THE EFFECT OF VITAMIN A IN HYPERKERATOTIC
MOUSE MUTANTS

by

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TABLE OF CONTENTS

I. INTRODUCTION.....	1
A. Vitamin A Deficiency.....	3
B. Clinical Use of Vitamin A in Dermatology.....	6
C. Description of Mouse Mutants	7
D. Treatment of Hyperkeratotic Mouse Mutants.....	11
II. MATERIALS AND METHODS:	
A. Mice.....	14
B. Vitamin A.....	15
C. Techniques and Routes of Administration.....	15
D. Gross Observations.....	17
E. Histology.....	18
III. EXPERIMENTAL RESULTS:	
A. Systemic Toxicity.....	20
1. Rhinos -	20
2. Hairless -	22
3. Normals -	26
B. Effects of Vitamin A on the Hair Growth Cycle in	
Mutant Mice.....	28
1. The First Hair Coat -	28
2. Subsequent Hair Growth -	30
C. Gross and Histological Observations on the Skin of	
Vitamin A-Treated Mice.....	34
1. Rhinos -	34

III. EXPERIMENTAL RESULTS: (CONT'D)	
2. Hairless -	37
3. Effect of Vitamin A on Depilation in Hairless Mice -	45
4. Effect of Biotin -	46
5. "Epidermal Warts" -	47
D. Effect of Sex and Pregnancy on Response to Vitamin A.	49
1. Untreated Mutant Mice -	49
2. Treated Hairless Mice -	49
3. Sex and Vitamin A-Induced Toxicity -	52
E. Congenital Defects Induced by Vitamin A.....	54
IV. DISCUSSION.....	61
A. Effect of Vitamin A on the Skin.....	61
B. Differences Between Rhino and Hairless Mice in Response to Treatment.....	65
C. Role of Vitamin A in Keratinization.....	66
D. Relation of Vitamin A to Mutation at the Hairless Locus in the Mouse.....	69
E. Sex Difference in Response to Vitamin A.....	71
F. Pregnancy and Skin Changes.....	73
G. Congenital Defects.....	75
V. SUMMARY AND CONCLUSIONS.....	84
Suggestions for Further Study.....	85
VI. ACKNOWLEDGEMENTS.....	88
VII. BIBLIOGRAPHY.....	89
VIII. PLATES.....	96

I. INTRODUCTION

The skin and its appendages in mammals provide an excellent medium for investigating the effect of experimental procedures on the development and morphology of a specific integrated system. Many mutations which affect the pilary system are known in various species of mammals (David,1932; Thigpen,1951). The most striking are those which result in hypotrichosis of varying degrees ranging from slight thinning of the pelage in some mutants to virtually complete hairlessness in others (Anon.,1917; Brooke,1926; Castle,1933; Castle et al, 1955; Carter and Phillips,1950; Crew and Mirskaia,1931-32; Dickie,1955; Dickie and Woolley,1950; Falconer et al,1950; Falconer and Snell,1952; Garber,1952; Green,1954; Howard,1940; Hutt and Saunders,1953; Letard, 1938; Loeffler,1934; Lyon,1956; Lebedevsky and Dauvert,1927; Roberts and Carroll,1936; Summer,1924; Wilder et al,1932). Mutations which involve faulty development of epidermal appendages are also known in birds (Abbott and Asmundson,1957; Danforth,1928; Hutt and Sturkie,1938; Sturkie,1942), and cases resembling hypotrichotic mutations in animals have been described in man (Albrechtsen and Svendsen,1956; Damste and Prakken,1954; Kylin and Dicker,1939).

In these forms of hereditary hypotrichosis the mutant genes appear to affect skin cells directly (Fraser,1946; Roberts,1939); usually keratinization is altered. Attempts to modify the effect of mutations affecting the pilary system have included, therefore,

therapeutic trial of substances known to have or suspected of having something to do with the process of keratinization. Thus, on the premise that it is the conversion of -SH groups to -S-S- cross-linkages that is deficient or abnormal, sulfhydryl-containing compounds such as cysteine and benzyl mercaptan have been used. The results of Martin and Gardner (1935), who reported hair growth in "hairless" rats fed cysteine, could not be confirmed, however, in subsequent investigations (Roberts, 1937; Roberts et al, 1940; Gershberg, 1939). Benzyl mercaptan had no effect in "Naked" mice, and caused exaggeration of the keratin defect in "hairless" mice (David, 1934). Among other "essential factors" assumed lacking or deficient, the use of cystine was without effect in "hairless" rats (Martin and Gardner, 1935; Roberts et al, 1940), and hair hydrolysates (cystine plus undetermined "keratin protein" precursors) had doubtful success in cases of alopecia areata in man (Flesch, 1954). The large number of investigations relating hair growth to humoral factors (reviews in: Baker, 1951; Chase, 1954; Flesch, 1954) provide virtually no information as to modes of action of hypotrichotic mutant genes.

The relation of vitamin A to keratinization is apparently more intimate than that involving SH-containing compounds. Inhibition of epithelial keratinization has been reported repeatedly (e.g., Kahn and Bern, 1950), and some investigators have attributed all epithelial metaplasia and hyperkeratinization to a local lack of or inactivation of vitamin A (Bern, 1954; McCullough and Dalldorf, 1937). A review

of the pertinent literature on the action of vitamin A on normal epithelial tissues (with emphasis on the skin) will serve as a useful introduction to investigations (including the present study) on the effect of this nutritional factor in hypotrichotic hyperkeratotic mouse mutants.

A. Vitamin A Deficiency

The consequences of vitamin A deficiency are extensive, in both animals (Wolbach, 1954) and man (Mason, 1954). In deficient animals, lesions occur in the eye and adnexa and in respiratory, intestinal and urogenital tracts. Epithelia of those organ systems which normally have a secretory function in addition to their role as covering layers undergo what Wolbach (1954) has described as a "keratinizing metaplasia." The sequence of events has been described as follows: "There is atrophy of the surface cells, followed by reparative proliferation of basal cells, and finally growth and differentiation of the new cells into a stratified keratinizing epithelium" (Wolbach and Bessey, 1942). The same type of epithelium apparently is produced in every region of the body involved, and appears to be comparable in all its layers with the epidermis of the skin. The basal cells, however, retain the capacity for their original functions throughout the period of metaplasia, for following vitamin A administration, they regenerate a normal epithelium characteristic for each particular region (Wolbach and Howe, 1933).

Bone formation in growing animals is affected adversely by

avitaminosis A (Mellanby,1941; Wolbach,1954). Injury to the central nervous system during experimental vitamin A deficiency has been ascribed to the effects of abnormal skeletal growth (Mellanby,1944; Wolbach,1954), although some investigators have reported degeneration of nerves and brain aberrations unrelated to skeletal malformations (Rigdon,1952; Lamming et al,1954). Defects in incisors, which grow continuously throughout life in rodents, have also been observed in vitamin A-deficient rats (Irving,1949).

