birth on clinical grounds, and histological examination of the spinal cord of other lambs of these types in the same groups showed that the clinical assessment was invariably supported by the histological findings. The control male lamb was castrated, and all four lambs had their tails removed with "Elastrator" rubber rings at two to three days of age. Before weaning, the lambs ran with their mothers in large covered boxes. At weaning, at three months of age, they were brought indoors into large boxes, together with the Blackface Border disease affected lambs described in Chapter VII. Side skin samples and blood samples were removed from each lamb at the ages shown in Table 5, and body weights and cannon bone circumferences were recorded.

The affected ram lamb born to the ewe which had been inoculated during a previous pregnancy with BVD/MD inoculum, had a severely retracted or undershot lower jaw. This disability and bullying by the other lambs in the group may have contributed to a loss of condition at about nine weeks of age. The group was sub-divided so that he shared a pen with some of the smaller ewe lambs, but in spite of /

of this, he developed diarrhoea, rapidly lost weight and became anorexic. The diarrhoea did not respond to anthelmintic treatment, or to intramuscular injections of antibiotic and cortisone. Subcutaneous glucose saline was administered every second day for ten days, but the lamb became progressively weaker, and was killed at twelve weeks of age. On post-mortem examination, no gross lesions were detected apart from a mild mucoid enteritis. Histological examination of the brain and spinal cord showed lesions characteristic of Border disease, but their contribution to the loss of condition of the lamb is impossible to assess.

As in Chapter IV, the histological parameters measured in skin samples from the lambs of Groups I and II were primary follicle and fibre size, fibre medullation and the $\frac{S}{P}$ ratios. In addition, secondary follicle medullation was recorded, as were the frequencies of "brush end" and "shed empty" follicles (Chapter III, p. 71).

Results

Of the lambs in Group I, all the controls were considered to be clinically normal. Eight of the nine experimental lambs showed a body tremor and an abnormally coarse and straight birthcoat, and one had an abnormal birthcoat but no body tremor. On histological examination of the spinal cord at three weeks of age, all nine lambs were shown to have central nervous system lesions characteristic of Border disease.

Of the four lambs in Group II, the two Border disease affected lambs had a body tremor and a coarse straight birthcoat. This fleece abnormality was apparent throughout the period of the experiment in the affected lambs (Plates 37 and 38). As in field reports (Shaw, 1962), most of the affected lambs in both groups were lighter and had finer long bones.

At birth, the percentages of medullated and heavily medullated primary fibres of all affected lambs were above the baselines of 40 per cent or 10 per cent respectively, derived as described in Chapter IV. These values were reached by only one control lamb which had 54 per cent primary fibres with very small medullae, but no primary fibres with large medullae (Figure 10). As in Chapter IV, the primary fibre medullation was more marked in central than in lateral primary fibres (Figure 10c and d).

Primary fibre medullation at three to six weeks was higher than at birth in both groups, but it was still very much greater in Border disease affected lambs than in controls (Plates 39 and 40). The occurrence of small medullae in the primary fibres of control lambs was frequent, but large medullae were seldom seen. In Border disease, many primary fibres contained large medullae, and again they /

they were more frequent in central than in lateral primary fibres (Figure 10c and d). Following a reduction in primary fibre medullation at about twelve weeks, its occurrence again increased at twenty-four weeks (Plates 41 and 42). The much greater frequency of medullation in Border disease continued to be evident.

Secondary fibre medullation was infrequent at birth, but increased to three to six weeks of age when it was 10 to 50 per cent (Figure 11). It remained at about this level to six months of age, showing no consistent difference between groups.

Primary follicle hypertrophy,* as measured by the largest bulb diameter, the longest follicle length and the largest central primary fibre diameter was consistently evident in Border disease affected lambs when compared with control lambs, throughout the experiment. In both groups, these parameters increased to three to six weeks of age, showed a reduction at about twelve weeks and rose again to twenty-four weeks of age (Figures 12, 13 and 14).

In both groups, the number of secondary follicles, as measured by the $\frac{S}{P}$ follicle ratio, tended to increase from birth to about six weeks of age (Figure 15a), and the number of fibre producing secondary follicles, as measured by the $\frac{S}{P}$ fibre ratio, increased rapidly to three weeks, and by six weeks almost all secondary follicles were producing fibres (Figure 15b). At all ages, the $\frac{S}{P}$ ratios were significantly lower in Border disease affected than in control animals, but their maturation rate was similar, since the proportions of secondary follicles producing fibres were similar in both groups, being about 60 per cent at birth, 90 per cent at three weeks, and almost 100 per cent at six weeks of age.

"Brush end" and "shed empty" follicles were observed only in the /

*Throughout the thesis, the term "primary follicle hypertrophy" is used to describe enlargement of the primary follicle.

the Border disease affected lambs. The affected ewe lamb had 3 per cent and 4 per cent shedding or shed primary follicles, at three and six months of age respectively, but the affected ram lamb had 12 per cent when killed at twelve weeks. At this age, he was moribund, and showed other changes in skin follicle activity which did not conform to the pattern described above. The occurrence of fibre medullation and primary follicle and fibre dimensions had fallen dramatically from six weeks of age (Figures 10, 12, 13, and 14). The $\frac{S}{P}$ ratios were not apparently affected (Figure 15).

Discussion

Every Border disease affected lamb had an abnormally coarse and straight birthcoat and fleece, compared with the finer, wavy or crimpy birthcoat and fleece of the control lambs (Plates 37 and 38). Although abnormal pigmentation has been described in field reports of Border disease (Hughes, Kershaw and Shaw, 1959; Shaw 1962; Bell, 1967b), in the present experiment, no pigmentation was observed in any animal, Border disease affected or control.

The main effect of Border disease, primary follicle hypertrophy with an increased frequency and degree of medullation, was apparent in all affected lambs except one ram lamb in the samples taken during its terminal illness. The difference in central primary follicle size is probably less apparent in the results than in fact, because in control lambs, the central primary follicles and fibres were often smaller than those of the lateral primary and secondary follicles (Plates 39, 40, 41 and 42). Thus, in measurements of the largest and longest follicles, the controls' secondary follicles may have been measured. Since the effect of Border disease would thus have been under-estimated, it was felt that the method was still valid for the purposes of the experiment.

The occurrence of very small medullae in the primary fibres of control lambs masks the effect of Border disease when medullation of any degree is considered (Figure 10a), so that the frequency of large medullae is a more useful indicator of abnormality in the follicle populations of lambs of this breed (Figure 10b; Plates 39, 40, 41 and 42). As in Chapter IV, the central primary follicles and fibres are affected to a greater degree than lateral primary fibres (Figure 10c and d), and in this respect the follicle population in Border disease resembles that of carpet fleeced breeds such as the Scottish Blackface (Burns, 1953).

