THE EXPRESSION AND INTERACTION OF HEREDITARY FACTORS AFFECTING HAIR GROWTH IN MICE

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I. INTRODUCTION

A. General Introduction.

Until about 1925, geneticists were mainly concerned with the demonstration, confirmation, modification and extension of Mendel's laws, and with their bearing on the problem of evolution. The genes were thought of as discrete units, and interest was focussed mainly on their identification and localization on the chromosomes, and the way in which they were combined, segregated and transmitted through the germ cells.

More recently the attention of geneticists has turned more and more from what Goldschmidt (1938) terms the 'static' towards the 'dynamic' aspects of genetic research; namely, the causal relationships existing between the genotype and the phenotype. It is realized that, in most cases at least, the primary action of the gene is connected with its phenotypic effect by a long series of developmental reactions, and that each series affects and is affected by many similar series stemming from other genes, so that the pattern of development is determined by an intricately branched and interlacing network of gene-initiated reaction chains.

Two fundamental questions must be answered: (1) what is the nature of the gene, and (2) what is its mode of action?

The ultimate solution of these questions will probably have to await further refinements in the techniques of micro-

chemistry and nuclear physics before a final solution is reached. Nevertheless, some progress has already been made along several lines of investigation, including: (a) an investigation of the physico-chemical nature of the chromosome, using ultra-violet and X-ray techniques and microchemical tests; (b) a study of the effects of alterations in the nature of the gene (e.g. the production of mutations by irradiation), in the nature of its genetic environment (position effects), and in its quantitative relationships with its genetic environment (e.g. dosage relationships).

Information as to the nature of the gene may also be obtained through studies of its mode of action, since knowledge of what a gene does may tell us something about what it is. The problem entails a determination of the chain of developmental reactions leading from the gene to its phenotypic effects, and it is with such investigations that the field of Physiological Genetics is concerned.

Since the normal action of a gene can only be inferred from a study of a mutant allele, the problem resolves itself into an investigation of the differences in the developmental patterns of normal and mutant organisms. Methods used for the detection of such differences include:

(1) direct observation of normal and mutant tissues using the techniques of histology, physiology and biochemistry.

(2) observation of the effects of experimentally induced alterations in environmental conditions on the development of of normal and mutant tissues. A study of the nature of the environmental agencies which can alter the expression of a gene, and of the kind of alterations produced, may give a clue as to the nature of the gene-initiated reaction chain involved.

This approach is exemplified by the large body of work on the production of phenocopies, in which the effects of mutations are reproduced in non-mutant organisms by altering the environmental conditions, and on the reverse process, where development towards the normal type is brought about in mutant organisms by the same means.

Methods of altering the environment include the subjection of the whole organism to various conditions of temperature, pressure, etc., treatment with various chemical substances, and also the techniques of transplantation and explantation.

The techniques outlined above for the analysis of genic action are the same methods used in experimental embryology to investigate the problems of organization and differentiation. In fact the questions asked in the two fields are very closely related. Both geneticists and

embryologists are concerned with the study of developmental chains of reactions, but whereas the geneticist tries to relate these chains specifically to genic action, the embryologist, at least so far, does not. Moreover, mutations may produce alterations in the course of development which, particularly in mammals, cannot be produced by experimental interference on the part of the investigator. Studies in physiological, or developmental genetics are therefore of importance not only in their relation to the mode of action of the gene but also with regard to their bearing on related problems in embryology, physiology, and biochemistry.

The mammalian hair, in view of its accessibility to observation and experimentation, and the many genetically controlled morphological variations which it exhibits, provides an excellent subject for studies of this kind. The investigation, by one or more of the methods outlined above, of conditions leading to the production of a genetically controlled abnormality in development of the hair may throw some light on the nature of the processes through which the gene has its effect. It may also reveal information concerning the related embryological and physiological problems of hair development and maintenance.

Types of hereditary hypotrichosis form one such class of genetically controlled abnormalities in hair-development. It is with the modes of expression of three mutant

genes ("rhino", "hairless" and "Naked") producing hypotrichosis in the house-mouse, and their interactions with one another and with another mutation ("waved") affecting the morphological development of the hair, that this paper is concerned.

B. Review of Literature.

(1) Normal hair development.

Hair growth in the normal house-mouse has been investigated by Oyama (1903), Dry (1926), Gibbs (1940) and others. A brief description of the normal development of the hair, based upon their reports, is presented below.

Follicle formation begins as a thickening of the basal layer of the epidermis. This soon develops into a cylindrical peg of epidermal cells extending down into the dermis. A concentration of dermal cells forms at the base of the young follicle and invaginates into it, forming the papilla. The epidermal cells surrounding the papilla form the follicle bulb, and cells proliferated from the latter region form the actual hair shaft which is pushed upward through the follicle to the surface of the skin. Pigment first appears in the cell layers immediately adjacent to the papilla, in the region of the hair bulb just above the zone of actively dividing cells (Kaliss, 1942). The sebaceous glands and arrector muscles appear soon after the invagination of the papilla. Elongation of the follicle continues for about a week after development has started, by which time the follicle extends through the the full width of the dermis down to the panniculus carnosus, and the layers of the inner and outer root sheaths are fully differentiated. The inner root sheath extends up only as far as the region of the sebaceuus gland. The region between this point and the surface of the skin is known as the hair

canal, and is lined only by the outer root sheath and the cells of the epidermis. The period from the beginning of development until growth ceases in the hair bulb is termed the Anagen phase (Dry, 1926).

The Catagen phase (Dry, ibid) is marked by a rapid decrease in size of the hair-root, the disappearance of the inner root sheath, and the formation of a broom-like hair club at the base of the hair shaft. The shortening of of the root brings the hair club and surrounding sheath to a level just below the sebaceous glands where it remains throughout the next stage, the Telogen phase, which is the latent period extending from the completion of the hair club until the initiation of the next Anagen phase.

(2) Reports of hereditary hypotrichosis.

Hereditary hypotrichosis is of widespread occurrence, having been reported in various forms in most of the domesticated mammals. Cases which do not appear to have been studied developmentally include occurrences as a simple recessive in the cat (Letard, 1938; Mellen, 1939), as a recessive lethal in cattle (Hadley, 1927; Regan, Mead and Gregory, 1935; Wipprecht and Horlacher, 1935), as a simple recessive in cattle (Craft and Blizzard, 1934), and in the dog as a simple dominant (Kohn 1910-1911; Anonymous, 1917; Prinzhorn, 1921).

A review of the rather confused literature on hypotrichosis congenita in humans is presented by Cockayne

(1933). The anhydrotic type of hereditary ectodermal dysplasia, characterized by limitation of the hair to a sparse downy fuzz, the absence of sweat glands, heat intolerance, a dry glossy skin, and associated tooth defects, has been described in reviews by Hill (1933) and Clouston (1939). Thannhauser (1936) has suggested that this condition may be correlated with an insufficiency of the adrenal medulla, and states that it is inherited as a sex-linked recessive. Clouston (1929) suggests that the hydrotic type of hereditary ectodermal dysplasia, which is inherited as a dominant autosomal factor and is characterized by a short, sparse, brittle hair coat, hyperkeratosis of the palms and soles, hyperpigmentation of the skin, and malformation of the nails, is due to changes in the anterior lobe of the pituitary gland. A similar case, involving a complete alopecia, has been successfully treated with pituitary hormones by Kylin and Dicker (1939).

The cases of hereditary hypotrichosis in which histological studies have been done show that genetic mutations can produce the final result - hypotrichosis - by affecting the normal developmental chains of reactions in a number of ways.

1. There may be a partial agenesis of the hair-follicles, so that functional follicles do occur, but are greatly reduced in number. Kislovsky (1928) reported a

recessive autosomal semi-lethal factor in rabbits, which was shown by David (1932a) to fall into this category. Follicles are few in number, irregular in direction, and reduced in size, but otherwise appear normal in structure.

Such a condition has also been described in swine (Roberts and Carroll, 1931; David, 1932b) inherited as a semi-dominant autosomal factor. Here, in addition to the reduction in the number of follicles the sebaceous glands were rudimentary, there was much diffuse pigmentation, and the hairs were often broken at the tips, possibly due to a lack of sebum.

The condition also exists in a normally hairless African rodent, Heterocephalus (Thigpen, 1940).

2. The primary abnormality may be a delay in the development of an otherwise normal follicle. Such a condition is reported by Mohr and Wriedt (1927) as occurring in cattle as a simple autosomal recessive lethal. Microscopic examination of the skin from affected calves shows that follicle development is retarded, but otherwise perfectly normal. The sweat glands, on the other hand, are not retarded in development, but form a continuous layer of elongated alveolar sacs below the follicles.

3. Premature keratinization of the hair follicles and sebaceous glands may prevent the eruption of all except

the guard hairs. In the furless rabbit (Castle, 1933; Drapeau, 1932-1933), the follicles and sebaceous glands keratinize after the overhairs, but before the underfur follicles have erupted, so that when the underfur hairs do develop they are unable to penetrate to the surface, but pass around the hardened glands, breaking through the outer root sheath, and may remain irregularly curled in the dermis or break erratically through the epidermis to the surface.

4. Imperfect keratinization of the hair shaft may cause the hair to break off sooner or later after its eruption. This type occurs in the house-mouse as the mutant 'Naked' (Lebedinsky and Dauvart, 1927; David, 1932 a; Tengbergen, 1939) and in the deer-mouse (Clark, 1939). The hydrotic type of hereditary ectodermal dystrophy described by Clouston (1929) probably falls into this group.

5. A normal coat is grown, and then falls out at the time of the first moult. Sometimes there is a partial regeneration of the hair coat, and there are varying degrees of cyst formation in the later stages. The mutant forms "hairless" and "rhino" in the house-mouse, with which this paper is largely concerned, fall into this group. The writer's observations on these forms show that the condition may be essentially a follicular hyperkeratosis, and it seems likely that this may apply also to similar conditions reported

elsewhere in the literature. Such a case is that occurring in Peromyscus (Sumner, 1924; David,1932 a; Clark, 1939),in which there occurs an irregular shortening of the follicles during the Catagen phase of the first hair generation, cyst formation in the hair canals, follicles and sebaceous glands, hypertrophy of the sebaceous glands and stratum corneum, and a sporadic regeneration of hairs. This condition appears to be rather like that of the hybrids between the hairless and rhino mutant types to be described below. A condition which is similar to the rhino condition to be described in this paper (David, 1932a) occurs in the rat (Roberts, 1924, 1925; Wilder, Bethke, Kick and Spencer, 1932; Feldman, 1935a).

Certain human skin diseases characterized by a follicular keratosis, and often showing a familial tendency, may also fall into this class. Darier's disease (keratosis follicularis), typical cases of which are described by Trimble (1912), Pels and Goodman (1939), Combes (1941) and Madden (1941) produces an eruption of scaly follicular keratotic papules, some of which coalesce to form plaques, especially on the feet and hands. Histologically there is a hyperkeratosis, especially of the pilosebaceous and sweat duct orifices, and characteristic round, deeply staining masses (the 'corps ronds' of Darier) in the epidermis.

Pityriasis rubra is also characterized by follicular papules and often shows a familial trend (Brunsting and

Sheard, 1941). A similar condition, inherited as a sex-influenced trait, is named 'keratosis follicularis spiralis' by Lehr (1939).

6. "Hypotrichosis juvenilis", a condition described by Loeffler (1934) in the house-mouse presents characteristics of both classes 3 and 5 in the classification outlined above. The condition resembles that described in the hairless house-mouse (David, 1932a) in that there are cyst-like widenings of the hair canals, filled with stratum corneum, but it resembles the furless condition in the rabbit (Drapeau, 1932-1933) in that there is a premature keratinization of the hair bulb. The striking feature of this abnormality is that it affects only the first hair generation. It would be interesting, in view of the author's findings to be reported later in this paper, to follow the transition from the abnormal first hair-coat to the normal second generation, but unfortunately Loeffler fails to describe this aspect of the matter.

The present investigation has been mainly concerned with the development of the mutant type "rhino" in the house-mouse, and with the interactions of rhino with other mutant genes ("hairless", "Naked", and "waved") producing changes in the normal development of the hair.

Mice whose descriptions correspond closely to that of the present "rhino" mutant have been reported several times

in the literature. Gaskoin (1856) described such an animal and stated that microscopic examination showed that there were no signs of hair follicles or "any recognizable vestige of their obliteration." He does not mention cyst formation. Campbell (1907) also describes such a mouse, and presents pictures which bear a striking resemblance to our "rhinos". Hair-loss, however, occurred later than in the present "rhino" stock; the six week old mouse in his photograph still retained a thin hair-coat on the head, at the base of the tail, and on the legs. This of course does not mean that this mutation was necessarily different from the present rhino mutation, since the difference in its expression may have been produced either by differences in the environmental conditions under which the mice were raised, or to differences in their genetic background.

The present rhino mutation (symbol hr^{rh}) was discovered in this laboratory by Howard (1940) who showed that it was a simple autosomal recessive, and allelic to hairless. Mice homozygous for the rhino gene grow a normal juvenile coat which begins to fall out around the eyes at about fourteen days, and depilation proceeds in a rather ill-defined anterior-posterior wave over the body, remaining longest on the tail, ears and feet. Older rhino mice develop a characteristic thickening and wrinkling of the skin, and a hyper-

trophy of the nails (Howard, ibid). The preliminary observations of Howard on the expression of the rhino gene have been confirmed and extended in the present study, and will be reported in more detail in a later section of this paper.

"hairless"(hr), a simple autosomal recessive (Crew, 1927), allelic to rhino (Howard, 1940), also causes depilation by a falling out of the hair. Depilation starts at about fourteen days around the eyes as in rhino, but the feet also lose their hair at this time, and there is a sharp demarcation between haired and non-haired areas of the body at all times (Howard, ibid). David (1932 a) reports that the first histologically observable abnormality is an irregularity in the shortening of the hair follicle at the time when the hair-formation is almost completed, and states that this irregularity leads to a failure of the hair club to form properly, which in return is responsible for the falling out of the hair (however, observations to the contrary are reported in this paper). She also describes the formation of varying numbers of cysts in the skin of the adult hairless mouse, and distinguishes four types of cyst: follicle-end cysts, sebaceous gland cysts (atheromata), hair-follicle cysts, and hair-canal cysts (utriculi). The degree of cyst formation is correlated with the degree of wrinkling and thickening of the skin. There is a hypertrophy of the nails similar to, but less extreme than that occurring in rhino mice.