Despite the effect of vitamin A deficiency on the epithelial tissues of most internal organs as well as in the eye, the skin and appendages have rarely been affected in vitamin A-deficient animals (Wolbach,1954). In rats fed diets deficient in vitamin A, Portman (1927) reported only a non-specific effect on the appearance of their hair coats (loss of smoothness, "unkempt fur"). Sullivan and Evans (1943), however, reported loss of hair and "hyperkeratinization" of the epidermis in their vitamin A-deficient rats. Pediculosis was reported in the latter's rat colony. The only other investigation describing effects of deficient diets on the skin and hair of rats was that of Moulton (1943), who reported thinning of the pelage and histological evidence of hyperkeratosis, with "keratotic plugs" blocking the hair canals. The experience with A-deficient diets in rats could not be duplicated in mice (McCarthy and Cerecedo,1952; Loewenthal,1956).

Physiological and pathological changes referable to vitamin A deficiency are also known in man (Mason,1954). Characteristic clinic-

al findings include nyctalopia, xerophthalmia, and in children, keratomalacia. Bone lesions have not been reported, and neural manifestations described in hypovitaminotic-A patients may or may not be a non-specific consequence of general inanition in individuals suffering from multiple nutritional deficiencies.

Although a few reports in the literature describe any cutaneous lesions in animals as a result of vitamin A deficiency, a large clinical literature has accumulated since Frazier and Hu (1931) first demonstrated this specific relation in man. These investigators described papular eruptions in undernourished Chinese soldiers, who in addition, exhibited various degrees of xerophthalmia. In a later report, Frazier and Hu (1936) extended their previous findings in studies of over 200 patients of all age groups. Histological examination of the skin biopsies of affected areas revealed follicular hyperkeratosis associated with hyperplasia of the epidermis adjacent to the follicles, and moderate hyperkeratinization of the surface epithelium extending beyond the papules. Sebaceous glands were absent in involved follicles, and sweat glands appeared to be atrophic (anhidrosis was reported in all patients). The observations of Frazier and Hu were independently confirmed by many other investigators (reviewed in Mason, 1954). In many of these reports, confirmatory evidence of vitamin A deficiency was provided by the presence of varying degrees of ocular manifestations (defective dark adaptation, xerophthalmia, keratomalacia), as well as by the response to therapy with vitamin A.

B. Clinical Use of Vitamin A in Dermatology

The cutaneous consequences of vitamin A deficiency described in man superficially resemble many dermatological disorders. This has prompted clinicians to employ vitamin A in the treatment of cutaneous diseases which involve faulty keratinization. Satisfactory clinical responses have been reported in common skin diseases such as acne vulgaris (Davidson and Sobel,1949), some eczemas (Gross,1941; Stroesser and Nelson,1952) and psoriasis (Schwartz,1950), as well as in other disorders characterized chiefly by follicular hyperkeratosis (Spies, 1947). Some patients with rare familial diseases such as Darier's disease (keratosis follicularis), Devergie's disease (pityriasis rubra pilaris) and ichthyosis have also responded favourably to vitamin A therapy (Leitner and Moore,1948; Leitner,1947; Keddle,1948; Rapaport et al,1942).

Low serum levels of vitamin A have been reported in some dermatologic patients (Chieffi and Kirk,1949; Leitner,1947; Lahiri and Scandrett,1954). The increased blood levels that followed intensive vitamin A therapy, however, were not always correlated with clinical responses in the same patients (Leitner and Moore,1948; Cornbleet,1954). In patients with Darier's disease or pityriasis rubra it has been reported that liver function is impaired (Leitner and Moore,1948; Leitner, 1947; Keddle,1948). This has led to speculations that those skin diseases reminiscent of vitamin A deficiency are due to defective liver function (Arons,1949). Other investigators, however, have found a

normal distribution of hepatic and blood vitamin A in patients with these same disorders (Cornbleet,1954). This has prompted postulations that the skin defect in these diseases involves a local and hereditary metabolic defect in the utilization of vitamin A by skin cells themselves rather than a secondary effect of a circulatory or hepatic dysfunction (Cornbleet,1954; Mason,1954). That very high doses of vitamin A are required for adequate therapeutic responses in dermatologic patients (10-100 times the daily dietary levels recommended for optimal physiological status,cf., HRC, 1953) would tend to support this latter speculation. It is curious that vitamin A has not been detected in the epidermis (Cornbleet and Popper,1942), even after massive dosing (Popper,1944). Minute amounts, however, were found in the dermal and subdermal fat layers (ibid).

C. Description of Mouse Mutants

It would be well at this point to describe the development of the rhino and hairless conditions. The author's observations of untreated animals corresponds, with only minor differences, to previous descriptions of these mutants (David,1934; Fraser,1946; Howard,1940; Montagna et al, 1952,1954; Steinberg and Fraser,1946).

From birth to approximately two weeks of age both rhino and hairless mice are indistinguishable from normal animals. At the age of 14-15 days, depilation begins in rhinos, in the region around the eyes, and spreads out first over the head and throat, and then posteriorly along the back and belly. Usually there is no clear-cut

line of demarcation between haired and non-haired areas in rhinos; rather there is a generalized thinning of the pelage followed by a diffuse "wave" of hair loss.

In rhinos, hair loss begins on the venter a bit later than on the dorsum but progresses more rapidly. By 23 to 24 days of age the body is usually bare, but hair loss is just beginning on the feet, tail and ears. Except for the vibrissae, most rhinos are virtually naked at five weeks of age; scattered hairs, however, may be retained on the hindpaws and tail of some animals up to seven weeks of age. Beginning at three weeks of age, there is a marked over-growth of the nails, and by six weeks these start to acquire the curved and spiral shape characteristic of older animals.

The pattern of loss of the juvenile pelage in hairless mice is basically similar to that of rhinos, but differs in certain details. Firstly, depilation begins a day or so earlier, usually at 12-13 days of age, and proceeds caudad a little more rapidly, so that by 21 days the backs of hairless mice are completely bare. In contrast to most rhinos, there is a definite line of demarcation between areas with and areas without hair. Secondly, hair loss on the forefeet begins at the same time as it does around the eyes and is complete by the same time the dorsal wave of depilation has reached the neck. At about the time depilation commences from the forelegs, loss of ventral body hair begins and is complete when less than one-half the dorsum is bare. Also, the hindlegs have lost their hair before the dorsum is bare (usually when

the dorsal wave has reached the level of the kidneys). At this time (ca 18 days) the hairless mouse has the slightly ridiculous appearance of wearing one-half of a furry bathing suit. By 21 days, most hairless mice are completely denuded; in several animals, a few hairs at the base of the tail may be retained for an additional week. Hypertrophy of the nails also occurs in hairless mice, but is less extreme than that found in rhinos.

Most hairless animals grow a sparse second pelage between five and six weeks of age; this falls out about three weeks later. A third and even sparser coat is usually produced (at ten weeks), but later hair generations have rarely been observed in older mice. After rhinos lose their juvenile pelage, regeneration almost never occurs.

Immediately following hair loss in rhino mice, the characteristic wrinkling of the skin (responsible for the name) begins to appear, initially as fine corrugations on the top of the head and the dorsum, and progresses to coarse and well-defined corrugations associated with noticeable skin thickening during the following two to three weeks. During the ninth and tenth weeks, the characteristic lateral folds, running between foreleg and hindleg, make their appearance, and the corrugations all over the body have become more intense. At about four months of age, small white lumps become noticeable in the thickened skin; these increase in size and number as the animals grow older and accentuate the "pasty" appearance of rhino skin. By six months of age, the claws are long, curved and/or spiralled, and the skin folds on the

head often develop to the point where they cover the eyes.