The /

The prenatal check described in Chapter IV was evident in newborn lambs of both groups in the present experiment, and for each group, values for follicle and fibre size and medullation are comparable with those obtained for the foetuses and lambs of the corresponding group in Chapter IV (Figures 10, 12, 13 and 14). Primary follicle and fibre size increased rapidly, and by three weeks of age were much greater than the maximal prenatal values in corresponding groups (Figures 4, 5, 6 and 7).

The replacement of birthcoat fibres with fibres of the adult fleece has been shown to occur at up to five months of age in the Wiltshire Horn (Slee, 1963) but Ryder and Stephenson (1968) consider that one to three months is more usual in other domestic breeds. However, in finer fleeced breeds, often only a small proportion of follicles can be identified as "brush-ends" (Burns, 1954b) and the relatively low number of shedding follicles reflects the tendency of their wool fibres to grow continuously (Slee and Carter, 1961; Ryder and Stephenson, 1968). Instead of shedding, some fibres seem to become thinner with a loss of medullation (Burns, 1953, 1954b). These observations might account for the reduction in primary follicle size, fibre diameter and medullation (Figures 10, 12, 13 and 14) observed here at three months of age in most lambs, and the simultaneous occurrence of a proportion of "brush-end" follicles in the Border disease affected lambs. The latter observation might be due to the presence in the birthcoat of heavily medullated fibres, many of which are probably kemps, which begin to shed at about seven weeks of age (Deshpande, 1953).

Because of the suppression of medullation peri-natally and at twelve weeks of age, these ages should be avoided when sampling for Border disease diagnosis. In view of the occurrence of small medullae in /

in controls, and the greater expression of medullation in central as opposed to lateral primary follicles in Border disease, it is suggested that, as baselines for the abnormal medullation of Border disease in this breed, five per cent primary fibres with large medullae and ten per cent central primary fibres with large medullae could be used. These could be applied to animals of this breed at all ages from about 115 days' gestation to at least six months of age, excluding the perinatal and birthcoat shedding phases.

The suppression of follicle activity and the increase in the frequency of "brush end" follicles in the moribund ram lamb illustrates that the effects of Border disease can be eliminated under some conditions. "Stress", by its effect on the pituitary gland and the adrenal cortex, is known to produce profound changes in the follicle population identical to those which occurred in the ram lamb (Chapter II, p. 51). Such changes might be expected therefore in severe cold, malnutrition and disease. The possibility that the enlarged primary follicles of Border disease could be affected by such factors should be borne in mind in diagnostic and experimental work, but it illustrates that affected follicles can respond to changes in the internal environment in a manner similar to that of normal follicles.

In Chapter IV, it was suggested that the effect of Border disease was on the primary follicle bulb or papilla. In the absence of any morphological or ultrastructural change in the cells of the bulb and papilla at the time of the effect of the agent, it could be argued that the effect is biochemical, and induces no cellular reaction in the surrounding tissue. The duration of effect from 115 days' gestation (Chapter IV) to at least six months of age shows that it persists through at least one growth cycle, and suggests that it might be an irreversible effect on cells of the follicle papilla during their differentiation.

In /

It may be that the Border disease process interferes with normal differentiation of the cells of the primary follicle papillae.

That the papillary cells not the bulb cells are affected seems likely as a result of the findings of McLoughlin (1963) and Rawles (1965) who have shown that the cells of the papilla usually determine the activity of the overlying bulb cells, and so determine the nature of the follicle product. The importance of the papilla in hair growth has been discussed in Chapter II. As a working hypothesis to explain these findings it is suggested that Border disease interferes with the production of an inhibitor substance, which could be a suppressor of follicle bulb growth or of mitotic activity in the cells of the bulb, and may have evolved during the domestication of sheep, with the selection for finer fleeced types. Interference with the production of this hypothetical substance could lead to the reversion to the more primitive pattern of follicle activity which seems to be a feature of Border disease in medium-fleeced and fine-fleeced breeds.

The permanency of the effect of Border disease may reflect the continued action of the agent on the cells of the papilla, such as might arise from a situation of "immune tolerance". This is a phenomenon in which antigenic material comes into contact with the cells of an antibody forming system at a stage in foetal life before the cells have reached maturity, with the result that suppression rather than stimulation of antibody formation against the particular antigen occurs (Weir, 1970). The validity of this hypothesis will be tested when the serological investigation of the immune response of the affected lambs to the Border disease agent has been made. However, if the agent is increased in phase with infected cells, it may remain intracellular, and provoke no immunological response, even without a situation of immune tolerance.

In /

In Chapter IV, it was shown that compared with controls, fewer secondary follicles developed in Border disease affected foetuses. The present experiment confirmed that there were fewer secondary follicles in newborn Border disease affected lambs, and indicated that the $\frac{S}{P}$ follicle ratio remained lower until at least six months of age. It has been reported that most secondary follicles in the Suffolk are initiated in the first few weeks after birth (Burns, 1954[b]), so that the $\frac{S}{P}$ follicle ratios at this age represent the potential adult $\frac{S}{P}$ ratios. The fluctuations in $\frac{S}{P}$ follicle ratio after three weeks of age seen in Figure 15a, probably represent experimental error, but their consistently lower values in the Border disease affected animals at and after this age suggest that this is a permanent effect of Border disease, although possibly a non-specific effect (Chapter IV, p. 102). Although the Border disease affected foetuses at term described in Chapter IV had a lower proportion of fibre-producing secondary follicles than their newborn controls, in the present experiment, there was no such difference between the groups of newborn lambs. The relative immaturity of the secondary follicles of the 145 day foetuses compared with those of the newborn lambs may have been, at least in part, due to the difference in age of two or three days between the groups, since maturation of secondary follicles occurred very rapidly at this time. The lack of difference in secondary fibre medullation between groups suggests that there is no difference in secondary follicle or fibre size, that is, the Border disease process does not alter secondary follicle size (Plates 39, 40, 41 and 42).

The evidence presented here indicates that the main effect of Border disease, primary follicle hypertrophy, and a reduced number of secondary follicles, possibly a non-specific effect of Border disease, are /

are not transient features of the disease. If the changes are permanent, they might be exploited in the detection of adult cases of Border disease which are almost certainly reservoirs of infection in the field. The accessibility of skin for sampling gives it obvious advantages, and together with serological studies, it might have real practical value. Although normal values for fibre medullation and $\frac{S}{P}$ ratio would have to be established for each breed the evidence presented here indicates that the differences can be very large, and that baselines could be derived from the study of relatively few animals.

Conclusions

1. In Border disease affected Cheviot x Dorset Hurn cross lambs, hypertrophy of primary follicles, especially central primary follicles with an increased frequency and degree of fibre medullation, continued to be evident after the first wool cycle, until at least six months of age.

