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'Nude', a new hairless gene with pleiotropic effects in the mouse

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1. INTRODUCTION

Cases of genetic hairlessness in the mouse may be classified into three categories on a histological basis: (*a*) partial suppression of follicle initiation, e.g. crinkled (Falconer, Fraser & King, 1951), ragged (Slee, 1957); (*b*) abnormal keratinization of the hair shaft, e.g. naked (David, 1932); (*c*) abnormal keratinization of the hair club and disruption of the cyclic activity of the hair follicles, e.g. hairless (Fraser, 1946). In class (*a*) the animals grow a coat which is noticeably sparse. In classes (*b*) and (*c*) the first coat is normal in density up to 12–14 days of age when hair loss occurs.

A new hairless mutant that differs phenotypically from previous cases forms the subject of this paper. Affected mice never grow a first coat and the adults are almost completely hairless. In addition to hairlessness, the new gene causes a variety of other abnormalities. Thus, it will be shown that the majority of affected mice die before weaning, that the survivors grow slowly, that the adults are poorly fertile and that they develop a liver disease which results in death.

According to Grüneberg's principle of the unity of gene action it should be possible to reduce the complex of abnormalities caused by the gene to a single primary defect of development. In particular, there should be a causal connexion between the failure of hair growth in young mice and the liver disease which develops in the adults. No connexion has, however, yet been found.

2. GENETICS

(i) *Origin*

The hairless mutant was found by Dr N. R. Grist of the Virus Laboratory, Ruchill Hospital, Glasgow, who sent it to Edinburgh for investigation. The mutation arose in closed but not deliberately inbred albino stock. One hairless animal, a male, was given to the present writer for investigation, together with two phenotypically normal mice, one male and one female, both thought to be heterozygous for the gene. It was supposed that a recessive gene was responsible for the abnormality and this supposition was confirmed later by the segregation data. The name 'nude', symbol *nu*, has been adopted.

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(ii) *Segregation data*

The original hairless male died without producing offspring. The normal coated pair were mated and produced two litters of twenty-three normal offspring. The offspring were intercrossed at random and one mating produced hairless animals. In this way two heterozygous parents were identified and they formed the basis of the nude stock. The segregation of nude from intercross and backcross matings is presented in Table 1. Nude mice can be classified at birth by the absence of vibris-

Table 1. *Segregation of nude*

Type of mating	No. of matings	Phenotype of progeny		Total	χ^2	P
		+	*nu*			
$+nu$ ♂ × $+nu$ ♀	122	3890	1349	5239	1·59	0·20
$nunu$ ♂ × $+nu$ ♀	4	16	14	30	0·13	0·70

sae. Because of the poor fertility of nude mice, the segregation data are derived mainly from intercross $+nu \times +nu$ matings. There is a bias towards an excess of nude mice as no mating was included unless it produced at least one nude offspring. The observed segregation, however, did not differ significantly from expectation. Four backcrosses gave normal and nude segregants in good agreement with the expected 1:1 ratio. Thus, *nu* was proved to be a single autosomal recessive gene.

(iii) *Linkage tests*

The nude gene was tested against the five multiple linkage stocks described by Carter & Falconer (1951). Two additional stocks were also used. The stocks were as follows:

Stock number	*Markers present*
I	*Va Sd Ra*
II	*Wh T N*
III	*p se fz v*
IV	*s wa-2 ln b a*
V	*ru je f* c^e
VI	*Re* W^v
VII	*Os So Sl*

Since most *nunu* mice are infertile the animal used for outcrossing to the marker stock was heterozygous nude + *nu*. Consequently, only half the F_1 mice carried the *nu* gene and these were identified only after testing them against known $+nu$ animals. The F_1 multiple heterozygotes were then either mated to normal + *nu* mice or mated *inter se*, depending on whether the gene against which *nu* was being tested was dominant or recessive. The deviation of *nu* from independent segregation with

all markers and the percentage recombination, together with its standard error, were estimated by the method of Carter & Falconer (1951).

It was soon evident that *nu* was not segregating independently of *Re*. The number of matings in this test was then increased in order to obtain further evidence. The results are given in Table 2. Only three phenotypes were recognizable in the

Table 2. *Results of linkage tests between* nu *and* Re, *from matings of* Re+/+nu × ++/+nu

Phenotypes and observed numbers of progeny				
Re+	++	*Renu* +*nu*	Total	Recombination frequency
623	407	383	1413	19·4 ± 4·6%

progeny because *Re* was indistinguishable from its normal allele in nude mice. The data show that *nu* is linked to *Re* in linkage group VII. The percentage recombination was 19·4 ± 4·6%.

Localization tests were then made to determine the linear order of *nu* and *Re* with respect to a third marker *Tr* in this linkage group. It was decided to backcross triple heterozygous *Re Tr*+/++*nu* females to ++*nu*/++*nu* males rather than to +++/++*nu* males because the increased amount of information obtainable from such matings outweighed the disadvantage of poor fertility amongst the ++*nu*/++*nu* animals. The results are shown in Table 3. The classes of progeny are

Table 3. *Results of triple backcross matings* ReTr+/++nu ♀♀ × ++nu/++nu ♂♂. *Phenotypes of progeny are arranged in pairs according to crossover type*

Type of crossover	Phenotypes of progeny	No. of progeny observed	
No crossover	*ReTr*+	21	44
	++*nu*	23	
Re	+*Tr*+	5	6
	Re+*nu*	1	
Tr	*Re*++	3	4
	+*Tr nu*	1	
nu	*ReTr nu*	0	0
	+++	0	
		Total	54

