The Skin Abnormality of 'Ichthyosis', a Mutant of the House Mouse

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WITH ONE PLATE

THE skin defect 'ichthyosis' of the house mouse is caused by a simple recessive gene (symbol *ic*) which arose as a spontaneous mutation. It was first described by Carter & Phillips (1950). 'Ichthyotic' mice have a thin coat of very short wavy hairs together with a scaly skin. There is, however, considerable individual variation in the severity of the defect. In badly affected animals the stratum corneum of the back is shed as large flakes, and the mice may be almost bald; but in less affected mice the skin is not very scaly and there is a complete though abnormal hair cover. The tail skin has a hard smooth appearance and is less flexible than normal. In some older mice a number of depressed rings form along its length, and a portion of the tail undergoes necrosis and drops off distal to one of the constrictions.

Female ichthyotic mice are usually sterile, as are some of the males. In the present investigation fertile *ic*/*ic* males were obtained from Dr. T. C. Carter, and these were outcrossed to normal CBA strain females from Professor H. Grüneberg's laboratory. An ichthyotic stock was maintained by back-crossing +/*ic* females to *ic*/*ic* males. These mice were generally less severely affected than those originally described by Carter & Phillips (1950).

The present paper describes the structure of the abnormal hairs and skin of the ichthyotic mouse, examined by routine and fluorescence microscopy (Jarrett, Bligh, & Hardy, 1956). Measurements of hairs and of the thickness of the tail epidermis are compared with measurements for the normal mouse.

INVESTIGATION

The coat

Hairs plucked from the backs of ichthyotic and normal mice were examined microscopically after mounting in Canada Balsam. Ichthyotic hairs were clearly distinguished from normal mouse hairs. The *awl*, *auchene*, *zigzag*, and *guard* hairs, as described by Dry (1926) for the normal coat, were not easily identified.

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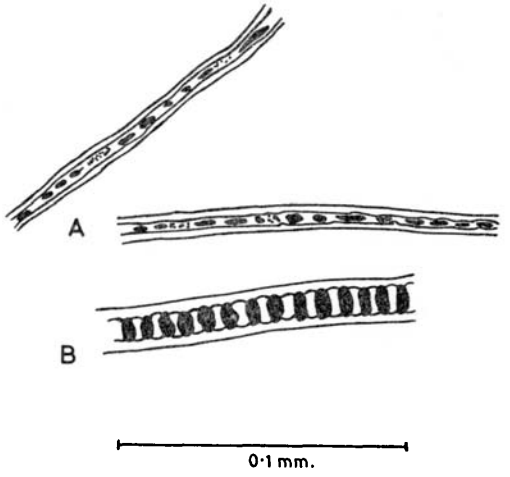
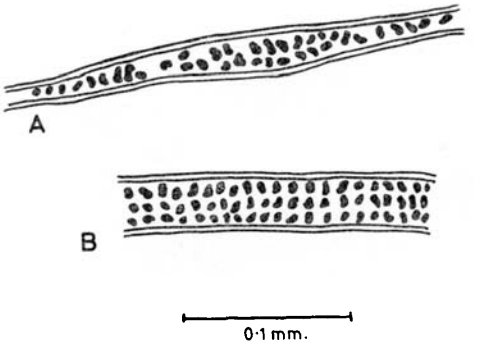
All ichthyotic hairs were wavy, and varied in thickness along their length due to alterations in size of the medulla. The most common type of unilocular hair of these animals (Text-fig. 1A) seems to correspond to the normal zigzag hair (Text-fig. 1B) and has a single row of air spaces; but in the ichthyotic hairs the medulla is compressed and there are no constrictions or angle bends. A smaller proportion of hairs have a multilocular medulla with air spaces varying from 1 to 3 in number, and they are arranged in a haphazard manner (Text-fig. 2A). This type probably corresponds to the normal awl and auchene hairs (Text-fig. 2B). Hairs approximating to normal awls were seen in some slightly affected animals. Long unilocular hairs possibly represent guard hairs. In some places in the medulla there were no melanin granules. There are gradations between the different types of hair, the most affected mice having the most deformed hairs.



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 1. A, ichthyotic mouse unilocular hairs from back showing compressed medulla. Camera lucida drawing. B, Normal mouse unilocular *zigzag* hair from back. Camera lucida drawing.

TEXT-FIG. 2. A, ichthyotic mouse multilocular hair from back, showing variation in width and haphazard arrangement of medullary melanin granules. Camera lucida drawing. B, normal mouse multilocular *awl* hair from back. Camera lucida drawing.

