

STUDIES OF SHEEP MOSAIC FOR FLEECE TYPE

I. PATTERNS AND ORIGIN OF MOSAICISM

By A. S. FRASER* and B. F. SHORT†

[Manuscript received December 17, 1957]

Summary

Sheep which are mosaic for fleece type are described with emphasis on the patterns of distribution of areas of the mutant fleece type over the body. Probable causes of the underlying mosaicism of skin tissues are discussed.

I. INTRODUCTION

Recently we have made a collection of sheep which are mosaic for fleece type. Most of these are Merinos or shortwools (Southdown and Dorset Horn), with fleeces of normal staple length over most of the body but which have patches of longer wool varying in area and position from sheep to sheep. The search for such sheep was stimulated by a description of a Merino fleece mosaic by Lang (1952) and Chapman, Moule, and Richards (1954). It was thought that sheep of this type could be useful in determining the biological control of the component characters of fleece weight and fleece type. The contribution which studies of mosaic fleeces can make to our understanding of fleece growth will be discussed in later papers; in this paper we wish to consider the genetic basis of mosaicism in sheep.

Mosaic individuals are those in which a normally monotypic cell lineage is split into two or more distinct types and it is pertinent to distinguish two types of mosaicism according to the way in which the mosaicism may have come about. These are (i) "developmental" mosaics in which the phenotypic differences in the cell lineage are not paralleled by genetic differences, and (ii) "genetic" mosaics in which a genetic mosaicism underlies the phenotypic one.

The first type includes individuals with broken colour patterns such as "English" rabbits, spotted mice, roan cattle, saddle-back pigs, and so on. Certain lambs which have hairy birthcoats also belong to this category. While it is not absolutely certain that such patterns are unaccompanied by genetic differentiation, there is no evidence that they are. In one instance there is evidence that they are due to something else. This comes from investigations of the white-mottled variegation series in the determination of eye color in *Drosophila melanogaster* where the variegation is due to a translocation or inversion shifting a gene into the heterochromatin. Although it is not possible to show that in such a mosaic the soma is not genetically differentiated, the patterns are nevertheless demonstrably controlled by genotypes which segregate normally in crosses and therefore the controlling factors are present in *all* cells, not just a fraction of them.

*Animal Genetics Section, C.S.I.R.O., University of Sydney.

†Division of Animal Health and Production, C.S.I.R.O., Sheep Biology Laboratory, Prospect, N.S.W.

Another class of mosaic, difficult to separate logically from these, appears to be due to a high rate of mutation of a gene or chromosome type. This mosaicism is supposed to be due to *back mutation* because the gene (*a*) has a mosaic expression, and (*b*) has a high mutation rate in the germ line. In these cases the genotype of the germ line is such that the soma to which it gives rise is liable to genetic differentiation by mutation at any stage. It can only be distinguished from the first type of mosaicism if the assumption is made that no genetic differentiation takes place in such cases of spotting or variegation.

Genetic mosaics other than those due to a predisposing genotype may originate as follows: (1) mutation of a gene in an early cell generation; (2) loss of a chromosome or part of a chromosome due to a faulty mitosis at an early cell generation; (3) addition of a chromosome or chromosome set due to a faulty mitosis at an early cell generation; (4) fertilization of the egg by more than one sperm; (5) fusion of two adjacent zygotes; (6) inclusion of cells from another individual (graft mosaics), e.g. erythrocyte mosaics in cattle (Stormont 1954), or sectorial mosaics in pigeons (Hollander 1949).

Conclusive demonstration of the mechanism underlying a genetic mosaic is difficult in organisms other than *Drosophila*, and therefore most analyses have been made in *Drosophila* (Morgan 1914; Sturtevant 1929; Stern 1936; Crew and Lamy 1938; Patterson and Stone 1938). These have shown that genetic mosaics are mostly caused by the elimination of all or a part of a chromosome at an early mitosis. Such analyses as can be made of the mechanisms of genetic mosaicism in sheep are much too limited to produce an unequivocal answer, and therefore this aspect has been given only cursory attention. The main aim here is to describe the essential features of our group of "mosaic" sheep and to discuss those which support the hypothesis that these are true genetic mosaics.