2. At all ages, baselines for abnormal medullation of five per cent primary fibres with large medullae and ten per cent central primary fibres with large medullae are proposed. Medullation of any degree is a less useful indication of primary follicle hypertrophy because of the high frequency of very small medullae which can occur in control animals.

3. Fewer secondary follicles developed in Border disease than in control animals, but there was no difference in their size or fibre medullation.

4. In both groups, there was a reduction in follicle activity, as measured by follicle and fibre size and fibre medullation during the pre-natal period and at the time of shedding of the birthcoat fibres at about three months of age. During these phases, the effect of Border disease on the follicle population was less apparent.

5. In a moribund Border disease affected lamb, a reduction of follicle activity occurred, so that the abnormalities of the primary follicle population were not apparent.

CHAPTER VI.

The effect of inoculation at various gestational ages.

Table 6: The results of histological examination of brain, spinal cord and maternal caruncles of foetuses from ewes killed 15 days after inoculation at various gestational ages (Group I)

	Foetus no.	Foetal age at maternal inoculation	Foetal age at slaughter	Presence of lesions, in the brain and spinal cord	Presence of lesions in the maternal caruncles
	1	50	65	±	+
	2	60	75	±	+
	3	60	75	±	+
	4	70	85	+	+
	5	70	85	+	+
Twins	6	70	85	+	+
	7	80	95	+	+
	8	90	105	+	+
	9	100	115	+	+
	10	100	115	+	+
	11	100	115	+	+
	12	110	125	+	+
Twins	13	110	125	+	+
	14	110	125	+	+
	15	120	135	+	+
	16	120	135	±	+
Twins	17	120	135	+	+
	18	120	135	+	+
	19	130	145	+	+
	20	130	145	+	+

\+ positive

± doubtful

Table 7: The clinical symptoms and results of histological examination of the brain and spinal cord of three week old lambs whose dams were inoculated with Border disease at various gestational ages (Group II).

Lamb No.	Foetal age at inoculation of dam (days)	Shaker	Conformation	Average fleece scores	Presence of lesions in the brain and spinal cord
21	uninoculated	-	normal	II	-
22	uninoculated	-	normal	I	-
23	130	-	normal	II	±
24	130	-	normal	III 4	-
25	120	-	normal	I	±
26	120	-	normal	I	±
27	110	-	normal	III 4	±
28	110	-	normal	I	+
29	110	-	normal	I	+
30	100	-	normal	I	±
31	100	-	normal	II	±
32	90	-	normal	I	+
33	90	-	normal	II	±
34	90	-	normal	N	N
35	80	+	normal	VI 6	+
36	60	+	short-boned	IV 4	+
37	60	+	short-boned	VI 6	+
38	50	+	short-boned	VI 6	+

\+ positive

± doubtful

\- negative

N not available for examination

CHAPTER VI.

Introduction

The timing of experimental inoculation has been shown to be of importance in the reproduction of Border disease. In Chapter I, p. 8, evidence has been presented which indicates that maternal inoculation before 16 and after 120 days' gestation results in the birth of apparently normal lambs.

This evidence suggests that the susceptibility of the developing primary follicle population to Border disease might vary during gestation. To test this hypothesis, the effect of inoculation at various gestational ages was studied, and is described in this chapter. It was thought that the study would also provide data from which the significance of the secondary follicle suppression after inoculation at 54 days' gestation could be assessed.

Materials and methods.

Approximately forty South Country Cheviot ewes, from a farm with no history of Border disease, and in lamb to Dorset Horn rams were inoculated at various stages of gestation from 50 days to 130 days with standard Border disease inoculum. Several of the ewes aborted or were found to contain mummified foetuses unsuitable for examination. The remaining ewes were divided into two groups.

Group I. Seventeen ewes were killed fifteen days post-inoculation, and the numbers of foetuses at each age were as shown in Table 6.

Group II. Fifteen ewes were allowed to lamb normally. The two un-inoculated control ewes from the 145 days group described in Chapter IV were included to provide control lambs. The lambs were killed at three weeks of age. The numbers obtained were as shown in Table 7.

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The brains and spinal cords of the perfused foetuses of Group I and of the three week old lambs of Group II were removed, as were maternal cotyledons from the ewes in Group I, and fixed in Baker's calcium formol, prior to histological examination for diagnosis of Border disease.

Skin samples from the Group I foetuses were removed as described in Chapter III, p. 59, but only trephine samples and skin strips from the side sites were examined. No assessment of the fleece was made. 0.6 cm. diameter trephine skin samples were taken from the side site of all but one 110 day inoculate lamb in Group II as soon as possible after birth. At three weeks of age a clinical assessment of both body conformation and the degree of any locomotor disturbance of these lambs was made (Table 7) and they were then killed and sampled. A 1 cm. diameter trephine side site sample, and a side site skin strip were removed from each lamb, as described in Chapter III, pp. 59-60. The lambs were then skinned, and the skins were laid flat on benches for several weeks, with weekly washes of ten per cent formol saline, until dry. The extent and degree of hairiness of each skin were assessed independently by several observers, including the author (Chapter III, pp. 60-61), and the average fleece scores were recorded (Table 7). Border disease diagnosis in the foetuses and lambs was based on histological examination of the brain and spinal cord, and, in Group I, maternal cotyledons were also examined. The results have been summarised in Tables 6 and 7. The methods used to estimate the frequency and degree of primary fibre medullation, $\frac{S}{P}$ follicle and fibre ratios and central primary follicle dimensions (bulb diameter, length and fibre diameter), have been described in Chapter III, pp. 70-72.

Results /

Results.

Border disease was confirmed in all the experimental foetuses of Group I (Table 6). Most lambs in Group II were also positive, but one 130 day inoculate lamb was negative and lesions were restricted in several other foetuses from ewes inoculated between 90 and 130 days' gestation (Table 7).

In Group II, the birthcoats of the 90 to 130 day inoculate lambs and the control lambs were fine, with fleece scores ranging from I to III. All 50 to 80 day inoculate lambs had coarse birthcoats, their fleece scores ranging from IV4 to VI6 and in this group the age at inoculation was not related to the degree of coarseness of the birth - coat.

To detect any abnormalities of the follicle or fibre population in the foetuses of Group I, a comparison was made with the control foetuses of Chapter IV. No abnormalities were detected in any of the parameters measured (Figures 4, 5, 6, 7, 8, 16, 17, 18, 19, 20).

In examination of the lambs of Group II, the normality of the follicle population was assessed by using the baselines of five per cent primary fibres with large medullae and ten per cent central primary fibres with large medullae derived from study of affected lambs whose dams were inoculated at 54 days' gestation (Chapter V). The 90 to 130 day inoculate lambs and the control lambs, i.e. the fine-fleeced lambs, had significantly less than these levels of primary fibre medullation at birth and at three weeks of age; in fact there were no large medullae in primary fibres at either age in any of these lambs, and they were considered to have normal follicle and fibre populations. Their primary fibre medullation is shown in Figure 21.