In contrast to the intense thickening and wrinkling of the skin which occurs in rhino mice, the skin of hairless mice remains smooth and shows only mild to moderate thickening. Between three and four months of age, the skin of hairless males also begins to acquire a "pasty" appearance, and a fine wrinkling is manifest on the head. In a large number of hairless females of the author's stock, however, even these moderate changes did not occur (see below, section III.D).

The fertility of rhino males appears to be normal, but that of rhino females is greatly reduced. In contrast to this, and to the reports of some investigators (Crew and Mirskaia, 1931-32; David, 1932), both hairless males and hairless females are vigorous and fertile (see below, section III.D).

Histologically, the first visible abnormality in the skin of both rhino and hairless animals is a follicular keratosis associated with widening of the hair canal, evident at about the time "club" formation of the hair occurs in normal mice (12-14 days). In normal skin the hair follicles (which contain club hairs) shorten at this time, and a new hair "germ" is set aside though remaining attached to the base of the follicle. The hair follicles in the skin of mutant mice, however, shorten only irregularly or not at all, and elongated cords of epithelial cells extend deep into the corium; club formation in most follicles is abnormal. During the following week, hair canals in mutant skin become wider, and a marked hyperkeratosis of the surface epithelium

occurs; both processes are more extreme in the skin of rhino mice. The widened hair canals become filled with keratinized debris, and in rhino skin they regularly develop into large cysts, or "utriculi", open to the surface. The hair canals of hairless mice only occasionally develop into utriculi, and in general, epidermal and follicular hyperkeratosis does not progress as rapidly, nor is it as marked, as in rhinos. The sebaceous glands (which appear to be normal at this stage) usually lie at the base of the widened follicles, or deeper in the corium attached to the extended epithelial cord. Between five and six weeks of age both the follicle-ends and sebaceous glands begin to develop into cysts, which enlarge rapidly in rhino skin during the next two months, but in hairless mice increase only moderately in size. In older rhino and hairless mice, sebaceous and follicle-end cysts increase in size and number until they practically fill the dermis, thus contributing to the skin thickening observed grossly. Beginning about two months of age, dermal fat stores (located just distal to the panniculus carnosus) in mutant skin diminish, until by the age of four months, they disappear in rhinos. Adipose tissue apparently does not completely disappear from the skin of hairless mice, however; some fat cells are evident even in animals one year and older (cf, Montagna et al, 1952).

D. Treatment of Hyperkeratotic Mouse Mutants

The skin of the mouse mutants "rhino" and "hairless" exhibits gross and histological changes similar to those reported in some normal

animals which were fed diets deficient in vitamin A, and to the cutaneous manifestations of vitamin A deficiency in man. Transplantation experiments have demonstrated that rhino skin when grafted onto normal hosts behaves autonomously, except in areas immediately adjacent to normal skin (Fraser, 1946). This suggests that a cell-diffusible substance produced by normal skin cells but not present in the blood stream is responsible for the maintenance of a normal cutaneous epithelium. On the assumption that this substance might be a metabolite of vitamin A, Fraser (1949) fed rhino mice a highly purified distillate of the natural esters in doses up to 5000 I.U. every other day for periods up to 48 weeks. Treated adults showed an exfoliative dermatitis associated with grossly pinker and oilier skins after only a few weeks at the higher dosage levels. Signs of systemic toxicity were observed, manifested externally by hyperirritability, loss of weight, emaciation and eventually, death. Gross examination of internal organs post-mortem revealed hyperemic, enlarged spleens and livers, both exhibiting white infiltrations. Histologically, the splenic changes were regarded as simple hyperplasia of the pulp, but the pathological picture in the liver appeared to be that of active cirrhosis. The lungs were congested and showed many petechial hemorrhages. When the dose level of vitamin A was adjusted periodically, rhino mice could be treated up to 50 weeks of age (treatment was begun at 17 days, just after hair loss had started) without external signs of systemic toxicity. Under these conditions, the skin of treated rhinos did not develop the

characteristic marked wrinkling observed in control animals, but remained smooth, thin and pink during the course of treatment. Histologically, the skin of treated animals was thinner and there was almost complete absence of dermal cysts which developed in the controls; treatment had no apparent effect on hair canal cysts. Treatment begun after cyst formation had commenced inhibited further development of the cysts, but did not cause their regression. Finally, there was no effect on the rate of loss of the juvenile pelage in rhinos fed vitamin A from birth.

Normal animals from the rhino strain fed 1250 I.U. vitamin A every other day from four weeks of age showed no effect of treatment on hair growth or skin histology, and external signs of systemic toxicity did not develop. The average daily dose used was comparable with that reported to be relatively non-toxic in various animal species by other investigators (Rodahl, 1950; Nieman and Klein Obbink, 1954; etc.).

In view of the fact that no other studies have been reported on the effect of vitamin A in hyperkeratotic mouse mutants, the present work was undertaken in order to extend the observations made in rhinos, as well as to investigate the effect of vitamin A in hairless mice. Since the hairless mutant is allelic (or at least cistronic) to the rhino mutant, and yet possesses certain distinctive differences in phenotype, it was also of interest to see whether there would also be differences in its response to vitamin A.

II. MATERIALS AND METHODS

A. Mice

Two lines of mice were used in the present investigation, RH and HR, both derived from stocks maintained in the mouse colony of the Department of Genetics at McGill University. The RH line carried the gene rhino (hr^{rh}), and was homozygous for genes determining black coat colour (B) and tan belly (a^t). The HR line carried the hairless gene (hr), an allele of rhino, and was segregating for a pair of alleles (full coat colour, S, and recessive piebald spotting, s) closely linked with the hairless locus, and for an independently segregating pair of alleles (black, B, and brown, b). All mice employed in the experimental studies to be described trace back to two ancestral litters, one from each stock; in the course of inbreeding, two sublines were established in each line.

The breeding system that furnished most of the mice for experimental purposes was mating heterozygous females (Hr/hr^{rh} , Hr/hr) to their homozygous mutant brothers (hr^{rh}/hr^{rh} , hr/hr). When mutant male littermates were not available, mutant sons from previous pregnancies were used; occasionally, it was necessary to use the next closest related males in the following generation ("nephews").

Many mutant females also proved to be fertile. Since mutant animals cannot be distinguished from their normal sibs until two weeks of age, litters obtained from this type of mating were especially use-

ful when paired littermate controls were necessary for experiments designed to study the effect of treatment during the period from birth to two weeks.

B. Vitamin A

The vast majority of animals were treated with the synthetic palmitate ester of vitamin A dissolved in corn oil. Dilutions were made with corn oil from a stock solution (1,850,000 International Units vitamin A/gm.) to concentrations providing 5000, 10,000, 25,000 or 50,000 units/cc., in order that the volume of material administered was usually about 0.2 cc., an amount easily swallowed by most mice at any age. Littermate control animals, paired by sex and weight, were given comparable volumes of corn oil vehicle.