Ichthyotic and normal mouse hairs were fluorochromed with 0·1 per cent. acridine orange and 0·1 per cent. rhodamine B. After dehydration and clearing the hairs were mounted in DePex, and examined by fluorescence microscopy under UV light in the manner described by Jarrett & Spearman (1957) for the mutant 'matted'. The acridine orange did not penetrate the ichthyotic hairs and fluoresce in the medula as in matted mouse hairs. This suggests that the hair cuticle is not defective. Hairs did not show splitting or breakage. The cortical keratin of ichthyotic hair fluoresced salmon pink in places with rhodamine B, unlike normal hair cortex, which was non-fluorescent. This indicates that the hair keratin is abnormal even though splitting is uncommon.

Plucked hairs from 12 ichthyotic and 12 normal mice were examined microscopically. Hair lengths and fibre widths were measured. The results are shown in Table 1, and it is evident that ichthyotic mice have finer and shorter hairs than normal mice.

TABLE 1

Comparison of mean hair measurements from middle of back for 12 ic/ic *and 12 normal CBA mice*

Type of hair	*Mean thickness in μ*	*S.D.*	*Mean length in mm.*	*S.D.*
Normal awl (multilocular fibre)	34·7	±3·0	6·1	±0·52
ic/ic (multilocular fibre)	18·1	±6·1	2·76	±0·56
Normal zigzag (unilocular fibre)	17·6	±2·6	5·3	±0·74
ic/ic (small unilocular fibre)	7·16	±0·8	2·3	±0·32

Dorsal skin from 3 bald ichthyotic and 3 normal mice was fixed in Bouin's fluid and then macerated in dilute acetic acid according to Dry's (1926) method. The panniculus muscle and fat were removed, and counts were made of the hair follicles in a given flat area of skin. No deviation from the normal was detected in follicle density. By reversing the slide the emergent hairs were examined, but again no numerical deviation was seen. The skin of 6 nearly bald adult ichthyotic mice was examined under the low power of a binocular dissecting microscope. Despite the hairless appearance, the skin was seen to be covered with short fine 'lanugo' hairs.

The hairless appearance of ichthyotic mice is, therefore, caused by the dwarfed nature of the hairs.

HISTOLOGY

The epidermis

Skin from the back and tail of 12 ichthyotic and 12 normal mice was fixed in 70 per cent. alcohol (Jarrett & Hardy, 1957) and cut at 7 μ in paraffin wax. Sections were stained in haematoxylin and eosin. Other sections for fluorescence microscopy were stained for 40 minutes in an aqueous mixture of 20 parts 0·02 per cent. congo red and 10 parts 0·1 per cent. titan yellow, washed in water, and then stained for 3 minutes in 0·1 per cent. thioflavine T. The congo red/titan yellow mixture was freshly prepared and the technique is fully described by Jarrett, Spearman, & Hardy (1959).

Normal mouse-tail skin had a granular layer around the hair follicles but not under the tail scales (Plate, fig. A). Ichthyotic mouse-tail skin had a patchy granular layer in both the follicular and scale regions, and the thickened tail epidermis had enlarged cells and many more active nucleoli than normal skin (Plate, fig. B).

Normal mouse stratum corneum in the follicular region of the tail and over the back fluoresced red with the congo red and thioflavine T technique, but the 'hard' keratin of the tail scales fluoresced blue (Jarrett *et al.*, 1959). Ichthyotic mouse follicular tail-skin keratin had the same blue colour fluorescence as the tail scales. The tail stratum corneum was thicker and more flaky than normal, but had no nuclear chromatin remains. The ichthyotic back horny layer fluoresced red by this congo red staining method.

Follicular structure

The structure of the hair follicles on the back of 3 ichthyotic and 3 normal mice was examined. Follicle growth was stimulated by plucking the hairs (Montagna, 1956); the mice were killed 26 days after plucking. Both ichthyotic and normal hair follicles were stimulated to the same phase of anagen development. Ichthyotic follicles did not appear abnormal, and the arrangement of the keratinized inner root-sheath was similar to that found in the normal mouse. Club hairs were seen in the resting areas of ichthyotic skin, as in the normal mouse skin.