Recombination frequencies: *Re–nu* = 6/54 = 11·1 ± 4·3%
nu–Tr = 4/54 = 7·4 ± 3·6%
Re–Tr = 10/54 = 18·5 ± 5·3%

grouped in pairs according to crossover type. The rarest type represents double crossovers, thus identifying the central of the three loci. There was no *nu* exchange and this proves that the order of the three loci is *Re–nu–Tr*. Falconer & Sobey (1953) found that the *Re–Tr* recombination frequency was 23 ± 2·6%. Combining the present data with that of Falconer and Sobey gives *Re–Tr* = 69/312 = 22·1 ± 2·35%. Then, if the *nu* recombination frequencies are scaled up in proportion, the following map is obtained:

Re–13–*nu*–9–*Tr*

3. MORPHOLOGY

(i) *External appearance*

At birth nude mice are classifiable by the absence of vibrissae. During the first week many die of general body weakness. Viable young animals fail to grow a first coat. At 5 days of age no hairs have erupted on the dorsum, although there is no delay in the thickening or pigmentation of the skin. Macroscopic examination of the skin at about 10 days of age shows that a few hair fragments are scattered on the dorsum and that short fine hairs are present on the head and feet. During the third week there is a pronounced reduction in skin thickness corresponding to the catagen stage of normal skin. At weaning, nude mice are much smaller than normals and are often in poor condition. Those animals which survive weaning show signs of sparse hair growth at about 5 weeks of age. An irregular band of sparse hairs about $\frac{1}{8}$ in. in length moves in a cephalo-caudal direction. These hairs are lost at about 6 weeks of age. Some adult nude mice undergo cyclic regeneration and loss of short fuzzy hair but others remain hairless for long periods.

Although vibrissae are absent at birth, 3-week-old nude mice usually have 6–12 vibrissae $\frac{1}{4}$–$\frac{1}{2}$ in. in length. These are shed at about 4 weeks. Older mice show repeated growth and loss of short wavy vibrissae. The toe-nails are frequently constricted and spirally malformed but they are not excessively elongated.

(ii) *Body growth*

The nude gene exercises an appreciable influence on body growth. Table 4 compares the mean weights of nude and normal mice at birth, 3 weeks and 6 weeks of age. There was no difference in the birth weights of nude and normal mice but at

Table 4. *The mean weights of nude and normal mice at birth, 3 weeks and 6 weeks of age*

		Nude		Normal	
		♀♀	♂♂	♀♀	♂♂
Birth	No. of mice	120	138	288	338
	Mean wt. (g)	1·5	1·6	1·5	1·6
3 weeks	No. of mice	42	65	103	115
	Mean wt. (g)	6·0	6·6	10·1	10·3
6 weeks	No. of mice	10	18	23	27
	Mean wt. (g)	14·6	18·4	22·6	26·7

3 weeks the difference was considerable; nude male mice were 3·7 g. lighter than normal males. These weight differences were also pronounced at 6 weeks; on a percentage basis nude female mice were only 64·6% of the weight of normals and nude male mice were 68·9% of the weight of normal males.

(iii) *Fertility*

The fertility of nude mice is very low. Out of forty-six nude females mated to normal males only four produced offspring. None of these females was able to suckle its young, and all four died 2–3 weeks after parturition. Vaginal smears indicated that the oestrus cycle of nude mice is irregular and many exhibit continuous dioestrus and metaoestrus phases. Dissections showed that the ovaries are much reduced in size. An attempt to induce ovulation in five adult nude mice by P.M.S. and chorionic gonadotrophic hormone injections was unsuccessful.

Occasionally, nude male mice are fertile. In seventy single pair matings between nude male and normal female mice, eighteen males were found to be fertile; seventeen males produced one litter each and one produced four litters. In most of the matings evidence of copulation was provided by the finding of vaginal plugs but microscopic examination of the sperm showed that many sperm were non-motile and had coiled tails.

(iv) *Mortality*

There is no evidence of increased pre-natal mortality of nude mice compared with normals; intercross $+nu \times +nu$ matings gave normal and nude mice in the ratio 3:1. Post-natal mortality, however, reached 100% by 25 weeks. Mortality records were maintained on 486 nude mice and 1393 normals, and the mortality curves are shown in Fig. 1. About 55% of nude mice died within 2 weeks after birth. Death is usually preceded by rapid loss in weight and an apparent inability to compete with

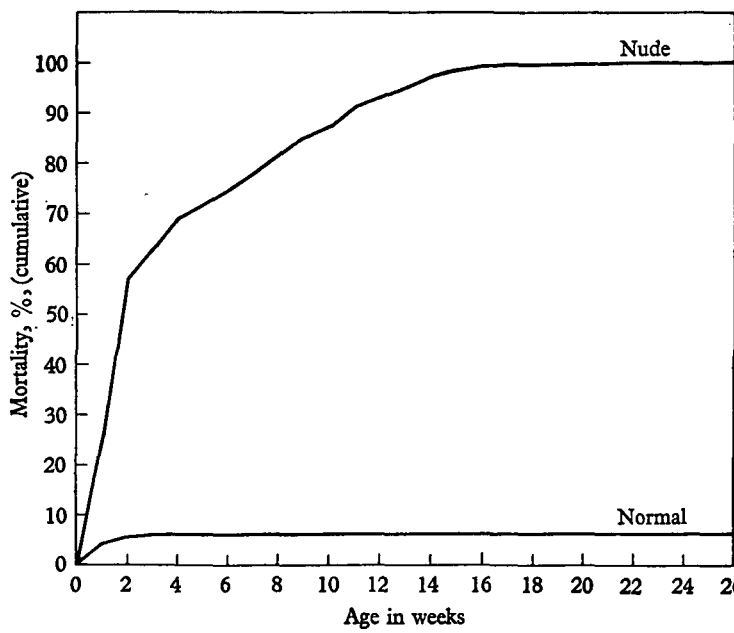

Fig. 1. Mortality curves of normal and nude mice.