An aqueous dispersion of synthetic vitamin A palmitate* was used in the studies of teratogenicity.

C. Techniques and Routes of Administration

1. Oral Administration - Mice were force-fed vitamin A or corn oil by means of a 1 cc. tuberculin syringe (calibrated in 0.01 cc.) to which was attached a short 22-gauge hypodermic needle broken half-way up its stem. The severed end of the needle was filed smooth to minimize the danger of injury when inserted deep in the mouth of the animal. The use of such an instrument was found to be a safe, convenient method of oral administration. *ACON, Endo Synthetic Vitamin A Palmitate, dispersed in distilled water and 30% glycerine, with 30% w/v polysorbate-80; 0.18% methylparaben plus 0.02% propylparaben added as preservative, plus flavouring and saccharin.

ient and rapid method of administering agents orally, and superior to stomach intubation. The technique of administration was as follows: With the mouse held firmly by the skin fold at the nape of the neck (which served to hold the jaws partly open during feeding), the needle was inserted into the mouth of the animal with its tip pressing gently against the tongue and the required amount of material was dispensed in one "shot". Only one mouse (a 10-day old rhino) among nearly 200 animals fed in this way for periods up to 7 months died through aspiration of the oily preparation into the lungs.

As a rule, mice were fed on alternate days, with a constant dose per unit body weight.

2. Parenteral Administration - Parenteral administration of vitamin A in corn oil was by intramuscular injection alternately into right and left thighs, usually every other day, using a tuberculin syringe and a No. 24 or No. 26 needle. As with the oral administration of the vitamin, the dose level was calculated on the basis of body weight. This method of administration was not continued with the preparation of vitamin A in corn oil since it was found that the chronic administration of the oily solutions by this route formed intramuscular depots of material from which absorption was very slow, and the effects produced were variable and inconsistent. The aqueous preparation of vitamin A, however, was relatively rapidly absorbed from injection sites, and showed little tendency for depot formation. (Since the vitamin A-free aqueous vehicle was not available, series of controlled experiments

with this preparation were not possible.)

D. Gross Observations

Mice were weighed before each dose of vitamin A was administered, and any changes in the appearance or behaviour of the animals recorded. When visible through the skin, the spleen in mutant animals was measured.

At the time the animals were sacrificed, or died as a result of systemic vitamin A toxicity, the appearance of the liver, spleen, lungs and kidneys was noted. Particular attention was paid to organ colour, presence or absence of gross lesions and/or hemorrhage, and any other alterations as compared with controls killed at the same time. The spleen and liver of each animal were weighed (on a triple-beam balance, to the nearest 0.01 gm.), and then fixed in either Bouin's Fluid or formol-calcium. In order to compare the effects of treatment on these organs among all age classes of treated animals, the ratio of wet weight of organ to body weight of the animal at the time of death was calculated.

Litters born to treated and control females were examined for gross congenital defects, and the number of offspring born alive or dead recorded. Because of the propensity of female mice to eat their grossly malformed or dead newborn soon after birth, most litters were collected "over wire". About the eighteenth day of gestation, each pregnant female was transferred to an isolation cage into which a basket fashioned from chicken wire had been inserted. The wire basket,

which was made to fit snugly, was held in place approximately two to three inches above the floor of the cage by wire strands looped over the upper edges of the cage. When the cage top was in place, the animal was not in any way restricted, and had access to food and water at all times. The gauge of the wire was large enough to allow the newborn to drop freely through to the sawdust-covered cage floor, but prevented the mother from reaching them. All newborn with gross defect(s) were fixed in Bouin's Fluid.

E. Histology

Skin biopsies, approximately 5 mm. square, were removed periodically from the mid-dorsal area of treated and control animals under light ether anesthesia. A post-mortem specimen of skin was taken from each experimental animal, usually from the same area. Skin specimens were fixed in either Bouin's Fluid or formol-calcium, dehydrated according to schedules employing either n-butyl alcohol or toluene, embedded in paraffin, sectioned at 10, 15 or 20 microns, and mounted on 75 x 25 mm. glass slides using Haupt's Adhesive and 4% formalin. Mounted sections were stained with either Harris' or Delafield's Hematoxylin and counterstained with alcoholic eosin.

Photomicrographs were taken with a specially-designed Kodak "Pony IV" 35 mm. camera (with a compensating lens replacing the conventional photographic eyepiece), coupled by an adapter to an American Optical "Microstar"* microscope with a trinocular body. No filters
*Series 4, equipped with fixed arm and focusable stage, an NA 1.25 Abbe condenser, built-in illumination and variable transformer.

were used. The camera-microscope assembly imparted a basic magnification of 2.5 X; achromatic microscope objectives gave magnifications of 3.5 X, 10 X and 40 X. With 4" x 5" positive enlargements of processed PanatomicX roll film, print magnifications of approximately 30 X, 90 X and 360 X were obtained.

III. EXPERIMENTAL RESULTS

Vitamin A was fed at doses ranging from 50 to 400 I.U. per gram body weight per day for periods of time of one to 30 weeks to 75 homozygous mutant mice (56 hairless and nineteen rhinos) and 50 normal heterozygous littermates; 37 mutants and nineteen normals served as controls (Table I).

A. Systemic Toxicity

1. Rhinos - It has been reported previously that at doses of vitamin A of the order of 5000 I.U. every second day, adult rhino mice manifest signs of systemic toxicity about a month after the beginning of treatment (Fraser, 1949). This dose level is similar to one of those used in the present investigation, viz., 100 I.U./gm./day, and the results observed were similar to those previously described. Thus, adult rhinos fed a constant dose of 100 I.U./gm. body weight/day became easily excitable and hyperirritable on handling during the fourth to fifth weeks of treatment. At this time the skin of all five vitamin A -treated adult animals appeared to be pinker and exhibited less rugosity dorsally than the three control rhinos fed the corn oil vehicle. Skin of treated animals sixteen to eighteen weeks old was also thinner than that of littermate controls, since the spleens of the former were still clearly visible and measurable, whereas in littermate control rhinos the outlines of this organ could not be seen clearly one week prior to this time. With continued

TABLE I

Numbers of Mice Treated Orally with
Vitamin A at the Dosage Levels Indicated

Treatment (I.U./gm./day)	HR*		RH**		Total
	Hairless	Normal	Rhino	Normal	
50	17	12	8	9	46
75	4	--	--	--	4
100	29	14	8	11	62
150	--	--	2	2	4
200	6	1	--	--	7
400	--	--	1	1	2
Total Vitamin A	56	27	19	23	125
Oil (control)	32	12	5	7	56
TOTAL	88	39	24	30	181

* HR line, carrying the gene hairless (hr)
 ** RH line, carrying the gene rhino (hr^{rh})

treatment, A-treated animals exhibited weakness, lassitude and cachexia, and the skin assumed a progressively yellow appearance. Treated rhinos usually became moribund and died two weeks following the first signs of toxicity; loss of body weight occurred in some but not in all treated animals immediately prior to death. During this terminal stage, the skin of vitamin A-fed rhinos was dry and covered with fine white flakes. At autopsy, greatly enlarged spleens and livers were found (Table II). The livers showed signs of fatty infiltration, either as discrete whiteish areas throughout some of the lobes, or as a fine white lacework covering the lobar surfaces; in some treated animals, entire hepatic lobes were cystic. Other pathological findings included hyperemic kidneys and, in some animals, congested lungs with petechial hemorrhages.