II. MATERIAL AND METHODS

(a) *Detection of Mosaic Sheep*

The search for mosaic fleece types has resulted in the discovery of some 30 mosaics, plus a number of animals with completely abnormal fleeces, presumably due to the action of mutant genes. In all, some 200 sheep have been examined in the field, and 40 of these, regarded as possible mosaics, were transferred to the Sheep Biology Laboratory, C.S.I.R.O., Prospect, N.S.W. Here they were closely shorn and penned individually to allow growth of a new fleece under controlled conditions.

The great majority of sheep inspected had regions of wool which was longer than normal. Only one sheep had regions with wool shorter than normal. The predominance of long-stapled mosaics may be due to observational difficulties since staples which project above the rest of the fleece are more noticeable than staples which lie between a majority of longer, neighbouring staples. Moreover, an increase of fibre length has usually resulted in a loss of crimp structure and uniformity of staple form, leading to a somewhat fuzzy, open staple. Earlier reports of fleece mosaicism (Ross 1933; Bosman 1935; Kelley 1939) also noted that the abnormal wool had longer and less discrete staples. These features are illustrated in Plate 1, Figure 1, which shows a

crossbred sheep with extensive mosaicism, and in Plate 2, which shows abnormal (left) and normal (right) staples from a number of fleece mosaics.

It is intended shortly to organize a search for "short-stapled" mosaics and this should indicate whether or not there is a real difference in the frequency of "long" and "short" staple fleece mosaicism.

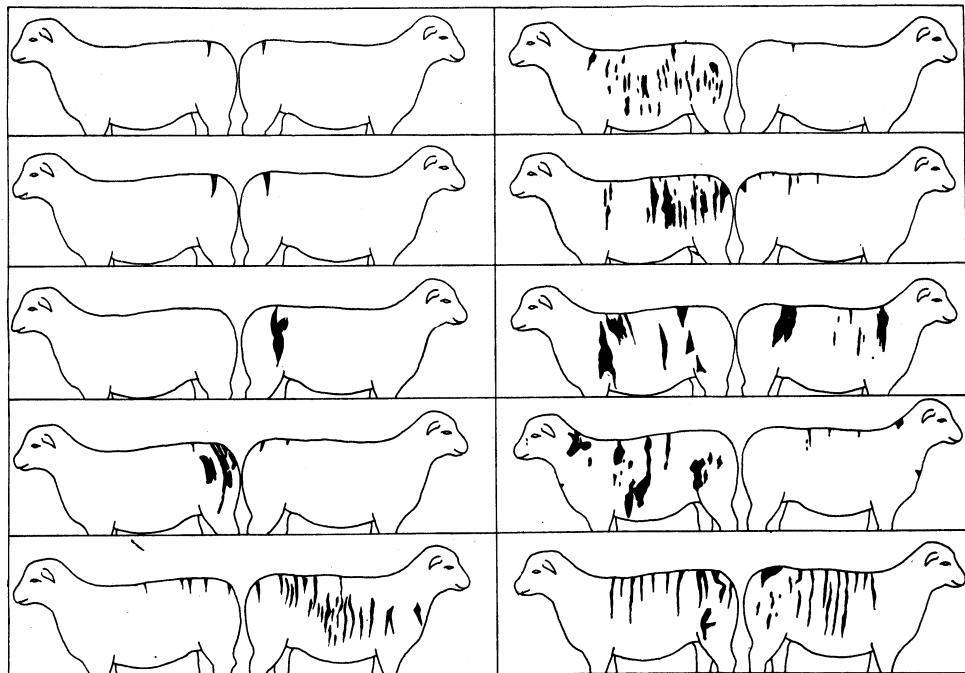


Fig. 1.—Patterns of fleece mosaicism.

(b) Patterns of Mosaicism

The patterns of mosaicism were sketched after regrowth of the fleece allowed clear identification of the abnormal and normal areas. Drawings were made on a standard sheep silhouette. The position, number, and relative size of abnormal regions was checked carefully by two independent observers. Although the position and size of abnormal regions was subjectively assessed, several sheep were checked by direct tracing, and the error did not exceed 20 per cent. of the measured area of any single abnormal patch.