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normal littermates for food. While many of the survivors at 2 weeks are weak some may live for considerable periods. In the present stock eight nude mice were viable at 15 weeks and one attained 26 weeks of age. The mortality of normal mice was 6%, no greater than could be expected under standard conditions.

(v) *Liver disease*

Failure to obtain viable adult mice prompted post-mortem examination of a few carcasses. All showed a severe liver disease; the liver lobes were atrophied and covered with red scars. The heart, lungs, spleen and kidneys appeared normal. Subsequently, all moribund or dead nude mice were examined for this defect. Table 5 gives the frequency of the liver disease at different ages. Only one animal

Table 5. *The frequency of liver disease among moribund or dead nude mice*

Age	No. of nude mice dissected	No. of nude mice with liver disease
Birth to 3 weeks	56	1
3 weeks to 6 weeks	31	16
6 weeks to 26 weeks	85	85

showed the disease before 3 weeks of age. About half of the nude mice found dead or moribund between 3 and 6 weeks of age showed the defect. All specimens older than 6 weeks exhibited the disease. Thus, it is seen that moribund or dead nude mice rarely show the liver abnormality between the ages of 3 and 6 weeks and that all of them are characteristically affected after 6 weeks.

The severity of the liver disease at time of death is variable. The defect ranges from small open scars scattered on the lobes of some animals to complete scarring and degeneration of all the hepatic tissue in other animals. Plate II*a* shows the degrees of severity of the defect. The milder form consists of small round reddish perforations on the lobes. A more severe form has large open scars with normal hepatic tissue interspersed between the scars. In extreme cases, no normal hepatic tissue remains; the lobes are atrophied and scarred. All degrees of severity have been found in dead mice.

The external symptoms associated with the liver disease are also variable. Nude mice may remain healthy for a variable number of weeks after weaning. They gradually become lethargic, the backs become arched and partial closure of the eyes accompanied by corneal lesions is observed. During the following 2–3 weeks a typical emaciation sets in, including absorption of subcutaneous fat and wastage of the muscles of the dorsum and legs. Such animals have completely degenerate livers. The disease develops insidiously and if animals are dissected at the first signs of illness a few scars are already present on the lobes.

Although the majority of nude mice become emaciated over a 2–3 week period some cases are more acute. Apparently healthy animals may suddenly become ill

and die within 2–3 days. There is little emaciation and the livers in these cases may be mildly or severely affected.

No case of the liver defect has been found in the coated littermates of nude mice. Normal littermates were killed intentionally at various ages and all the livers were normal.

4. HISTOLOGY

In this section the histological effects of the nude gene on the skin and liver are described.

(i) *Skin histology*

The failure of nude mice to grow a first coat could be attributed to one of two possible causes: (*a*) there may be no follicle initiation or differentiation; (*b*) follicle initiation and differentiation may occur but the follicles may not produce hair. If the hairlessness was due to a complete absence of follicle initiation nude mice would represent a new type of hereditary hypotrichosis. Alternatively, if follicles were formed but produced abnormally keratinized hair the nude gene would resemble naked (*N*).

The skin histology was studied in order to decide between these alternatives. Samples of dorsal skin were obtained from normal and nude littermates at birth and at 3, 6, 9, 15 and 21 days of age. Samples were fixed in Bouin's solution, dehydrated in alcohol and cleared in methyl benzoate. Serial paraffin wax sections were stained with haematoxylin and eosin.

No histological abnormality was observed in the skin of nude mice at birth. The epidermis and dermis are then fully differentiated. The follicles are at different stages of development, many consisting of aggregates of epidermal cells while others have already formed a bulb and papilla.

Histological defects become obvious as the follicles continue to grow downwards through the dermis and as the hair tip reaches the epidermis. Skin sections of 6-day-old normal and nude mice are compared in Plate I*a* and *b*. Whereas the hairs of normal mice have erupted through the epidermis, the hairs of nude mice are bent and coiled in the upper dermis and they fail to penetrate the epidermis. There are no obvious structural differences between normal and nude mice in the appearance of the outer root sheath, dermal papilla, bulb and sebaceous glands.

Non-eruption of hair in nude skin could be due either to imperfect keratinization of the hair shaft or to increased resistance of the epidermis to the erupting hair tip. The following observations indicated that the first interpretation is correct. In normal skin the hair cells undergo keratinization in the middle third of the follicle and these cells are basophilic. The fully keratinized hair in the upper third of the follicle is characteristically picrophilic. In nude skin the hairs were found to be basophilic in the middle third of the follicle but eosinophilic in the upper third and showed poor affinity for picric acid. In addition, the hairs were noticeably thin; the cuticle and cortex were much reduced and the hairs consisted mainly of medullary substance. The thickness and structure of the epidermis of nude mice was

normal and, in particular, the stratum corneum appeared to be normally keratinized.

It was concluded that the failure of nude mice to grow a coat is due to the abnormal keratinization of hair in the follicles and not to failure in follicle initiation or abnormal structure of the epidermis.