Epidermal thickness

Tail epidermal thickness was measured in 12 normal CBA mice and 12 ichthyotic mice. Ten measurements were made for each animal in the mid-scale regions. The width was measured from the basal layer to the bottom of the horny layer in haematoxylin and eosin stained sections. The mean thickness of the normal tail epidermis was 26·4 $\mu \pm$S.D. 2·0, but the thickness of the ichthyotic tail epidermis was 59·8 $\mu \pm$S.D. 15·0. The variation in thickness of the mutant epidermis reflects the different degrees of severity of the defect.

Histochemistry

Sections of skin from 3 ichthyotic and 3 normal mice were examined by a modified Baker's acid haematin method for phospholipids (Jarrett *et al.*, 1959). Although some lipids and absorbed sebum were removed during processing, normal tail skin showed a reaction in the scale keratin layer, but not around the follicles. It is thought that this is due to protein-bound phospholipids. Ichthyotic mouse-tail stratum corneum had a uniform reaction for phospholipids in both follicular and scale regions.

In normal alcohol fixed tissue only nuclear proteins fluoresce with thioflavine T. The epidermal cytoplasmic RNA was examined in sections of tail skin stained with 0·1 per cent. thioflavine T after removal of the DNA with the enzyme DNAase. Ichthyotic tail epidermis showed a stronger yellow colour fluorescence than normal mouse skin, indicating a higher content of RNA. This technique has been described by Jarrett (1958) and Jarrett *et al.* (1959). The findings of a high cytoplasmic RNA content, together with active nucleoli in ichthyotic epidermis, suggests that protein synthesis is increased (Davidson, 1960).

Normal epidermal keratin fluoresces blue with thioflavine T, but after oxidation of sections in 3 per cent. peracetic acid for 25 minutes it fluoresces yellow with this fluorochrome. Peracetic acid has little effect on the unkeratinized epidermis, and it is thought that this acid specifically oxidizes the cystine of the keratin (Alexander & Hudson, 1954) and renders it stainable (Fraser & Rogers, 1955; Jarrett *et al.*, 1959). Therefore, keratins having a high cystine content (as in hair) fluoresce a brighter yellow than those with a lower cystine content. Normal mouse-tail skin fluoresced a deeper yellow in the scales than in the keratin around the follicles. Ichthyotic tail keratin, however, fluoresced strongly in both regions after oxidation. This suggests that the keratin around the tail-hair follicles of the ichthyotic mouse has an abnormally high cystine content. Sections of skin were also examined for protein-bound sulphydryl groups by the method of Barrnett & Seligman (1952). Normal tail-epidermal keratin showed a reaction only in the scales, but the mutant tail had a uniform reaction for bound sulphydryl groups in both the scale and perifollicular horny layers.

The tail constrictions

Tail-skin containing deep depressed rings was examined histologically in 3 mice. Sections were stained in haematoxylin and eosin, and by the congo red/thioflavine T technique. The epidermis appeared to be drawn down against the panniculus carnosus muscle and the dermal tissue was missing in this region (Plate, fig. C). No alteration was detected in the epidermal cells of the depressed ring, either by routine staining or by fluorescence microscopy. Constrictions were, therefore, not due to overlying keratin pressing down on the epidermis, as had been suggested as a possible cause (Carter & Phillips, 1950). It has been shown by Jarrett *et al.* (1959) that compressed epidermal cells undergo a form of keratinization, and that even slight pressure causes the liberation of nucleic acids from the nucleus into the cytoplasm. It is likely that local atrophy of the dermis produces ringing. Pressure on blood-vessels in the affected region would then lead to necrosis and shedding of the distal portion of the tail. Ichthyotic mice were found especially liable to impetiginous skin infections, and the epidermis at the constrictions often became secondarily infected.

COMPARISON OF THE TAIL-SKIN OF MOUSE MUTANT 'ICHTHYOSIS' WITH HUMAN ICHTHYOSIS AND PSORIASIS

Sections of ichthyotic mouse-skin were compared with sections of skin from subjects suffering from ichthyosis vulgaris and psoriasis. These are human disorders with abnormal keratinization, thought to have a genetic basis. Both defects, as in ichthyotic mouse-tail skin, have an abnormally high content of sulphydryl groups and protein-bound phospholipids in the stratum corneum (Jarrett *et al.*, 1959). Human ichthyosis has a thin atrophic epidermis, but psoriasis, like the mutant mouse-tail, has an abnormally thick epidermis containing numerous active nucleoli: stainable nuclear remains are absent from

the horny layer in human ichthyosis and in the mutant mouse, but in psoriasis there is a parakeratotic horny layer with altered nuclei.