Howard (1940) states that F_1 animals from a cross of rhino x hairless mice resemble hairless mice both in the pattern of hair loss and the smooth skin of the adults, i.e. that the hairless factor is dominant to the rhino allele (however, see below).

"Naked" (N) is an autosomal dominant, semi-lethal when homozygous (David, 1932 a). When heterozygous it causes an imperfect keratinization of the hair which produces regions of weakness along the hair shaft and causes the hair to break off sooner or later after emerging from the skin. This is the reason for the short, rough, juvenile coat characteristic of young heterozygous Naked mice, for the dingy, smutty appearance of the skin after depilation, and for the periodic waves of regenerated hair which pass back along the body at regular intervals. Depilation starts around the eyes at about ten days, and passes in an anterior-posterior wave over the body, but leaves the feet and tail unaffected. Histological examination shows that some hairs fail to penetrate the stratum corneum, and remain coiled in the hair-canal. Most hairs erupt normally but later break off at points of weakness. There is no sign of cyst formation comparable to that in hairless mice.

In homozygous Naked mice the condition is much more extreme; there is a complete absence of vibrissae at birth, and if such mice survive at all they never grow more

than a few scattered hairs, since nearly all the hairs are too weak to penetrate the stratum corneum, but remain as irregularly coiled masses in the hair canal, giving the skin a highly pigmented appearance. The nails are soft and malformed (David, ibid).

Tengbergen's evidence (1939) that the abnormal hair growth in Naked mice is due to a lack of some substance transmitted in part, at least, through the mother's milk is of highly questionable value. The reciprocal difference in lethality which she obtained in offspring of crosses between homozygous and heterozygous Naked parents is of dubious significance in view of the fact that NN females are poor mothers anyway (ibid, p.382). The fact that some of the normal mice which she fostered on NN mothers showed a short, rough, juvenile coat is also not significant, since 13 of the 56 mice so fostered died. Tengbergen apparently did not run any control experiments using normal foster-mothers, but the results of large numbers of fosterings carried out in our laboratory with non-Naked mice indicate that her mortality rate using NN mothers was much higher than it would have been using normal mothers. This leads the writer to believe that the mice fostered by Tengbergen on NN mothers were probably undernourished and ill-cared-for, and would therefore have had short, dull, rough coats anyway.

(3) Reports on 'waved' mutations.

Mutations causing a waviness of the hair coat have been reported in man (Mohr, 1932; Schokking, 1934), mice (Crew and Auerbach, 1939), rabbits (Pickard, 1941), rats (King, 1932; Feldman, 1935), and swine (Rhoad, 1934). The mutant type concerned in the present investigation (waved-1) has been described by Crew (1935). waved-1 is an autosomal recessive which produces characteristic hooked vibrissae identifiable shortly after birth, and a coat which develops a relatively loose but distinct wave at seven to eight days which increases to seven or eight weeks of age, and then gradually disappears. A similar but more extreme type (waved-2) has been described by Keeler (1935).

(4) Interactions of Naked, hairless and waved.

In mice homozygous for hairless and heterozygous for Naked (Nn; hr/hr) the expression of both mutants is exaggerated (Snell, 1931; David, 1932 a). These mice show a retardation in growth a few days after birth; their juvenile coat is shorter and rougher than that of Nn mice and is shed earlier - in fact the most retarded individuals may never acquire a complete hair-coat. The shedding occurs according to the Naked pattern on the body, and leaves the the skin pigmented and highly wrinkled, but shows the characteristic "gloves" of the hairless pattern on the feet. The "utriculi" or hair-canal cysts in adult mice of this type are especially large and numerous.

Mice heterozygous for both mutations (Nn; hr/Hr) develop characteristic "goggles" (ring-like hair-formations around the eyes) and also show an increase in utriculi (David, 1932a) - a surprising fact in view of the completely recessive nature of the hairless mutation on a "normal" genetic back-ground.

David (1937) has described an interaction between a waved mutant (she does not state which one) and hairless. In mice homozygous for both these mutants depilation is delayed, and there is a regeneration of a short woolly coat (at the time when hairless mice regenerate only a few scattered hairs) and an increased wrinkling in older mice due to an increase in the number of hair canal cysts, apparently caused by the pressure of the bent hairs on the follicle walls (David, ibid).

A different situation occurs in the case of heterozygous Naked, homozygous waved (Nn; wa/wa) mice (David, ibid). In these animals there was little sign of a waved coat, only a few animals showing a more or less strong wave. She explains this by assuming that the curvature of the follicle in waved mice is due to a poor keratinization of the hair, which causes it to bend at the time of eruption, thus producing a curvature of the follicle. That the Nn; wa/wa hybrids show little waving is explained by the fact that the poorly

keratinized hairs of the Naked phenotype do not have strength enough to bend their follicles, and therefore remain straight. This explanation, however, does not seem to be consistent with the fact that in heterozygous Naked mice most of the hairs do bend at the time of eruption (David, 1932) but do not appear waved except sometimes at the tip. Moreover, the vibrissae of waved mice often show bends at irregular intervals along the shaft, in regions which must have been formed after the vibrissae erupted. Since the bends are at irregular intervals they cannot be due to growth of the hair in a permanently misshapen follicle, and since they occur in regions of the vibrissae formed after eruption they cannot be due to a temporary bending of the follicle at the time of eruption.

(5) Experimental work on hereditary hypotrichosis.

(a) Transplantation experiments.

Crew and Mirskaia (1931) did epidermal transplants from haired to hairless adult mice, and succeeded in getting grafts to stay on for a period of from fourteen to thirty days. They also described a regeneration of hair on the back of the host two weeks after grafting, which was shown to be due to the operational procedure, not to an influence of the graft tissue, since the same response was evoked by excising a piece of skin from the back of a hairless rat, reversing its direction, and replacing it. David (1934)

obtained one successful graft from a hairless mouse to a haired host, and one from a haired donor to a Naked host, and found that the grafts behaved autonomously in both cases. Here also evidence of the liberation of "growth-stimulating substances" due to the grafting procedure is reported. Roberts (1937) did skin transplants from haired to hairless rats, and again the graft behaved autonomously. Gershberg (1940) also did transplantation experiments on the hairless rat, and showed that grafts both from hairless to normal and normal to hairless rats behaved autonomously. Again there was stimulation of a secondary growth of hair over the bodies of operated rats, due to the effects of the operation. In all these experiments no attempt was made either to inbreed the animals used or to transplant between closely related individuals; hence difficulty was experienced in getting grafts to survive in a healthy condition long enough to justify any final conclusions about their autonomy.

(b) Attempts at treatment of hypotrichosis.

Attempts to treat cases of hereditary hypotrichosis with various substances have met with little success. Klauder (1933) claims to have produced a regrowth of hair in a case of complete alopecia by the administration of cystine in the form of hydrolysed wool. However, since spontaneous regrowths occasionally do occur in such cases, this report is of little significance without further evidence.

Martin and Gardner (1935) fed hairless rats with cystine and cysteine, and reported a regeneration of the hair coat, but Roberts (1937), who repeated the experiment with cysteine, failed to stimulate any such regrowth. No explanation was offered for this discrepancy, but it seems possible that the answer lies in the fact that although Martin and Gardner used hairless rats from the stock of Roberts, a considerable period elapsed between Martin and Gardner's experiment and that of Roberts. During that time Roberts' stock was crossed with that of Wilder et. al. (1932). Although the stocks are stated to be "genetically the same" (Roberts, 1937) it is possible that Wilder's mutation may have been an allele of Roberts' but with a more extreme effect (the relation being similar to that between the hairless and rhino mutations in the house-mouse - see below), and hence may not have been susceptible to the same treatment.

Gershberg (1940) fed cysteine to normal rats on which hairless skin had been grafted and found no increase in the amount of hair on the graft. The feeding of potassium iodide, and of thyroid also failed to have any effect on hair growth in the hairless rats (Roberts, Quisenberry and Thomas, 1940).

Emery (1935) attempts to show that the lack of fertility and lactation in hairless rats is due to a mal-

function of the regulatory mechanism of the pituitary gonadotropic hormone on the production of theelin. However, in view of the fact that he used an unrelated strain of rats as controls (therefore many of the differences shown to exist between hairless and normal rats may not have been related to the hairless condition at all), and that rats heterozygous for hairless have a normal oestrous cycle although they show similar differences in pituitary function, his conclusions seem hardly justified. Moreover, Roberts, Quisenberry and Thomas (1940) report no significant differences between normal and hairless rats in weights of the pituitary, testes, spleen or thyroids, and no essential structural differences in the pancreas, thyroids or adrenals.

David (1934) treated hairless and Naked mice with benzyl mercapton to test whether the loss of hair was due to a deficiency of the growth-stimulating sulphydryl group. There was no effect of this treatment on hair-growth in either Naked or hairless mice, but in some of the hairless individuals there was a premature appearance and increase in number of hair-canal cysts. However this may have been due to irritation caused by the treatment, and need not necessarily have been a result of the growth-stimulating properties of the - SH group.

Several cases have recently been reported in the medical literature in which types of follicular keratosis in

man have shown a decided response to vitamin A therapy. Peck, Chargin and Sobotka (1941) report four cases which were diagnosed as having Darier's disease (keratosis follicularis) and which all showed a low concentration of vitamin A in the blood. Treatment with vitamin A gave a decided symptomatic improvement, and a corresponding rise in the blood level of vitamin A. This, as the authors point out, indicated that the condition was due to an inability of the affected persons to maintain an adequate concentration of vitamin A in the blood, either because of faulty absorption of the vitamin from the intestinal tract or an inability to convert the provitamin (carotene) into vitamin A. Welton (1943) reports a similar case also diagnosed as Darier's disease, and showing a low concentration of vitamin A in the blood. Treatment with large doses of vitamin A had given a rise in the blood level of vitamin and a moderate symptomatic improvement at the time of writing.

Pityriasis rubra, another disease in which the elementary lesion is a keratotic follicular papule, has also been shown to respond to vitamin A therapy. Brunsting and Sheard (1941) report three cases of pityriasis rubra all of which showed high dark-adaptation levels (indicative of a vitamin A deficiency) and in which the skin condition improved slowly on treatment with vitamin A. One of these cases showed an excess of carotene in the blood, which the authors

regard as an indication that there is either an inability to metabolize carotene or an increased demand for vitamin A.

(6) Factors affecting normal hair growth.

It is interesting to note that a condition similar to the follicular keratoses described above can arise as the result of a deficiency of vitamin A in the diet. This condition, characterized by the appearance of spinous follicular papules, loss of hair, and dry skin due to plugging of the sebaceous and sweat glands, has been described by Frazier and Hu (1931), Nicholls (1933) and Loewenthall (1933, 1935). The pathology of the condition has been reviewed by Keil (1938). There is a hyperplasia of the epithelium and a marked hyperkeratosis of the follicle opening, which is filled with a dense mass of cornified cells, occasionally containing the remains of atrophic hairs. The sebaceous glands atrophy, and there are degenerative changes in the sweat glands. Vitamin A therapy gives involution of these signs in seven to eight weeks.

Lehman and Rapaport (1940) suggest that many of the names used more or less synonymously for skin conditions involving a follicular keratosis (keratosis follicularis, ichthyosis follicularis, keratosis pilaris, lichen spinulosus, etc.) are merely descriptive terms for cutaneous manifestations of a vitamin A deficiency.

Bessey (1942) reviews the effects that a vitamin A deficiency in rats produces on epithelial tissues other than the skin. There may be a metaplasia of the epithelia of the salivary glands, the respiratory tract, the genito-urinary tract, and the eyes and paraocular glands. The deficiency of vitamin A leads first to atrophy of these tissues, then to a reparative proliferation of the basal cells, and finally to a growth and differentiation of these cells into stratified squamous epithelium. Certain normally stratified squamous epithelia may show an increase in growth rate and become hyperkeratotic - evidence that these epithelia normally have specialized functions which are inhibited by the A deficiency (Bessey, ibid). The fact that many different kinds of epithelium are all converted into the stratified keratinizing type by a deficiency of vitamin A leads Bessey to assume that vitamin A is in some way necessary for the normal differentiation and life of these epithelia. Repair of the epithelia when vitamin A is again added to the diet is initiated by focally distributed basal cells which multiply, spread beneath the keratinized epithelium, and finally form a continuous epidermis-like layer, which proliferates to form epithelium of its original type. The keratinized cells are removed by vacuolar degeneration and leucocytic infiltration.

Moult (1943) shows that a vitamin A deficiency has a similar effect on the skin of rats. A diet completely

deficient in vitamin A produces a distention of the follicle neck within one week after the beginning of treatment. After one month atrophy of the follicle roots has begun, the upper one-third of the follicle is filled with a stratified mass of keratinized material, and the sebaceous glands are atrophied. There is no mention of cyst formation.

Although many other factors affect the hair and skin there is surprisingly little knowledge of their mode of action. Hair growth can be stimulated by irritation or injury of the skin, either by plucking (Collins, 1918-1919) or by the application of irritants (Butcher, 1940), or by grafting (Crew and Mirskaia, 1931; David, 1934; Butcher, 1935-1936). This is probably due simply to an increase in the blood supply of the skin.

The injection of thyroxin also causes a precocious regeneration of hair in under-fed rats, and the combination of thyroxin with irritants has an even stronger effect (Butcher, 1940). The secretion of the adrenal cortex evidently inhibits the effect of the thyroid hormone (Butcher, 1937). The 'hairless' malady of Smith (1917) and Hart and Steenbock (1918) in pigs has been shown to be due to a foetal athyrosis resulting from a lack of iodine in the mother's diet.