The nude gene does not interfere with the cyclic activity of the follicles. During the hair growth phase the follicles become distended with masses of twisted hairs and these are forced on to the skin surface by growth pressure from the lower follicle regions. At the end of the growth phase the skin decreases in thickness and the follicles shorten as in normal skin. Resting phase skin sections of 21-day-old normal and nude mice are shown in Plate I *c* and *d*. In normal skin the follicles are sloped and parallel to one another and the sebaceous glands are closely adjacent to the hair shaft. The follicles of nude skin are grossly distorted and widened, containing hair and cornified material. The sebaceous glands are abnormally located either at the base of the hair canals or embedded in the dermis. In adult nude mice the hair follicles show cyclic activity at variable intervals and the hair abnormalities described above are repeated.

(ii) *Skin histochemistry*

The next step in this investigation was to seek an explanation for the hair keratin defect in terms of an underlying biochemical abnormality. In the normal follicle the main feature of keratinization is the localization and concentration of a keratin precursor in the mid-follicle region. This precursor consists of cysteine complex with free sulphydryl (—SH) groups (Giroud & Bulliard, 1930). During keratinization the sulphydryl groups of cysteine are oxidized to form disulphide bonds (—S—S—) of cystine. Thus, the fully keratinized hair in the upper follicle region is rich in cystine and contains no free sulphydryl groups. The disulphide bonds contribute largely to the strength and rigidity of the hair fibre. It was therefore considered important to compare the follicles of nude mice with those of normals in order to determine whether nude follicles are deficient in sulphydryl groups or whether sulphydryl groups are present in normal concentration but not oxidized

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EXPLANATION OF PLATES

Plate I

a. Dorsal longitudinal skin section from a normal mouse at 6 days of age showing follicles with fully keratinized hairs. H. and E. stain, × 85.

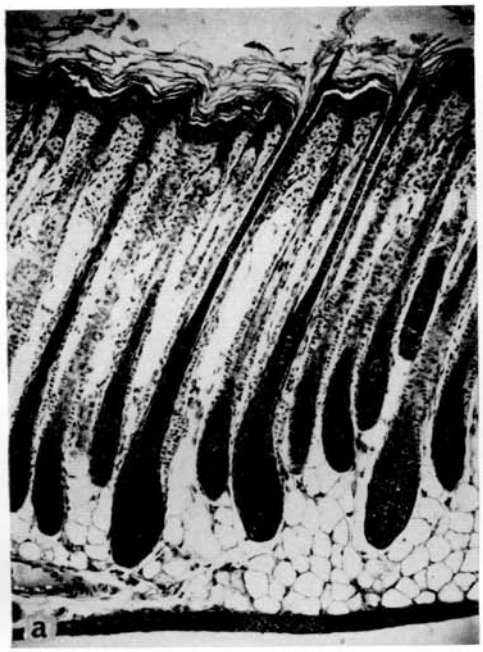
b. Skin section from nude mouse at 6 days of age showing follicles with abnormally keratinized hairs which bend in the upper dermis. H. and E. stain, × 85.

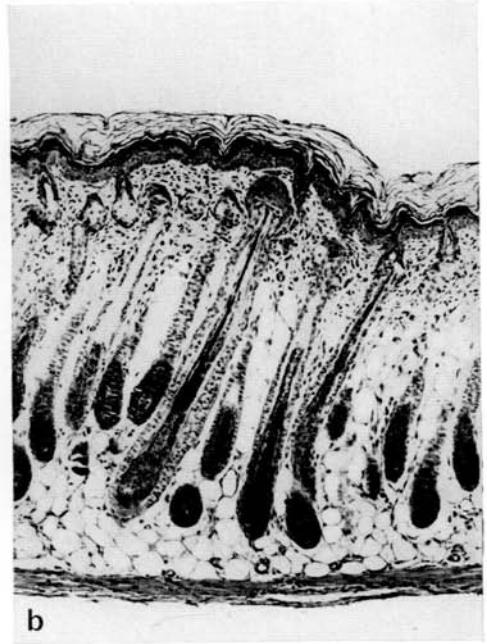
c. Normal mouse skin at 21 days of age showing hair follicles during the resting stage in the upper dermis. H. and E. stain, × 85.

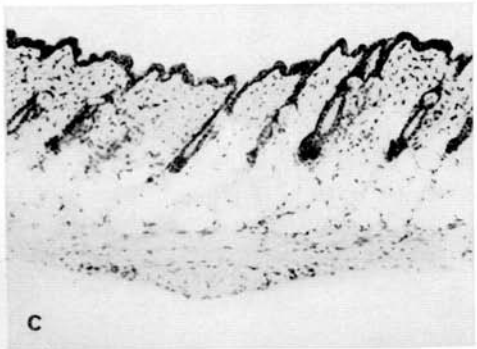
d. Nude mouse skin at 21 days of age. The follicles are grossly malformed and distended with coiled hairs and cornified material. H. and E. stain, × 85.

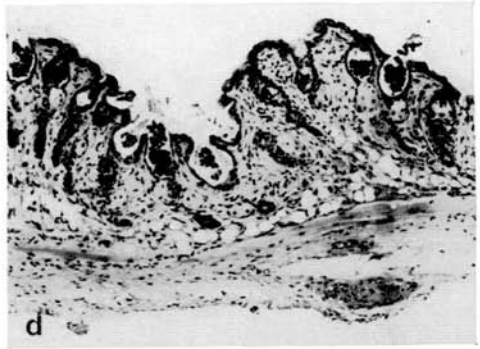
e. Normal mouse skin at 6 days of age. The mid-follicle region reacts intensely with sulphydryl reagent. The reaction ends suddenly above this region. Bennett's sulphydryl reagent stain, × 95.