III. RESULTS AND DISCUSSION

The patterns of distribution of the areas of abnormal fleece type are illustrated in Figures 1 and 2 for 15 sheep from our collection. It can be seen that the abnormal wool nearly always occurs in more than one patch. This separation of the abnormal wool into a number of separate areas is a characteristic feature of fleece mosaicism. It might have been expected from text-book references to genetic mosaics that the abnormal area would be symmetrical and unitary, though several detailed descriptions of genetic mosaicism have previously demonstrated the inaccuracy of this idea. Many

coat-colour mosaics in mice, rats, rabbits, and guinea pigs have involved a single patch of abnormally coloured fur (Castle 1922, 1929; Pincus 1929; Fisher 1930; Bittner 1932) but several with more than one abnormally coloured area have been described (Wright and Eaton 1926; Dunn 1934; Feldman 1935).

Sturtevant (1929) concluded from his extensive studies of genetic mosaics in *Drosophila simulans* that the first cleavage patterns are indeterminate since there was no definite pattern among these mosaics. This indicated that cleavage nuclei are distributed to the blastoderm differently in different embryos. Detailed analyses showed that a complex pattern of migration was involved in the determination of the

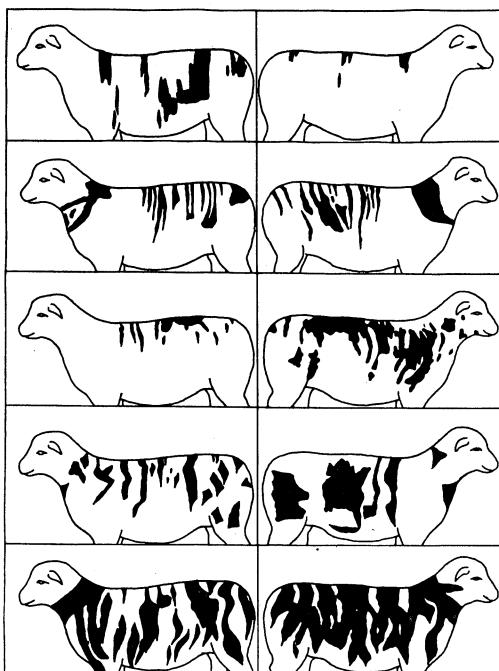


Fig. 2.—Patterns of fleece mosaicism.

imaginal discs, and of the relation of the imaginal discs to the surface of the adult. Patterson and Stone (1938) agreed with Sturtevant's conclusions. The main point of the evidence from *Drosophila* mosaics in so far as sheep mosaics are concerned is their clear demonstration that initially unitary mutational events may lead to widely separated mutant areas.

Although the differences between normal and abnormal wool in our sheep mosaics vary slightly from region to region, this variation is in accord with the normal gradients of expression of wool characters which occur over the body. These slight variations do not conflict with the close similarity of the basic differences in several regions; regardless of their anatomical position abnormal patches all differ from normal regions in the same ways and to essentially the same degree. This is illustrated in Plate 1, Figure 2, a Southdown mosaic in which the marked increase of length of

the wool on the abnormal regions has been shown to be associated with an equally marked decrease in the density of the follicle population. This similarity from region to region agrees with the hypothesis that the separate areas have a common origin in a single region of abnormal cells which later forms separate regions due to cell migrations.

These patterns point to the occurrence of two distinct processes during development: (1) an extension in the anteroposterior direction which is accompanied by intermigrations, along dorsoventral axes, of cells of the abnormal and normal regions. This process is deduced from the occurrence of the abnormal regions in separate patches aligned in the anteroposterior axis; (2) an extension, unaccompanied by any marked migration, which results in the separate regions being "stretched" in the dorsoventral direction. It is possible that this latter mechanism is responsible for the similar dorsoventral disposition of skin folds in some Merino sheep (Plate 1, Fig. 3).

The allometry of the sheep foetus has not been studied earlier than 45 days of gestation. Stephenson (personal communication) has shown that variation of allometric constants after this age is small. It may therefore be concluded that the processes of extension and migration deduced from the patterns of fleece mosaicism occur before 45 days of gestation. Regardless of the mechanism of the separation of an initial single region into a number of separated areas in the adult, it is clear that such separation does not constitute an argument against the hypothesis that fleece-type mosaicism is genetic in origin.

(a) Extent of the Abnormal Areas

The total areas of the abnormal regions on each sheep, though subjectively assessed, indicate a mutational origin. These areas are given as percentages in Table 1.