The correlation between cycles of hair growth and ovarian activity (Butcher, 1928; Gibbs, 1941) does not

signify that hair growth is controlled by the gonads (since the cycles continue in ovariectomized animals) but rather that both receive a stimulus from a common source. That this may be a secretion of the basophil cells of the anterior lobe of the pituitary gland is suggested by Gibbs (1941). The report of Gardner and De Vita (1940) that oestradiol inhibits hair growth in the dog conflicts with that of Hu and Frazier (1940) that ovarian hormones and urinary estrogens in the rabbit stimulate growth of the hair.

Several of the B-complex vitamins have been shown to be essential to hair-growth. A lack of inositol in the the diet of the mouse (Woolley, 1940, 1941) causes an "abrupt" loss of hair on the body, scaliness and then redness of the denuded areas, and eventual death. A similar effect is produced by a deficiency in pantothenic acid (Morris and Lippincott, 1941-1942) which causes a thinning of the hair, scaliness of the skin, paralysis of the hind legs, and loss in body weight. Histologically this condition has been described as a hyperkeratotic atrophic and desquamative dermatosis (Lippincott and Morris, 1941-1942). Antopol and Unna (1939) have shown that a deficiency of vitamin B6 in the rat causes a severe dermatitis within six to nine weeks, accompanied by denudation and edema of the paws, scaliness of the ears, and a roughness and redness of the lower abdomen. The histological picture is

characterized by hyperkeratosis, intercellular edema, polymorphonuclear infiltration, and atrophic changes in the epidermis and follicles.

The relation of the endocrine glands to hair-growth in humans is reviewed by Cooper (1930), but too little accurate knowledge is available to justify a discussion of the subject here.

II. EXPERIMENTAL WORK

A. Materials and Methods.

Stocks carrying the mutants studied were being maintained in this laboratory when the present investigation was begun. The appropriate crosses were made and observations on mice of the various phenotypes described were made at intervals throughout life, either with the naked eye or with the aid of a dissecting microscope. Skins of mice of typical stages were preserved.

The stocks used for observation of the mutant types were not closely related, and had not been inbred prior to the beginning of the present experiments. Although there was some variation in the expression of the mutants studied it was not considered worth-while to inbreed the stocks for the purpose of eliminating such part of this variation as was due to residual heredity, since (apart from the fact that sufficient time was not available) the differences between the various types observed were in most cases sufficiently marked to preclude any possibility of overlap. Moreover, since (1) much of the variation that occurred was obviously correlated with differences in the nutrition and general health of the mice, and (2) no diminution was noticed in the amount of variation in rhino and hairless stocks during the course

of six generations of brother-sister matings, it seems probable that a large part of the variation was due to environmental differences.

For most of the histological work pieces of skin were removed from the mid-dorsum of animals which had been killed by severing the spinal cord at the base of the skull. In a few cases, when the animals were needed for breeding purposes, or for further observation, a small piece of skin was removed under ether anaesthesia and the wound was sutured. Healing occurred within a few days, leaving only a small scar. No observations which could have been affected by this procedure were made on such mice.

Skins were fixed in Allen's B-15 fixative, embedded in paraffin, and cut in serial sections at various thicknesses, ten and twenty micra being found the most suitable. Sections were stained with Harris' modification of Delafield's alum-haematoxylin, and counterstained with aqueous eosin. Heidenhain's iron-haematoxylin, and Mallory's Azan triple-stain were also used, and for cellular detail in the epidermal layers where the cytoplasm is highly basophilic, a combination of Feulgen's nuclear stain with Orange - G and Aniline Blue was found useful.

Further details of technique will be given when necessary in the following section of the paper.

B. Observations

(1) rhino.

(a) macroscopic. The observations on the development of the rhino condition reported in this paper confirm and extend the preliminary observations published by Howard (1940). No macroscopic difference between rhino and normal mice can be distinguished until fourteen or fifteen days of age, when rhinos show a thinning of the hair coat around the eyes. The pattern of hair-loss on the head and body is rather variable; it may range from a condition resembling the hairless pattern, with a fairly sharply-defined wave of depilation progressing caudad and leaving relatively few scattered hairs in the denuded area (Fig. 1), to a generalized thinning of hair all over the body, with the anterior-posterior gradient very poorly defined (as illustrated by Howard). The pattern often varies on the same mouse, being well-defined in the early stages, and becoming more diffuse as it progresses towards the posterior regions of the body.

In general the wave of depilation spreads from the region around the eyes out over the head and throat, and caudad along the back and belly. Hair-loss starts on the belly at about the time when the dorsal wave of depilation has reached the ears (18-19 days) but proceeds more rapidly than it does on the back, being complete except for a few

scattered hairs at twenty to twenty-one days, whereas the dorsal wave does not reach the tail until twenty-two to twenty-five days of age. At this time the feet, tail, and ears are still haired. The hair on the forefeet may begin to thin out somewhat at twenty to twenty-one days, but may not be completely lost until from thirty to forty days. Loss of hair on the hind-feet is complete a few days after that on the forefeet, and the hairs of the ears are also lost during the fifth and sixth weeks. The eye-sensory hairs fall out at from twenty-three to twenty-nine days, but are replaced within ten to fifteen days. The vibrissae also fall out prematurely (30-40 days), but by the time the last of the long first-generation vibrissae have disappeared, the first few shafts of the next generation are visible. The vibrissae tend to become fewer in number and more irregular in older rhino mice.

Elongation of the nails can be noticed in some mice as early as twenty to twenty-five days of age, and by five or six weeks of age the nails begin to acquire the characteristic curved or spiral shape seen in older rhino mice. The effect seems greatest on the inner toes and next greatest on the outer toes, with the hind-feet usually showing a somewhat more pronounced effect than the fore-feet. The teeth appear normal.

The wrinkling occurring in young rhino mice is the

result of two different factors: (1) Well-defined folds of skin, particularly in the neck region, occur in normal (and rhino) mice as the result of changes in the relative growth-rates of the skin and the body during the growth of the first hair coat,and to some extent during later periods of hair growth (David, 1934). This wrinkling can be seen by removing the hair from normal mice, or by observing heterozygous Naked mice, in which the skin can easily be observed through the short rough hair coat. (2) The wrinkling due specifically to the rhino condition begins to appear at about three weeks of age as a number of fine corrugations on the head, and to a less extent on the body. At five to six weeks of age the skin becomes noticeably thickened, the folds on the back of the neck are more pronounced than in normal and heterozygous Naked mice of this age, and the corrugations on the head and back are coarse and prominent (Fig. 14). At about nine weeks the characteristic lateral folds, running from foreleg to hindleg, make their appearance. By the age of six months all the manifestations of the fully developed rhino condition are present. The skin is intensely wrinkled and contains small, hard, white lumps, which increase in number and size with increasing age. The claws are long, and curved or spiralled, the skin folds over the eyes have developed to a point where such a mouse is usually blind, and the lateral folds may be so enormous that they drag on the floor of the cage when the mouse walks (Fig.2).

When the skin of such a mouse is cut open and examined under the dissecting microscope it is seen to be filled with large numbers of round or oval vesicles of various sizes, which are filled with a thick white pasty material.

The fertility of rhino males seems normal, but that of the females is greatly reduced. Rhino females are also unable to suckle their young, which therefore have to be fostered.

(b) <u>histological</u>. For simplicity's sake, the following account of the histological changes accompanying the development of the rhino condition is based on observations which, unless otherwise stated, have been made on skin removed from the mid-dorsum.

Signs of abnormal development in this region can be seen in rhino mice as early as fourteen days of age, at the time when depilation is just detectable as a slight thinning around the eyes, and when the hair on the body appears perfectly normal to the naked eye. At this time the hair on the mid-dorsum is in the early "Catagen" phase of Dry (1926), where pigment has ceased to pass upward from the bulb, and the follicle is commencing to shorten.

In the normal mouse of this age the epidermis no longer shows the well-differentiated layers characteristic

of younger stages. The stratum germinativum consists of a single layer of cuboidal cells with round or oval nuclei, the stratum intermedium is also one cell layer thick, the stratum granulosum is composed of one or two layers of darkly staining flattened cells, and the stratum corneum consists of a thin layer (4-5 cells thick) of squamous keratinized cells. In the follicle necks the epidermal layers are even less differentiated, consisting of two or three rows of cuboidal, oval-nucleated, epithelial cells, a hardly distinguishable stratum granulosum, and about two rows of squamous keratinized cells (Fig. 16).

In rhino mice of this age, however, the epithelial layers are well differentiated, and are beginning to show signs of hyperplasia. The cells of the stratum germinativum of the surface epithelium are elongated in a plane perpendicular to the surface, and often appear columnar rather than cuboidal in type. The stratum intermedium is from one to three layers thick, the stratum granulosum is composed of two or three layers of cells with pyknotic nuclei and granular cytoplasm, and the stratum corneum is much thicker than that of normal mice of the same age, consisting of about eight to ten layers of squamous keratinized cells.

Signs of increased growth activity are also evident in the epithelium of the follicle neck. The stratum germinativum of the wall of the follicle neck is only two or

three layers thick but its cells are crowded towards the periphery of the follicle. Inside the basal layers there is a well-defined, deeply-staining stratum granulosum several cell-layers thick. The hair canal is enlarged, leaving a space filled with loosely packed squamous cells around the hair shaft. This enlargement of the hair canal is most evident at the distal end of the follicle where (at fourteen days) the canal may contain 6 to 8 layers of squamous cells which appear to be continuous with those of the stratum corneum. Lower regions of the follicle neck are less affected, and at the base of the hair canal the follicle wall appears normal (Fig. 17).

During the next few days the normal follicle shortens rapidly, and the hair club is formed. The shortening of the follicle brings the hair club, with its surrounding sheath, up to a level just below the sebaceous glands (Fig. 18).

In the skins of rhino mice during this period, the widening of, and proliferation of cells into, the hair canal continues in the upper part of the follicle and extends down to the region of the sebaceous glands. At eighteen days the whole canal is extended to over twice its normal size. Shortening of the follicle occurs irregularly, leaving elongated cords of epithelial cells extending down into the corium, and many of the hair clubs are malformed (Fig. 19). Although macroscopically the wave of depilation does not seem to have reached the mid-dorsum at this age, it can be seen in the histological sections that many of the hairs have already fallen out. By the age of

twenty-five days (Fig. 20), the hair canals are over three times their normal width and only occasionally contain a hair. There is a marked hyperkeratosis of the surface epithelium, and cords of cells from the irregularly shortened follicles still extend down almost to the panniculus carnosus.

During the next few weeks enlargement of the hair canals continues until at the age of five to six weeks they appear as large cysts, oval in shape, open to the surface, filled with masses of keratinized cells, and lined with stratified squamous epithelium, the basal cells of which are now elongated in a plane parallel to the cyst wall (Fig. 21). The sebaceous glands have become displaced, and lie at the base of these "utriculi", or associated with the irregularly shortened follicle ends lower in the dermis.

At about thirty to thirty-five days small vacuoles can be seen in these follicle ends, representing the first stages in the formation of follicle-end cysts. Similar vacuoles appear in the sebaceous glands shortly afterwards (Fig. 21). These vacuoles increase in size until by the age of fifteen to sixteen weeks they have developed into cysts as large as those developing from the hair canals. The cysts arising from the follicle ends are filled with varying amounts of stratum corneum-like material, and are lined with stratified

squamous epithelium like, but not as thick as, that of the surface. As the cysts grow older the lining becomes more and more compressed until it consists of a single row of basal cells with deeply staining nuclei elongated in a plane parallel to the cyst wall, and one or two rows of squamous cells. The cells of the walls of the sebaceous gland cysts retain for a time their characteristic appearance (large, round,light-staining nuclei, and vacuolar, somewhat basophilic cytoplasm) but eventually become elongated and compressed, due presumably to pressure within the cyst. In the older stages the walls of the sebaceous cysts are indistinguishable from those of the follicle-end cysts. The above description applies to cases in which the two types of cyst remain separate, but this situation seems to be the exception, rather than the rule in rhino mice. In most cases the cysts arising from the sebaceous glands and the end of a particular follicle seem to be connected, and as enlargment progresses they both become part of a single cyst, the walls of which contain both types of cell.

At the age of about seven to eight weeks, the fat stores in the lower regions of the dermis begin to diminish, and by the age of seventeen to eighteen weeks they have disappeared completely (Fig. 22).

In later stages of the development of the rhino condition (after about three months of age) the size of the utriculi no longer increases and may even decrease, due presumably to the fact that the enlargement of the openings of these cysts to the surface provides an opportunity for some of the cellular debris within the cysts to be extruded. The hair-follicle and sebaceous gland cysts on the other hand, do continue to enlarge, until by the age of nine to ten months they almost completely fill the dermis, giving it the appearance of an open-mesh network. Some of the largest cysts extend almost the complete width of the dermis, being of the order of 1 mm. in diameter (Fig. 23).

The hyperkeratosis of the follicle neck and widening of the hair canal also occurs in the follicles of the vibrissae, but since the vibrissa has a greater proportion of its shaft situated below the sebaceous gland than does the ordinary hair, it does not fall out until pushed out by the succeeding vibrissa.

(2) hairless.

(a) <u>macroscopic</u>. Macroscopic observations on the development of the hairless condition correspond in general with those reported by David (1932 a). The pattern of depilation in hairless mice differs from that in rhino mice in that: (1) Depilation begins on the feet almost as soon as it does around the eyes, and is complete by the

time the dorsal wave of depilation has reached the ears (Fig.3). (2) The line of demarcation between haired and non-haired regions is usually more sharply defined in hairless than in rhino mice. (3) There is a sparse regeneration of somewhat irregular and usually unpigmented hair at about five weeks which falls out again about three weeks later and recurs at increasingly irregular intervals throughout life. (4) Although hairless mice show various degrees of thickening and wrinkling of the skin with increasing age, they never approach the extreme "rhinoceros" appearance of rhino mice of corresponding ages (Figs. 4 and 5).