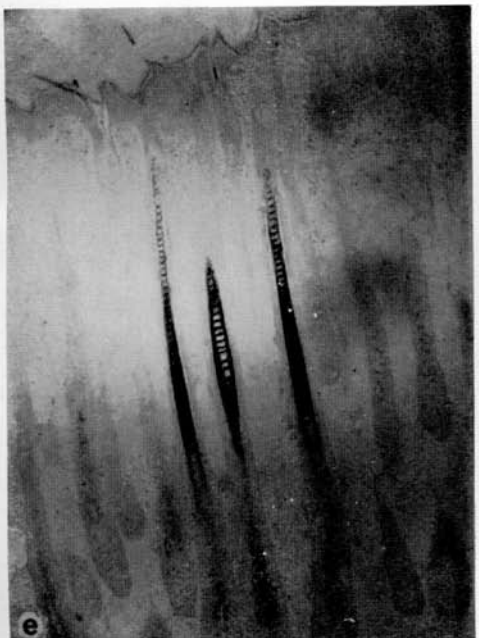
f. Nude mouse skin at 6 days of age. The strands of pigment indicate the outline of the follicles. The follicles reacted negatively with sulphydryl reagent. Bennett's sulphydryl reagent stain, × 95.

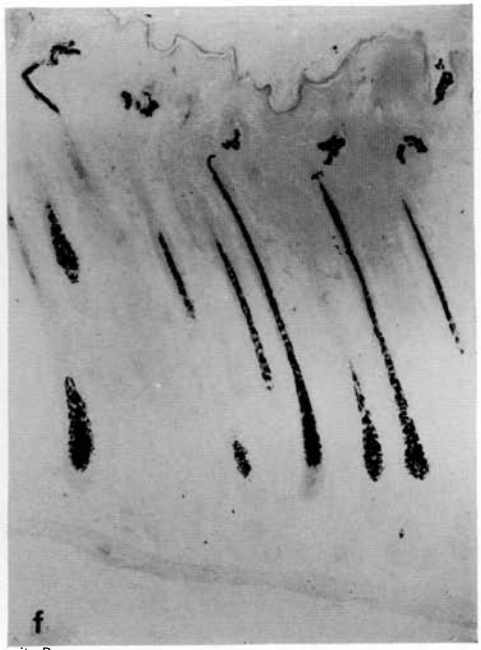












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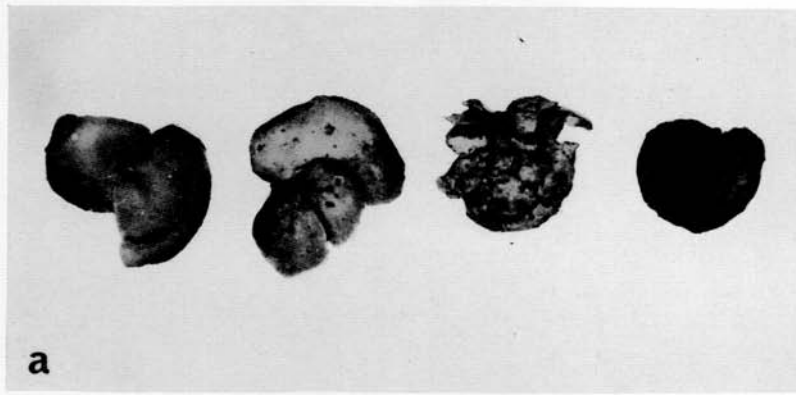

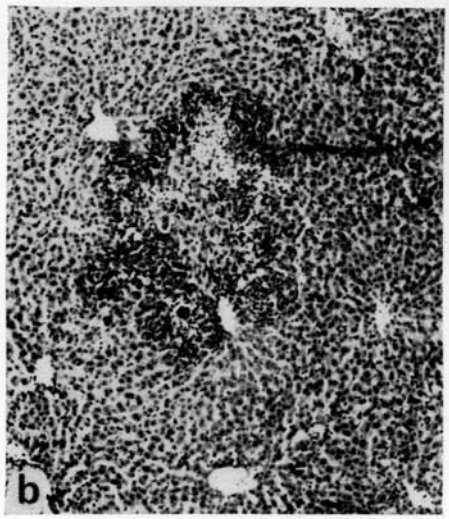

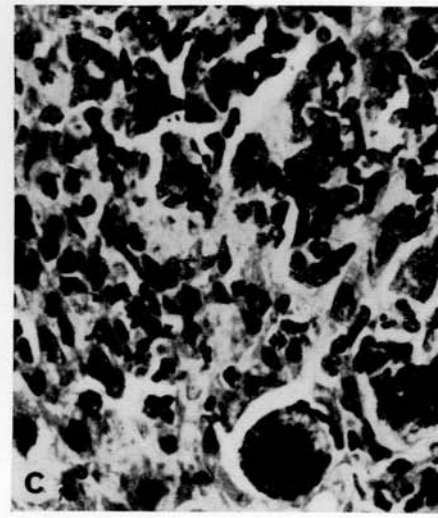

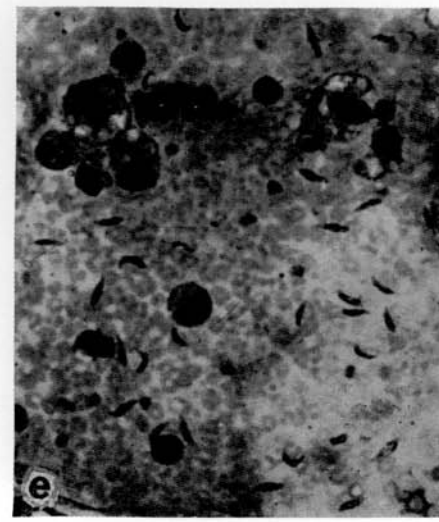

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to form disulphide bonds. Paraffin wax sections of dorsal skin from 6-day-old normal and nude mice were stained with sulphydryl reagent after the method of Bennett (1951), as modified by Mescon & Flesch (1952). The reagent used was 1-(4-chloromercuriphenylazo)-naphthol-2.

