The expression of the gene asebia in the laboratory mouse

I. Epidermis and dermis

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SUMMARY

Mice homozygous for the *asebia* mutation (*ab/ab*), which have defective sebaceous glands, display abnormalities in several other aspects of the integument. Histological sections showed that hyperplasia of the cellular layers and the stratum corneum of the epidermis is apparent at birth and increases markedly with age. Enlarged intercellular spaces are also noted in the epidermis. The thicker dermal layer of the asebic mice is characterized by increased vascularity, increased cellularity and the abnormal morphology of a large proportion of the 'fibroblast' population. Electron microscopy demonstrated the many abnormalities in the dermal fibroblasts as well as large amounts of cellular debris in the surrounding matrix. Collagen and elastin show alterations at the light microscopic and ultrastructural levels. Many features of the asebic dermis resemble those found with mild inflammation and with the lysosomal storage diseases. Changes in the dermis of asebic foetuses were noted prior to epidermal alterations and may mediate the latter.

1. INTRODUCTION

Mice carrying the '*asebia*' (*ab/ab*) mutation originated spontaneously from the sib-mated colony of BALB/c strain maintained by Dr A. H. Gates at Stanford University and were first reported by Gates & Karasek (1965). Because the affected mice showed 'no evidence of sebaceous gland development, the one pathological feature thus far found to be present throughout life' the name '*asebia*' meaning 'without sebum', was coined. These authors reported that the alteration of a single autosomal recessive gene was responsible for the dry skin, the sparse hair coat and the absence of sebaceous glands in the homozygous (*ab/ab*) mice. Mice heterozygous (+ /*ab*) at the *asebia* locus showed no sign of these integumentary defects.

We have observed that abnormalities were already apparent in the epidermis, dermis and hair follicles of homozygous asebic mice by the time the sebaceous gland rudiments appeared, as they do in normal mice, in the late stages of foetal development. Since the epidermis differentiates from a simple epithelium as a result of interactions with the dermis (Wessells, 1967), and since the maintenance of the properties of mammalian epidermis and its derivatives depends on the

continuance of specific influences from the dermis (Billingham & Silvers, 1967), it is possible that the persistent effects of *asebia* on epidermis and its derivatives are secondary to its effects on the dermis, or to some systemic factor affecting the dermis. This is the first of three papers describing the development and adult structure of the skin of asebic mice and providing the basis for discussion of the above hypothesis. The second paper (Josefowicz & Hardy, 1978) deals with hair follicles and the third (Josefowicz & Hardy, 1978) with sebaceous glands. Some preliminary observations have been reported (Josefowicz & Hardy, 1974). Skeletal and endocrine abnormalities in asebic mice have been described elsewhere (Josefowicz, 1975).

2. MATERIALS AND METHODS

The mice used in this study were derived from the *chinchilla-asebia* (CA) stock, which carried the additional *chinchilla* (c^{ch}) colour gene, and which was developed from the BALB/cCrglGa *albino* substrain line (Gates & Karasek, 1965). They were obtained from Dr Gates in 1970.

The standard matings of heterozygous ($+/ab$) females to homozygous recessive (ab/ab) males, yielding equal proportions of heterozygous ($+/ab$: phenotypically normal) and homozygous (ab/ab: phenotypically asebic) litter-mates, provided the majority of the animals studied. However, because the asebic and normal litter-mates could not be distinguished macroscopically until six or seven days after birth (Gates & Karasek, 1965), $ab/ab \times ab/ab$ as well as $+/+ \times +/+$ matings were used to provide animals of known genotype prior to this age.

Approximately 80 homozygous asebic (ab/ab) mice, 60 heterozygotes ($+/ab$) and 20 homozygous normal ($+/+$) mice, were examined in detail during the course of this study. These animals carried 2, 1 or no c^{ch} genes. The male and female mice of these genotypes which were examined ranged from 16 days post-conception to approximately two years post-natal. The descriptions of external features by Grüneberg (1943) and Rugh (1968) were used to adjust the estimated chronological age of the pre-natal mice examined to true developmental age. The gestation period of the substrain was 20–21 days.

Paraffin serial sections were prepared from skin samples from the mid-dorsal region fixed in Zenker's fluid, and were stained according to the following procedures:

Mayer's haemalum, eosin and picric acid (Carter & Clarke, 1957)

Periodic acid Schiff with Mayer's haemalum counterstain (McManus & Mowry, 1965)

Alcian Blue–Feulgen stain (McManus & Mowry, 1965)

Mallory's triple connective tissue stain (McManus & Mowry, 1965)

Acid orcein–Giemsa stain (Pinkus, 1944).

Measurements of epidermal and dermal thickness were made from skin sections cut vertical to the skin surface in the direction of the antero-posterior axis. Sections were selected in which the hair follicles were cut longitudinally and the hair cycle stage (Dry, 1926) was either anagen (the period of active growth) or

telogen (the resting period). The catagen stage of the hair cycle was excluded because of the lack of synchrony between follicles at that stage. A Leitz Prado projector provided images enlarged 3350 times on which the thickness of the stratum corneum, the remainder of the epidermis, the dermis and the hypodermis were measured to the nearest millimetre. Five measurements, made from different fields on a slide containing 10–20 sections, provided mean thickness values for each mouse studied.

Two male litter-mates 8 days old and two 30 days of age provided material for electron microscopy. One member of each pair was *ab/ab*, the other, *+/ab*. Samples from the mid-dorsal skin, measuring 1 mm³, were pre-fixed for two hours in 3 % glutaraldehyde in 0·2 M-Sorenson's phosphate buffer and post-fixed for two hours in 1 % OsO_4 in phosphate buffer with 0·2 M-sucrose. Fixation and dehydration were carried out at 4 °C and the tissues embedded in Epon (Luft, 1961). One-micron sections stained with 0·5 % Toluidine Blue O in 1 % sodium borate were used for light microscopic observations. Thin sections of gold or silver iridescence were stained with methanolic uranyl acetate and lead citrate (Reynolds, 1963) and observed with a Philips 200 electron microscope. Those sections used to study the periodicity of collagen fibres were stained with uranyl acetate alone.

The dermis of asebic and normal mice was studied at the level of the sebaceous glands by electron microscopy. Measurements of collagen fibre diameter were made from printed fields of sharply defined cross-sectioned fibres at a magnification of 167 700 times.

The skin vascularity of two- to four-month old, male and female, *+/ab* and *ab/ab* mice was determined by the examination of paraffin sections and whole skin preparations. For the latter, mice were sacrificed by ether administration and skinned. The skins were gently stretched and pinned on to paraffined trays with the hypodermal side up, thus exposing the vascular network. Skin preparations of homozygous *hairless* (*hr/hr*) mice were also prepared to observe the effect of hairlessness on vascularity in a different mutant. A blueing test was used as an indicator of vascular permeability. The lateral tail vein was injected with 0·1 ml of 1 % Pontamine Sky Blue 6BX (1 mg/33 g body weight; Wells & Eyre, 1972). The mice were sacrificed and whole skin preparations examined at time intervals ranging from 15 min to 1 h after injection.