The totals range from 0·2 to 44 per cent. and there is an indication that they form a binary sequence such as would occur if a mutational event happened at the first, second, third, etc. cell division. However, the sums of the areas of abnormal regions need not necessarily follow a binary sequence, for if the mutational event is *bidirectional*, therefore affecting both daughter cells, the cell lineages will comprise three genetic types: a normal and two mutant types. Non-disjunction of a chromosome at mitosis is a typical bidirectional mutation—it can result in one daughter cell being trisomic, the other monosomic. The cell lineage will then contain trisomic, disomic, and monosomic cells. Since mosaicism comprising three different variants of a tissue have not been observed either here or elsewhere it must be concluded that *either* the mutational basis of genetic mosaicism is unidirectional, so affecting only one of a pair of daughter cells, *or*, if it is bidirectional, then one of the mutant cells lineages is indistinguishable from normal or is inviable.

In the event of a third cell type being inviable, the total areas of mosaic regions will not follow a binary sequence. The percentages of abnormal tissue would follow the series 100, 33·3, 14·2, 6·7, 3·2 per cent., etc. as compared with the series 50, 25, 12·5, 6·3, 3·1 per cent., etc. It would be difficult to distinguish these series except in the second term, i.e. when the mutation occurred during the second cell division. In the bidirectional case this would result in 33·3 per cent. of abnormal tissue, whereas in the unidirectional case it would result in 25 per cent. of abnormal

tissue. It is impossible to decide between these two alternatives on the data from our present group of mosaics, but the occurrence of one with 44 per cent. and three with 21–24 per cent. indicate fairly strongly that the causal basis is unidirectional.

The most probable types of unidirectional mosaicism are (i) gene mutation, and (ii) chromosome breakage. The latter is initially bidirectional, but since the fragment lacking a centromere would soon be lost, it is effectively unidirectional.

(b) *Boundaries between Abnormal and Normal Regions*

Regional variation of fleece structure seldom, if ever, exhibits sharp boundaries. The fleeces of some carpet-type sheep have an obvious division into an anterior region growing long wool and a posterior region growing carpet wool. In a detailed examination of such an animal the authors have found that the boundary is quite diffuse, occupying at least several centimetres over which a gradation occurred from long wool to carpet wool.

TABLE 1
PERCENTAGE AREA OF ABNORMAL WOOL TYPE (STANDARD SILHOUETTE AREA = 100)

Sheep No.	Left Side	Right Side	Mean	Sheep No.	Left Side	Right Side	Mean
1	37.0	51.8	44.4	12	4.2	3.2	3.7
2	26.8	22.0	24.4	13	0.1	7.3	3.6
3	17.0	26.4	21.6	14	0.0	5.6	2.8
4	27.2	15.7	21.4	15	5.4	0.1	2.7
5	15.8	3.2	9.5	16	2.4	2.4	2.4
6	16.7	1.8	9.2	17	4.1	0.2	2.2
7	8.0	7.4	7.7	18	0.9	3.4	2.1
8	8.4	6.4	7.4	19	0.2	3.4	1.8
9	10.4	1.1	5.6	20	0.0	3.1	1.6
10	5.2	5.3	5.3	21	0.5	0.6	0.6
11	7.6	0.7	4.2	22	0.2	0.1	0.2

The boundary between abnormal and normal regions in mosaic fleeces is dramatically sharp—in the architecture of the staple, in the population of skin follicles and glands, and in the structure of the follicles. These differences are illustrated in the photographs of wool and vertical sections of skin taken across the normal-abnormal boundary of a Merino mosaic (Plate 3, Figs. 1 and 2).

The sharpness of the boundaries between abnormal and normal regions supports the argument that these mosaics are genetic in origin even though in laboratory animals genes are known which cause "developmental" mosaicism of coat and skin colour with equally sharp boundaries (e.g. the spotting gene in mice).

(c) *Inheritance of Fleece Mosaicism*

Genetic analyses in sheep can rarely be concluded in less than 4 years, and usually take much longer. Therefore a rigorous test of the inheritance of fleece mosaicism is unlikely to be concluded until well after the death of the animals concerned. However, some tests have been made. Ten lambs have been produced

from matings between mosaic-fleeced parents, approximately 40 lambs have been produced from matings of mosaic rams and normal, unrelated ewes, and 13 lambs have been produced from mosaic ewes mated to normal rams. All of these lambs grew normal birthcoats and first-year fleeces. Such results would not disprove the inheritance of mosaicism as a pattern of the spotting type, but they argue strongly that it is unlikely.