The distribution of sulphydryl groups in normal and nude skin is shown in Plate I*e* and *f*. In normal skin the mid-follicle region reacted intensely with sulphydryl reagent indicating that sulphydryl groups were concentrated in this part of the follicle. At the distal end of the mid-follicle region, the reaction ended suddenly and the fully keratinized hair in the upper follicle was unreactive. This indicated that free sulphydryl groups were absent from the upper follicle, the groups having been oxidized to form disulphide bonds. These observations agree with the findings of Eisen *et al.* (1953) and Ryder (1958). Plate I*f* shows that there was no intense sulphydryl reaction in the follicles of nude mice. The follicles are marked in the figure by the pigment in the hairs and hair fragments. Many of these reacted weakly with sulphydryl reagent and others reacted negatively. The absence of an intense sulphydryl reaction in the mid-follicle region suggests that the hair follicles of nude mice are deficient in keratin precursor.

(iii) *Liver histology*

The purpose of examining the liver histology was to determine the course of events leading up to degeneration and atrophy of the hepatic tissue. After distinguishing the anatomical form of the initial lesions and the subsequent reactions of the hepatic tissue it may then be possible to reduce tentatively the number of causative agents. The liver histology was also studied in order to find a unifying factor relating the liver disease to the keratinization abnormality in the hair follicles and to see if the disease resembles any of the hepatic disorders of man.

Random samples of apparently healthy, mildly ill and moribund nude mice were killed and the livers fixed in formol saline. Paraffin wax sections were stained with haematoxylin and eosin and with basic fuchsin. With each nude specimen, liver

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Plate II

a. Progressive liver degeneration in nude mice. From left to right: normal liver; liver with small open lesions scattered over the lobes; liver at an advanced stage with normal hepatic tissue interspersed between large open scars; atrophied liver from a moribund nude mouse and having no normal hepatic tissue. Actual size.

b. Liver section from a healthy nude adult mouse. Cell degeneration involving 2–3 adjacent lobules is observed. This is an early stage of the liver disease. H. and E. stain, × 63.

c. Liver section from nude mouse showing a 'balloon' cell in open space (bottom of picture). The 'balloon' cell contains numerous basophilic bodies. There is extensive infiltration of the surrounding necrotic tissue with polymorphonuclear leucocytes and monocytes. H. and E. stain, × 520.

d. Liver section from nude mouse at time of death. The hepatic tissue has lost its lobular structure and the central and peripheral veins are abnormally adjacent to one another. A few islets of normal cells remain, H. and E. stain, × 63.

e. Blood smear of necrotic liver of nude mouse showing the banana-shaped organisms, *Toxoplasma gondii*. Wright's blood stain, oil immersion.

sections of a normal littermate were also examined. Normal littermates, however, showed no histological abnormality.

The liver disease of nude mice commences with the appearance of lesions at widely separated points throughout the lobes. The early stage of one of these lesions is shown in Plate II*b*. The parenchymal cells of a lobule become necrotic and the necrosis spreads rapidly until most of the lobule is destroyed. The necrosis may occur at any point within a lobule. There is no definite tendency for the necrosis to be central or peripheral. With progressive cell degeneration whole lobules are destroyed and the lesions gradually link up. At this stage affected areas have lost their lobular structure and the central veins are abnormally adjacent to one another. A prominent feature of the lesions is the common occurrence of 'balloon' cells containing basophilic bodies (Plate II*c*). The balloon cells are circular shaped and situated in open spaces. The possible significance of these cells will be mentioned later. There is extensive infiltration of the necrotic areas with polymorphonuclear leucocytes and monocytes which engulf the cellular debris. Degeneration of the hepatic tissue proceeds until little normal tissue remains (Plate II*d*). The atrophied lobules assume a glassy appearance as phagocytosis reaches an advanced stage. A few islets of normal cells remain and they are very much enlarged. Large masses of cytoplasm devoid of nuclei are found.

Experiments carried out to relate the liver disease to the hair abnormality are outlined in the next section.

5. STUDY OF THE PLEIOTROPY

The pleiotropy of the nude gene involves a range of tissues and characters—the hair follicles, body growth, ovaries, sperm and hepatic tissue. The hair follicles form abnormally keratinized hairs and it was found that the follicles were deficient in sulphydryl groups. As regards body growth, it can be supposed that the absence of the coat results in increased heat loss from the body surface and that considerable amounts of energy are dissipated which would otherwise be converted into muscle and fat. Absence of the coat, however, may not be the only factor retarding body growth. The nude gene appears to have an intrinsic effect on general body development as seen by the reduced ovary size and reduced number of eggs shed. The latter defects explain the very low fertility of female nude mice. The poor fertility of male animals is due to their high proportion of non-motile sperm.