The rate of development and the severity of systemic toxicity to vitamin A treatment in rhinos varied with the dose level at which the vitamin was administered, as well as with the age of the animal when treatment was begun. For instance, four older rhinos (six to nine months of age) on dosages of 100 or 150 I.U./gm./day died after only five to seven weeks of treatment, whereas two younger animals (two to four months old) fed at comparable dosages appeared healthy even after nine to fifteen weeks of treatment. In the two rhinos given 50 I.U./gm./day for periods up to 20 weeks, no signs of systemic toxicity were observed.

2. Hairless - Adult hairless mice fared much better than

TABLE II

Effect of Feeding Vitamin A to Adult Mice of RH
and HR Stocks on Weights of Liver and Spleen

Stock	Phenotype	Treatment (I.U./gm./ day)	Duration (wk.)	Liver		Spleen	
				No.	Ratio*	No.	Ratio*
RH	rh	100	6-12	4	13.05	4	3.01
		150	7	1	12.39	1	2.35
		(Control)	---	1	5.99	1	0.61
	+	100	6-30	4	6.26	5	1.04
		150	7	2	9.84	2	0.34
		(Control)	---	---	----	---	----
HR	hr	100	20-28	3	7.57	3	1.21
		(Control)	---	2	6.45	2	0.62
	+	100	11-27	8	5.35	8	0.66
		(Control)	---	1	5.79	1	0.41

*Average of $\left(\frac{\text{wet weight}}{\text{body weight}} \times 100 \right)$

rhinos when treated with similar doses of vitamin A. For instance, at 100 I.U./gm./day, hyper-excitability and yellowing of the skin did not appear in any treated hairless mouse before fourteen weeks of continuous treatment, and generally much later (19 to 23 weeks). Signs of systemic toxicity similar to those exhibited by rhinos occurred after five to six weeks treatment in the three hairless adults fed 200 I.U./gm./day. Hairless mice became moribund four to five weeks after the appearance of signs of toxicity, but except for enlarged livers and spleens (Table II), no gross lesions were found in most animals. Gross changes in the appearance of the skin of treated hairless mice are described below (section III. B. 2.).

Hairless mice fed vitamin A in doses of 100 I.U./gm./day from birth (five animals) or weaning age (five to six weeks), (three animals), did not show any evidence of systemic toxicity, even after six months of continuous treatment (three animals). No pathological changes in internal organs were found, and the livers and spleens constituted the same percentage of whole body weight as did those of controls (Table III).

At dosages of 200 I.U./gm./day, however, five of the six young animals treated with vitamin A died within two months. The one exception was a female that lasted seventeen weeks; both the liver and spleen were obvious through the exceedingly thin skin of this animal just before it disappeared (presumably eaten by littermates). The cause of death in the other hairless mice that succumbed during treat-

TABLE III

Effect of Vitamin A on Weights of Liver and Spleen in Mice of RH and HR Stocks Fed from Birth or Weaning

Stock	Phenotype	Treatment (IU/gm/day)	L I V E R				S P L E E N			
			D u r a t i o n (wk.)							
			2-13		14-27		2-13		14-27	
			No.	Ratio*	No.	Ratio*	No.	Ratio*	No.	Ratio*
RH	rh	50	3	4.25	1	7.03	3	0.65	1	2.32
		100	---	----	2	6.50	---	----	1	3.79
		400	1	4.91	---	----	1	2.86	---	----
	+	50	2	4.78	3	7.72	2	0.82	3	0.65
		100	---	----	4	6.60	---	----	4	1.05
		400	1	4.42	---	----	1	2.08	---	----
		(Control)	---	----	5	5.97	---	----	5	0.66
	HR	hr	50	2	2.68	6	6.51	2	0.14	6
75			---	----	1	7.21	---	----	1	0.47
100			12	4.16	2	4.39	8	0.85	2	0.51
200			3	3.68	---	----	3	0.31	---	----
(Control)			9	5.26	4	6.12	5	0.79	4	0.58
+		50	1	4.17	6	5.59	1	0.12	7	0.41
		100	1	6.67	4	6.47	1	0.89	4	0.41
		200	---	----	1	7.94	---	----	---	----
		(Control)	2	5.85	6	6.07	2	0.46	6	0.29

*Average of $\left(\frac{\text{wet weight}}{\text{body weight}} \times 100 \right)$

ment could not be determined; no significant gross pathological changes were found in the internal organs of three young treated animals that died after nine weeks of treatment at this dosage level (Table III).

3. Normals - Normal adult mice of both the RH and HR lines, fed vitamin A at a dosage of 100 I.U./gm./day, exhibited signs of hyperirritability (spontaneous twitching) and episodes of pulmonary difficulty (gasping) after five to seven weeks of treatment. The most significant changes observed in these mice were those involving the pelage and skin, and were similar to the effects described in hypervitaminotic rats (Wolbach, 1954). At this dose level, the hair coat of all nine normal adult mice began to thin out dorsally during the fourth week of treatment. With continued treatment (for two to three months), the pelage became thinner and "scruffy" (ruffled and ragged). In some animals, bald patches appeared on the pate, neck and dorsum and, in a few mice, marked hair loss also occurred on the rump and belly. After about three months of treatment, normal mice were extremely nervous and irritable; a few seemed to have great difficulty in breathing (e.g., one female, killed after eleven weeks of treatment, had developed sonorous rales which had begun one week previously). After four months of continuous treatment, marked cutaneous erythema and exfoliative dermatitis were observed in most animals; these epidermal changes were especially obvious in areas denuded of hair. Skin samples removed from these animals were grossly very thin and tore easily. The four mice which survived six months of treatment exhibited marked alopecia;

the skin was "paper-thin" and peeled easily from the body. The livers and spleens of two animals killed at this time exhibited, grossly, the same white surface lacings observed in mutant mice treated for comparable lengths of time. No other abnormal signs were found in the internal organs of normal animals that died after five months of treatment. Although the external and internal effects of vitamin A treatment were generally similar in normal mice of both lines, spleens of treated RH normals tended to weigh more (Tables II, III).

Normal mice still alive after six months of treatment at dosages of 100 I.U./gm./day were killed one month later. Gross changes similar to those described above were found.

In five normal mice fed higher dosages of vitamin A (150-200 I.U./gm./day), rles and ataxic behaviour developed during the first week of treatment in two animals (RH females); alopecia and cutaneous exfoliation were observed in all treated mice during the second week. When the dosage was decreased to 100 I.U./gm./day in these two RH females, wheezing and ataxia disappeared or were minimal, but the skin changes persisted. Mice killed after seven weeks of treatment exhibited, internally, gross pathological changes similar to those found in normal animals treated constantly at 100 I.U./gm./day for longer periods of time.

At 50 I.U. vitamin A/gm./day, no toxic effects attributable to treatment were observed in five normal mice, even after six months of continuous treatment. Livers of RH normals tended to weigh more

than those of HR normals (Table III).