A number of our mosaic sheep have come from small flocks of shortwools in which, if mosaicism were due to a single gene, other similar animals would be expected. In every case, the mosaic sheep was the only known case in the history of the flock.

The number of Merino sheep in our collection of fleece mosaics allows an estimate of the frequency of mosaicism in this breed. We have collected 12 mosaics in 2 years from a population of the order of 100 million giving an estimate of the frequency of mosaics in the order of 10^{-7} . If mosaicism is determined by a single gene then the frequency of mosaics would be expected to be of the order of mutation frequencies if the gene is completely lethal. There is no indication from our sheep of any effect on viability either in themselves or in their progeny. This does not exclude a lethality imposed by selection against mosaics as parents. However, such selection is not practised universally since eight of the 11 rams in our collection were in use as stud or flock rams at the time of their identification. Three of these were Merinos, 1 Polwarth, and 4 shortwools (2 Southdown, 1 Dorset Horn, and 1 Ryeland). Selection against mosaicism in ewes would be much less stringent than in rams. It can be concluded that selection against mosaicism is not marked and it follows that if it is determined by a single gene then the expected frequency would be much higher than that actually found, i.e. the expected frequency on the hypothesis of a single gene would be greater than 10^{-5} - 10^{-4} rather than the value of 10^{-7} actually found, which, even allowing our present figures to be a considerable underestimate of the frequency of mosaics, agrees with that expected on a hypothesis of somatic mutation.

IV. CONCLUSIONS

It is not possible to demonstrate that fleece mosaics have a somatic genetic basis but there is strong circumstantial evidence to suggest that this is the correct interpretation, namely: (1) the probable binary sequence of the areas of abnormal regions; (2) the extremely sharp boundary between the normal and abnormal wool types and the underlying skin; (3) their frequency of occurrence being of the order of mutation frequencies; and (4) the indication that mosaicism is not inherited. As this project continues and more data are obtained, it is probable that accurate evaluation of (1) will allow more confidence to be placed in our interpretation.

Interest in sheep mosaic for fleece type lies primarily in their use as tools for the analysis of correlations between components of the fleece. Previously the presence or absence of such correlations has been determined from studies of groups of related sheep, i.e. of the same breed in a single flock. This is the "between sheep" level of comparison (Turner 1956). A weakness of this method is that correlations between characters may not be due to developmental interaction or to a common genetic basis, but rather to a common environmental effect.

A second approach has involved comparisons "between groups" of animals of different breeds, and here it is assumed that averaging over groups reduces or removes environmental effects which might affect correlations between characteristics of the fleece (see Daly and Carter 1956; Fraser, Short, and Carter, unpublished data).

Another approach involves the estimation of genetic correlations in a large group using individual pedigrees (Morley 1953; Schinckel, personal communication), or between groups which have been selected from a single original population in different directions for the traits in question (Morley 1953, 1955; Turner 1956). The latter method, utilizing selection lines, suffers from the disability that each pair of selection lines effectively allows only a single comparison, regardless of the numbers of sheep involved.

Studies of sheep mosaic for fleece type are useful since in comparisons between areas growing normal and abnormal wool, environmental differences can be regarded as trivial. Further, the genetic causation is identical in the separate abnormal regions and so, by comparing the differences between adjacent normal and abnormal areas in various regions of the body, it is possible to detect the effects of variation in the normal level of expression of a particular character, e.g. differences due to a gradient of fibre density over the body.

In later papers of this series comparisons of normal and abnormal wool from a number of mosaic sheep will be detailed and discussed on the assumptions (a) that fleece mosaicism is "genetic" in causation, and (b) that the mutation is probably a deletion.

V. ACKNOWLEDGMENTS

We wish to thank all flock owners who have donated mosaic-fleeced sheep for this investigation, and many others who assisted generously in their identification and collection. Our particular thanks are due to Messrs. S. Power of Moree, N.S.W., and P. Hyland and D. Cannon, Department of Agriculture, Victoria, whose enthusiastic cooperation has been responsible for a large proportion of our mosaic flock, and to Mr. T. Dagg for the photography.