The 50 to 80 day inoculate lambs at birth, with one exception, had more than the baseline values for abnormal medullation at birth and at three /

three weeks of age. These lambs were thus considered to have the abnormal primary follicle and fibre populations associated with Border disease. The one lamb of the 50 to 80 day inoculate group which did not fall into this latter category was a 60 day inoculate lamb which, although it had primary fibre medullation characteristic of Border disease at three weeks of age, showed no medullation at birth (Figure 21).

At birth, the $\frac{S}{P}$ follicle ratios of the coarse fleeced lambs tended to be lower than those of the normal fleeced lambs, and the mean values were 2.6 and 3.0 respectively (Figure 22a). As in Chapter IV the proportion of secondary follicles which was mature at birth was lower in the coarse fleeced lambs, since these had about 70 per cent fibre producing secondary follicles compared with 80 per cent in the normal lambs; their $\frac{S}{P}$ fibre ratios were 1.8 and 2.4 respectively (Figure 22b).

At three weeks of age, the difference in mean $\frac{S}{P}$ ratios was still marked, being 3.5 for the normal lambs and 2.8 for the coarse fleeced lambs. The mean $\frac{S}{P}$ fibre ratios were 2.5 and 3.4, so that almost all secondary follicles were mature, in both groups (Figure 22). The values for the normal and coarse fleeced lambs correspond to those for the control and Border disease affected lambs respectively, described in Chapter V (Figure 15).

Maximal follicle lengths were similar in all lambs at birth and at three weeks of age (Figure 24), but maximum bulb diameter tended to be greater in the coarse fleeced lambs than in those with fine fleeces, particularly at three weeks of age (Figure 23). With the exception of the 60 day inoculate lamb at birth mentioned above, all coarse fleeced lambs at both ages had considerably greater maximum fibre diameters than the fine fleeced lambs (Figure 25). In these measurements of follicle and fibre dimensions, the coarse fleeced lambs once again resembled the 54 day inoculate Border disease affected lambs, and the normal lambs resembled the controls described in Chapter V (Figures 12, 13 and 14).

Discussion /

DISCUSSION.

Only the parameters altered by Border disease in post-natal life were measured in the assessment of the normality of skin samples. As shown in Chapter V, these parameters were primary fibre medullation, primary follicle size and the $\frac{S}{P}$ ratios.

In Chapter Iv, it was shown that no abnormalities of the follicle population in Border disease could be detected at 105 days' gestation or less. Thus, although in the present study the follicle populations of the Group I foetuses at 65 to 105 days' gestation were similar to those of the control foetuses in Chapter IV, abnormalities characteristic of Border disease would not have been expected. The older foetuses, aged 115 to 145 days' gestation also had follicle populations similar to those of control foetuses, so that their follicle populations seemed to be unaffected by Border disease inoculation fifteen days prior to sampling, i.e. at 90 to 130 days' gestation. The birthcoats of the 90 to 130 day inoculate lambs were also normal, as were the results of histological examination of their skin for the frequency and degree of medullation, primary follicle and fibre dimensions and $\frac{S}{P}$ ratios.

The birthcoats of all 50 to 80 day inoculate lambs were abnormally coarse, but there seemed to be no correlation between the degree of coarseness and the age of inoculation. At three weeks of age the increases in the histological parameters characteristic of Border disease were very significant. Although these parameters were smaller at birth than at three weeks of age, at birth they were still significantly higher than normal in all but one 60 day inoculate lamb which had no medullation at this age. As in Chapters IV and V, the pre-natal check suppressed the expression of the follicle abnormalities at birth, particularly in one 60 day inoculate lamb. This confirms the observation

made /

made in Chapter V that peri-natal skin sampling is less likely to be useful in Border disease diagnosis than sampling at three weeks of age. The 60 day inoculate lamb (36) with a fleece score of IV4 (Table 7, Diagram 6) showed some anterior reduction in the degree of birthcoat coarseness, and the sample may have been taken from a site which was too low to represent the birthcoat abnormality. In the lambs of Group II, the frequency of medullation was fairly closely correlated with the fleece score (Table 7, Figure 21), and again showed no correlation with gestational age at inoculation.

With the evidence from the Group I foetuses, showing that inoculation at 90 days' gestation or over had no effect on the follicle population fifteen days later, the absence of follicle abnormalities in the 90 to 130 day inoculate lambs of Group II suggests that the primary follicle population was no longer susceptible to the effects of the agent following inoculation at 90 days' gestation. In Chapter IV it was shown that primary follicles at 90 days' gestation were at stages of development from F2a to F4 (Figure 1). If the effect of the inoculation on individual follicles occurs immediately after inoculation, then it can be assumed that primary follicles at stage F2a or more are not susceptible to the Border disease process. However, at 80 days' gestation, primary follicles were at stages of development of F1 to F2b, and it has been shown in this Chapter that the 80 day inoculate lamb had a primary follicle population as severely affected as the 50 to 60 day inoculate lambs. Thus primary follicles at stage F2b would seem to be susceptible to the Border disease agent. These contradictory conclusions suggest that the effect of the agent may not be immediate.

It is feasible that the agent acts, after a latent period, on the differentiating follicle population at a stage when /

when all primary follicles are at, or are developing to a susceptible stage. This stage of development is likely to be less than F8, since this normally represents a stage of full differentiation, and more than F2b, since follicles at this stage in the 80 day inoculate lamb were affected.

These results show that as in other viral foetal infections, such as BVD/MD virus in bovine foetuses (Scott, Kahrs,de Lahunta, Brown, McEntee and Gillespie, 1973), the stage of gestation at which infection occurs is important in the pathogenesis of the lesions of Border disease. The Border disease follicle abnormalities are only produced following maternal inoculation up to 90 days' gestation, and when this observation is related to the results of Chapter IV, some interesting possibilities arise. It is likely that the cells of the papillae are the target for the Border disease effect, and since these are differentiating until at least 105 days' gestation when the follicle is mature, they must pass through a crucial phase during which their course of differentiation can be altered by Border disease. If this modification interferes with their production of an inhibitor substance, as proposed in Chapter V, p. 121, this would fit the hypothesis made in that chapter that lack of the inhibitor substance leads to primary follicle hypertrophy.

Alternatively, the lack of susceptibility of primary follicles at 90 days' gestation and over may be due to the development of immune competence in the foetus to the Border disease agent at about this time. Since placental lesions and neurological lesions can be demonstrated even after inoculation up to and at 120 days' gestation (Barlow, 1972a; Gardiner and Barlow, 1972), this seems unlikely, although it could be argued that any immune response which developed at 90 to 120 days' gestation was incomplete.