The hairless mice in this laboratory, in contrast to those described by Crew and Mirskaia (1931-1932) and David (1932 a), are fertile and vigorous, and females usually suckle their young, although their ability in this respect is somewhat subnormal. It is probable that these differences are due to changes in the residual heredity of the hairless stocks, especially since Crew and Mirskaia were able to improve the viability and fertility of their stock by backcrossing and selection.

(b) <u>histological</u>. The histological changes occurring in the skins of hairless mice just prior to, and during depilation are very like those described above for

corresponding stages in rhino mice. A distinct hyperkeratosis of the surface layers of the epidermis and of the follicle neck can be seen at fourteen to fifteen days of age (Fig. 24). At this stage there is little, if any, difference between hairless and rhino mice in the degree of hyperplasia. At eighteen days the distal end of the hair canal is extended to about twice its normal width, so that as the hair shortens during the Catagen phase the shaft lacks the support of the normally tight-fitting follicle neck and eventually falls out. The shortening of the follicle during the Catagen phase occurs irregularly, as in rhino mice, leaving cords of cells extending down into the dermis (Fig.25). The hyperplasia of the epidermis of the surface and follicle neck does not, however, progress as rapidly as it does in rhino mice. The hair canal usually shows little or no increase in width beyond that reached at eighteen days; in later stages the majority of the follicles are filled with a compact plug of stratum-corneum-like material, and in relatively few follicles does hypertrophy continue towards the formation of a typical "utriculus". Since follicle organization is, for this reason, presumably interfered with to a lesser degree than it is in rhino mice, the capacity for hair formation is often retained, and some follicles do produce hairs at the time when the second hair

generation normally appears. This capacity is progressively reduced with increasing age. Such hairs usually erupt through the hair canal and are generally very irregular in direction and shape, but are otherwise normal in structure. No detailed study of the fate of hairs which fail to erupt has been undertaken in this investigation, but in sections of skin from a one and a half month old hairless mouse several cases have been observed where hairs have broken through the follicle wall and have grown irregularly through the dermis. (David (1932 a) describes this process in detail.)

The first stage in the formation of follicle-end cysts can be seen at about thirty to thirty-five days, as in rhino mice; the sebaceous gland cysts begin to develop shortly afterwards. The enlargement of these cysts and consequent thickening of the skin in the later stages of development of the hairless condition is never as extreme as that occurring in rhino mice. In old hairless mice the walls of many of the cysts, particularly in the lower regions of the dermis, become reduced to a single layer of squamous cells, and such cysts, presumably as the result of compression, become reduced in size and irregular in outline, resembling large fat-cells (Fig. 26). As in rhino mice there is a reduction and eventual disappearance of the fat deposits of the dermis in the skins of older hairless animals.

(3) Naked

The writer's observations on the expression of the Naked factor (both when heterozygous and homozygous) agree closely with those reported by David (1932 a).

(a) macroscopic. Depilation in heterozygous Naked (Nn) mice is the result of a breaking-off, rather than a falling-out of the hair. The juvenile pelage is short and rough. Depilation begins around the eyes at about ten to twelve days, spreads in a wave over the head and caudad along the body in a pattern similar to that of rhino, leaving many scattered hairs in the depilated areas (Fig.6). The denuded skin (in non-albino mice) immediately after depilation contains many pigmented hair remains which give it a dirty grey colour. These are gradually eliminated during the next few days. The feet remain haired at all times. At, or shortly after, the time when the dorsal wave of depilation has reached the tail (ca. 3 weeks) a new hair generation appears on the head and passes back along the body. Before this growth of hair has reached the tail depilation has already begun again around the eyes, and in this way more or less well-defined "waves" of hair pass anterior-posteriorly along the body at increasingly irregular intervals throughout life.

In mice homozygous for the Naked mutation, the effect on the hair coat is much more extreme than that in

heterozygous Naked animals. The vibrissae are absent at birth and throughout life. The skin becomes highly pigmented during the periods when hair growth normally takes place, but never produces more than a few short, curled hairs, since most hairs either fail to erupt, and remain coiled in the skin, or break off immediately upon reaching the surface. The claws are soft and malformed. Such mice show a decided retardation in growth rate during the first few weeks of life, and usually die within the first ten days after birth. In crosses observed by the author between animals heterozygous for the Naked factor, 46 offspring have been classified as homozygous Naked on the basis of a complete absence of vibrissae at birth. Of these only two (one male, one female) survived to maturity. These mice, after maturity was reached, appeared healthy and vigorous, and the male, at least, was fertile.

(b) <u>histological</u>. No intensive histological investigation of the expression of the Naked gene has been carried out by the writer, since a thorough study of the histology of this mutant has already been made by David (1932 a). However, in the present investigation sufficient observations have been made to confirm the conclusion of David that the primary abnormality is an imperfect keratinization of certain regions of the hair shafts, which causes them

to break off sooner or later after erupting from the skin. In some cases the weakness of the hair shaft is so great that the hair fails to penetrate the stratum corneum, but buckles within the follicle and may pierce the follicle wall and protrude into the dermis. In other cases the hair may succeed in erupting, but in so doing the resistance of the stratum corneum may cause a considerable distortion and bending of the shaft and a consequent irregularity in shape of the follicle. In regions where this results in pressure on the follicle wall, a mild hyperkeratosis of the follicular layers often results (Fig. 27).

(4) Interactions between rhino, hairless, Naked, and waved.

(a) <u>hairless/rhino</u>. Offspring of crosses of hairless x rhino mice (hr/hr^{rh}) lose their hair according to the hairless pattern as described by Howard (1940), and show a sparse regeneration of irregular, often unpigmented hairs over the body at about forty to fifty days of age. However the rhino mutant is not completely recessive to the hairless allele as stated in Howard's (ibid) preliminary report, since at from six to nine weeks of age the hairless/rhino hybrids begin to develop a wrinkling which is characteristically rhino in pattern, but which is finer and less extreme than that of rhino mice of corresponding ages (Fig. 7). The time at which these mice can be distinguished by their wrinkling

from hairless mice of the same age is rather variable, but the difference has always been distinct by nine to ten weeks of age in the crosses observed by the writer. With progressing age the wrinkling of the skins of hairless/rhino hybrids gets coarser and thicker, and although it never approaches the degree of wrinkling shown by rhino mice, such animals can always be distinguished from the normally smooth-skinned hairless animals (Cf. Fig. 8, a hr/hr^{rh} mouse 180 days old, with Fig. 4, a hr/hr mouse of the same age). Hairless/rhino mice are fertile and are able to suckle their young.

Histologically the hairless/rhino hybrids show the same epidermal and follicular hyperkeratosis described for rhino and hairless mice, but the condition appears to be intermediate in degrees between the latter two types. Fig. 28, a section of skin taken from a hairless/rhino hybrid twenty days old, shows the characteristic widening of the hair canal and irregular shortening of the follicle end. It can be seen that here the follicular hyperkeratosis is more advanced than that in hairless skin at twenty-one days (Fig. 25), but less extreme than that in rhino skin at eighteen days of age (Fig.19). In later stages the majority of follicles do produce hair-canal cysts, but much smaller ones than those occurring in rhino skins of the same age.

(b) Naked; rhino. The interaction of the Naked and rhino mutations in heterozygous Naked; homozygous rhino (Nn; hr^{rh}/hr^{rh}) mice is similar to that described by David (1932 a) for the Naked; hairless (Nn; hr/hr) compound. The expression of each mutant is intensified when in combination with the other.

Mice of the required genetic constitution (Nn; hr^{rh}/hr^{rh}) were obtained by crossing Naked (Nn) females with rhino (hr^{rh}/hr^{rh}) males and crossing such of the F_1 animals as were phenotypically Naked (i.e. Nn; $hr^{rh}/+$) inter se. From this cross it was expected, on a priori grounds, that there would be six phenotypic types: (1) NN; hr^{rh}/hr^{rh} animals, showing a combination of NN and hr^{rh} hr^{rh} characteristics; (2) NN; $hr^{rh}/+$ and NN; $+/+$ mice phenotypically NN in appearance (mice of types (1) and (2) could be distinguished from other classes by their complete lack of vibrissae at birth); (3) mice of the constitution nn; $hr^{rh}/+$ or nn; $+/+$, phenotypically normal, and therefore separable from other types at 15 days; (4) Nn; $hr^{rh}/+$ or Nn; $+/+$ animals, characteristically Nn in appearance; (5) nn; hr^{rh}/hr^{rh} mice showing the usual rhino syndrome, and (6) the Nn; hr^{rh}/hr^{rh} class showing characteristics of both the Nn and hr^{rh}/hr^{rh} conditions. Mice of all six types were obtained in offspring of the above cross. To test whether

the classification of the Nn; hr^{rh}/hr^{rh} type was accurate several males of this type were out-crossed to normal females. The presence of Naked (Nn) animals among the offspring of these crosses showed that the tested males were heterozygous for the Naked mutant. It was not considered necessary to test these animals for the presence of the rhino factor, since the mice were so obviously "rhino" in phenotype.

Nn; hr^{rh}/hr^{rh} mice show a retardation in growth shortly after birth. The first hair coat may not appear until eight to ten days of age, and is very short and rough - much more so than that of Nn mice of the same age. Hair loss begins around the eyes at twelve to thirteen days, and is usually complete except for a few scattered hairs by twenty-two days, leaving the skin with a dirty, smutty appearance due to the presence of pigmented hair remains (Fig. 9). There is an intense wrinkling of the skin during the period of depilation, and for several months wrinkling is more extreme than that in rhino mice. During the fourth or fifth month, however, the difference in wrinkling becomes less distinct, and eventually the two types can be distinguished only by the presence of spots of pigment which remain in the skins of Nn; hr^{rh}/hr^{rh} mice throughout life. There is no regeneration of hair after depilation has occurred.

Histologically, characteristics of both the Naked and rhino conditions can be seen in intensified form in skins of Nn; hr^{rh}/hr^{rh} mice. A hyperkeratosis of the follicle neck is visible at twelve days of age. The pressure exerted on the follicle walls by the irregularly bent hairs of the Naked type apparently exaggerates the hyperplastic tendencies inherent in follicles which are genetically rhino, leading to a great increase in the rate of formation of hair-canal cysts. There is often a hyperkeratosis of the follicle wall below, as well as above, the region of the sebaceous glands, so that the utriculi involve most of the follicle, rather than the hair canal alone. The characteristics of the Naked condition are also exaggerated in mice of this type, the bending and buckling of the hair shafts being much more extreme than that in heterozygous Naked mice (For an illustration of these characteristics see Fig. 29 showing similar features in Nn; hr/hr^{rh} skin).

Observations on Nn; $hr^{rh}/+$ mice show that the rhino mutant remains recessive to the normal allele when in combination with the Naked factor. There is no indication in our stocks of "goggle" formation analagous to that described by David (1932 a) for Nn; hr/+ mice.

(c) Naked; hairless/rhino. Mice of the constitution Nn; hr/hr^{rh} were obtained by crossing Nn; hr/hr females with Nn; hr^{rh}/hr^{rh} males and selecting animals showing the short, rough coat characteristic of Nn mice before depilation.

As would be expected on the basis of the observations described above for Naked; rhino mice, the characteristics of both the hr/hr^{rh} and Nn types are also exaggerated in the Nn; hr/hr^{rh} compound. Depilation is of the Naked type on the body, but on the feet hair loss occurs according to the hairless pattern, and by fifteen days the skin on the feet is clean and pink. Wrinkling is more extreme than that in hr/hr^{rh} animals (Fig. 10 - compare with Fig.7), but less extreme than that in hr^{rh}/hr^{rh} mice during the first few months. In later stages (three to five months) wrinkling becomes typically rhino in character and degree, but the skin retains, throughout life, pigmented spots like those described in Nn; hr^{rh}/hr^{rh} animals.

Histologically the condition resembles that in Nn; hr^{rh}/hr^{rh} mice as described in the preceding section, but is less extreme (Fig. 29).

One female of the constitution NN; hr/hr^{rh} (offspring of a cross of Nn; hr/hr x Nn; hr^{rh}/hr^{rh}) has

been raised to maturity in this laboratory. This mouse (Fig. 11) showed an even greater intensification of the hr/hr^{rh} characteristics than did the Nn; hr/hr^{rh} type described above. It was completely lacking in hair, including the vibrissae, but the skin was intensely pigmented in the younger stages, and retained many dark, pigmented granules throughout life. At the time of depilation, and for a few weeks afterwards, wrinkling was somewhat more extreme than that in rhino mice, but during the third month the difference became less and less distinct, and eventually disappeared.

No histological studies have as yet been done on mice of this type.

(d) Naked; hairless. The author's observations on the interaction of the Naked and hairless mutations in the Nn; hr/hr compound agree closely with those reported by David, but will be presented briefly here for purposes of comparison with other types.

Mice of the constitution Nn; hr/hr show a decided retardation in growth shortly after birth. Their juvenile coat is short, sparse and rough, and loss of hair may begin around the eyes as early as twelve days after birth. Depilation on the body follows the Naked pattern, and leaves the skin moderately wrinkled. The feet, however,

show the typical "gloves" of the hairless pattern. The skin remains distinctly wrinkled throughout life (to about the same degree as that of the hairless/rhino hybrids), and contains many dark granules (presumably pigmented hair remains) which can be clearly seen in Fig.12.

One mouse of the constitution NN; hr/hr (offspring of a cross of Nn; hr/hr x Nn; hr/hr) has been observed. This mouse resembled the NN; hr/hr^{rh} animal described in the preceding section. It was very small, completely lacking in vibrissae or other hair, and showed intense pigmentation and wrinkling of the skin (Fig.13). Unfortunately the animal died under anaesthesia while a piece of skin was being removed for histological examination, so no observations on later stages of its development were obtained.