VI. REFERENCES

- BITTNER, J. J. (1932).—*J. Hered.* **23**: 421–3.
BOSMAN, V. (1935).—*Ondersteepoort J. Vet. Sci.* **5**: 517–18.
CASTLE, W. E. (1922).—*Publ. Carneg. Instn.* **320**: 51–4.
CASTLE, W. E. (1929).—*J. Exp. Zool.* **52**: 471–80.
CHAPMAN, R. E., MOULE, G. R., and RICHARDS, M. (1954).—*Qd. J. Agric. Sci.* **11**: 37–42.
CREW, F. A. E., and LAMY, R. (1938).—*J. Genet.* **37**: 211–28.
DALY, R. A., and CARTER, H. B. (1956).—*Aust. J. Agric. Res.* **6**: 476–513.
DUNN, L. C. (1934).—*J. Genet.* **29**: 317–26.
FELDMAN, H. W. (1935).—*J. Hered.* **30**: 383–8.
FISHER, R. A. (1930).—*J. Genet.* **23**: 77–81.
KELLEY, R. B. (1939).—*Aust. Vet. J.* **10**: 2–11.
HOLLANDER, W. F. (1949).—*J. Hered.* **40**: 274–7.
LANG, W. R. (1952).—*J. Aust. Inst. Agric. Sci.* **18**: 108–9.

- MORGAN, T. H. (1914).—*Proc. Soc. Exp. Biol., N.Y.* **11**: 171-2.
- MORLEY, F. W. H. (1953).—*Aust. J. Agric. Res.* **4**: 204-12.
- MORLEY, F. W. H. (1955).—*Aust. J. Agric. Res.* **6**: 77-90.
- PATTERSON, J. T., and STONE, W. (1938).—Univ. Tex. Publ. No. 3824.
- PINCUS, G. (1929).—*J. Exp. Zool.* **52**: 439-41.
- ROSS, I. C. (1933).—*Aust. Vet. J.* **9**: 32-4.
- STERN, C. (1936).—*Genetics* **21**: 625-730.
- STORMONT, C. (1954).—*J. Anim. Sci.* **13**: 94-8.
- STURTEVANT, A. H. (1929).—*Z. wiss. Zool.* **135**: 323-56.
- TURNER, H. N. (1956).—*Anim. Breed Abstr.* **24**: 87-118.
- WRIGHT, S., and EATON, O. H. (1926).—*Genetics* **11**: 333-51.