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The data also allow an appraisal of the significance of secondary follicle suppression in Border disease. In Chapter IV, it was suggested that this suppression could result from primary follicle hypertrophy or poor foetal nutrition in late gestation. In the present experiment, reduced $\frac{S}{P}$ follicle and fibre ratios were evident in 50 and 60 day inoculate lambs but not in 80 to 130 day inoculate animals (Figure 22). Primary follicle hypertrophy occurred only in the 50 to 80 day inoculate lambs, and the placentitis was demonstrated in all ewes in Group I (Table 6), and therefore probably also in the Group II ewes. With the exception of the 80 day inoculate lamb, primary follicle hypertrophy and secondary follicle suppression were closely associated, so that, as suggested in Chapter IV, the increased primary follicle competitive efficiency or the production of less secondary follicle organiser may have led to a reduction in the number of secondary follicles which developed and matured. The pathology of the placental lesions varied according to the gestational age at inoculation, and this has been described by Barlow (1972a). Basically, the ewes inoculated at 54 days' gestation had a severe diffuse type of placentitis, whereas those inoculated later tended to have more lesions which were much less diffuse. It may be that the severity of the placentitis determined any reduction in foetal-maternal exchange, and that this was reflected in secondary follicle suppression in the earlier inoculated ewes.

The relative importance of these two possible factors affecting secondary follicle development therefore remains unresolved, particularly in view of the small number of animals in each group.

Conclusions

1. The differentiating follicle population was susceptible to the effects of Border disease inoculation at 50 to 80 days' gestation, but not between 90 and 130 days' gestation.

2. The susceptible phase of primary follicle development was at stage F2b or over.

CHAPTER VII.

Border disease in the Scottish Blackface

Introduction

In Chapter V it has been shown that Border disease, in a normally medium fine-fleeced breed, can produce a coarse birthcoat which resembles macroscopically that of carpet-fleeced sheep such as the Scottish Blackface and the N-type New Zealand Romney. It is important to establish whether or not the morphology of the underlying follicle populations is similar.

In this Chapter the development of the follicle population of control Scottish Blackface foetuses is studied, and compared with that of Border disease affected foetuses of the same breed at 115 days' gestation, and the follicle populations of control and affected lambs in the first year of life are compared. The findings are contrasted with those for Cheviot x Dorset Horn cross animals described in Chapter IV.

Materials and methods:

Of thirty-six Scottish Blackface ewes, thought to be pregnant and from farms with no history of Border disease, six aborted or were found to be non-pregnant, and these were excluded from the experiment. Of the remainder, twelve were inoculated at 54 days' gestation with standard Border disease inoculum, and eighteen were not inoculated, the two groups providing Border disease affected and control offspring respectively. Sixteen control ewes were killed at various ages of gestation from 95 to 135 days, and six inoculated ewes were killed at 115 days' gestation. Skin samples were removed from the side site of the foetuses as described in Chapter III, p. 59. Of the remaining seven inoculated and five un-inoculated ewes which were allowed to lamb normally, three and two ewes respectively produced twins; all other ewes produced single lambs. One experimental ewe produced stillborn twins, and one produced a still-born lamb with a live twin.

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Table 8: The numbers of foetuses and lambs sampled at each age.

	Control	Border disease
Age of gestation (days)	Number of foetuses	Number of foetuses
95	1	-
100	5	-
105	-	-
115	9	7
125	3	-
135	4	-
Post-natal age (weeks)	Number of lambs	Number of lambs
newborn	7	10
3	7	7
6	6	7
12	6	7
24	6	7
36	6	6
48	6	6

From all lambs, skin samples were removed soon after birth, at three week intervals to twelve weeks of age, then at twelve week intervals to forty-eight weeks of age, as described in Chapter III, pp. 59-60. Blood samples were also taken, and the bodyweight, cannon bone circumference, and horn length of each lamb were recorded. The numbers of foetuses and lambs sampled at each age are shown in Table 8 .

The control lambs were born out of doors and were brought indoors on weaning at twelve weeks of age. The experimental lambs were housed indoors throughout the experiment and were also weaned at twelve weeks of age. At three days of age, the three ram lambs of the control group were castrated and the tails were removed from all lambs. For both procedures, "Elastrator" rubber rings were used. The three ram lambs from the Border disease group were left entire. The husbandry of the lambs was standard, and included an injection of Nemicide Injection V (I.C.I.), an anthelminthic, at about sixteen weeks of age.

A control lamb, one of twins, contracted acute bronchopneumonia at four weeks of age, and died. A Border disease affected ram lamb was killed at twenty-four weeks of age because of severe urolithiasis. The two other Border disease affected ram lambs also developed urolithiasis at the same time, but responded to treatment with Depropanex Injection V (Merck, Sharp and Dohme), and surgical removal of the veriform appendix of the penis. It was subsequently discovered that the water supply for these lambs was unpalatable, and so dehydration may have been a major factor in the causation of the outbreak. The condition did not recur when the lambs were moved to an adjacent pen.

At eight months of age the control and experimental lambs were divided into batches of three or four, so that each batch contained one or two lambs from each group. The Border disease affected entire ram lambs were grouped with a castrated control. It had been intended to separate /

separate the rams before they reached breeding age, but one Border disease affected ewe lamb aborted a dead foetus about 120 days later.

Horizontal and vertical Sacpic stained sections of skin were prepared from each sample (Chapter III, pp. 61-65), and in addition, vertical sections of skin from the 115 day foetuses were stained by the Mercury Orange method. The histological parameters measured were those described in Chapter V, and have been described in Chapter III.

Results:

All Border disease affected lambs had locomotor dysfunction at birth, which varied from a fine body tremor to severe incoordination with difficulty in standing. In all cases these symptoms lessened with age, and by six months of age most were apparently normal, although two ewe lambs still showed a fine tremor of the head. At all ages, the Border disease affected lambs were smaller and lighter than the controls, with finer long bones, as measured by cannon bone circumference, and the ewe lambs grew narrow horns which tended to curve towards the neck. There was no difference in the horn lengths between groups, although the Border disease affected entire ram lambs tended to have much thicker and longer horns than any other lamb. The birthcoats and fleeces were not markedly different except perhaps at about six months of age, when some of the experimental lambs seemed to have curly tips at the end of the wool staples, especially on postero-ventral parts of the body, in contrast to the straight staples of the control lambs (Plates 43 and 44).

In the control Blackface foetuses the ages of initiation of follicles and their rate of development conformed to the pattern described in Chapter IV for Cheviot x Dorset Horn cross foetuses (Figure 1), but the occurrence of primary fibre medullation was much greater, and primary follicle size was greater than in the control Cheviot cross foetuses (Figures 4, 5, 6, 7, 26, 28, 29 and 30). Primary fibre medullation /

medullation occurred frequently during gestation (Figure 26), particularly in central primary fibres (Figure 26 c and d), and many of these were heavily medullated (Figure 26 b and d, Plate 46). Thus there is a similarity between the frequency of medullation in the control Blackface and that of the Border disease affected Cheviot x Dorset Horn cross foetuses (Figure 4). Although in the Border disease affected Cheviot cross foetuses, central and lateral primary fibres were often medullated to a similar degree, in the Blackface lateral primary fibres tended to show less medullation than centrals.