(e) rhino; waved. Several animals of the constitution hr^{rh}/hr^{rh}; wa$_1$/wa$_1$ were obtained by crossing waved-1 females to rhino males and breeding the F_1 hybrids inter se. In such a mouse the pattern of depilation is delayed, and is much more diffuse in character than that in non-waved; rhino mice; scattered hairs may remain in the skin until four or five weeks of age. Wrinkling in mice of this constitution is somewhat more extreme during the first few months of life than it is in non-waved; rhino mice, but the difference gradually diminishes as the mice

grow older. There is no regeneration of hair analagous to that occurring in hairless; waved mice (David, 1937; the author, unpublished).

(5) Transplantation experiments.

In order to discover whether the rhino condition is due to an effect of the rhino mutation specifically on the skin cells, or whether it is a secondary manifestation of an effect on some other part of the body (e.g. the endocrine system), reciprocal ectodermal transplants between rhino and normal mice were performed.

The technique used was that described by Reed and Sander (1937). Operations were done on the day of, or the day after birth. The pair of mice to be operated on were etherized. Small rectangles (about 3 x 5 mm. in size) of skin were interchanged between litter-mates. Each graft was then covered with a piece of cellophane a little larger than the graft. Over this was placed a strip of adhesive tape wide enough to cover the graft, and long enough to almost encircle the mouse's body. The bandage usually fell off three to five days after the operation.

For the purpose of the present experiment it was necessary (a) that the grafts should be successfully

incorporated on the host (for this reason reciprocal grafts had to be made between animals whose ancestors had been inbred by brother-to-sister matings for at least three or four generations so that the genetic constitution of the stock was sufficiently homogeneous to allow compatibility of host and graft tissue), and (b) that graft tissue could be distinguished with certainty from host tissue (e.g. by a difference in pigmentation of the hair) at all times. To this end, the "black-and-tan" mutant (a^t) was introduced into the rhino stock. Non-albino mice carrying this gene have a cream or tan-coloured belly and a darker back, the colour of which depends on the other coat-colour factors involved. Reed and Sander (1937) have shown that this dorso-ventral differentiation is determined early in embryonic life, and hence grafts of dorsal skin to a ventral environment and vice versa behave autonomously with regard to their pigmentation except for occasional "invasion hairs" within the borders of such grafts which receive at least their pigment cells from the host (Reed, 1938 a; Reed and Henderson, 1940). There is, moreover, a morphological dorso-ventral differentiation, dorsal hairs being longer, coarser and more closely spaced than ventral ones. Grafts from dorsal to ventral tissue behave autonomously with regard to this differentiation (Reed, 1938 a) as well as

in regard to pigmentation, with the possible exception of the "invasion hairs" mentioned above.

Black rhino males were crossed with females homozygous for the black-and-tan gene and also carrying factors for albino (c), dilute (d) and brown (b). F_1 animals were crossed inter se, and in following generations Black-and-tan rhino males were crossed with sisters which were heterozygous for the rhino factor and carried the black-and-tan mutant, together with various combinations of the other colour factors involved. Since the presence or absence of the rhino factor cannot be detected at birth, reciprocal grafts were made at random on the offspring of F_3 and later generations of this stock. Provided that both members of a pair survived the operation, both host and graft could be classified as to their colour and whether or not they were homozygous rhinos at fifteen days, at which time depilation has begun in rhino mice. In this way grafts from rhino to non-rhino, from non-rhino to rhino, from rhino to rhino, and from non-rhino to non-rhino were obtained.

Of the total of 74 grafts in which both members of a pair survived the operation, there were 25 which were considered successful "takes" (Table 1, column 1). These grafts showed little or no scab formation after the

operation, grew a healthy coat of hair, and remained in good condition for at least several months (the first of these is still healthy at 10 months of age - see below).

Table 1

Donor	Host	Successful "takes"	Partial "takes"	Graft grew hair but fell off within one month	Graft fell off without growing hair	Total
rhino	non-rhino	5	3	0	7	15
non-rhino	rhino	4	4	4	2	14
rhino	rhino	5	8	5	3	21
non-rhino	non-rhino	11	3	2	8	24
Total		25	18	11	20	74

Column 2, Table 1, represents 18 grafts in which patches of donor-type hairs were grown and remained on the graft for at least several months, but in which there were varying degrees of scab formation and replacement by host tissue. 11 grafts grew a complete coat of hair, but degenerated and sloughed off at about one month (column 3, Table 1). 20 grafts degenerated and fell off without growing hair at all (column 4, Table 1). The most successful grafts seemed to be of ventral to dorsal or dorsal to dorsal tissue, but the numbers in each class are too small to allow any significant conclusions on this matter, and hence will not be reported here.

The grafting operation delayed hair growth on the transplant. Hair was not produced by the donor tissue for anywhere from 6 to 17 days, the average time of appearance being about twelve days after birth.

The operative procedure also delayed depilation in grafts of rhino skin to either rhino or non-rhino hosts. First signs of thinning on successful rhino grafts occurred at from 21 to 27 days of age, but usually at about 25 days. This delay in depilation was to be expected in view of the delay in hair growth in such grafts, since rhino depilation does not occur until the later stages of the growth cycle.

In grafts of either rhino or non-rhino skin to rhino hosts there was also a delay in depilation of host hair around the borders of the transplant. In such cases host hairs remained around the borders of the graft for from 30 to 45 days, well after depilation was complete on the rest of the body.

Grafts of non-rhino skin to rhino hosts behaved autonomously. When such grafts were successful they grew a normal coat of hair and showed no signs of rhino depilation. Fig. 14 shows a graft of skin from the back of a Black-and-tan non-rhino, female which was transplanted at birth to the back of an albino rhino brother. At the

time of writing (150 days after the operation) the hair on the graft is still in good condition.

No effect of graft on host tissue or vice versa could be distinguished in this or similar cases. There was no delay in host depilation other than that attributable to effects of the operation as mentioned above. There was, however, a narrow strip on the outer border of these grafts in which the graft hair was lost, but this was probably due to degeneration and the formation of scar tissue in the zone of healing, and can not be considered an effect of the rhino tissue on the non-rhino graft.

Grafts of rhino skin to non-rhino hosts yielded results of an unexpected nature. Their behaviour indicated that rhino skin behaves autonomously when grown on a non-rhino host except in regions where it comes into close contact with cells of the host epidermis.

Indications of this phenomenon were obtained during the early stages of the investigation from cases where such grafts were only partially incorporated on the host and where there was considerable replacement of graft by host tissue (column 2, Table 1). For example, a piece of dorsal skin from a Black-and-tan rhino male was grafted to the belly of a brown-and-tan non-rhino female. The

graft was largely replaced by host tissue, but a small patch (about 1-2 mm. in diameter) of black, dorsal-type hairs remained, completely surrounded by the tan ventral-type hairs of the host. This patch of hair contained within it several (ca. 6) brown dorsal-type hairs - evidently "invasion hairs" (Reed, 1938 a) which had received at least their pigment cells from the host. The fact that these hairs were brown, rather than tan, showed that the tissue in which they were growing was dorsal in character (Reed, ibid) and therefore rhino in constitution. Hence the black hairs in this area must have been genetically rhino, and since they remained in the skin for over 119 days, their behaviour was non-autonomous, presumably due to some influence of the normal tissue with which they were in contact.

A more clear-cut example of this influence was provided in the case of ♀ 1313 (Fig. 15), a Black-and-tan non-rhino female on the back of which a piece of ventral skin from a Black-and-tan rhino littermate had been grafted at one day after birth. Hair was first visible on the graft at 12 days of age, and by 22 days there was a luxuriant growth of tan hair all over the graft area. During the next few days loss of hair began, first in the middle of the graft, and then spreading towards the sides. By 26 days there were a few scattered hairs in the center,

and a border of tan hairs around the edges of the graft. At the time of writing this mouse is nine months old, and still has well-defined bands of tan, ventral-type hairs on the right and left sides of the graft, a thin line of tan hairs on the anterior edge, and a few scattered tan hairs on the posterior border. There was a mild wrinkling and moderate thickening of the graft skin which was detectable at about 35 days, but which was never as extreme as that occurring in rhino mice at corresponding ages.

Histological examination of a small piece of skin removed from the edge of the graft at 166 days showed that there was a characteristic follicular keratosis and epidermal hyperkeratosis in the graft tissue, but that the hyperplasia was less extreme than that in rhino mice of the same age; the development of follicle-end and sebaceous-gland cysts was also less advanced. The thickness of the epidermal layers changed quite sharply in the transition zone between non-rhino and rhino skin, and so did the appearance of the follicle necks, but cyst-formation in the lower parts of the follicle in the rhino epidermis appeared definitely retarded in the zone adjacent to the non-rhino skin (Fig.30).

A similar case was one in which a piece of dorsal skin from an albino rhino male was grafted at birth to the back of a Black-and-tan non-rhino female. Bands of white hair still remain around the edges of this graft at

the time of writing, 118 days after the operation. In the three other cases in which successful rhino to non-rhino grafts were obtained, only irregular groups of graft-type hair remained around the borders of the graft. The eldest of these three mice has retained such groups of hairs for 235 days to date.

A similar influence of host on donor skin has been demonstrated in epidermal transplants from hairless to normal mice.

During the course of the grafting experiments some incidental observations were made concerning the effects of the grafting operation on the morphology of the hair. It was noticed that both graft and host hairs, when situated near the borders of a graft, were often irregularly curved or bent, like the hairs of genetically "waved" mice. It seemed probable that this appearance was due to alterations (such as tensions due to the formation of scar tissue after the operation) in the physical forces acting on the hair during its formation and growth. To test this assumption, some of the transplants when placed on the hosts were deliberately rotated through 180° from their original direction.

The behaviour of the hair on such grafts seemed to be affected by three factors: (a) a tendency of hair

within the graft to grow in its original direction, (b) effects of irregular tensions set up in the skin as the result of scar-tissue formation, and (c) a tendency for the hair to conform with the general anterior-to-posterior direction of the host hairs. For instance in the first coat grown by such a graft, the hairs on the anterior portion of the graft usually pointed anteriorly and showed varying degrees of irregularity and waviness. As time went on, these hairs gradually swung around until they were pointing posteriorly, and showed a marked "wave" pattern which gradually disappeared in later months. Hairs in the center of the graft usually stood more or less upright in the early stages, and showed much bending and irregular curvature. They too in later months became oriented in an anterior-to-posterior direction, and their irregularity decreased with age. On the posterior and right and left edges hair direction and form were highly variable and seemed to depend on the quantity and location of scar tissue in these regions, but again with increasing age, their irregularity decreased, and they tended towards an anterior-to-posterior orientation. It seems clear, therefore, that in transplantation experiments phenotypically waved hairs can be produced in genetically non-waved follicles merely as the result of the grafting operation.

This fact throws considerable doubt on Reed's (1938 b) interpretation of the results of his experiments involving transplantation of skin from waved to non-waved and from non-waved to waved mice, where he states that waved tissue influences the adjacent genetically non-waved hairs to become phenotypically waved. In the light of the author's observations reported above, it seems clear that if there is such an influence of waved on non-waved tissue, it will have to be demonstrated by some method other than that of transplantation.

(6) Effects of vitamin A treatment on rhino mice.

Because: (1) the rhino and hairless conditions seem to be essentially the result of a follicular keratosis. (2) a follicular keratosis can be produced in rats, humans and other animals as the result of a vitamin A deficiency, and (3) certain cases of inherited follicular keratoses in humans have been cured by massive doses of vitamin A, it was decided to investigate the effects of treatment with vitamin A on rhino mice. Unfortunately the results were rather confusing, and will only be reported briefly here, since no definite conclusions could be drawn from them.

Young rhino mice were fed 1-4 drops of Ling-liver oil (containing 250,000 International Units of

vitamin A per gram) daily. This treatment (roughly 10,000 - 40,000 units per day) unexpectedly produced an exfoliative dermatitis, usually within the first week, which was followed by a retardation in growth rate, weakness, emaciation and eventual death after about one month if treatment was continued. Treatment of young non-rhino controls produced a similar, but less extreme effect, accompanied by a thinning of the hair coat and the appearance of bare patches on the posterior portions of the body. It was thought that these unusual effects might be due to some toxic substance (not associated with vitamin A) in the Ling-liver oil, so similar treatments were tried using a Halibut-liver oil concentrate (containing 64,000 International Units of vitamin A per gram) instead. In this case the daily dose was roughly 2,500 - 10,000 units. Results were less extreme than, but essentially similar to those produced by the Ling-liver oil treatment.

Older mice (after 2 - 3 months of age) showed a much less marked response to treatment. There was no emaciation, weakness or death after such treatment. One rhino male, seven months of age, was fed 4 drops of Halibut-liver oil for a month and a half, but showed no reduction in weight and remained healthy and fertile. However the treatment did produce a desquamation of white

powdery flakes from the skin of the head and body, and wrinkling of the skin seemed to progress less rapidly in this mouse than in the untreated rhino control. Preliminary histological examination of skin from this mouse and from an untreated rhino litter-mate showed a reduction in size of the utriculi in the treated mouse. Skin from a 6½ month old rhino treated with 2 drops of Ling-liver oil daily for 11 days showed a similar effect, indicating that there may indeed be a relation between the rhino follicular keratosis and the concentration of vitamin A in the body tissues. However it must be born in mind (a) that the small number of cases observed does not completely preclude the possibility that the reduction in size of utriculi was due to causes other than the vitamin A treatment, and (b) that this reduction may have been the result of a generalized retardation in the body functions due to the treatment - a retardation expressed as a reduction in growth rate in young mice, but not readily distinguishable in fully-grown animals.

III. DISCUSSION

For the purposes of discussion the material presented in this paper may be conveniently divided into two sections: (A) the descriptive study of the developmental changes occurring in the skins of rhino and hairless mice (and their compounds with Naked), prior to and following depilation, and (B) an experimental attempt at analysis of the mode of action of the rhino and hairless mutations.