3. RESULTS

No differences were found between homozygous normal (+/+) and heterozygous asebic (+/ab) mice in any of the features examined. Descriptions are therefore based mainly on a comparison of *ab/ab* mice with their *+/ab* littermates which will be described as 'normal' (Plate 1, figs. 1, 2). The *ab/ab* mice had a short sparse hair coat and a dry scaly skin. The degree of expression of these characters varied more between families than between or within the litters of one family. The *chinchilla* allele (c^{ch}) did not appear to affect any of the structures observed except the melanocytes and melanin pigment granules of hair follicles.

(i) *Epidermis*

While some differences between asebic and normal litter-mates in the epidermis were observed at pre-natal stages, the epidermis of asebic mice was consistently different from that of the normal mice from six days to four months after birth. Hyperkeratosis was expressed by a greatly increased thickness of both the combined cellular or 'living' epidermal layers (stratum germinativum, stratum

Table 1. *Measurements in microns of epidermal thickness in normal (+ /ab) and asebic (ab/ab) mice*

Age	Stage of hair cycle*	Cellular layers (μm)		Stratum corneum (μm)	
		+ /ab	ab/ab	+ /ab	ab/ab
6 days	A	$28 \cdot 0 \pm 0 \cdot 3$ (3)†	$37 \cdot 1 \pm 0 \cdot 2$ (3)	$59 \cdot 2 \pm 8 \cdot 0$ (3)	$83 \cdot 8 \pm 9 \cdot 9$ (3)
8 days	A	$10 \cdot 6 \pm 2 \cdot 2$ (2)	$23 \cdot 9 \pm 3 \cdot 0$ (2)	$36 \cdot 6 \pm 6 \cdot 0$ (2)	$49 \cdot 0 \pm 4 \cdot 8$ (2)
11 days	A	$10 \cdot 6 \pm 1 \cdot 7$ (2)	$17 \cdot 5 \pm 4 \cdot 3$ (3)	$18 \cdot 7 \pm 1 \cdot 1$ (2)	$37 \cdot 0 \pm 10 \cdot 7$ (3)
20 days	T	$15 \cdot 3 \pm 3 \cdot 4$ (2)	$22 \cdot 0 \pm 1 \cdot 1$ (2)	$4 \cdot 7 \pm 0 \cdot 2$ (2)	$27 \cdot 6 \pm 6 \cdot 7$ (2)
37–39 days	A + T	$8 \cdot 4$ (1)	$25 \cdot 1 \pm 3 \cdot 6$ (3)	$5 \cdot 4$ (1)	$31 \cdot 5 \pm 0 \cdot 9$ (3)
4 months	A + T	$7 \cdot 8$ (1)	$25 \cdot 7$ (1)	$3 \cdot 6$ (1)	$46 \cdot 3$ (1)
11 months	A + T	$14 \cdot 5 \pm 3 \cdot 6$ (3)	$59 \cdot 2 \pm 0 \cdot 6$ (2)	$9 \cdot 4 \pm 1 \cdot 8$ (3)	$59 \cdot 3 \pm 7 \cdot 4$ (2)
21–23 months	A + T	$9 \cdot 0 \pm 0 \cdot 8$ (4)	$42 \cdot 7$ (1)	$1 \cdot 5 \pm 1 \cdot 1$ (4)	$50 \cdot 1$ (1)
Overall total		$14 \cdot 0 \pm 1 \cdot 8$ (18)	$30 \cdot 4 \pm 3 \cdot 3$ (17)	$18 \cdot 9 \pm 5 \cdot 2$ (18)	$48 \cdot 5 \pm 5 \cdot 3$ (17)

* A, anagen; T, telogen. † Mean (μm) $\pm$ standard error of the mean, followed by the number of mice (in brackets).

spinosum and stratum granulosum) and the non-living corneum (Table 1; Plate 1, figs. 3, 4; Text-fig. 1). Large intercellular spaces were found in all layers of the asebic epidermis, but were particularly prevalent in the two lower layers. As well as being thicker than normal, the stratum granulosum of the asebic skin contained coarser keratohyalin granules (Fig. 4). The cells of the thicker stratum corneum of the asebic mice were irregularly packed and their boundaries were less sharply defined. The surface of the epidermis and often the entire epidermis of asebic mice was characteristically thrown into sharp ridges.