In human ichthyosis and in the ichthyotic mouse there is a thin poorly developed granular layer, but this is entirely absent in true psoriasis. Psoriasis has an abnormally increased dermal vascularity, which produces a clinical reddening of the skin, but no such vascular change is seen either in mouse or human ichthyosis. In neither of the human disorders is there any alteration in hair structure.

In the human disorders, similar histochemical changes in the stratum corneum can be produced either by an atrophic or by a physiologically active epidermis: the mouse mutant has features resembling both human ichthyosis and psoriasis. The epidermal horny layer resembles human ichthyosis in that there are no nuclear remanents, but the increased epidermal activity is more allied to psoriasis.

DISCUSSION

The thinness of the ichthyotic mouse-coat is due to the abnormal nature of the hairs. There does not appear to be a suppression of follicle growth as occurs in the mutants *crinkled* (Falconer *et al.*, 1951) and *ragged* (Slee, 1957). Hairs do not fragment as in *naked* (David, 1932, 1934) or *matted* (Searle & Spearman, 1957). Widespread depilation of whole hairs as in *hairless* (Fraser, 1946) was not seen.

Normal mouse-tail skin has a flexible 'soft' stratum corneum around the hair follicles with a more rigid 'hard' keratin in the tail scales (Jarrett *et al.*, 1959). This arrangement allows freedom of movement of the tail. Ichthyotic tail skin has a rigid type of horny layer in both follicular and scale regions, and this would interfere with tail movements.

The rigid type of horny layer in ichthyotic tail skin is formed from a patchy granular layer. This is unusual because in normal skin a granular layer is associated with the formation of flexible keratinized cells. Ichthyotic epidermis is active and it is possible that the rate of cell movement from basal to horny layer is greater than normal. If the rate of cell turnover is increased, there may be too little time for enzyme systems thought to be concerned in flexible keratin formation to act before the epidermal cells become cornified (Jarrett *et al.*, 1959). In these circumstances one might expect the formation of an abnormal keratin layer. It is interesting that no keratin abnormality of the back skin was detected by fluorescence microscopy. It is possible that ichthyotic mice with more severely affected backs than those examined might show an altered keratinization.

The fact that both hairs and epidermis are affected indicates that the primary abnormality lies in the skin, as was suggested by Grüneberg (1952). The dermis is thought to control the pattern of epidermal development, and epidermal cells in tissue culture become organized into epidermis only when dermal cells are

present (Montagna, 1956). Possibly there is an induction of abnormal epidermis by the dermis in the ichthyotic mouse-tail. The peculiar ringing which takes place in the ichthyotic mouse-tail skin appears to be due to atrophic changes in the dermal connective tissue. The immediate cause of these changes is not clear.

In the human race an abnormality known as Ainhum occurs among African negroes. In this defect there is a similar formation of depressed rings around the digits (Sutton, 1956; Clarke, 1959). The cause of this disorder is uncertain but it has been suggested that it has a genetic basis. Keratoma mutilans referred to by Gates (1946) as a dominant gene defect in man may be a form of Ainhum. The epidermis in Ainhum is reported to be drawn down in a furrow against the underlying bone and spontaneous amputation later occurs as in the ichthyotic mouse-tail.

In the condition known as ring tail (Worden & Lane-Petter, 1957), which occurs in new-born rats, depressed rings form along the tail and the part distal to a constriction eventually drops off as in the ichthyotic mouse and in Ainhum. The pathology of this defect does not seem to have been investigated and the cause is uncertain.

SUMMARY

1. Hair and skin from the mouse mutant 'ichthyosis' have been studied by routine and by fluorescence microscopy. Ichthyotic mouse-hairs from the back were shorter and finer than normal. All hairs are wavy, and have an unevenly compressed medulla with an irregular distribution of melanin granules. Hairs do not fragment. No deviation from normal was found in hair follicle counts even in bald areas, but the dwarfed hairs are unable to form an adequate hair cover.

2. The tail epidermis was thicker and more active than normal, and in contrast to normal tail skin there was a patchy granular layer in the scale regions.

3. The stratum corneum around the hair follicles of the tail was histochemically similar to the hard keratinized scales of the normal mouse. There was also hyperkeratosis of both the follicular and tail scale regions. No abnormality was detected in the epidermis of the back by the methods employed.

4. Depressed rings form along the tail and the epidermis is drawn down against the panniculus muscle. This is probably due to dermal atrophy. The defect simulates a syndrome known as Ainhum, which occurs in African negroes.

5. The keratinization defects are compared in mouse ichthyosis and in the human disorders ichthyosis vulgaris and psoriasis.