A. Descriptive.

It may be well at the beginning of the discussion to review briefly the outstanding characteristics of the mutations with which this study is concerned. It will be remembered that the presence of either the rhino or hairless allele in the homozygous condition causes the juvenile pelage to fall out, and that the presence of the rhino factor produces a subsequent wrinkling of the skin, whereas in hairless mice the skin remains relatively smooth. The pattern of hair loss is different in the two types, depilation being more diffuse and loss of hair on the feet occurring later in rhino than in hairless mice. The expression of both mutations is intensified by the presence of the mutant Naked, a dominant factor which causes imperfect keratinization of the hair shaft, a consequent breaking-off of the hair after erupting from the skin, and varying degrees of bending and buckling of the hair within the follicle.

The data derived from the descriptive study of the rhino and hairless syndromes, and the information thus obtained

concerning the mode of expression of these mutant factors will, for the sake of convenience, be discussed under several headings. It must be emphasized, however, that this division is made purely for the sake of discussion, and does not imply an actual independence of the various factors involved.

(1) Cause of depilation.

In normal mice, the hair (which is formed by a proliferation of cells from the follicle bulb and is pushed upwards through the follicle to the surface) is, during the growth-period, continuous at its proximal end with the follicular tissues. After the formation of the hair club during the Catagen stage, however, the hair becomes a separate unit with no direct connection with the follicle. At the end of the Catagen phase (when shortening of the follicle brings the hair club to a level just below the sebaceous glands) the hair is held in position presumably by: (1) the frictional forces existing between the hair shaft and the surrounding cells of the close-fitting wall of the hair canal, and (2) by the pressure due to the existence of an enlarged hair club at the base of the narrower cylinder in which the hair rests.

The first visible abnormality in the skin of both rhino and hairless mice is a hyperplasia of the stratified squamous epithelium of the skinsurface and follicle neck, and a widening of the hair canal, both of which are first evident at the time when the follicle has just started to shorten. That the widening of the hair canal is the result of a mechanical

stretching of the follicle wall due to a crowding of cornified cells into the hair canal is argued against by the fact that these squamous cells are loosely packed, showing no signs of being under pressure. It seems more likely that the widening is due to an increase in the growth activity of the outer layers of the wall of the follicle neck, leading to a lateral expansion and consequent increase in the circumference of these layers.

At any rate, it is reasonable to suppose that the widening of the hair canal reduces the resistance offered by the follicle neck to any outward movement of the hair shaft. It is lack of the support normally supplied by the tight-fitting follicle neck at the end of the growth period (when the hair is no longer directly attached to the follicle) which is considered to be the immediate cause of depilation. The subsequent irregular shortening of the follicle and malformation of the hair club are, in the author's opinion, very probably a result of the disruption of the normal developmental processes consequent on the hyperkeratosis and widening of the follicle neck.

These conclusions are in disagreement with those of David (1932 a) who states that loss of hair in the hairless mouse is due to a failure of the hair club to form properly. David does not describe any follicular keratosis previous to depilation, and states that "following shedding, until new hairs are formed ..... the upper portion of the follicle

appears normal in all respects." She does, however, mention that although the stratum corneum in the hair canal appears normal after depilation, it gradually increases in amount until it appears as a rather compact plug in old animals. Unfortunately she does not illustrate the stages previous to shedding, but her illustrations of sections of hairless skin at the time of the first regeneration of hair (4-5 weeks) show a definite follicular keratosis which must have developed before this stage.

It is possible that David is right in assuming that the immediate cause of hair loss is a malformation of the hair club; the difference between the hairless stocks of David and of the writer in the time of appearance of the hyperkeratosis could be explained by a difference in the residual heredity of the two strains. On the other hand, in view of the facts (1) that the entire hairless (and rhino) syndrome is characterized by a tendency towards hyperplasia of the epidermis and its derivatives, (2) that it has been clearly demonstrated in the present investigation that in our hairless stock shortening of the follicle is preceded by hyperplastic changes in the wall of the follicle neck, and (3) that the malformation of the hair club is variable, and even in David's illustrations does not always seem great enough to appreciably reduce its "hair-retaining" powers, it seems more reasonable to conclude that the hyperplasia gives rise to the irregular follicle shortening, rather than that the two effects are separate branches of the original gene-initiated reaction-chain.

(2) Pattern of hair loss.

The difference between hairless and rhino mice in the pattern of hair loss may be at least partially explained on the following assumptions. The time at which any individual hair is lost depends on (1) the rate at which widening of the hair canal occurs, and (2) the time at which the hair club is formed and shortening of the follicle takes place. If enlargement of the hair canal is relatively slight (as in hairless mice) the hair presumably will not fall out until the end of the Catagen phase, when the hair club is raised to a level just below the sebaceous gland and offers a minimum of resistance to outward movement of the hair. The pattern of hair loss will therefore correspond to the pattern in which the hairs of the juvenile pelage reach the Telogen phase. That it actually does so is shown by a comparison of the hairless pattern of depilation with the pattern in which the juvenile pelage reaches Telogen, as diagrammed by Dry (1926). Both the area of depilation and the area in which the hairs have reached Telogen appear first on the throat and around the eyes and spread in an anterior-posterior wave along the body, proceeding more rapidly on the feet and belly than on the back. The boundaries of these areas in both cases are relatively well-defined.

If, on the other hand, there is a marked widening of the hair canal as in rhino mice it is likely that, although

the general pattern remains the same, some hairs will fall out before shortening of the follicle is complete, resulting in a more diffuse thinning of the hair coat instead of a sharp line of demarcation between haired and non-haired areas.

No explanation can be offered at the present time for the delay in depilation on the feet of rhino mice, since no observations were made on the behaviour of the follicles in this region during the period of depilation.

(3) Cyst formation.

Another point of disagreement with David (1932 a) is in regard to the formation of hair-follicle and hair-canal cysts, which she considers to be due to "pressure exerted by the growing hair when its progress is impeded by some obstruction." This refers to hairs of the second generation which, in hairless mice, develop in irregularly directed follicles and press against the sides of the hair canal during their progress towards the surface. In rhino mice hair-canal cysts develop in follicles which have never contained an irregularly shaped hair shaft; the shaft of the first-generation hair is straight and rhino follicles never produce hairs after the juvenile pelage is lost. Apparently the utriculi arise as an expression of the hyperplastic tendencies of the rhino epidermal tissues, as do the cysts of the sebaceous glands and follicle ends. It seems reasonable to suppose that utriculi can also develop in hairless mice as a result of the hyperplastic tendencies inherent in hairless skin, particularly since the writer has

observed utriculi in the necks of follicles which show no signs of hair regeneration. It is possible, however, that in some cases utriculi do arise as the result of pressure on the hair canal wall, since the presence of irregularly bent hairs in the follicles of Naked; hairless mice is associated with an increase in utriculus formation (see below).

(4) Wrinkling.

The degree of wrinkling in the skins of mice carrying various combinations of the hairless, rhino and Naked factors is directly proportional to the extent of utriculus formation. Wrinkling is apparently the result of an increase in surface area of the skin caused by the widening of the hair canals. Thickening of the skin on the other hand is evidently a result of cyst formation in the lower parts of the follicles.

The gradations of wrinkling found in mice carrying the hr and hr^{rh} mutations in various combinations with the Naked factor are determined by two processes. (1) There is an inherent tendency towards hyperplasia in the epidermal tissues of hairless, rhino and hairless/rhino mice. The hr^{rh} factor when homozygous has a stronger effect than the hr factor, while the interaction of the two mutations in the hr/hr^{rh} hybrids produces an intermediate effect. (2) The degree of hyperplasia in mice of any of these types, although primarily determined by what factors are present at the Hr locus, is intensified if such animals are also carrying the Naked mutation, the

exaggeration being greater in NN than in Nn mice. This effect may be due to mechanical stimulation of the follicle walls by the pressure of irregularly bent or coiled hairs of the Naked type. (Hypertrophy in response to irritation is characteristic of stratified squamous epithelia.) On the other hand, the retardation in growth-rate and the intensification of the Nn characteristics before follicle widening occurs in such mice indicate that there may also be an earlier interaction of the hr (or hr^{rh}) and N reaction-chains.

Thus various combinations of the three mutants (hr, hr^{rh} and N) can give nine possible degrees of wrinkling. Eight of these types have been observed; in approximate order of increased intensity of wrinkling they are: hr/hr hr/hr^{rh} = Nn; hr/hr Nn; hr/hr^{rh} NN; hr/hr = hr^{rh}/hr^{rh} NN; hr/hr^{rh} Nn; hr^{rh}/hr^{rh}. (The NN; hr^{rh}/hr^{rh} compound, in which wrinkling would probably be even more intense, was not observed.)

This seriation is based on observations made during the first few months after depilation. In later stages there is evidently an upper limit to the amount of wrinkling the skin can display. Hence in the compounds in which the degree of wrinkling approaches this upper limit, differences in wrinkling between the different types become less distinct with increasing age, and may eventually disappear.

(5) Dominance relations of the hr and hr^{rh} factors.

The similarity of the early stages in development of the rhino and hairless conditions, in conjunction with the

fact that the hr^{rh} and hr factors are alleles, indicates that the two mutants affect the same reaction-chain. Macroscopically the hairless factor appears to be dominant to the rhino allele in the early stages of the condition, since hr/hr^{rh} hybrids lose their hair according to the hairless pattern, and regenerate a sparse, fuzzy hair coat at about six weeks of age. In older mice, on the other hand, the rhino factor appears (in the present stocks) to be partially dominant, since these mice develop a wrinkling less extreme than, but similar in pattern to that of rhino mice. Histological observations have shown, however, that the character of hairless/rhino skins is intermediate between that of the rhino and hairless types at all stages. That depilation occurs according to the hairless pattern in these mice is evidently because widening of the hair canal does not occur rapidly enough to result in the more diffuse rhino type of shedding (see section (2) above). There seems to be no fundamental change in the pattern of development corresponding to the change in "dominance" observed macroscopically; the"dominance" of one allele or the other at different stages is more apparent than real.

The writer's macroscopic observations are at variance in this respect with those of Howard (1940)who stated that "F_1 animals resemble hairless mice both in the manner of hair loss and in the smooth skin of the adults." The discrepancy is very probably due to a change in the residual heredity of the hairless and/or rhino stocks between the times at which

Howard and the writer made their observations (ca. 3 years). Howard's notes describe hr/hr^{rh} hybrids at eight months of age which showed no wrinkling on the back and only a few mild wrinkles on the nose, whereas the hybrids observed by the author often show definite signs of wrinkling at six to seven weeks of age (cf. Fig 7). Evidently the expression of the hairless and rhino factors is subject to alteration by genetic modifiers. This is not surprising in view of the range of variability shown by various strains of hairless mice in the past (e.g. the variation in degree of cyst-formation described by David, and the differences in fertility, vigour and suckling ability between the stocks of Crew, David and the author).

The evidence derived from the descriptive study of the hairless and rhino mutant types concerning the mode of expression of these mutations may be summed up as follows. The first observable abnormality in mice homozygous for either of these factors is a hyperkeratosis of the follicle neck accompanied by a widening of the hair canal, beginning at the time when active growth ceases in the follicle. The hyperkeratosis and widening of the hair canal (which is evidently the immediate cause of depilation) is considered to be one manifestation of a general tendency towards hyperplasia of epidermal tissues homozygous for the hairless or rhino factors - a tendency of which cyst formation in the follicle ends and sebaceous glands, hyperplasia of the surface layers of the epidermis, and

overgrowth of the nails are further manifestations. The rhino factor has a more extreme effect than the hairless mutant, and the hairless/rhino combination is intermediate between the two, indicating that the alteration in the normal developmental pattern brought about by these mutants is one of degree, rather than an "all-or-none" type of reaction.

B. Experimental.

(1) Transplantation results.

The object of the transplantation experiments was to discover how rhino tissue behaved when transplanted into a normal environment and vice versa. To do this it was necessary to have some criterion - in this case hair colour and form - by which graft skin could be distinguished from host skin. A possible complication was introduced by the occurrence in the grafts of "invasion hairs", which Reed (1938 a) and Reed and Henderson (1940) described as possessing host-type pigment but determined as to their dorsal or ventral character by the nature of the graft, rather than of the host. For instance dorsal tissue from a Black-and-tan mouse grafted to the belly of a brown-and-tan animal produced black hairs, with a few brown "invasion hairs" near the border of the graft. The brown hairs may have arisen either (1) by a migration of epithelial cells from the host into the graft, and the formation by them of complete hairs, which contained their own type of pigment but were dorsal in structure because they developed in dorsal-type tissue, or (2) by a migration of melanoblasts from the host into

the graft and their subsequent entrance into dorsal-type follicles of the graft where they formed brown pigment according to their dorsal environment.

The experiments of Reed and Henderson (1940), although they rule out the possibility of the migration into the "invasion hairs" of melanophores (in which the pigment granules are already formed), do not present conclusive evidence in favour of either of the above possibilities. Nevertheless Reed seems to favour the first theory - that "invasion hairs" arise from follicles produced by epithelial cells which have migrated from the host. In the author's opinion the second hypothesis is the better of the two. It seems more reasonable to assume that melanoblasts migrate through the dermis and become incorporated in a developing graft follicle than that a group of epidermal cells from the host pushes its way between the cells of the donor epidermis into the graft and then proceeds to form a a complete hair, particularly since Rawles (1940) has clearly demonstrated that pigment-producing cells of the mouse epidermis can migrate extensively in the coelomic cavities of chick embryos.

If complete follicles can be formed by epidermal cells migrating into a graft it should be possible to demonstrate this process by utilizing a mutation that produced a morphological abnormality of the hair shaft, provided that the mutant tissue behaved autonomously. The presence, in grafts of normal skin to mutant-type hosts, of "invasion hairs" showing both host-type

pigment and the morphological variation, would be definite proof that these hairs were formed by epithelial cells from the host. On the other hand, the occurrence of "invasion hairs" with host-type pigment but not showing the morphological abnormality would not disprove this theory, since there would be no way of showing that the mutant tissue, when growing in a normal environment, did not produce phenotypically normal hairs(i.e. was non-autonomous).