The above mentioned characteristics observed in the skin of young asebic mice were greatly exaggerated in the skin of older mice between 11 and 23 months (Table 1; Text-fig. 1). With increasing age, the epidermis of normal mice became extremely thin, often composed of one or two cell layers with little or no stratum
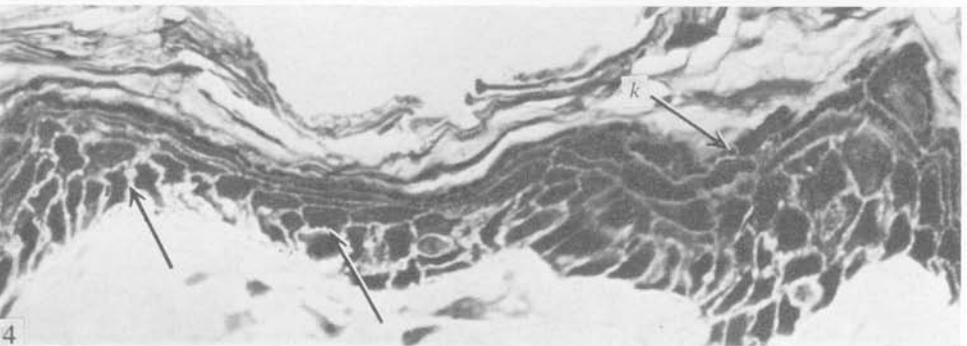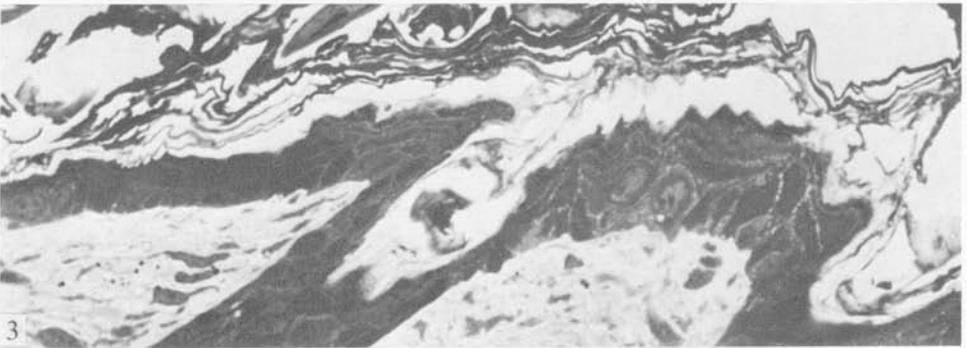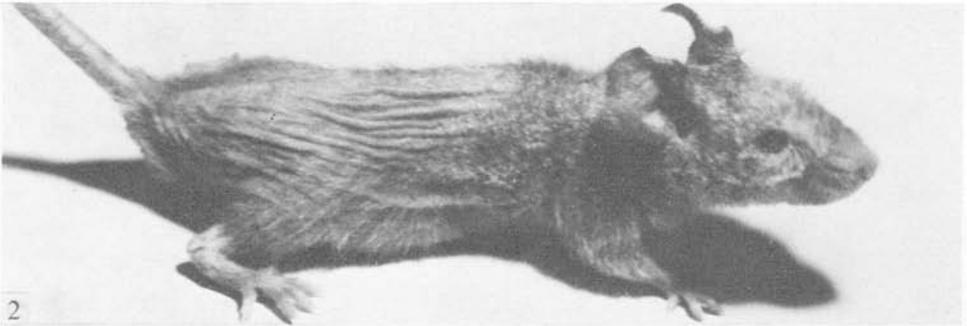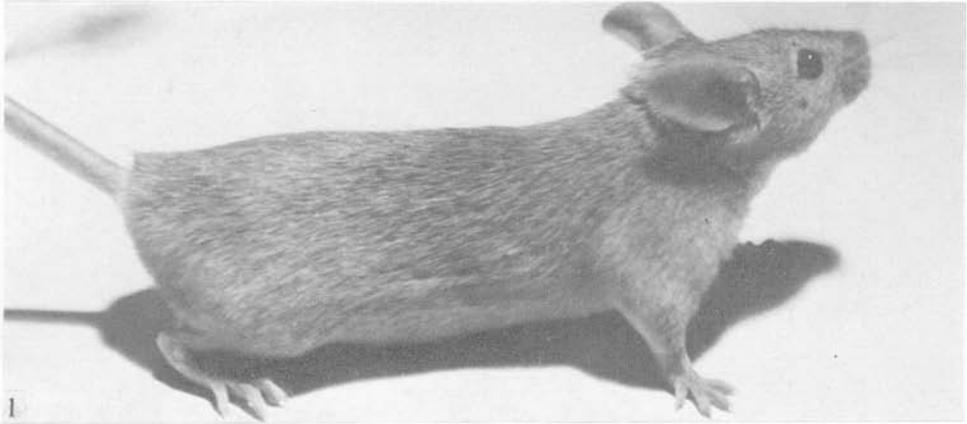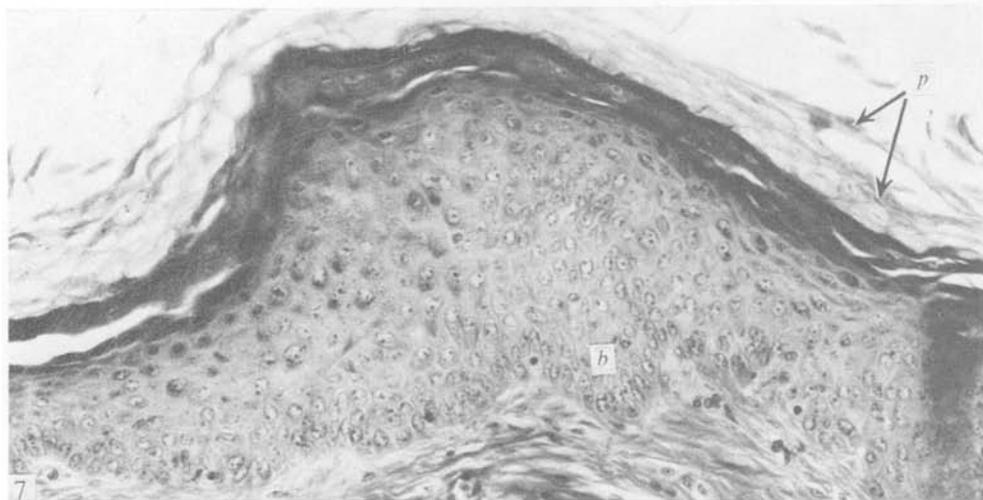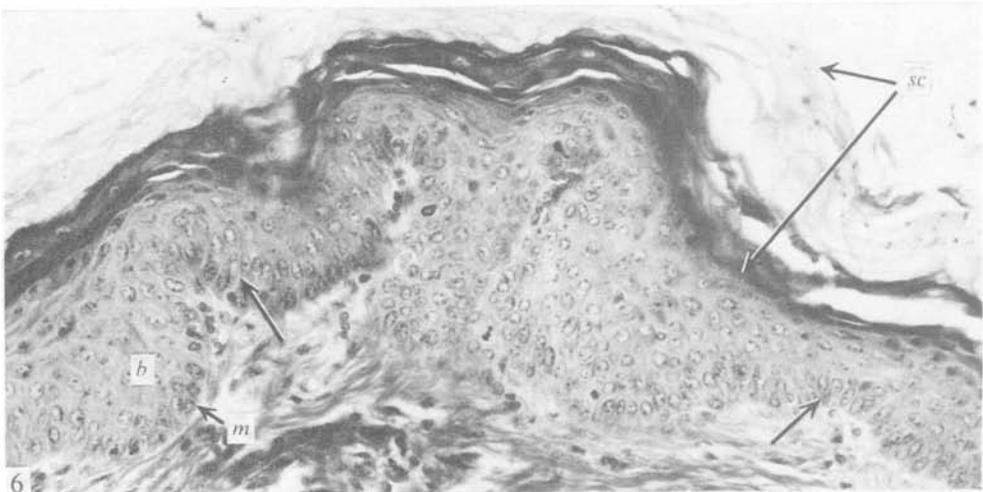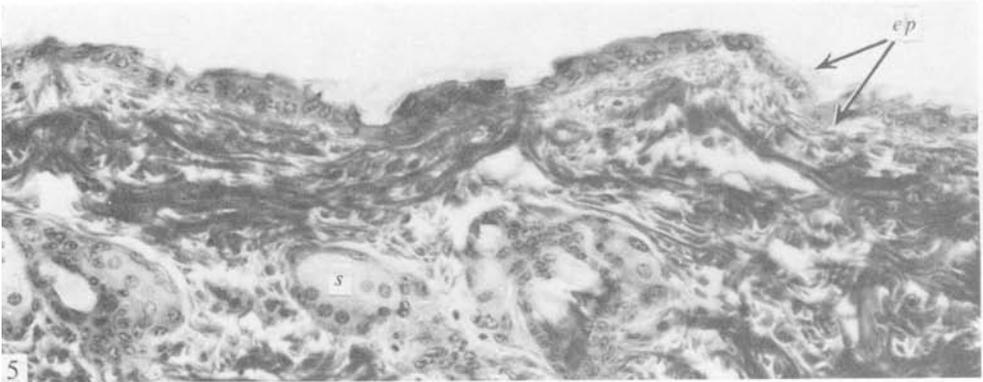

PLATE 1

Fig. 1. Heterozygous 'normal' (+ /ab c/c^{ch}) adult female mouse.