STUDIES OF SHEEP MOSAIC FOR FLEECE TYPE. I

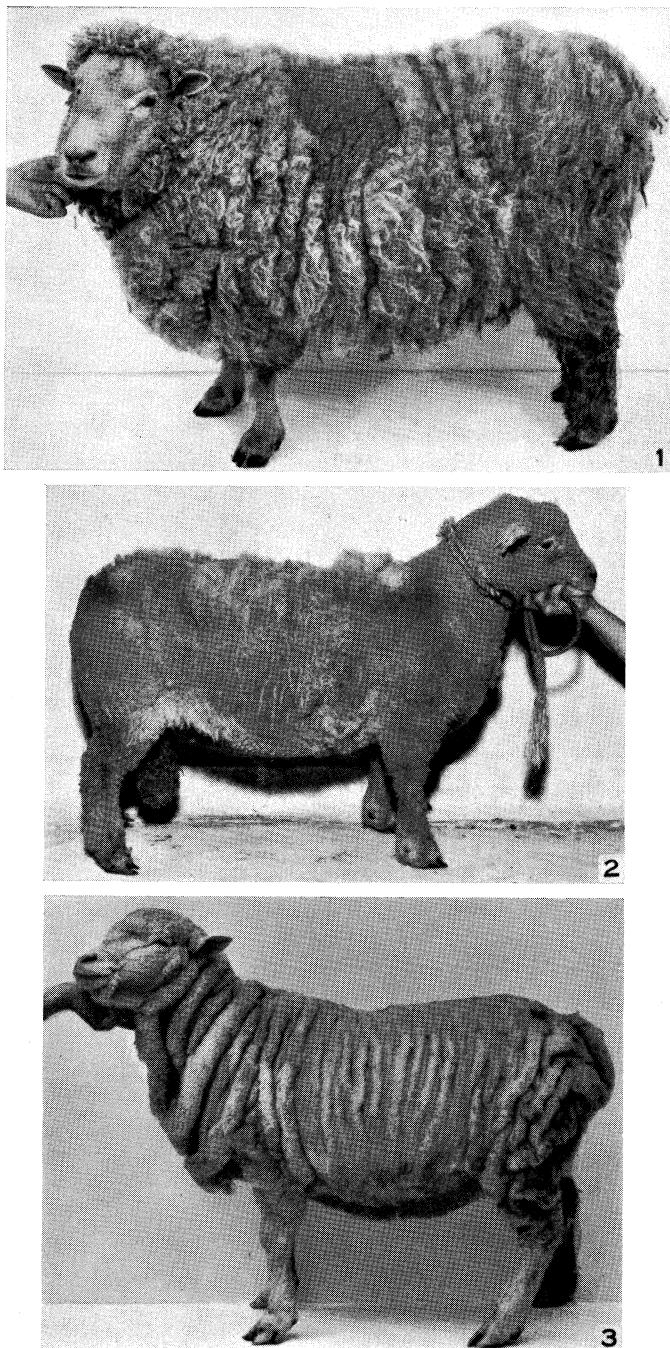
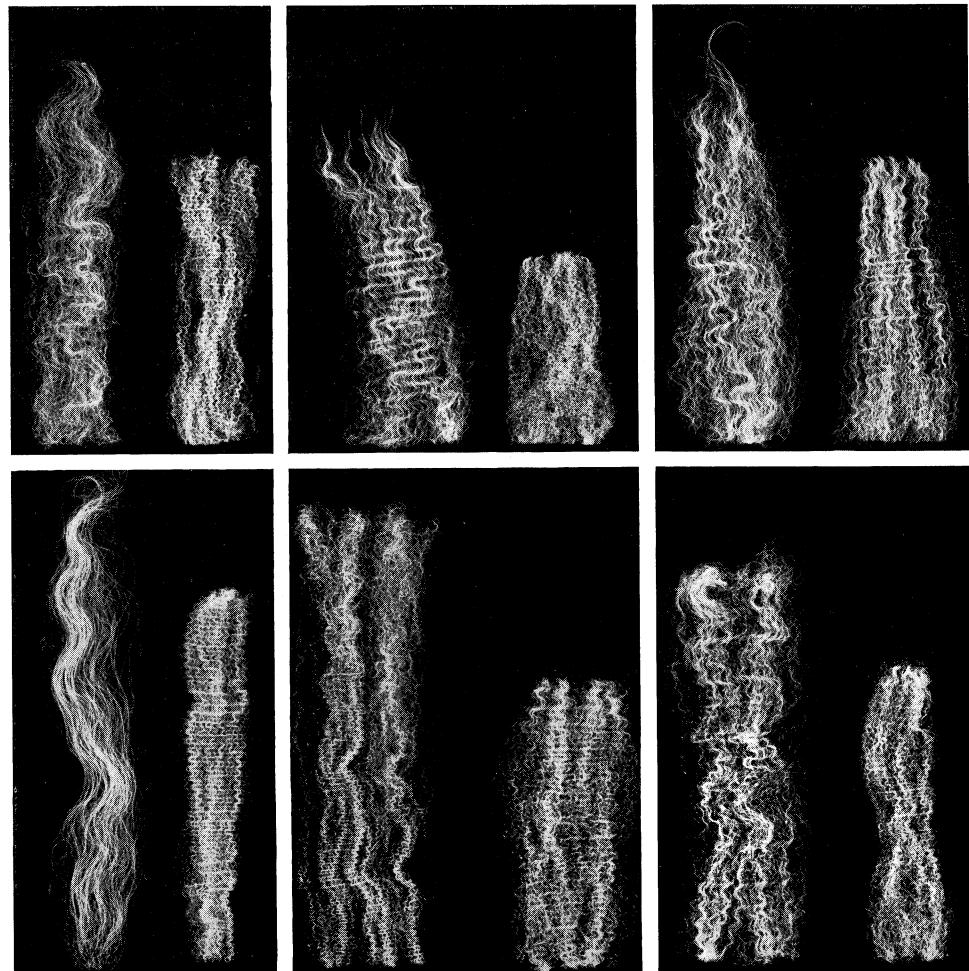


Fig. 1.—Crossbred sheep showing extensive (44 per cent.) fleece mosaicism.

Fig. 2.—Southdown fleece mosaic showing similarity of abnormal wool in different regions.