As far as the conclusions to be drawn from the author's grafting experiments is concerned, it makes little difference which explanation one accepts for the presence of "invasion hairs", since at no time have these "invasion hairs" occurred with a frequency sufficient to create any difficulty in distinguishing graft from host tissues.

Results of the grafting experiments reported in this paper show quite definitely that rhino epidermal tissues, when in close proximity to those of normal epidermis, behave non-autonomously. Apparently there is something present in the epidermis (but not in the blood-stream) of non-rhino mice which influencesgenetically rhino epidermis adjacent to it to develop normally instead of according to the rhino pattern.

Two cases which clearly demonstrate this effect were described in detail in the Experimental section of this paper. The first was the case in which dorsal tissue from a Black-and-tan rhino donor was grafted to the belly of a

brown-and-tan non-rhino host. The graft was largely replaced by host tissue, but the small area of graft tissue that survived grew a tuft of black, dorsal-type hairs. Among these there appeared a few dorsal-type brown hairs, evidently "invasion hairs" which had received at least their pigment cells from the host, but which were dorsal because they were growing in dorsal tissue. The black hairs, therefore, must certainly have been formed from tissue that was dorsal, and therefore genetically rhino. It is hardly possible that this patch of skin could retain its dorsal organization if all the hairs in it were formed from cells migrating from the host, hence the possibility that the black hairs arose as a result of black pigment from the donor tissue becoming incorporated into follicles formed of host tissue is ruled out. Since the black hairs were genetically rhino, and since they remained in the skin for many months, their behaviour was non-autonomous.

The case of 1313 in which ventral skin from a Black-and-tan rhino mouse was grafted to the back of a Black-and-tan non-rhino sister demonstrates even more clearly the non-autonomous character of rhino skin when in close proximity to normal epidermis. Here the tan hairs on the borders of the graft did not fall out, although those in the interior of the graft did. The objection might be raised that these persistent hairs could have developed from cells which had migrated from the host tissue but were ventral in nature because of their proximity to the ventrally organized graft tissues. That such is

not the case is indicated by (1) the fact that the hairs around the graft borders developed just as soon as, or sooner than the other hairs of the graft; if they had been "invasion hairs" they would presumably have shown a delay in development (see Reed, 1938 a) and (2) in all the grafting experiments described by Reed or observed by the author the "invasion hairs" were present only as scattered individual hairs, never in well-defined rows as they were in the case of 1313. Moreover, in the case where dorsal albino rhino skin was grown on the back of a Black-and-tan non-rhino host, if the hairs that remained on the edges of the graft had been "invasion hairs" they would have been black instead of white.

The non-autonomous behaviour of rhino and hairless epidermal tissue when in close proximity to normal skin shows that the mutant skin cells are supplied by the normal epidermis with something (let us call it the H factor) necessary for the maintenance of epidermal stratified squamous epithelium in its normal non-proliferative condition. The mutant epidermal tissues can evidently utilize the substance H, but apparently cannot produce it, at least in quantities sufficient to enable the skin to remain normal. Moreover, the H factor must be something produced intracellularly, and not present in the blood stream of normal mice. (Its utilization may well be correlated in some way with the phsyiological changes occurring in the follicle during its transition from a state of active growth in the Anagen phase to one of rapid involution during

Catagen.). The mode of action of the rhino and hairless mutations, therefore, may be to interfere in some way with the reaction-chain leading to the production of H. If such is the case, it might be possible to enable mutant tissue to develop normally not only by supplying the H factor itself, but by raising the concentration of a precursor of H sufficiently to allow production of H in normal quantities by mutant cells

(2) Results of treatment with vitamin A.

In view of the similarity between the histological appearance of the skins of rhino and hairless mice and the cutaneous manifestations of hypovitaminosis A, it was thought that the action of the hr^{rh} and hr mutations might be to interfere in some way with the metabolism of vitamin A by the cells of the epidermis. This was the reason for the vitamin A treatments, but unfortunately the results are very inconclusive. The desquamative dermatitis, hair-thinning (in normal mice), and emaciation resemble the lesions produced by feeding large doses of various vitamin A concentrates to rats (Eddy and Dalldorf, 1941; Rodahl and Moore, 1943), but it is not yet certain that the toxicity of these vitamin A concentrates is due to vitamin A itself; it may be due to some other substances contained in the concentrates.

However, the retardation obtained in the rate of utriculus formation, and the fact that treatment seems to affect rhino and hairless mice more strongly than it does

normal animals is a further indication of a possible relationship between the development of the rhino and hairless syndromes and the metabolism of vitamin A.

It would be of interest to carry out further investigations along the following lines: (1) to make a more detailed study of the effect of vitamin A concentrates on rhino and hairless mice and of the variation in effect with size of dose, age of mouse and length of treatment, (2) to study histologically the changes taking place in the skins of normal mice fed large doses of vitamin A concentrate, and (3) to study the effect of pure vitamin A and of carotene on rhino and hairless mice.

Further studies along these lines, however, will, as far as the author is concerned, have to be postponed for the duration of the war.

IV. SUMMARY

1. A study has been made of the expression of the mutation rhino (hr^{rh}), an autosomal recessive the present of which causes a falling-out of the juvenile pelage and a subsequent wrinkling and thickening of the skin and hypertrophy of the nails,

and of its interaction with two other factors affecting hair-growth, namely:

hairless (hr) an autosomal recessive, allelic to rhino, the presence of which also causes the juvenile pelage to fall out but leads to little, if any, wrinkling of the skin, and

Naked (N), an autosomal dominant, semi-lethal when homozygous, which in the heterozygous condition produces an imperfect keratinization of the hair, a consequent bending and buckling of the hair shaft within the follicle, and a breaking off of the hair after it has erupted.

2. The rhino mutation in the homozygous condition is expressed as a tendency towards hyperplasia of the epidermal tissues and their derivatives. The hyperplasia first becomes evident as a hyperkeratosis of the epidermis and follicle neck wall at the time when active growth of the hair ceases and the follicle begins to shorten. Development of the follicular keratosis is associated with a widening of the hair canal due to a lateral expansion of the hyperplastic layers of the follicle neck, and

a subsequent irregular shortening of the follicle. The cause of hair loss is considered to be the widening of the hair canal and consequent lack of the support supplied by the normallly tight-fitting follicle neck when shortening of the follicle raises the base of the hair to a level just below the proximal end of the hair canal. The hyperplastic tendencies of the epidermal derivatives are further expressed by (a) the development of hair canal cysts (utriculi) which leads to an increase in surface area and consequent wrinkling of the skin, (b) the formation of sebaceous-gland and follicle-end cysts which cause the thickening of the epidermis, and (c) over-growth of the nails.

3. The expression of the hairless mutation resembles, in general, that of the rhino factor, but the hyperkeratosis is less extreme. Widening of the hair canal in hairless mice is sufficient to allow loss of the hair, but few follicles develop utriculi. Sebaceous-gland and follicle-end cysts develop more slowly than they do in rhino mice, and many follicles retain, for a time, the capacity to regenerate hairs.

4. Although hairless/rhino hybrids lose their hair according to the hairless pattern and in later life develop varying degrees of rhino-type wrinkling, histological observations show that the character of the skins of these mice is intermediate between the rhino and hairless types at all stages.

5. The presence of the Naked factor in either rhino or hairless mice or their F_1 hybrids exaggerates the follicular keratosis, leading to an increase in number and rate of formation of utriculi, and an consequent increase in the degree of wrinkling. This interaction may be partly due to a mechanical stimulation of the follicle wall neck by the irregularly bent, Naked-type hairs, but there are indications of an interaction of the Naked and rhino (or hairless) reaction-chains previous to the appearance of the hyperkeratosis.

6. Transplantation experiments have shown that rhino epidermal tissues, when in close proximity to normal epidermis, behave non-autonomously, indicating that rhino skin cells are unable to produce some substance necessary for the maintenance of normal stratified squamous epithelium, but are able to utilize the substance if it is supplied. Similar results were obtained with hairless mice.

7. Results of the treatment of rhino and hairless mice with vitamin A, although complicated by a possible hypervitaminosis, indicate that the presence of the homozygous rhino or hairless mutation may interfere in some way with the metabolism of vitamin A by the epidermis and its derivatives.

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VII. DESCRIPTION OF PLATES

PLATE I.

Fig. 1. rhino, 19 days. Depilation is rather more advanced than usual, but the feet are still haired. (Photograph by Howard).

2. rhino, 10 months. Note wrinkling of skin, and overgrowth of nails. (Photograph by Howard).

3. hairless, 17 days. The feet are completely bare (cf. Fig. 1).

4. hairless, 6 months. Note smooth skin, some overgrowth of nails.

PLATE II.

Fig. 5. hairless, 13 months. Skin only slightly wrinkled, nails elongated.

6. Naked, (Nn) 19 days.

7. hairless/rhino, 2 months. Note mild rhino-type wrinkling.

8. hairless/rhino, 6 months. Compare with smooth-skinned hairless mouse of same age (fig. 4).

PLATE III.

Fig. 9. Naked; rhino, (Nn; hr^{rh}/hr^{rh}), 19 days old. Note wrinkling, skin pigmentation, scattered hairs remaining in depilated areas. Compare with rhino of same age (fig. 1).

PLATE III.

Fig. 10. Naked; hairless/rhino (Nn; hr/hr^{rh}) 2 months. Wrinkling more intense than that of hairless/rhino of same age (fig. 7).

11. homozygous Naked; hairless/rhino (NN; hr/hr^{rh}), 2 months. Wrinkling more intense than in Nn; hr/hr^{rh} of same age (fig. 10). Note also the reduction in size.

12. Naked; hairless (Nn; hr/hr), 6 months. Note wrinkling and pigment grains in skin. Compare with hairless of same age (fig. 4).

PLATE IV.

Fig. 13. homozygous Naked; hairless (NN; hr/hr), 18 days. Note extreme wrinkling, pigmentation and reduction in size.

14. A graft from the back of a Black-and-tan non-rhino mouse to the back of an albino rhino litter-mate. 52 days.

15. A graft of ventral skin from a Black-and-tan rhino mouse to a Black-and-tan non-rhino litter-mate. 141 days old. Note tan hairs on borders of graft.

PLATE V.

Fig. 16. Normal, 13 days. Follicles in late Anagen. Note close-fitting follicle neck. (The irregular bending of the follicles is an artefact.) X 70.

17. rhino, 14 days. Note follicular keratosis. X 70.

18. Normal, 18 days. Follicles in late Catagen. X 140.

19. rhino, 18 days. Note hair-canal widening, irregular follicle-shortening, hair club malformation. X 140.

20. rhino, 25 days. X 140.

21. rhino, 47 days. Showing typical utriculi. Arrows indicate (1) a young sebaceous-gland cyst, and (2) a young follicle-end cyst.

PLATE VI.

Fig. 22. rhino, 4 months. Showing well-developed cysts, disappearance of fat-stores in dermis. X 70.

23. rhino, 14 months. X 50.

24. hairless, 15 days. Note follicular keratosis. X 140.

25. hairless, 21 days. X 140.

26. hairless, 15½ months. X 50.

27. Naked (Nn), 17 days. Note irregularity of hairs. X 70.

PLATE VII.

Fig. 28. hairless/rhino, 20 days. Note follicular keratosis. Compare with rhino at 18 days (fig. 19) and hairless at 21 days (fig. 25). X 140.

29. Naked; hairless/rhino, 24 days. Showing exaggerated follicular keratosis, very irregular hair growth. X 70.

30. Graft of rhino to normal skin, (see fig.15). Normal follicles can be seen at the right of the picture. The follicular keratosis and cyst formation increases as the distance away from the normal skin increases.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