Fig. 2. Homozygous asebic (ab/ab c/c^{ch}) adult female mouse.

Fig. 3. Epidermis of the mid-dorsal region from + /ab c/c 8-day-old male. 1 μm Epon section stained with Toluidine Blue O. × 700.

Fig. 4. Epidermis of the mid-dorsal region from an ab/ab c/c 8-day-old male litter-mate of the above. Note the coarse keratohyalin granules (*k*), and the wide intercellular spaces (arrows) as well as the less dense dermal matrix. 1 μm Epon section stained with Toluidine Blue O. × 700.

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corneum (Plate 2, fig. 5). At the same age, the skin of older asebic mice had an extremely thick cellular epidermis (having 8–10 cell layers) and a thicker stratum corneum (Plate 2, figs. 6, 7). Small areas of more extreme hyperkeratosis and of parakeratosis (Fig. 7) were found in the asebic epidermis, and were possibly related to the skin irritation and scratching which occurs frequently among these animals. The upper layers of nucleated cells also appeared to be sloughing off in many areas. In some mutants, the base of the epidermis appeared irregular where bulges extended into the dermis (Fig. 6).
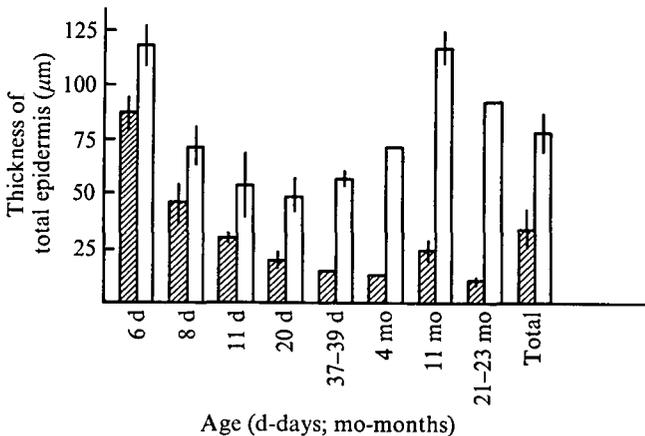
Text-fig. 1. Histogram showing total epidermal thickness in normal mice ($+/ab$; shaded bars) and asebic mice (ab/ab; unshaded bars) at different ages and hair cycle stages. (Total epidermal thickness is the summation of the thickness of the cellular layers and the stratum corneum as presented in Table 1.) The vertical lines represent standard errors of the means.

(ii) *Dermis and hypodermis*

The dermis of asebic mice was consistently thicker than that of normal mice after birth, and from four months onwards was roughly twice as thick (Table 2).

Like the hypodermis of normal mice (Chase, Montagna & Malone, 1953) that of asebic mice underwent cyclic changes in thickness corresponding to the hair growth cycles. However, the hypodermis of the mutants was usually thicker than that of normal mice at any given stage of the cycle. The fat cells of the asebic

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PLATE 2

Fig. 5. Thin epidermis (*ep*) of the mid-dorsal region from a $+/ab$ female, 21 months old. *s*, sebaceous gland. Mayer's haemalum, eosin and picric acid. × 300.

Fig. 6. Epidermis of the mid-dorsal region from an ab/ab male, 22 months old. Note the thick epidermis, including a thick stratum corneum (*sc*), above the basement membrane (*m*). Intercellular spaces (arrows) are conspicuous in the lower layers of the epidermis, and bulges (*b*) extend into the dermis. Mayer's haemalum, eosin and picric acid. × 300.

Fig. 7. Epidermis of the mid-dorsal region from an ab/ab female, 11 months old. Note the thick cellular epidermis and stratum corneum with some haematoxylin-stained nuclei (*p*), giving evidence of parakeratosis. *b*, bulge. Mayer's haemalum, eosin and picric acid. × 300.

hypodermis often appeared smaller and more irregularly shaped than those of the normal hypodermis.

At all ages studied the blood vascular system of the skin of asebic mice was more extensive than that of normal mice. An increased number of arterioles, venules and capillaries, many of which were found more superficially in the dermis, prevailed in the skin of all asebic mice. The appearance of increased vasodilatation at 19 and 20 days postconception and in young postnatal mutant mice became more noticeable with increasing age. The whole skin preparations confirmed this

Table 2. *Thickness in microns of dermis in normal* ($+$/ab) *and asebic* (ab/ab) *mice*

| Age | Stage of hair cycle* | Thickness of dermis (μm) | |
		$+$/ab	ab/ab
6 days	A	$79 \cdot 1 \pm 5 \cdot 8$ (3)†	$114 \cdot 2 \pm 11 \cdot 4$ (3)
8 days	A	$63 \cdot 8 \pm 5 \cdot 6$ (2)	$96 \cdot 0 \pm 4 \cdot 7$ (2)
11 days	A	$82 \cdot 3 \pm 2 \cdot 9$ (2)	$137 \cdot 6 \pm 37 \cdot 2$ (3)
20 days	T	$94 \cdot 3 \pm 10 \cdot 7$ (2)	$159 \cdot 7 \pm 10 \cdot 4$ (2)
37–39 days	A + T	$234 \cdot 9$ (1)	$289 \cdot 7 \pm 69 \cdot 2$ (3)
4 months	A + T	$218 \cdot 5$ (1)	$388 \cdot 1$ (1)
11 months	A + T	$169 \cdot 9 \pm 22 \cdot 5$ (3)	$266 \cdot 4 \pm 17 \cdot 1$ (2)
21–23 months	A + T	$119 \cdot 1 \pm 17 \cdot 1$ (4)	$343 \cdot 3$ (1)
Overall total		$119 \cdot 9 \pm 13 \cdot 1$ (18)	$200 \cdot 0 \pm 26 \cdot 2$ (17)

* A, anagen; T, telogen.   † Mean (μm) $\pm$ standard error of the mean, followed by the number of mice (in brackets).

finding and revealed that the major veins and arteries were enlarged to more than twice the diameter of vessels of normal mice. This effect was most apparent in the dorsal cervical and thoracic region of the asebic skin. While the whole skin preparations of *hairless* (hr/hr) mice also revealed an increased number of smaller blood vessels in this region, the major blood vessels were apparently less dilated than those of either their asebic (ab/ab) or normal ($+$/ab or $+$/$+$) counterparts. This would suggest that the increase in blood vessel size observed in the asebic skin was not merely a result of decreased hair coat.