The liver disease of nude mice is outstanding in a number of ways. Firstly, the coexistence of the hairless condition and the liver abnormality as a result of a single genetic change is surprising. In the course of the breeding experiments the two defects never became dissociated in the segregating litters. It is difficult to explain how the liver damage can be related to an epidermal structure, the hair follicle, and ultimately to the abnormal synthesis of keratin. Secondly, the liver disease appears to be a secondary effect of the hairlessness. While the keratinization defect may be observed 3–6 days after birth, abnormal livers are very rarely found before 3 weeks of age (see Table 5). Thirdly, balloon cells containing basophilic bodies are a characteristic feature of the liver lesions. Since these bodies were never observed in

normal littermates of nude mice it was at first considered unlikely that they represented pathogenic organisms. Later observations showed, however, that this conclusion was probably wrong.

(i) *Sulphur metabolism*

Since the hairlessness was associated with a deficiency of sulphydryl groups in the follicles, the liver abnormality was investigated to see whether it could be shown that this defect is also associated with abnormal sulphur metabolism in the cells of the hepatic tissue. In this study the amino acids, methionine, cysteine and cystine were particularly relevant. Methionine cannot be synthesized in the animal body and must be provided in the diet for the promotion of growth and for the maintenance of nitrogen balance in adults. Methionine alone can satisfy all the sulphur requirements of the body since it is readily convertible to cystine. This reaction occurs in liver tissue. Methionine is first converted to homocysteine which then condenses with serine to form cysteine. The cysteine is then oxidized to cystine.

In view of the deficiency of cysteine in the hair follicles and also on account of the reduced growth rate, it seemed possible that the nude gene may cause a block in the metabolic pathway whereby methionine is converted to cystine. Also, it has been shown experimentally that cystine deficiency causes massive hepatic necrosis (Glynn *et al.*, 1945). To test this hypothesis fifty-one nude mice were divided into five groups; four groups each received a supplement of methionine, cysteine, cystine and cystine + methionine, while the remaining group received no supplement. Each group had a control group of normal mice receiving no similar supplements. The supplement in all groups comprised 0·8% of the diet (Long, 1961).

The amino-acid supplements failed to ameliorate the liver defect. All the nude mice died after a variable number of weeks and there was no difference between the length of survival of supplemented and non-supplemented nude animals. All nude animals showed the liver disease. Normals showed no ill effects. From the results it was concluded that the liver disease is not caused by a deficiency of cystine.

(ii) *Urine chromatography and liver-extract analyses*

Further attempts to reduce the liver abnormality to biochemical terms were made by examining urine chromatograms and liver extracts so that pathological excretion of amino-acids and deviant amino-acid concentrations in the hepatic tissue could be detected. One millilitre urine samples were collected from normal and nude mice and passed through a cation exchange column of N HCl-washed Amberlite IR 120 resin. After washing the column with distilled water the amino acids were displaced with 2N NH_3. Samples of 50 μl. of effluent were applied to the papers which were developed in a 4:1:5 n-butanol–acetic acid–water system. For liver extract analyses, livers of normal and nude mice were weighed and homogenized in 10 ml. 3% sulphosalicylic acid, centrifuged and freeze dried. The extract was diluted in distilled water and 0·5 ml of solution was made up to 1 ml. by adding 0·1 ml. N HCl, 0·1 ml. α-amino β-guanidino propionic hydrochloride and 0·3 ml. distilled water. One liver

extract from a normal mouse and two from nude mice were run through an amino-acid autoanalyser.

The results of both experiments were negative. The chromatograms did not reveal pathological excretion of amino acids in nude mice and there was no difference between normal and nude specimens in the positions of the amino acids on the papers. With regard to liver extracts there was no difference, qualitatively or quantitatively, between the series of peaks produced on the charts by normal and nude samples. The peaks of methionine, cysteine, cystine and serine were not abnormal in nude samples.

(iii) *Identification of a pathogenic organism*

When the above tests revealed nothing abnormal in amino-acid metabolism attention was focused on the balloon cells in the liver sections. The appearance of these cells gave the impression that they might possibly be cysts containing pathogenic organisms. Three moribund nude mice were given to Dr J. G. Campbell, British Empire Cancer Campaign, Poultry Research Centre, Edinburgh, and the carcasses were examined. A parasitic protozoan, *Toxoplasma gondii*, was identified in histological sections. The balloon cells of the liver were interpreted as pseudo-cysts containing large numbers of the parasite. The cysts were also present in the brain. Liver smears of twenty nude mice were stained with Wright's blood stain and the free form of the parasite was found in one animal. This case is illustrated in Plate II*e*. Thus, while *Toxoplasma* cysts were commonly observed in liver sections, the free organism was found only rarely. This indicated that the infection was chronic rather than acute.

It was not within the scope of this study to inquire fully into the pathology of *Toxoplasma*. Suffice it to say that the organism is commonly harboured by mice, cats, dogs and sheep (Jacob, 1953). In chronic infections the organism is present in pseudo-cysts which lie dormant in the brain and liver (Frenkel, 1953). After initial infection the animals develop immunity and may survive in a healthy condition for a considerable period. If infection is acute ascitic tumours develop and death occurs within 5–10 days. The free organisms are readily observed in smears of peritoneal fluid. Intra-uterine infection is the only natural mode of transmission of *Toxoplasma* known with assurance. The organism can be transmitted across the placenta from a chronic tolerant carrier to the foetus (Weinman, 1952). The congenital toxoplasmosis may result in death of the foetus, or the foetus may be born but structural changes may take place afterwards (Feldman, 1953).