B. Effect of Vitamin A on the Hair Growth Cycle in Mutant Mice

1. The First Hair Coat - As indicated above (section I. C.), untreated rhino and hairless mice begin to lose their juvenile hair coat at about two weeks of age; depilation is complete seven to ten days later. The first signs of hair loss around the eyes in 40 of 47 untreated rhino mice appeared between fourteen and sixteen days of age, with a mean of 14.87 days, whereas in the majority of 33 untreated hairless mice, depilation began one to two days earlier, the mean being 13.73 days (Table IV). The means are significantly different, the difference (1.14 days) being 5.45 times the standard error of the difference (0.21 days). No sex differences were found. In most of the presumptive rhino mice, a "raked" appearance of the pelage on the dorsum (running antero-posteriorly) was observed one to two days before hair loss began around the eyes; this raking may represent the first signs of depilation in rhinos. Raking of the pelage was not observed in hairless mice prior to, or after the commencement of, actual hair loss.

Of nine rhinos fed vitamin A (50 to 100 I.U./gm./day) from birth on which observations with respect to rate of depilation were made, hair loss began in two animals on the thirteenth postnatal day, in four on the fourteenth day, and in the remaining three animals on the fifteenth day, the average age at which depilation began being 14.11 days. Two of the four rhinos fed the corn oil vehicle (these were not

TABLE IV

First Signs of Depilation in Untreated
Rhino and Hairless Mice

Age (days)	rhino (19 litters)		hairless (11 litters)	
	Males (No.)	Females (No.)	Males (No.)	Females (No.)
12	---	---	2	1
13	3	2	3	5
14	8	5	6	11
15	10	4	2	3
16	9	4	---	---
17	1	1	---	---
TOTALS	31	16	13	20
MEAN AGE $\pm$ S.E. (days)	14.90	14.81	13.62	13.80
	14.87 $\pm$ 0.15		13.73 $\pm$ 0.14	
S.E.d (days)	0.21			

littermates of the treated rhinos) started to lose their juvenile pelage on the thirteenth day of life, one started on the fourteenth and one of the fifteenth day, the average age for the four "controls" being 13.75 days. Vitamin A had been fed during pregnancy to the mothers of three of the four rhinos (two treated and two oil-fed) that started to lose their hair on the thirteenth day.

Hair loss in treated hairless mice also appeared to start earlier than in treated rhinos. The average age at which signs of depilation were first noticeable among 37 hairless mice fed vitamin A in doses ranging from 50 to 200 I.U./gm./day from birth was 12.54 days (Table V). In 26 littermates fed corn oil, depilation also began at about the same age (average, 12.58 days). The rate of depilation among vitamin A-treated hairless mice, i.e., the mean number of days required for depilation to be complete, did not differ from controls (Table VI).

2. Subsequent Hair Growth - Untreated hairless mice, but not rhinos, grow a sparse second "pelage" between five and six weeks of age (Fraser, 1946; Montagna *et al.*, 1952); this is lost about two weeks later. Another "generation" of body hair may appear subsequently at ten weeks of age.

Among 20 hairless mice in nine litters fed vitamin A, second hair growth did not appear in twelve animals (by twelve weeks of age), and only very few pelage hairs were observed in the remaining eight animals (Table VII). All but one of eleven littermate controls fed

TABLE V

First Signs of Depilation in Treated
Hairless Mice (15 litters)

Treatment (Vitamin A, I.U./gm./day)	Sex	Number of Mice	Depilation began, day:				Means (days)	S.D. (days)
			11	12	13	14		
50	♂	6		2	4		12.67	12.47
	♀	9	1	5	2	1	12.33	
75	♂	2			1	1	13.50	13.67
	♀	1				1	14.00	
100	♂	11		8	3		12.27	12.35
	♀	6		3	3		12.50	
200	♂	1			1		13.00	13.00
	♀	1			1		13.00	
Total vitamin A		37					12.54	0.93
Oil (controls)	♂	14		7	6	1	12.57	12.58
	♀	12	1	6	2	3	12.58	

TABLE VI

Rate of Depilation in Treated
Hairless Mice (15 litters)

Treatment (Vitamin A, I.U./gm./day)	Sex	Number of Mice*	Days required for depilation				Means (days)	S. D. (days)
			8	9	10	11		
50	♂	6		5	1		9.17	9.20
	♀	9	1	5	3		9.22	
75	♂	-					--	(8.00)
	♀	1	1				8.00	
100	♂	10		5	2	3	9.80	9.67
	♀	5	2		2	1	9.40	
200	♂	1			1		10.00	(9.00)
	♀	1	1				8.00	
Total vitamin A		33					9.37	0.88
Oil (controls)	♂	11	1	2	5	3	9.91	9.55
	♀	11	2	6	2	1	9.18	

*Differs from total number in Table V; 8 animals (4 treated, 4 control) were killed between 15 and 22 days of age.

TABLE VII

Effect of Vitamin A on "Secondary" Hair Growth
in Hairless Mice.*

Dose (I.U./gm./day)	No. of Mice	Sex	Secondary Hair	
			Appeared	Did Not Appear
50	10	♂	1	3
		♀	4	2
75	2	♂	-	-
		♀	1	1
100	7	♂	1	4
		♀	1	1
200	1	♂	-	-
		♀	-	1
Total Vitamin A	20	♂	2	7
		♀	6	5
(Control)	11	♂	4	1
		♀	6	-
TOTAL MICE:	31 (9 litters)	$\chi^2 = 5.608$ $P = 0.02$		

*Observations up to 12 weeks of age.

corn oil exhibited a second hair generation at an age expected in untreated hairless mice. In the exceptional animal, hair growth had not appeared at ten weeks of age; a littermate, fed 100 I.U. vitamin A/gm./day from birth, exhibited a sparse growth at seven and one-half weeks of age.

A comparison of the proportion of treated hairless mice in which second hair growth occurred (8/20) with the proportion in corn oil-fed controls (10/11) gave a X^2 of 5.608, the difference therefore being significant at the 2% level.

C. Gross and Histological Observation on the Skin of Vitamin A-Treated Mice

Gross observations of the effect of vitamin A on the skin of rhino and hairless mice (and their normal littermates) were supplemented with histological examination of skin samples from the mid-dorsal area.

1. Rhinos - The histology of skin specimens taken from the dorsum of rhino mice treated for increasing lengths of time at various dosages did not differ essentially from that previously described (Fraser, 1949). Changes induced in the skin of these animals appeared to be closely associated with the appearance and severity of the general systemic toxicity produced by vitamin A.