The apparently swollen dermis and the infiltration of polymorphonuclear

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PLATE 3

Fig. 8. Electron micrograph of portion of a fibroblast in the dermis of a $+$/ab male, 8 days old. The mitochondria (m) are rounded and the RER is compact. n, nucleus. $\times 24\,000$.

Fig. 9. Electron micrograph showing portion of an altered 'fibroblast' in the dermis of an ab/ab male litter-mate to the above. Note the abundance of swollen cisternae of RER. $\times 26\,500$.

Fig. 10. Electron micrograph of another abnormal cell found in the dermis of the above ab/ab mouse. Many pinocytotic vesicles (p) are present as well as dense lysosomal bodies (l) with associated microfilaments (arrows). $\times 45\,500$.

Fig. 11. Electron micrograph of an abnormal cell typical of many observed in the asebic dermis. Vacuolated mitochondria (m) and sharply demarcated tubular spaces (arrows) associated with dense amorphous material are prominent. $\times 35\,000$.
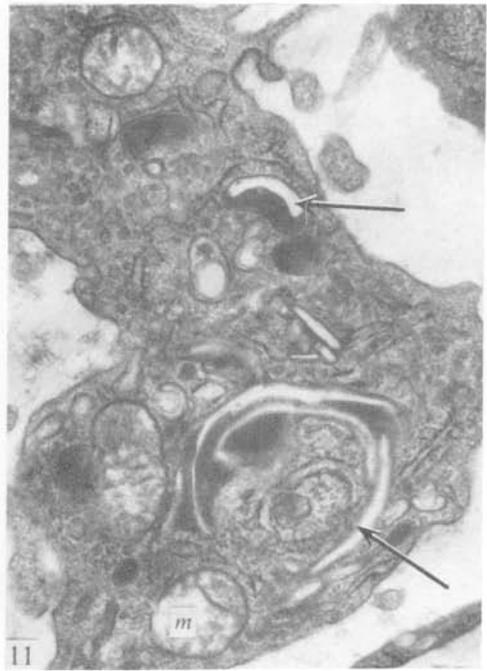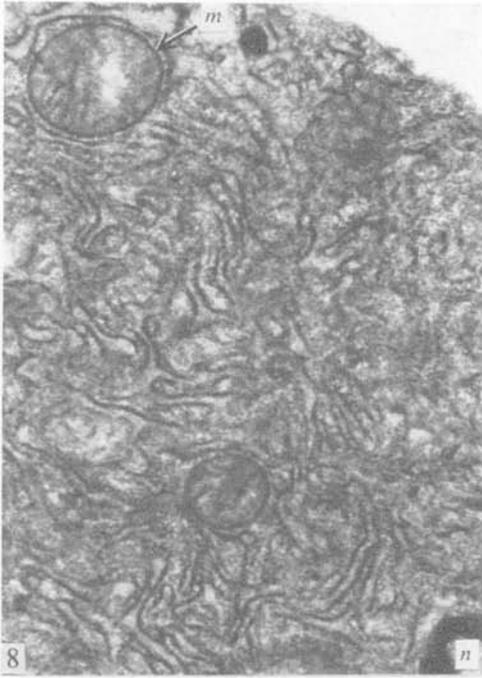
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leucocytes frequently found in asebic skin, both in areas of follicle degeneration and also in areas without obvious necrosis, prompted the investigation of vascular permeability. The results of the blueing tests showed that at the macromolecular level (for molecules larger than the dye, Pontamine Sky Blue 6BX, M.W. = 992·8) the blood vessels of the asebic skin were not more permeable than those of the normal skin. On the other hand, tissue damage induced by pinching the dorsal skin initiated the expected blueing of the extravascular spaces in both normal and mutant mice. The presence of dye also revealed, in the skin of mutant mice only, small foci of irritation which could have been initiated by scratching, biting or internal factors.

The morphological characteristics of the dermis and hypodermis of young asebic mice prevailed in older mice. However, the increased vascularity common in young mutants was greatly exaggerated with age, and large congested blood vessels were often observed in both tissues.

Cellular components

At 16 days post-conception the dermal cells of asebic mice resembled the dermal fibroblasts of non-mutants, and had the typical orientation parallel to the epidermis, especially at the more superficial level. However, the mutant (*ab/ab*) dermis had a higher density of cells than that of normal (+/+) foetuses of the same age. After birth and throughout postnatal life, increased cellularity remained characteristic of the asebic dermis.

Altered morphology of the dermal cells in asebic mice, suggesting altered cellular activity, was first seen at 18 days postconception when many of these more numerous cells were smaller and rounded with densely stained nuclei. Additional indications of the abnormality of dermal cells were seen soon after birth. While almost all dermal cells of normal mice were stained red with the acid fuchsin component of Mallory's stain, large numbers of asebic dermal cells were stained orange and yellow with the Orange G component. Many cells were found in paraffin sections of asebic skin which, not being identified as mast cells, polymorphonuclear leukocytes, eosinophils, macrophages or cells of the nervous system, would normally have been classified by light microscopy as 'fibroblasts'. However, the electron microscope revealed that the majority of these dermal cells were atypical or modified fibroblasts. On the other hand, in normal skin samples a vast majority of the 'fibroblasts' identified with the light microscope appeared as typical fibroblasts at the electron microscope level, containing an extensive system of compact rough endoplasmic reticulum (RER) and rounded mitochondria (Plate 3, fig. 8).

Examples of the abnormal cells found in the dermis of eight-day-old asebic mice are shown in Figs. 9–11 (Plate 3). Fibroblasts with extremely dilated cisternae of RER were relatively common in the dermis of asebic mice (Fig. 9). A large number of dermal cells contained abnormal mitochondria and large clear or flocculent vacuoles. These 'fibroblasts' showed many pinocytotic vesicles, and many of them also contained densely stained amorphous inclusions resem-

bling the products of secondary lysosomes (Fig. 10). Although some lysosomes were found in the fibroblasts of the normal ($+/ab$) mice, the large and irregular lysosomes of the dermal cells of asebic mice were very different. Another very bizarre cell type, frequently found in the asebic dermis, contained sharply outlined spaces which appeared to be straight or curved 'tubes' (from less than 10 nm to 72 nm in diameter) surrounded by densely stained material, possibly lysosomal in origin (Fig. 11).

The asebic dermis was not only characterized by abnormal cellular morphology but also by large tracts of cellular debris which contained degenerating organelles, dark granules, possibly of lysosomal origin, large clear membrane-bound vesicles and membranous debris. There were also large numbers of degenerating and lysed polymorphonuclear leucocytes, and even more eosinophils. When compared with the small amount of cellular degeneration typical of the dermis of normal mice, the extent of cytolysis occurring in the dermis of asebic mice was very striking.