RÉSUMÉ

Les Anomalies de la peau chez le mutant 'ichthyosis' de la Souris domestique

1. Le poil et la peau de la Souris portant la mutation 'ichthyosis' ont été étudiés au microscope ordinaire et au microscope à fluorescence. Les poils du

dos des Souris ichthyosis sont plus courts et plus fins que normalement. Tous les poils sont ondulés. Ils présentent une moelle inégalement comprimée et une distribution irrégulière des grains de mélanine. Les poils ne se fragmentent pas. Le nombre des follicules pileux n'est pas modifié par rapport au nombre normal, même dans les régions chauves, mais les poils nains sont incapables de former une fourrure adéquate.

2. L'épiderme de la queue est plus épais et plus actif que l'épiderme normal. Il existe dans les écailles des mutants une couche granuleuse morcelée, alors que celle-ci est absente chez la Souris normale.

3. Histochimiquement, le stratum corneum autour des follicules pileux est semblable à celui des écailles de kératine dure de la peau normale. On ne constate, avec les méthodes employées, aucune anomalie de l'épiderme dorsal.

4. La queue des mutants porte une série de constrictions annulaires, au niveau desquelles l'épiderme est appliqué contre le muscle du pannicle. Cela est probablement dû à une atrophie du derme. Cette malformation mime l'ainhum des Nègres africains.

5. Les anomalies de la kératinisation chez la Souris mutante ichthyosis sont comparées aux affections humaines ichthyosis vulgaris et psoriasis.

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REFERENCES

ALEXANDER, P., & HUDSON, R. T. (1954). *Wool, its Chemistry and Physics*, p. 265. London: Chapman & Hall.

BARRNETT, R. J., & SELIGMAN, A. M. (1952). Histochemical demonstration of protein-bound sulphydryl groups. *Science*, **116**, 323–7.

CARTER, T. C., & PHILLIPS, R. S. (1950). Ichthyosis, a new recessive mutant in the house mouse. *J. Hered.* **41**, 297–300.

CLARKE, G. H. V. (1959). *Skin Diseases in the African*, p. 46. London: H. K. Lewis.

DAVID, L. T. (1932). The external expression and comparative dermal histology of hereditary hairlessness in mammals. *Z. Zellforsch.* **14**, 616–719.

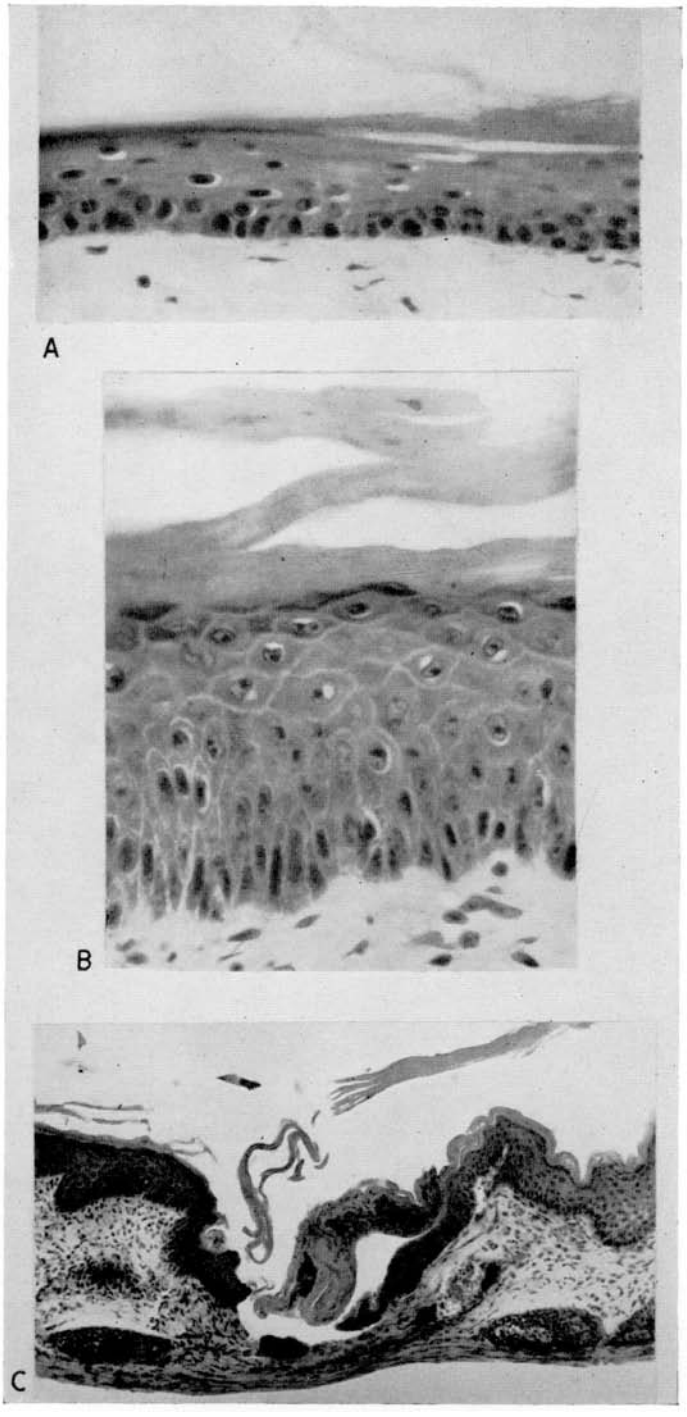
—— (1934). Studies on the expression of genetic hairlessness in the house mouse. *J. exp. Zool.* **68**, 501–18.