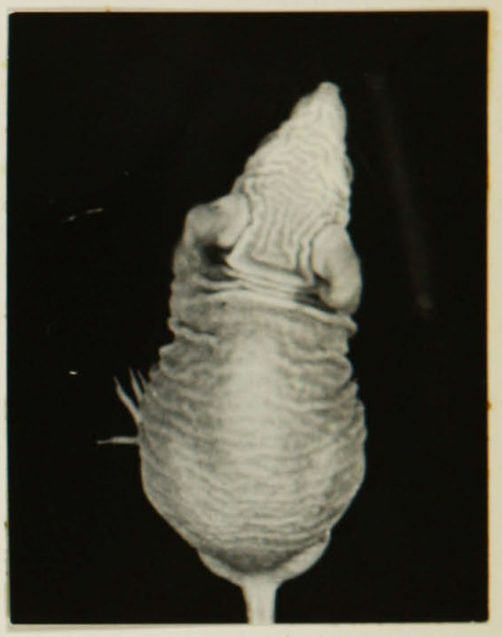


Fig. 10

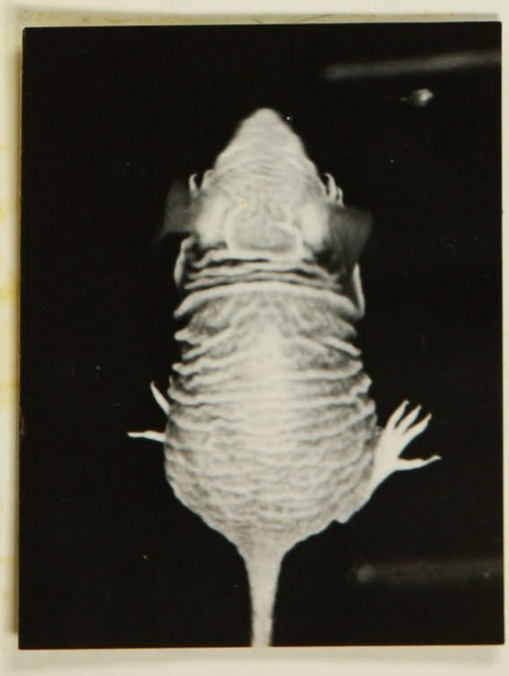


Fig. 11

Fig. 12

Fig. 13

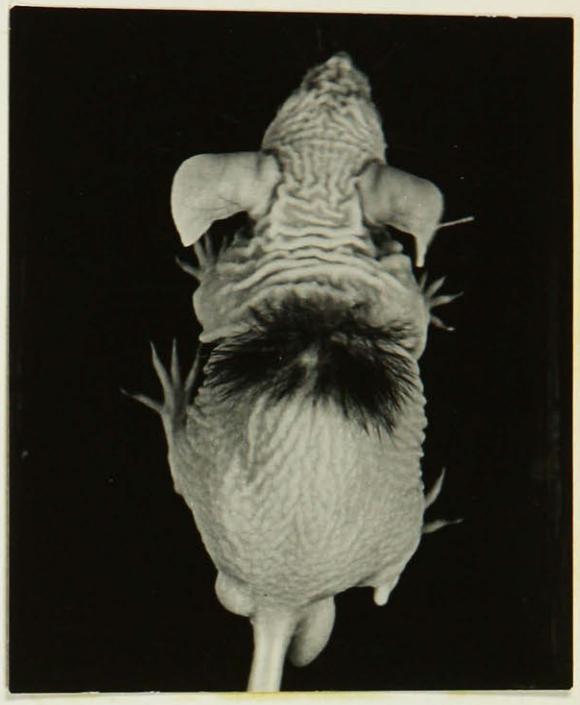


Fig. 14

Fig. 15

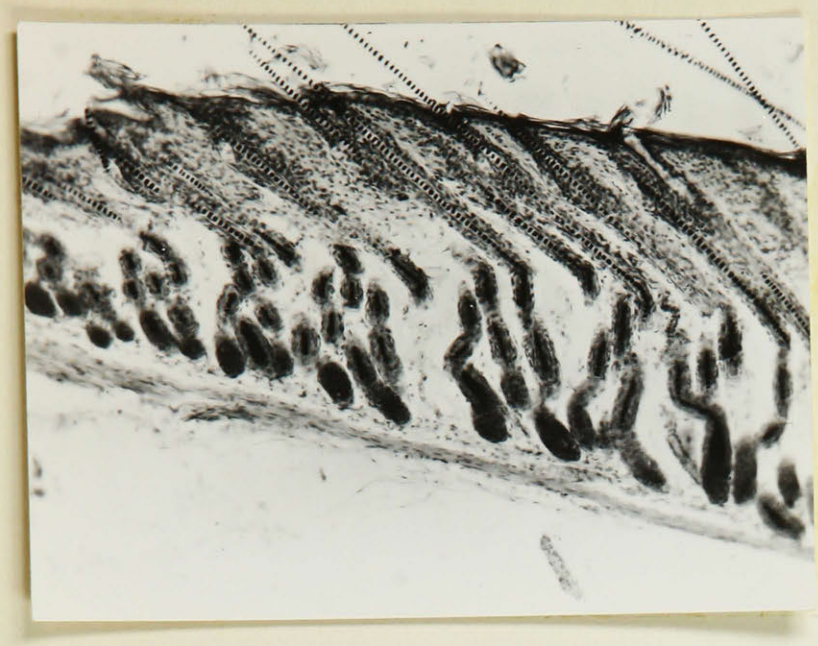
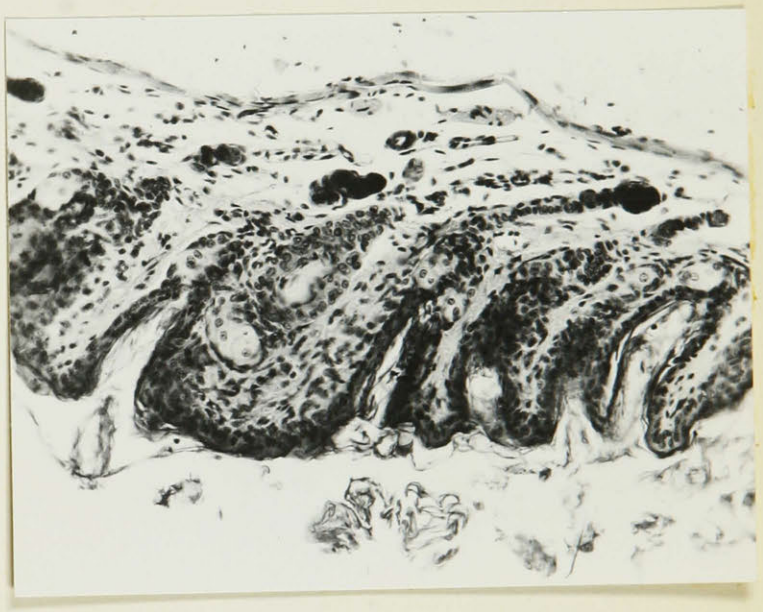


Fig. 16

Fig. 17

Fig. 18



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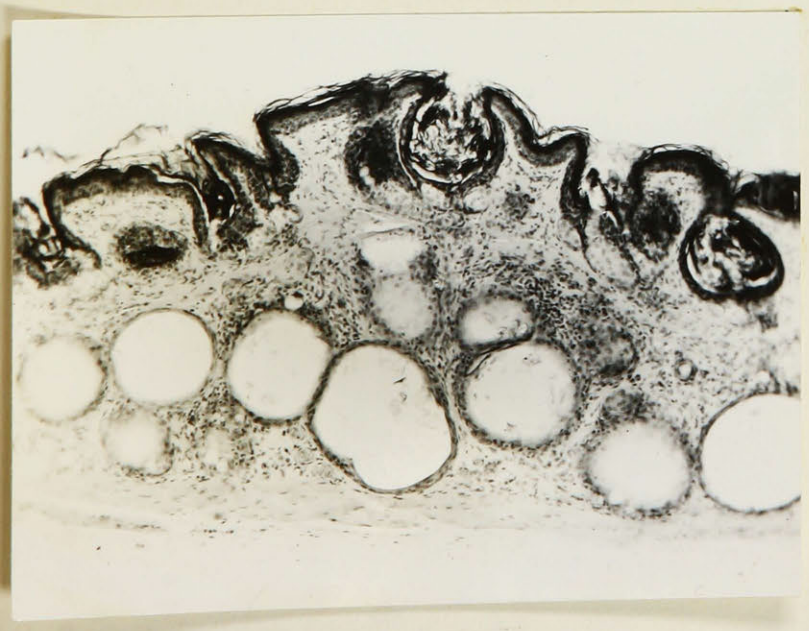


Fig. 20

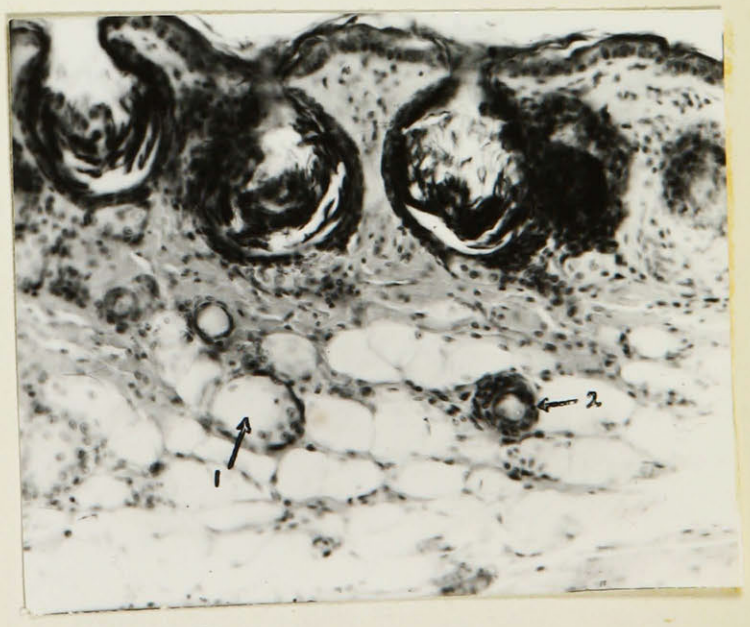
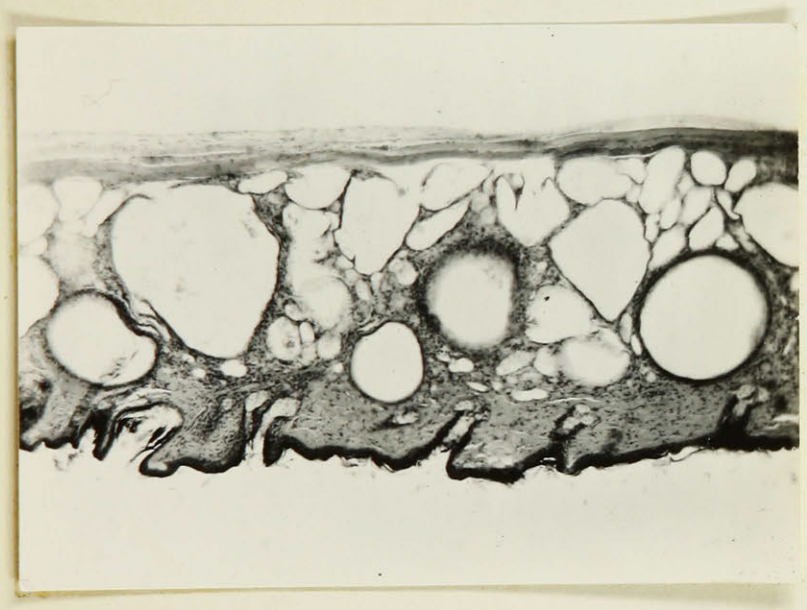


Fig. 21

Fig. 22

Fig. 23



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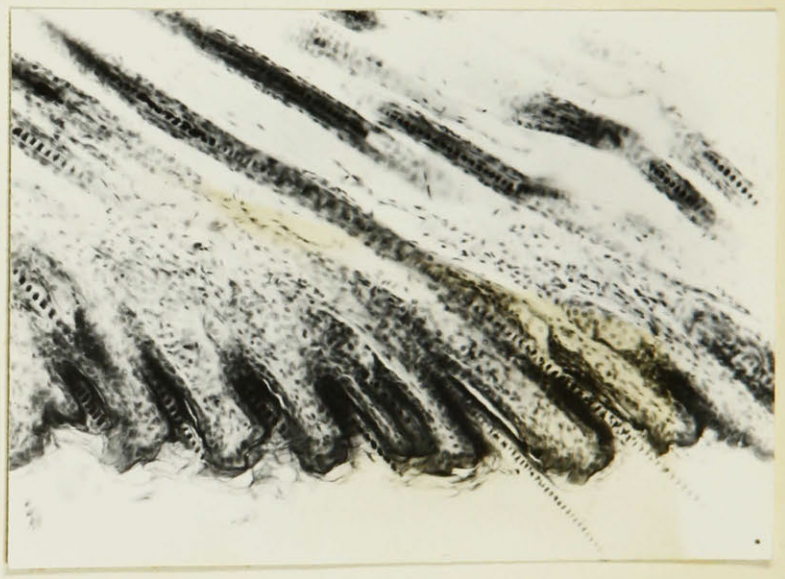

Fig. 25

Fig. 26

Fig. 27

Fig. 28

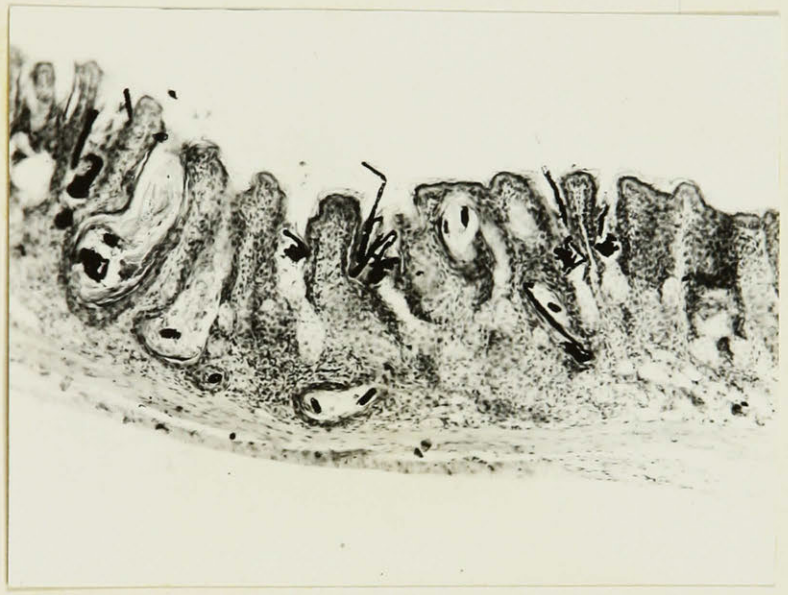

Fig. 29

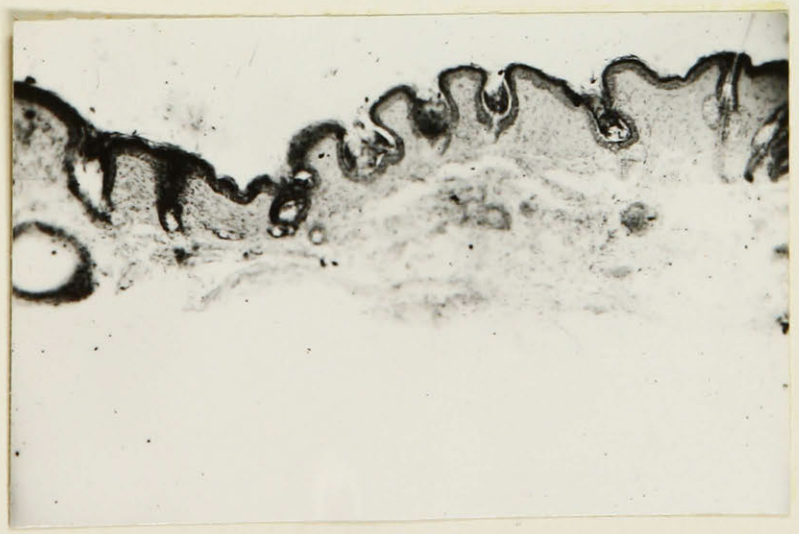

Fig. 30