Extracellular matrix

At the light microscope level, it was seen that from the 16th day postconception the dermis of asebic mice contained a less dense packing of finer collagen bundles than the dermis of normal mice, giving the impression of less collagen per unit area. This difference persisted at birth and throughout life (Plate 4, figs. 12, 13). The electron microscopic images of dermis from normal and asebic mice at 8 and 30 days conveyed the same impression, and also showed that the individual collagen fibres were on the average finer in asebic dermis. The results of measurements of individual collagen fibre diameters at eight days and 30 days after birth are presented in Text-figs. 2 and 3. At both ages the mean diameter of the collagen fibres in the asebic mice (32·8 nm at 8 days and 54·5 nm at 30 days) was less than that of the normal (40·5 nm at 8 days and 68·1 nm at 30 days). From the ratios of the variances and the t test these differences were highly

PLATE 4

Fig. 12. Mid-dorsal skin from $+/ab$ male, 8 days old, showing collagen bundles (arrow). s, sebaceous cell. 1 μm Epon section stained with Toluidine Blue O. $\times 600$.

Fig. 13. Mid-dorsal skin from an ab/ab male litter-mate, 8 days old. Note the less dense packing of finer collagen bundles (arrow). 1 μm Epon section stained with Toluidine Blue O. $\times 600$.

Fig. 14. Mid-dorsal skin from a $+/ab$ male, 20 days old. The upper arrow points to the sheath of elastic fibres around a telogen follicle and the lower arrow points to the bracing fibres. Other elastic fibres (e) are seen in the remaining dermis. dp, dermal papilla; h, hypodermis. Paraffin section with acid orcein-Giemsa stain. $\times 270$.

Fig. 15. Mid-dorsal skin from a $+/ab$ male, 20 days old. Note the distribution of elastin (arrows). Paraffin section with acid orcein-Giemsa stain. $\times 500$.

Fig. 16. Mid-dorsal skin from an ab/ab male litter-mate, 20 days old. Note the more sparse distribution of elastin, the finer elastic fibres and the amorphous masses of orcein-positive material (arrow). Paraffin section with acid orcein-Giemsa stain. $\times 500$.

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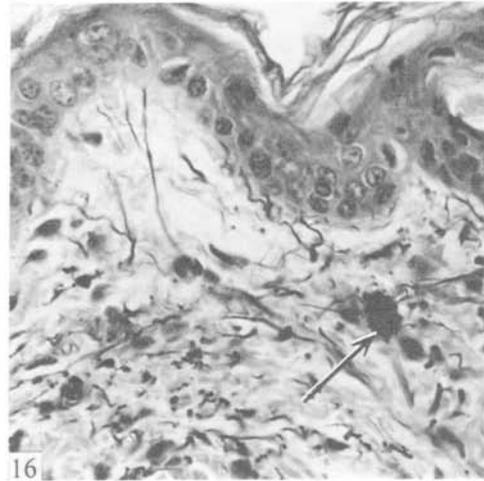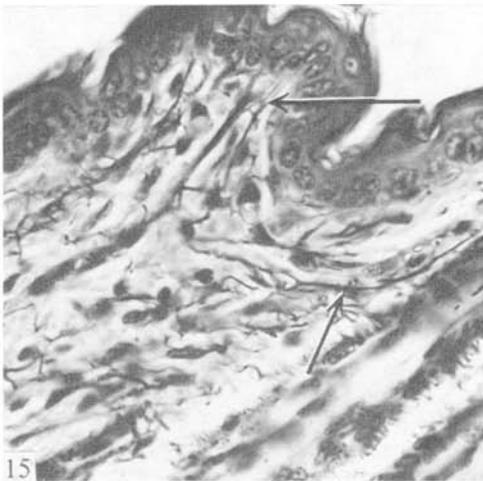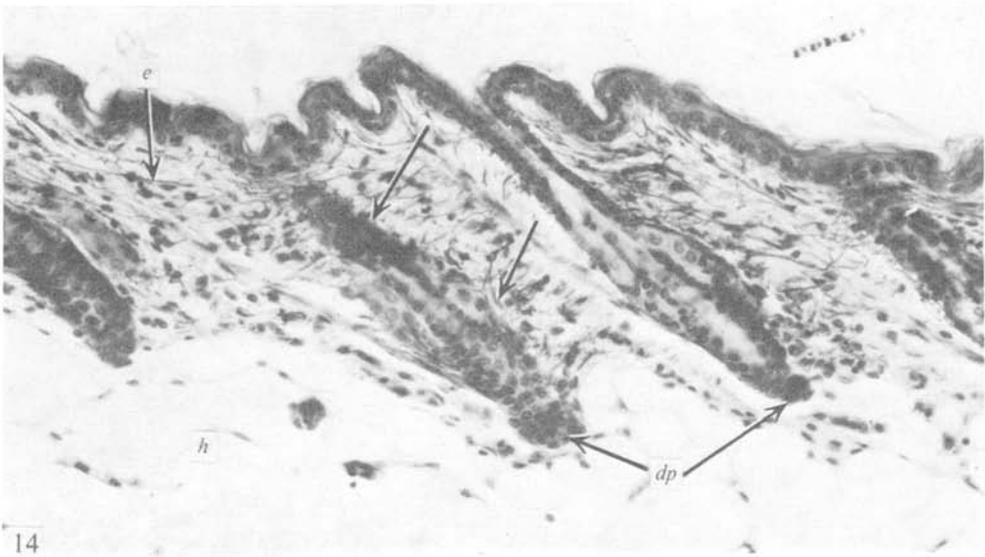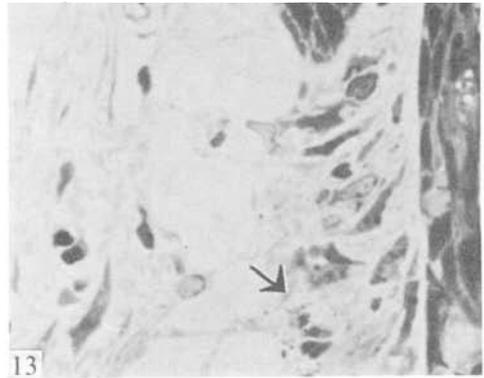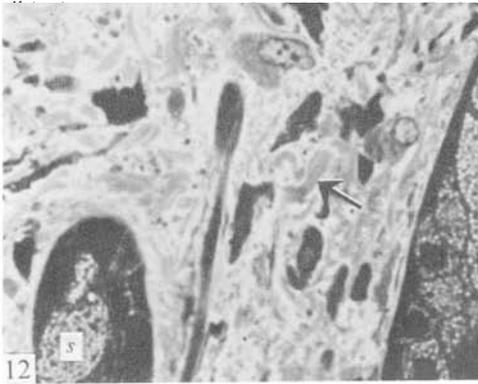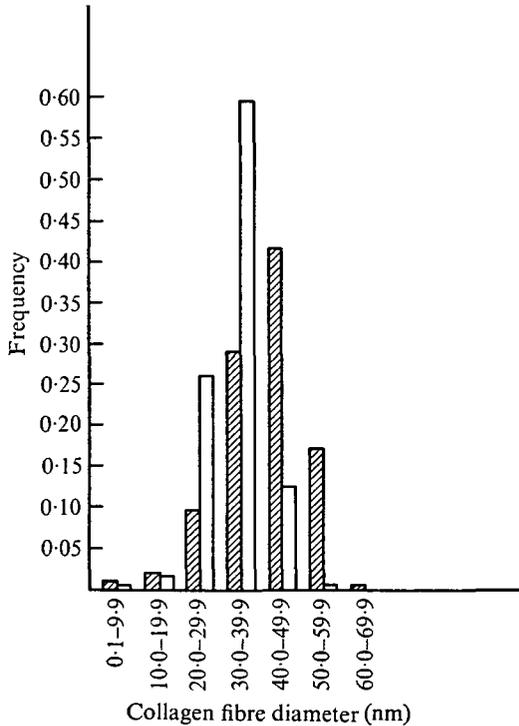
Text-fig. 2. Histogram showing collagen fibre diameter in a normal (+ /*ab*; shaded bars) and an asebic (*ab*/*ab*; unshaded bars) mouse, 8 days after birth. Measurements were made on 665 fibres from the normal and 309 fibres from the asebic mouse.
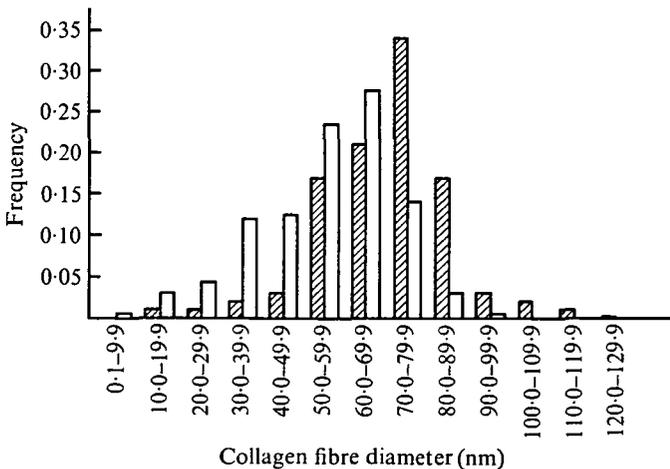
Text-fig. 3. Histogram showing collagen fibre diameter in a normal (+ /*ab*; shaded bars) and an asebic (*ab*/*ab*; unshaded bars) mouse, 30 days after birth. Measurements were made on 899 fibres from the normal and 1141 fibres from the asebic mouse.