DAVIDSON, J. N. (1960). *The Biochemistry of the Nucleic Acids*, p. 231, 4th ed. London: Methuen.

DRY, F. W. (1926). The coat of the mouse. *J. Genet.* **16**, 287–340.

FALCONER, D. S., FRASER, A. S., & KING, J. W. B. (1951). The genetics and development of *crinkled*, a new mutant in the house mouse. *J. Genet.* **50**, 324–44.

FRASER, F. C. (1946). The expression and interaction of hereditary factors producing hypotrichosis in the house mouse: histology and experimental results. *Canad. J. Res.* (D), **24**, 10–25.



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FRASER, R. D. B., & ROGERS, G. E. (1955). The bilateral structure of wool cortex. *Proc. Inst. Wool Text. Res. Conf. (Australia)*, pp. 151–5.

GATES, R. R. (1946). *Human Genetics*, vol. 1, p. 336. New York: MacMillan.

GRÜNEBERG, H. (1952). *The Genetics of the Mouse*, p. 102, 2nd ed. The Hague: Martinus Nijhoff.

JARRETT, A. (1958). The Chemistry of inner root sheath and hair keratins. *Brit. J. Derm.* **70**, 271–83.

—— & HARDY, J. A. (1957). The value of alcohol for fixation of skin. *Stain Tech.* **32**, 225–33.

—— & SPEARMAN, R. I. (1957). The keratin defect and hair cycle of a new mutant (matted) in the house mouse. *J. Embryol. exp. Morph.* **5**, 103–10.

——, SPEARMAN, R. I., & HARDY, J. A. (1959). The histochemistry of keratinization. *Brit. J. Derm.* **71**, 277–95.

——, BLIGH, A., & HARDY, J. A. (1956). Fluorescent microscopy of the human skin. *Brit. J. Derm.* **68**, 111–19.

MONTAGNA, W. (1956). *The Structure and Function of Skin*, pp. 240, 332. New York: Academic Press.

SEARLE, A. G., & SPEARMAN, R. I. (1957). 'Matted', a new hair mutant in the house mouse: genetics and morphology. *J. Embryol. exp. Morph.* **5**, 93–102.

SLEE, J. (1957). The morphology and development of 'ragged'—a mutant affecting the skin and hair of the house mouse. I. Adult morphology. *J. Genet.* **55**, 100–21.

SUTTON, R. L. (1956). *Diseases of the Skin*, p. 1027, 11th ed. London: Kempton.

WORDEN, A. N., & LANE-PETTER, W. (1957). *UFAW Handbook on the Care and Management of Laboratory Animals*, p. 366, 2nd ed. London: U.F.A.W.

EXPLANATION OF PLATE

FIG. A. Normal mouse-tail scale epidermis stained with haematoxylin and eosin. No granular layer is present. Sagittal section. × 600.

FIG. B. Ichthyotic mouse-tail scale, showing the thickened epidermis containing enlarged cells with active nucleoli. In contrast to the normal tail scale a granular layer is present under the hyperkeratotic stratum corneum. H. & E. stained sagittal section. × 600.

FIG. C. Ichthyotic mouse-tail skin, showing a sagittal section through a tail constriction. The dermis is atrophied beneath the groove formed by the epidermis. Secondary impetiginous infection has occurred and inflammatory cells are seen in the stratum corneum. H. & E. stained section. × 140.