A pair of rhino males will illustrate the gross and associated microscopic changes induced in rhino skin by vitamin A. Starting at ten weeks of age, rhino ♂ 1358 was fed 200 I.U. vitamin A/gm./day

(as single doses of 400 I.U. vitamin A/gm., administered every second day); ♂ 1359, a rhino littermate, was given equal volumes of corn oil. After nine days of treatment, the characteristic skin rugosities of rhino mice (which begin to appear in untreated rhinos at about eight weeks of age) seemed to be less prominent in the treated animal, and the skin a shade pinker than in the control. These differences were more obvious after two weeks of treatment, at which time the spleen of ♂ 1359 could no longer be seen clearly through the skin, but was still visible in ♂ 1358 (and remained visible during the following two weeks of treatment). On the fifteenth day of treatment, the dosage level was decreased to 100 I.U./gm./day (200 I.U. vitamin A/gm. given every other day) to avoid killing the animal which began to show, at this time, signs of systemic toxicity. During the following seven weeks of treatment at the reduced dosage, the skin of ♂ 1358 continued to appear noticeably pinker and smoother than that of ♂ 1359. From this time, however, the skin of the treated animal began to get progressively yellower, and showed xerosis and an exfoliative dermatitis ("dander"). After a further three weeks of treatment, the skin of the treated animal was dry, leathery and distinctly yellowish, but also very much smoother, than that of its oil-fed littermate. The treated male died during the twelfth week of treatment (at 22 weeks of age).

Histological examination of a dorsal skin sample taken from the control, ♂ 1359, exhibited all the features characteristic of the

skin of 22 week-old untreated rhinos (Fig. 1, see PLATES). The dermis contains numerous large sebaceous cysts, many filled with keratinized debris. Other striking features are the increase in thickness of the stratum corneum, the gaping follicle canals ("utriculi") and plugged follicle-ends, the latter undergoing cyst formation. The subdermal fat layer, characteristic of the skin of normally-haired mice, is absent.

Dermal sebaceous cysts in the skin of the treated mouse, ♂ 1358, do not appear to have been affected by vitamin A (Fig. 2). The entire skin (stratum corneum to panniculus carnosus) is much thinner than that of the control, whereas the size and number of the utriculi, follicle-end and sebaceous gland cysts appear to be the same as those found in the control skin. The connective tissue of the corium is denser (more fibrous); there is no evidence of fat cells.

The effect of vitamin A on the skin of younger rhino mice fed vitamin A at a dose level of 100 I.U./gm./day starting at two to three weeks of age and for a similar period of time (i.e., eleven to twelve weeks) was similar to that described above for older animals. In most treated young rhinos, the skin exhibited a jaundiced appearance, but the marked yellowing observed in older treated rhinos did not develop. The skin of one treated rhino male, ♂ 417-3, however, maintained a very pink appearance up to the time of death (at thirteen and one-half weeks of age); flaking of the skin began to appear at six weeks of age (after four weeks of treatment), and became progressively more profuse

during the last two weeks of treatment. At autopsy, the appearance of soft tissues was grossly similar to that found in untreated and control rhinos of comparable age. The liver of this animal weighed 0.7 gram, which corresponded to a ratio to body weight at the time of death of 4.36 (normal for this age). Histologically, the most significant feature of the skin of ♂ 417-3 is the almost complete absence of dermal cysts (Fig. 3), which are characteristic of the skin of untreated (and oil-fed) rhinos at this age (Fig. 4).

In general, the effect of vitamin A on the skin of male and female rhinos is similar. At doses of 100 I.U./gm./day, the development of sebaceous cysts in the corium of females treated from two weeks of age is almost completely inhibited (Fig. 5). At lower dosages, however, vitamin A appears to have little or no effect on cystic development in rhino skin (Fig. 6).

2. Hairless - As described above (section III. A. 2.), adult hairless mice appear to be more resistant than adult rhinos to the toxic effects of vitamin A administered for comparable lengths of time at similar dosage levels. In addition, it was noted that the cutaneous response of hairless mice to vitamin A appeared to be different from that of the morphologically similar rhino mice (see below).

Vitamin A was fed to four adult hairless mice (18 to 22 weeks old) at a daily dosage of 100 I.U./gm./day; four littermates were given corn oil. The records of one pair of male littermates, ♂ 1372 and ♂ 1373, are presented in detail to illustrate the typical effects

of vitamin A administered at this dose level for six months, and the differences in the response to treatment of these mice as compared to that of rhinos treated at a comparable dosage.

The skin of untreated hairless mice at eighteen weeks of age is thinner and much less corrugated than that of rhinos of the same age. In both the treated (hairless ♂ 1372) and control (hairless ♂ 1373) animals, the spleen was clearly visible and measurable at the time treatment was begun, but during the first week of treatment its outlines could not be made out clearly (two days after the beginning of treatment for ♂ 1372, seven days for the control). During the following seven weeks no gross differences were observed between treated and control animals, but at about the beginning of the eighth week, the skin of ♂ 1372 appeared to be slightly pinker than that of the control, ♂ 1373. There was little or no change for the next four months. During the twenty-third week of treatment, the skin of the treated animal became noticeably yellowish; the oil-fed animal was "normal" (compared to untreated hairless mice of comparable age) in all respects. Three weeks later the yellowish colouration of the treated animal had increased strikingly, but the wrinkling of the skin which appears in untreated hairless mice at this age (ten months) was noticeably less than in the control. After 27 weeks treatment, ♂ 1372 became moribund (hunched over and cold to the touch) and was killed (45 weeks of age). The liver of the treated animal weighed 2.93 grams (compared to 1.50 grams for the liver of the control) and exhibit-

ed white infiltrations; most of one of the larger lobes was composed of a hard white matter. The spleen of ♂ 1372 was lighter in colour than that of the control and weighed 0.35 gram (compared to 0.13 gm. for the spleen of ♂ 1373). A section of skin from the mid-dorsum of the treated animal (Fig. 7) was thinner than that from the same area of the control (Fig. 6), but otherwise differs little histologically from that of the control skin. Groups of fat cells are evident in the dermis of the specimens from both animals; these cells are clearly differentiated in sections from the control (Fig. 6) but appear to be fewer in number in treated skin (Fig. 7; see also Fig. 9, a non-littermate adult hairless male, treated for 20 weeks at the same dosage level). There appears to be less hyperkeratinization in treated skin. Finally, vitamin A treatment apparently has had little effect on follicle-end and follicle canal cysts.

The response to vitamin A of hairless females of comparable age appears to differ in certain respects from that observed in hairless males. For example, even before treatment was started, the skin of both ♀ 1374 and ♀ 1375 (littermates to the pair of males described immediately above) was grossly pinker and thinner than that of their male littermates; outlines of the spleen were clearly visible for at least two weeks after such definition was lost in the males. During treatment (at 100 I.U./gm./day), no differences were discernible in the appearance of the skin between the treated female, hairless ♀ 1375, and the control, hairless ♀ 1374, until the fourteenth week of treat-

ment, when a yellowish tinge was observed in ♀1375. At this time, the skin of both treated and control females was smoother and pinker than either the control or treated male littermates, respectively ♂1373 and ♂1372. This sex difference was maintained until the twenty-third week of treatment (at which time the treated male also started to turn yellow). During the following three weeks, a large number of cutaneous petechiae developed over the dorsum and venter of the treated female and, subsequently, marked exfoliation in the form of fine white flakes was observed. After 29 weeks of treatment both treated and control females were killed (eleven months of age). The liver of the treated female appeared to be grossly normal, and weighed 1.52 grams (compared to 1.95 grams for that of the control female). The spleen of the treated female, however, exhibited a delicate white lacework over its surface and weighed over twice that taken from the control (0.5 gm. vs 0.2 gm.). No other gross differences between treated and control females were found internally. Histologically, sections of skin from the treated animal (Fig. 10) have fewer and smaller dermal cysts than those from the control (Fig. 11); other than this, treated and control skin sections exhibit little or no histological differences. Dermal fat cells are evident in sections from both treated and control animals. Debris which appears in and around some sebaceous cysts in treated skin (e.g., Fig. 10) may represent evidence of petechial hemorrhages observed macroscopically.