significant ($P < 0.001$). At both ages the range of the fibre sizes was narrower in the asebic than in the normal mice. The distribution of collagen fibre diameters in the 30-day-old asebic dermis was skewed when compared with the more normally distributed population in the normal $+/ab$ dermis. However, the periodicity of the collagen fibres in asebic mice was the same as that of fibres in normal mice at both ages.

Differences between normal and asebic mice were found in the distribution and composition of elastin. The extracellular orcein-positive fibres seen with the light microscope and considered to be elastin were usually abundant in the dermis and scarce in the hypodermis of normal mice. An extensive sheath of elastic fibres surrounded the entire hair follicle after it had resorbed into the dermis and entered the telogen stage. Large anchoring elastic fibres appeared to be bracing the hair clubs in the dermis (Plate 4, fig. 14), while the resting dermal papillae were linked to the hair club by elastic fibres. Most of the remaining elastic fibres found in the dermis, but not associated with the hair follicles, were roughly parallel to the epidermis. These fibres were short and broad, and finer branching fibres were seen extending from them towards the epidermal basal cells (Plate 4, fig. 15). The elastin fibres of normal 8-day mice were seen in the electron microscope to be composed of amorphous material and microfilaments.

In the asebic mouse a less extensive sheath of elastic fibres enclosed those telogen follicles which resorbed to the level of the dermis. No elastin was associated with the clubs of those distended telogen follicles which remained deep in the hypodermis. In the remaining dermis of the asebic skin, somewhat thinner and shorter elastic fibres were distributed more sparsely than in the normal animals. However, the most striking feature was the presence of masses of amorphous orcein-positive material scattered throughout the asebic dermis (Plate 4, fig. 16). The electron microscope images of the elastin found in the asebic dermis revealed that in the 'fibres' of light microscopy, the microfilaments were usually lacking, and only the amorphous component was present.

No significant or consistent differences between normal and asebic litter-mates in the staining properties of the ground substances were observed with any of the histological methods used.

4. DISCUSSION

Gates & Karasek (1965) and Gates, Arundell & Karasek (1969) reported hyperkeratosis of the epidermis in adult asebic mice. We have found that both the living layers and the stratum corneum were greatly thickened from birth onwards, but that this feature was most pronounced in older mice. Since hyperkeratosis was already established at birth, it was not primarily a response to the sparse hair covering, which was not apparent until 6 days postnatal. More probably there was an alteration in the complex regulating mechanism for epidermal proliferation which involves cyclic AMP, cyclic GMP and glucocorticoids (Voorhees, Marcelo & Duell, 1975). Preliminary histological findings on abnormalities in the adrenal cortex of asebic mice (Josefowicz, 1975) suggest that glucocorticoids may be implicated in this condition. Reduced intercellular contact in the epidermis

was indicated by large intercellular spaces in the deeper layers, and reduced adhesion was indicated by occasional sloughing of the cells of stratum granulosum, and irregular packing of the cells of the stratum corneum.

The increased cellularity of the dermis, first observed at 16 days postconception, and the altered morphology and staining properties of dermal cells, first observed at 18 days postconception, were consistent features of asebic mice throughout life. The electron microscopic study of skin from 8-day and 30-day postnatal asebic mice showed profound changes in the morphology of the fibroblast population and the collagen fibre population. However, since the periodicity of collagen fibres was normal, a fault in the control mechanism of fibrogenesis or fibre breakdown is more probable than a structural defect in any of the components of the growing collagen fibre complex (Jackson, 1968). Elastin fibre formation was also abnormal, since there was a deficiency both ultrastructurally in the proportion of microfilaments to amorphous substance, and also in the number of fibres visible in the light microscope. The large amorphous masses of orcein-staining material may have been amorphous elastin. Unfortunately no information on differences in the 'ground substance' has come from the procedures employed.

The increased cellularity of the asebic dermis, the high levels of pinocytosis and lysosome production in many dermal cells, the altered metabolism of collagen as well as the increased cutaneous vascularity and infiltration of polymorphonuclear leucocytes, all support the suggestion that the dermis of asebic mice is in a state of mild but chronic inflammation (Willoughby & Di Rosa, 1971). However, blueing tests for increased vascular permeability, an integral part of the immune response, were negative and did not support the hypothesis of an autoimmune reaction. Because some integumentary abnormalities in the asebic mice were apparent at least by the 16th day postconception, it is doubtful that they were initiated by either humoral or cellular immunological mechanisms, which are believed to function later – just prior to and after birth (Decker & Sercarz, 1975). However, chronic irritation and inflammation could develop later in response to an abnormal condition in the dermis, whatever its cause, and were in fact sufficiently severe to be visible to the naked eye.