At 115 days' gestation, medullation and central primary follicle and fibre size were similar in control and Border disease affected Blackface foetuses (Figures 26, 28, 29 and 30), but secondary follicles of the latter were less well developed (Plates 45 and 46).

To summarise, it seems that the birthcoat coarseness in affected Cheviot cross lambs has a slightly different basis in the activity of the follicle population than that of Blackface lambs. In the former, primary follicle hypertrophy may affect most or all primary follicles, particularly central primary follicles, while in the Blackface, although the primary follicles are large, the central primary follicles are often very much larger than the laterals.

The pre-natal check was less marked in the Blackface than in the affected Cheviot cross foetuses at 135 days' gestation, and in both groups, follicle activity was increasing soon after birth (Figures 10, 12, 13, 14, 26, 28, 29 and 30).

The Mercury Orange method showed that the keratogenous zone of primary follicles occupied about one-fifth of the follicles' length, and began about one-third of its length from the bulb (Plates 47 and 48). This is similar to the relative position and length of the keratogenous zone in the Border disease affected primary follicles of the Cheviot cross at 115 days' gestation (Plate 28), described in Chapter IV.

Medullation /

Medullation, and primary follicle and fibre dimensions of both groups increased from birth to three weeks, when they tended to be lower in Border disease (Plates 49 and 50; Figures 26, 27, 28, 29 and 30). The primary medullation frequencies fell to six weeks of age and varied widely thereafter from one sampling age to another. In the controls, the frequency of medullation tended to rise to three months of age but then showed a steady fall (Plate 51) to nine months, before rising again at one year of age. In Border disease affected lambs the pattern differed, and primary medullation fell from six weeks to three months of age, rose at six months (Plate 52), fell at nine months and rose again at one year of age, although this recovery was much less evident than in the controls. The occurrence of "brush ends" tended to be negatively related to the frequency of medullation (Figures 26 and 32) and together the figures suggest that shedding of the fibres of the birthcoat occurred at about six weeks of age in the controls, and at about twelve weeks of age in the Border disease affected lambs. Comparisons with shedding in the Cheviot x Dorset Horn cross are probably of little value because of the small number of Cheviot cross lambs and the great variation between individual animals within groups. However, throughout the post-natal period, the ranges of primary follicle and fibre dimensions and primary medullation were similar in control and affected Blackface and affected Cheviot cross lambs, although follicle size in the latter tended to be smaller than in the Blackface.

The dimensions of central primary follicles and fibres tended to vary with central primary fibre medullation, as one might expect. Although follicle bulb diameter was less closely related to medullation, it may be that the presence of many "brush end" follicles at three months and over interfered with the accuracy of the method.

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The $\frac{S}{P}$ follicle ratio in the controls tended to rise throughout gestation from 95 days, with two waves of formation, at about 95 days' and just before term (Figure 31a), as in the control Cheviot cross foetuses (Figure 8b and d). Post-natally, secondary follicles continued to be initiated to about six weeks. The maturation of secondary follicles, indicated by $\frac{S}{P}$ fibre increases, continued to twelve weeks, when almost all secondary follicles were producing fibres above the skin surface (Figure 31b). In the Cheviot cross, secondary follicle initiation and maturation were also complete at about twelve weeks of age (Figure 15). Although there was no significant difference in the number of secondary follicles between Border disease affected and control Blackface foetuses at 115 days' gestation, post-natally there were fewer at all ages in the affected lambs (Figure 31a). The reduced $\frac{S}{P}$ follicleratio was most apparent in the ewe lambs, and less so in the entire ram lambs, a sex difference which contributed to the large variation in the $\frac{S}{P}$ values between affected lambs shown in Figure 31. The number of mature secondary follicles in Border disease was correspondingly lower than in the control animals (Figure 31b), but by twelve weeks of age, again almost all were producing fibres. The tendency for the $\frac{S}{P}$ ratio of both groups to fall towards forty-eight weeks of age was perhaps due to the formation of "brush-ends" in secondary follicles. These, which were occasionally seen and included in the counts, may have been above the level at which counts were made in some cases. Burns (1949) and Doney and Smith (1964) also noted a tendency for the $\frac{S}{P}$ ratio to decrease after reaching a maximum value at twelve to twenty-eight weeks of age, and could offer no explanation.

There was considerable variation between lambs within groups from six weeks of age in all the parameters measured. Sex differences were only apparent in the $\frac{S}{P}$ ratios. Differences between twins and singletons /

singletons were also confined to $\frac{S}{P}$ ratios, which tended to be lower in twins at birth, but not significantly so. This difference had disappeared by three weeks of age.

Discussion:

Although the Border disease affected Blackface lambs showed the body tremor, conformation and poor thrift characteristic of Border disease, there were no gross changes in their birthcoats, which were straight and coarse.

The modification of the development of the follicle population of twins during gestation is confined to suppression of secondary follicle development (Short 1955b; Doney and Smith, 1964), and have not been considered in this experiment since about half of the lambs in each group were twins, and comparisons between groups are therefore valid. Since there seem to be no reports of sex differences in the skin follicle populations of rams, ewes and castrated males, it was thought that inclusion of the ram lambs in the experiment was also valid. It was borne in mind that testosterone administration has been shown to increase wool growth (Slen and Connell, 1958), although it is possible that this effect was mediated via the pituitary and adrenal glands (Ryder and Stephenson, 1968).

The pattern of prenatal follicle development in the control Blackface was similar to that of the control Cheviot cross, but there was a much greater frequency of medullation and a smaller prenatal check (Figures 4, 5, 6, 7, 26, 28, 29 and 30).

At 115 days' gestation, there was no apparent difference in the primary follicle populations of the two groups (Figures 26, 28, 29 and 30). The study of follicle development in control Blackface foetuses showed that the primary follicles were approaching maximal size at this age, and by analogy to the Cheviot cross foetuses, affected animals might have been expected to show the increased frequency and degree of medullation which characterised the disease in the Cheviot cross. However, this was not evident at 115 days' gestation or at any subsequent age examined.

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This lack of effect suggests some fundamental difference between the two breeds studied in the susceptibility of the "target" cells, probably in the papilla, to the effects of the Border disease agent. Their lack of susceptibility in the Blackface could possibly be due to the lack of an "inhibitor substance" such as that proposed in Chapter V, (p. 121) as the cause of the reduced size of primary follicles in finer fleeced breeds. If the coarse fleece type is more primitive than that of finer fleeced breeds (Ryder, 1969), the inhibitor may have evolved during the selection of sheep for their finer fleece type.