The question which immediately arises is whether the liver disease of nude mice is caused by *Toxoplasma* or by an inborn error of metabolism. Thus, there are two possible alternatives: (*a*) *Toxoplasma* may have a specific affinity for nude mice thereby causing liver disease, and be absent from normals. (*b*) *Toxoplasma* may be present in dormant cysts in both normal and nude mice. The occurrence of this latent form of the organism may be merely coincidental to some defect in intermediary metabolism which results in liver disease. No experiments have been carried out to decide which interpretation is the correct one but the alternatives may

be discussed briefly on the merits of the observations. Dormant pseudo-cysts were not observed in the livers of normal mice but they may be present in the brains which were not examined and which are characteristic sites of the cysts. Histological demonstration of cysts in the brains of normals would disprove the first alternative. Liver lesions of the type described are not generally associated with toxoplasmosis. The cysts usually lie dormant in the tissues but the necrosis and extensive monocytic infiltrations in the livers of nude specimens are exceptional. The second alternative is more probable. In view of the ubiquitous nature of *Toxoplasma* both normal and nude mice may carry the organism. If this is true, then an inborn error of metabolism in nude mice may provide suitable conditions for the organism to manifest itself more clearly than in normal mice.

Obviously, further research is necessary to clarify the relationship between nude and the protozoan *Toxoplasma*.

6. DISCUSSION

The main interest of the nude gene lies in the manifold effects that it causes. The abnormalities were studied in morphological terms and the principle of the unity of gene action (Grüneberg, 1943) was taken as a convenient guide. This principle states that "the primary morphological effect of the gene must be shown to be either cell-specific or tissue-specific". However, the primary effect of the nude gene has been shown to be neither cell nor tissue specific. The hairlessness is caused by abnormal keratinization of hair in the follicles; low fertility is due to non-motile sperm, small ovaries and low egg counts; the cause of the liver disease has not been determined but the defect has been traced to its initial stage, i.e. necrosis of small areas of parenchymal tissue at various points throughout the liver. Other cases have also been found to be irreducible to a single primary defect in development, notably the two mutants, dominant spotting W and its viable allele W^v (Russell, 1949). Hence the nude phenotype raises the question again whether the methods employed were sufficiently sensitive to reduce the series of changes to one primary defect, or whether the gene is acting differently in the different tissues. The observed deficiency of sulphydryl groups in the hair follicles led to an investigation of the liver disease in terms of sulphur metabolism. The one gene-one enzyme theory suggested that the nude gene may cause a block in the biosynthetic sequence of reactions leading to the formation of cystine in the liver cells. Although experiments with amino-acid supplements, urine chromatography and liver extracts were carried out, nothing abnormal in amino-acid concentrations, especially methionine, cysteine and cystine, was found. Further research involving protein electrophoresis and isotopes is being planned in order to reduce the liver disease to biochemical terms. The problem of the parasite *Toxoplasma*, which was found to be superimposed on the nude pleiotropy, will also be studied further.

The abnormal keratinization of the hair means that the nude gene is an additional example of that group of hairless genes typically represented by naked. The hairs are incompletely differentiated and consist of thin strands of medullary substance which bend and coil in the upper dermis. The defect was traced to a deficiency of

sulphydryl groups in the mid-follicle region. Normally, these sulphydryl groups are concentrated in this region of the follicle and are attached to a cysteine complex, a precursor of keratin. They are required for oxidation to the disulphide bonds of keratin. It appears that follicles of nude mice synthesize inadequate amounts of keratin precursor. The distribution of sulphydryl groups in the follicles of hairless mice has not previously been reported. The nude gene, like naked, does not affect the cyclic activity of the hair follicles.

The liver disease is interesting both from genetic and pathological viewpoints. Hereditary liver disease in mice has not previously been reported. In nude mice the reaction of the liver tissue to initial injury in the form of lesions can be readily witnessed and, thus, the abnormality offers suitable material for the study of liver diseases in man. The histology shows that the necrosis is massive in form rather than zonal, as relatively large areas of tissue degenerate while adjacent to such areas extensive tracts of tissue remain temporarily normal. (In zonal necrosis all lobules are affected simultaneously.) Once a region is affected there is no recovery and the necrosis progresses towards an inevitable conclusion, namely, degeneration of all the hepatic tissue.

It is obvious that much further research is necessary in order to understand the action of the nude gene. This study has clarified the genetics of the new mutant and has revealed the morphological abnormalities in the different tissues. It is expected that an understanding of the metabolic process underlying the morphological changes can be obtained by using the biochemical techniques already mentioned.

SUMMARY

1. Nude is a new recessive gene causing hairlessness in the mouse. It is linked to rex and trembler in linkage group VII. The order of the three loci and the recombination frequencies are as follows:

$$Re\text{–}13\%\text{–}nu\text{–}9\%\text{–}Tr$$

2. In addition to hairlessness the new gene causes reduced body growth rate, very low fertility and a liver disease causing death. Nude mice may be classified at birth by the absence of vibrissae.

3. The hairlessness is due to abnormal keratinization of hair in the follicles. The skin histology resembles that of naked mice. The hair follicles were found to be deficient in free sulphydryl groups.

4. The majority of nude mice die of general body weakness within 2 weeks of birth. The survivors grow slowly and may live for a considerable period. But all nude mice eventually die, usually between 3 and 14 weeks of age.

5. The livers of dead or moribund nude mice are covered with lesions and scars. The defect has been traced histologically to its initial stage, namely, necrosis of small areas of tissue.

6. Attempts to relate the deficiency of sulphydryl groups in the hair follicles to abnormal sulphur metabolism in the liver were unsuccessful.

7. Pseudo-cysts of a parasitic protozoan, *Toxoplasma gondii*, were identified in

the liver and brain of nude mice. In one case the free form of the organism was found.

8. The possible relationship between the liver disease and the pathogenic organism is discussed.

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