Many characteristics of the cells seen in the asebic dermis are typical of lysosomal storage diseases. The cells containing large vacuoles, some filled with flocculent material, were similar to those found in fibroblasts in mucopolysaccharoidosis (Hers, 1973). The cells with tubular inclusions were similar to the macrophages in human patients with either of the two lipid storage diseases, globoid leucodystrophy (Stern, Novikoff & Terry, 1972; Suzuki & Suzuki, 1973) or Gaucher's disease (Brady & King, 1973). The large amount of debris in the asebic dermis could be accounted for by cell lysis or extensive cell defecation, both results of overloading of the lysosomal system with residual material. The accumulation of lysosomal products within a cell may upset the balance of synthetic and other hydrolytic activity (Muir, 1973; Lloyd, 1973). If this occurred in asebic mice it could account for abnormalities in the fibrous component of the dermis as well as the symptoms of mild inflammation.

Some of the abnormalities of the epidermis seem to have been initiated before birth. Preliminary observations on foetuses of known genotype showed differences in the epidermal thickness in asebic mice as early as 18 days postconception. However, differences in the dermis of asebics were observed even earlier, at 16 days post-conception, and may have been responsible for mediation of the epidermal changes. The relationship of dermal to epidermal changes will be discussed in the third paper of this series.

REFERENCES

BILLINGHAM, R. E. & SILVERS, W. K. (1967). Studies on the conservation of epidermal specificities of skin and certain mucosas in adult mammals. *Journal of Experimental Medicine* 125, 429–446.

BRADY, R. O. & KING, F. M. (1973). Gaucher's disease. In *Lysosomes and Storage Diseases* (ed. H. G. Hers and F. Van Hoof), pp. 381–394. New York and London: Academic Press.

CARTER, H. B. & CLARKE, W. H. (1957). The hair follicle group and skin follicle population of Australian Merino sheep. *Australian Journal of Agricultural Research* 8, 91–108.

CHASE, H. B., MONTAGNA, W. & MALONE, J. D. (1953). Changes in the skin in relation to the hair growth cycle. *Anatomical Record* 116, 75–81.

DECKER, J. & SERCARZ, E. (1975). Antigen binding cells and the generation of diversity. *American Zoologist* 15, 189–197.

DRY, F. W. (1926). The coat of the mouse (*Mus musculus*). *Journal of Genetics* 16, 287–340.

GATES, A. H. ARUNDELL, F. D. & KARASEK, M. A. (1969). Hereditary defect of the pilosebaceous unit in a new double mutant mouse. *Journal of Investigative Dermatology* 52, 115–118.

GATES, A. H. & KARASEK, M. A. (1965). Hereditary absence of sebaceous glands in the mouse. *Science, N.Y.* 148, 1471–1473.

GRÜNEBERG, H. (1943). The development of some external features in mouse embryos. *Journal of Heredity* 34, 88–92.

HERS, H. G. (1973). The concept of inborn lysosomal disease. In *Lysosomes and Storage Diseases* (ed. H. G. Hers and F. Van Hoof), pp. 147–171. New York and London: Academic Press.

JACKSON, D. S. (1968). Biological function of collagen in the dermis. In *Advances in Biology of Skin. Vol. X. The Dermis* (ed. W. Montagna, J. P. Bentley and R. L. Dobson), pp. 39–48. New York: Appleton-Century-Crofts.

JOSEFOWICZ, W. J. (1975). *The Development and Expression of the Asebia Mutation in Mice.* M.Sc. Thesis, University of Guelph, Guelph, Canada.

JOSEFOWICZ, W. J. & HARDY, M. (1974). The development and expression of the mutation *asebia ab/ab* in mice. *Proceedings of the Canadian Federation of Biological Societies* 17, 173.

JOSEFOWICZ, W. J. & HARDY, M. H. (1978a). The expression of the gene *asebia* in the laboratory mouse. II Hair follicles. *Genetical Research* (in press).

JOSEFOWICZ, W. J. & HARDY, M. H. (1978b). The expression of the gene *asebia* in the laboratory mouse. III Sebaceous glands. *Genetical Research* (in press).

LLOYD, J. B. (1973). Experimental support for the concept of lysosomal storage disease. In *Lysosomes and Storage Diseases* (ed. H. G. Hers and F. Van Hoof), 173–195. New York and London: Academic Press.

LUFT, J. H. (1961). Improvement in epoxy resin embedding techniques. *Journal of Biophysical and Biochemical Cytology* **9**, 409–414.

McMANUS, J. F. A. & MOWRY, R. W. (1965). *Staining Methods, Histological and Histochemical.* New York, Evanston and London: Harper and Row.

MUIR, H. (1973). Structure and enzymic degradation of mucopolysaccharides. In *Lysosomes and Storage Diseases* (ed. H. G. Hers and F. Van Hoof), pp. 79–104. New York and London: Academic Press.

PINKUS, H. (1944). Acid Orcein-Giemsa stain (modification of Unna-Taenzer method); useful routine stain for dermatologic sections. *Archives of Dermatology and Syphilology* **49**, 355–356.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–225.

RUGH, R. (1968). *The Mouse, Its Reproduction and Development.* Minneapolis: Burgess Publishing Co.

STERN, J., NOVIKOFF, A. B. & TERRY, R. D. (1972). In *Sphingolipids, Sphingolipidoses and Allied Disorders* (ed. B. Volk and S. M. Aronson), p. 651. New York: Plenum.

SUZUKI, K. & SUZUKI, K. (1973). Krabbe's disease. In *Lysosomes and Storage Diseases* (ed. H. G. Hers and F. Van Hoof), pp. 395–410. New York and London: Academic Press.

VOORHEES, J. J., MARCELO, C. L. & DUELL, E. A. (1975). Cyclic AMP, cyclic GMP, and glucocorticoids as potential metabolic regulators of epidermal proliferation and differentiation. *Journal of Investigative Dermatology* **65**, 179–190.

WELLS, P. W. & EYRE, P. (1972). The pharmacology of passive cutaneous anaphylaxis in the calf. *Canadian Journal of Physiology and Pharmacology* **50**, 255–262.

WESSELLS, N. K. (1967). Differentiation of epidermis and epidermal derivatives. *New England Journal of Medicine* **277**, 21–33.

WILLOUGHBY, D. A. & DI ROSA, M. (1971). A unifying concept for inflammation: a new appraisal of some old mediators. In *Immunopathology of Inflammation* (ed. B. K. Forscher and J. C. Houck), pp. 28–38. Amsterdam: Excerpta Medica.