With their exposure to a greater variety of environmental stimuli in post-natal life, the lambs' follicle populations showed greater variation within groups than pre-natally. Differences between groups are therefore very difficult to interpret. However it is clear that in post-natal life, as at 115 days' gestation, Border disease did not cause primary follicle hypertrophy in the Blackface. In fact during the first few weeks of life, the primary follicles and fibres seemed smaller than those of the controls (Plates 49 and 50), and medullation tended to be less (Figure 26). The smaller size of the follicles and fibres could be explained by the reduced intake of milk which might have been a consequence of their body tremor, and it has been widely reported that poor nutrition can reduce the length and diameter of wool grown (Ryder and Stephenson, 1968).

After a period of increased activity at about three weeks, primary follicle and fibre size and medullation frequencies fell during the phase of birthcoat fibre shedding, and increased again before a second wave of shedding and follicle activity in the winter months, which was followed by increased activity in early spring. As in the controls in this study, birthcoat shedding has been found by other workers to occur at about one to three months of age in the Blackface (Burns, 1953; Doney and Smith, 1964). Birthcoat shedding is considerably

delayed /

delayed by poor nutrition (Slee, 1963; Doney and Smith, 1964), and so the poor thrift of the Border disease affected lambs might explain their delayed shedding. Some effects of light on the shedding of the birthcoat have been studied by Slee (1965), who showed that dim lighting delayed shedding. The control lambs were out of doors, and the affected lambs were housed indoors for the first twelve weeks after birth, so that this too could have contributed to the delayed shedding of the affected lambs. The curly tips of the staples of most of the affected lambs is probably due to the retention of many of the curly-tipped fibres of the birthcoat in the fleece at this age (Plate 45).

Burns (1953) observed that the second wave of shedding began in October, in lambs aged about twenty-eight weeks. In the present study, the winter shedding in the controls which increased steadily from about twelve weeks, was most evident at about thirty-six weeks of age, in November and December, "Brush-end" fibres, not observed in secondary follicles by Burns but identified in shed fibres by Doney and Smith (1964), were positively identified in skin sections at this age. In Border disease, the second wave of shedding was more marked than in the controls, as was the accompanying reduction of medullation and follicle size, but this effect may have been due to the long intervals between sampling, so that a corresponding phase in the controls could have been missed. As in the controls, the winter suppression of wool growth was most marked at about thirty-six weeks of age, in November - December.

It may be that the apparently slow recovery of the Border disease lambs' follicle populations from their relative inactivity in winter is a reflection of their poor thrift, as perhaps was the delay in birthcoat shedding. Perhaps differences in the follicle populations will be apparent in the second year of life, and this will be investigated.

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Although in some breeds such as the Merino, all secondary follicles have been initiated by a few weeks of age (Schinckel, 1955a; Short, 1955a), Burns (1953) found that, in the Blackface, secondary follicles continued to be initiated up to about six weeks of age, an observation which was confirmed in the present study. Thus, the maturation of the secondary follicle population of the Blackface is particularly liable to modification by post-natal as well as prenatal nutrition (Doney and Smith, 1964). However, Doney and Smith found that poor prenatal and post-natal nutrition delayed maturation but did not reduce the number of secondary follicles which developed, so that by forty-eight weeks of age the $\frac{S}{P}$ ratios were normal. The results presented here indicate that both secondary follicle initiation and maturation are reduced in Border disease, although this effect was less evident in entire ram lambs. Thus a factor other than poor nutrition may be involved in the Border disease $\frac{S}{P}$ ratio reduction. In Chapter IV, possible causes were proposed; more effective competition from the hypertrophied primary follicles may mean that less space and substrate are available for secondary follicle development; the hypertrophied follicles may produce less follicle "organiser", or the placentitis of Border disease, by interfering with foetal-maternal exchange, may reduce the number of secondary follicles which develop and mature. Primary follicle hypertrophy can be ruled out as a cause of secondary follicle suppression in the Blackface, since this is not a feature of Border disease in this breed. No doubt the placentitis which is a feature of Border disease in Cheviot ewes occurred also in the Blackfaces, since abortions and stillbirths occurred, but the comparison of 115 days' gestation Blackface foetuses, control and affected, showed that secondary follicle initiation was not reduced, although maturation was significantly delayed (Figure 31, Plates 45 and 46). Thus no one explanation /

explanation serves to explain the reduction in the number of secondary follicles or their delayed maturation, but it is possible that the placentitis of Border disease and the poor condition of the lambs combine in having this effect. A comparison between the degree of effect on secondary follicles in Cheviot cross and Blackface lambs would not be useful because of the small numbers of Cheviot cross lambs used.

That the secondary follicle population is not directly affected by Border disease is suggested by the lack of effect on secondary follicle size or medullation (Figure 27).

In Chapter IV it was observed that, in Border disease affected Cheviot cross foetuses at 115 days' gestation, there was a "distal shift" of the keratogenous zone of primary fibres. In the Blackface, the relative length and position in the follicle of the keratogenous zone of control and Border disease affected foetuses did not differ (Plates 47 and 48). The position in the follicle was very similar to that of the affected Cheviot cross foetuses, and suggests that this zone may be situated relatively further distally in larger follicles, reflecting the greater growth rate of fibres from these follicles.

All the comparisons made above illustrate the similarity of the follicle activity in control and affected Blackface and affected Cheviot cross lambs, particularly in primary fibre size and medullation. The implications of these observations will be discussed in Chapter VIII.

Conclusions

1. There was no macroscopic difference between the birthcoats of control and Border disease affected Blackface lambs, although at 24 weeks of age, some affected lambs had curly-tipped staples.
2. Histologically, at 115 days' gestation, the secondary follicles of affected foetuses were less well developed than those of control foetuses but the $\frac{S}{P}$ follicle ratio was not significantly different. It is important to note that there was no primary follicle hypertrophy in affected foetuses. Post-natally, the $\frac{S}{P}$ ratio was reduced in Border disease, and primary follicle and fibre size were less than those of controls at 3 weeks of age.
3. Shedding of the birthcoat was delayed in Border disease affected lambs compared with controls, occurring at about 12 weeks and 6 weeks respectively. This and the poor thrift of the affected lambs resulted in such variation within groups that after 6 weeks of age interpretation of the results became difficult.
4. The development of the follicle population in control and affected Blackface foetuses was similar to that of affected Cheviot cross foetuses, although in the latter, lateral primary fibres tended to show relatively more medullation in relation to central primary fibres than they did in the Blackface foetuses, both Border disease affected and control, and the primary follicles were subject to a much greater prenatal check.
5. Postnatally, the follicle and fibre dimensions of the control and affected Blackface lambs tended to be larger than those of the affected Cheviot cross lambs.
6. There was no difference in the relative length and position of the keratogenous zone of primary fibres between control and affected Blackface and affected Cheviot cross foetuses at 115 days' gestation.