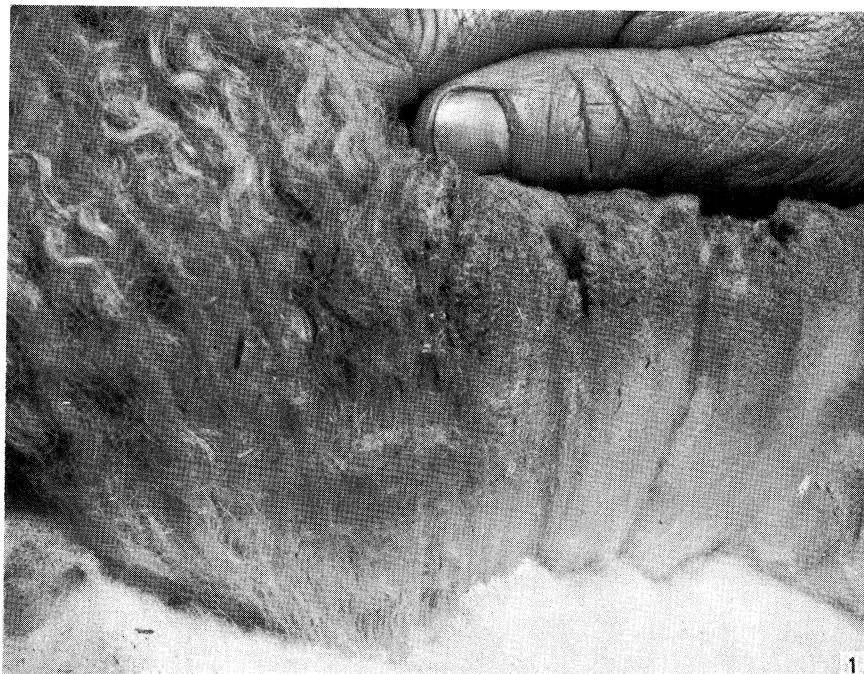
Fig. 3.—Merino sheep showing dorsoventral disposition of skin folds.

STUDIES OF SHEEP MOSAIC FOR FLEECE TYPE. I

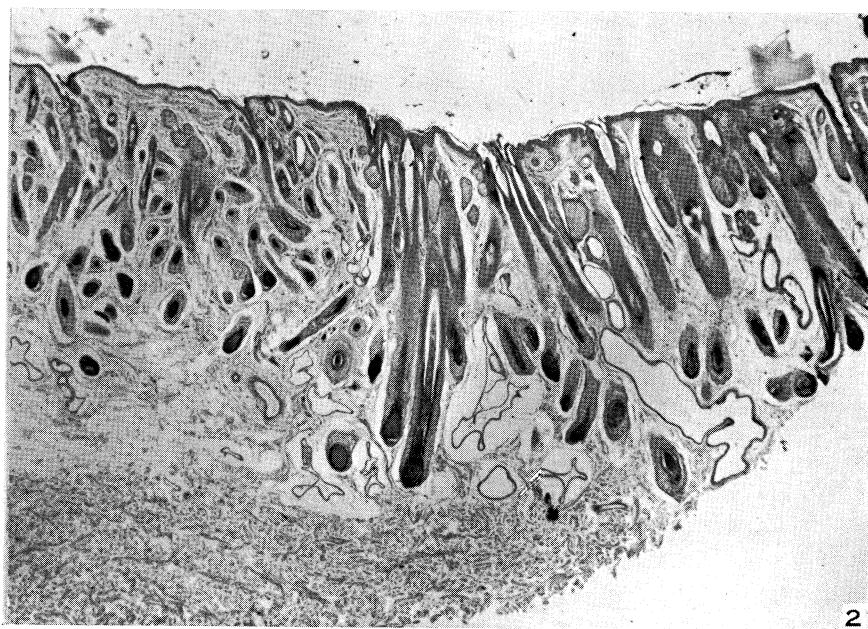


Wool staples from mosaic-fleeced Merino sheep. Abnormal staples on the left, normal staples on the right of each pair.

STUDIES OF SHEEP MOSAIC FOR FLEECE TYPE. I



1



2

Fig. 1.—Wool staple differences in a Merino fleece mosaic.

Fig. 2.—Photomicrograph of a vertical section across the boundary between normal and abnormal regions of skin. Note difference in follicle depth and size of suderiferous glands.