CHAPTER VIII

General Discussion.

Study of the development of the abnormal follicle population in Border disease, using the medium fine fleeced Cheviot x Dorset Horn cross, has occupied a large part of this Thesis. The main feature of the diseased follicle population is primary follicle hypertrophy, with an increased frequency and degree of primary fibre medullation. The mechanisms causing the primary follicle hypertrophy are intriguing. It has been proposed in the text that inhibitors normally involved in the control of primary follicle growth and differentiation in medium and fine fleeced sheep do not function in Border disease. An extension of this hypothesis which fits the observations made in the text is that the inhibitor is derived from the cells of the papilla (Chapter IV) at a developmental stage of F2b or later (Chapter VI) and has a permanent effect on the morphology of the follicle and fibre (Chapter V).

The failure of function of an inhibitor substance complies with the general concept that foetal malformations essentially result from defective differentiation (Smith and Jones, 1968), and the hypomyelinogenesis and small long bones of Border disease are examples of such malformations.

In Chapter VII, it was shown that the differentiation of primary follicles in Border disease in the Cheviot cross, and in control Blackface animals was similar. This suggests that an inhibitor may not be involved in follicle differentiation in the Blackface, and would explain the lack of effect of Border disease on the primary follicles of this breed.

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The evidence presented here indicates that Border disease causes

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a move towards a more primitive type of wool growth by interfering with the production or effect of an inhibitor involved in the control of primary follicle development. This hypothesis has already been suggested by Carter, Terlecki and Shaw (1972) who stated that the follicle abnormalities of Border disease were "a phenocopy of certain fleece variations whose genetic basis is well established.

The concept of genetic mimicry in Border disease is supported by comparing it with the N-type variant of the New Zealand Romney. Of the two non-allelic genes which have been identified as giving rise to the abnormally coarse fleece (Chapter II, pp. 53-54), the effect of the recessive nr gene resembles Border disease in that it causes primary follicle hypertrophy. This is also a feature of animals with the N gene, a small proportion of which are also reported to show signs of defective myelination (Cockrem, 1963). However, excessive growth of horns is a pleiotropic effect of the gene N which has no parallel in Border disease.

The inoculation of sows with Border disease affected lamb tissues resulted in the production of piglets with stripes of coarse hair distributed longitudinally along the sides of their bodies in a wild piglet pattern (Derbyshire, 1975), and this gives strong support to the hypothesis that Border disease causes a tendency for follicle development to occur in a more primitive manner.

Two aspects of secondary follicle development are altered by Border disease; the numbers which develop are reduced, and their maturation is delayed.

The most likely hypothesis to explain the reduced numbers is that the hypertrophied primary follicles produce less secondary follicle organiser. Burns (1953) suggested that the dermal concentration of such an organiser determined the number of secondary follicles /

follicles which developed, and that its concentration was determined by primary follicle density. However, this hypothesis does not fit the observations made in Chapter IV, that in Border disease, primary follicle density is not altered but secondary follicle numbers are reduced. If this idea is adapted, by proposing that the amount of organiser produced by a primary follicle is inversely proportional to its size, then one has a neat hypothesis to explain the reduced number of secondary follicles in Border disease. The fact that throughout the range of fleece types in different breeds, primary fibre coarseness and $\frac{S}{P}$ ratios are negatively correlated (Ryder and Stephenson, 1968) provides additional support.

It has been suggested that the greater competitive efficiency of primary follicles might interfere with secondary follicle development, or that this might be due to impaired foetal nutrition, a possible effect of the placentitis of Border disease. These are unlikely to account for the reduced number of secondary follicles in Border disease for the following reasons. The competition theory (Fraser, 1952) cannot be justified in terms of present knowledge of the adaptability of blood supply to hypertrophied tissue, and there is no convincing evidence that poor foetal nutrition will permanently reduce the number of secondary follicles which develop.

The retardation of secondary follicle development which occurred in Border disease is probably due to the effects of the placentitis. The extent and severity of the placental lesions are likely to interfere with maternal-foetal exchange, although such an effect has not yet been proved. Reports of poor maternal nutrition during gestation and of the effects of intrauterine competition by siblings suggest that suboptimal foetal nutrition does delay secondary follicle maturation. Further evidence that this occurs in Border disease is provided /

provided in Chapter VII, in which secondary follicle development retardation was apparent in Border disease in spite of the fact that primary follicle hypertrophy did not occur, and increased competition by the primary follicles could not have been a factor in the retardation of secondary follicle development.

Finally, what are the practical applications of this work?

Firstly, it seems that in some situations, Barlow's (1972b) suggestion that baselines of abnormal medullation could be established is justified. The enormous amount of work which would have to be done in establishing baselines for each breed could be reduced substantially if the parameter measured was the frequency of central primary fibres with medullae or large medullae. The highly significant difference between the value of this parameter in control and affected Cheviot cross lambs suggests that guidelines could readily be established for breeds of this fleece type, and probably also for the fine fleeced breeds such as the Suffolk and Dorset Horn. The lack of effect of Border disease in the skin of the Scottish Blackface is a strong indication that skin sampling would be of no use in coarse fleeced breeds.

Ideally, sampling should be carried out at about three weeks of age, and it should be avoided during the perinatal and shedding phases, and the winter months, when follicle activity in both control and affected animals is reduced. As in control animals, the follicle populations of Border disease affected lambs can be modified by chronic debility, and follicle activity can be greatly reduced. Many Border disease affected lambs tend to become emaciated and often die within a few months of birth (Shaw, 1962), and these animals are therefore unsuitable for diagnostic examination of the skin.

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The use of skin samples would be particularly valuable in the identification of adult affected animals which may be carriers of the disease, as well as being of benefit in diagnosis of Border disease in lambs when neurological examination is impracticable or uninformative.

The Thesis would not be complete without reference to the occurrence of pigmentation. Abnormal pigmentation has been reported in many field outbreaks of Border disease, and personal observations confirm that affected lambs of breeds which can have some pigmentation normally, sometimes have more pigmented patches than unaffected lambs. However, abnormal pigmentation was not a feature of the disease in the breeds studied and described here. The Cheviot x Dorset Horn cross has no tendency to develop pigmentation in the fleece and none was induced by Border disease. The Blackface can have pigmented patches normally which are not affected in Border disease. This is not surprising since there seemed to be no direct effect of the disease in the skin of this breed. The material therefore does not allow an appraisal of the situation with regard to pigmentation, but based on the studies of the Cheviot cross and observations in the field, it seems unlikely that abnormal pigmentation occurs in breeds with normally unpigmented fleeces.

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