A total of 60 hairless mice were fed vitamin A from birth or

from the first week of life. Because their responses to treatment differ in some respects from the above descriptions of the results of treatment, the records of one litter (No. 389) of nine hairless mice (from a hairless x hairless mating) are presented to illustrate the typical responses in younger animals during a six-month period of treatment.

Hairless δ 389-5 was started at 100 I.U. vitamin A per gram body weight per day (200 I.U./gm. fed every second day) from 2 weeks of age; δ 389-4 served as control (fed an equal volume of corn oil vehicle). During the second week of treatment, depilation, which normally begins at two weeks of age in hairless mice, was proceeding at approximately the same rate in both treated and control animals. During the following three weeks, little or no gross differences were observed, but in the fifth week of treatment, moderate exfoliation (flaking) was observed in the treated animal. At this age (35 to 45 days) a second hair "generation" is observed in most untreated hairless animals; none, however, was observed in the treated male, whereas a good growth occurred in the control animal. Furthermore, the progressive "pasty" appearance of the skin which is observed in untreated mutant mice five to six weeks old was also obvious in the oil-fed control, but the treated animal retained its juvenile pinkness until killed during the eighth week. Histologically, skin taken from the mid-dorsum exhibits very little effect of vitamin A treatment (compare biopsy section from the control, Fig. 12, with the post-mortem specimen

from the treated animal, Fig. 13). The treated skin appears to be thinner, and to exhibit a slightly denser dermal connective tissue. In both treated and control skins, a few fat cells are observed (Figs. 12 and 13, however, do not show this).

Skin taken at eight weeks from treated hairless females of the same litter, as well as specimens from young animals of both sexes in other litters after the same period of treatment, did not differ either macroscopically or histologically from that described above in ♂ 389-5.

Another male from this litter, ♂ 389-1, fed at the same dosage level (100 I.U./gm./day) from two days of age, developed epidermal warts at four weeks of age; these outgrowths gradually disappeared during the following two weeks of treatment. Beginning at fourteen weeks the skin of this animal took on a "glossy" (smooth) appearance in contrast to the "pasty" appearance of that of the control litter-mate, ♂ -4, and also appeared to be thinner (the spleen was visible through the skin).

From fourteen weeks on, no further changes were observed in the remaining treated mice of this litter (three males, one female) up to the time all were killed in good condition after 25 weeks of continuous treatment. At autopsy, internal organs (livers, spleen, etc.) showed no evidence of vitamin A toxicity, such as was found in the treated rhinos described above. Histologically, sections of skin from the mid-dorsum demonstrated several beneficial effects of vitamin

A on some of the elements of the hyperkeratotic process which normally develops in untreated hairless mice (Figs. 14-17). Sebaceous gland cysts, which are large and numerous in the control (Fig. 14), are smaller and fewer in number in treated skin (Fig. 15). Dermal connective tissue is denser in treated skin, i.e., it appears to be more fibrous and less cellular. In total thickness (panniculus carnosus to stratum corneum), sections of skin from treated and control animals are similar, but the number of Malpighian layers differ. Thus, in sections from δ -1, (treated), the Malpighian layer is composed of an average of seven to eight cell layers, compared to the four to five cell layers in sections from the control. Furthermore, keratin lamellae from the stratum corneum are less prominent in the treated skin. In sections from the skin of δ -2, treated for the same length of time (6 months) but at a dosage level of 50 I.U./gm./day, the increase in cell layers in the Malpighian layers over the control is also evident (Fig. 16). The histological character of the corium, i.e., its fibrous density and the number and size of dermal cysts, is intermediate between that seen in skin sections from animals treated at the higher dosage of vitamin A and that in sections from the controls fed the vehicle.

All hairless mice treated from birth or before weaning at daily doses of 50 to 100 I.U./gm. for periods up to six months manifested grossly pinker skins than controls. Histologically, cystic development in the dermis was not entirely suppressed by treatment, but cysts

that did develop were smaller and fewer in number than those seen in control skins. Exfoliation ("dandruff" and flaking) was observed grossly in treated animals late in treatment.

Not all treated hairless mice exhibited histological evidence of the favourable effects on the skin induced by vitamin A, but in most animals treated at doses of 50 or 75 I.U./gm./day, certain responses to long-term treatment were usually found. Thus, ♀ 402-5, treated at a level of 75 I.U./gm./day for five months (from the first week of life) exhibited a pinker and smoother skin than a control littermate, ♀ 402-6, but only after sixteen weeks of treatment. Whereas a large number of epidermal warts appeared in the control at five weeks of age, none were observed in the treated female at any time. Sections of skin from ♀ -5, taken post-mortem after 20 weeks of treatment, compared to those from the control, showed: 1) a decrease in the number and size of sebaceous cysts; 2) an increased density (increased amount of fibrous tissue) of the connective tissue of the corium; 3) minor changes in the Malpighian layer of the epidermis; and, 4) decreased hyperkeratinization. Total skin thicknesses were similar in treated and control sections.

Hairless mice treated with vitamin A at a dosage level of 200 I.U./gm./day from the first week of life showed few of the beneficial effects of treatment, since at this dose level, most of the animals died within two months. One hairless animal, however, ♀ 366-4, was treated for seventeen weeks, starting at two weeks of

age. During the eighth week of treatment, the skin of this female was dry and large flakes were being shed, but within the following two weeks, the skin became much oilier and pinker. Both the liver and the spleen were clearly visible through the exceedingly thin skin, up to the time the animal disappeared at the age of nineteen weeks. No second hair growth and only a few small epidermal warts appeared during treatment.

Other mice treated at 200 I.U./gm./day manifested similar responses. For example, no gross changes in the skin were observed during four weeks of treatment in 6460-6, fed vitamin A from the age of one week. There was no second hair growth, and epidermal warts did not develop, whereas both were observed in the control,

6460-5. The skin of the dorsum of the treated animal was very thin and showed fine powdery flaking. The treated animal died during the fifth week of treatment. The cause of death was not readily apparent; there were no notable gross pathological changes found internally, and the post-mortem appearance of liver, spleen, kidneys and lungs was similar to that found in the control. Histological sections of dorsal skin corroborate the gross findings (Figs. 18, 19). Cross-sections of active hair follicles are evident in sections from the control (Fig. 19).

3. Effect of Vitamin A on Depilation in Hairless Mice - A total of five litters from hairless females (which had been mated to their hairless brothers) were used to determine the effects of vitamin A on the histological characteristics of the skin before, during

and after depilation. Dorsal skin biopsies were taken at intervals of two to four days up to 23 days of age, and at five to seven day-intervals thereafter (to 45 days of age in two mice). In the majority of animals (20/25), two to four biopsies could be obtained usually from each animal; in five mice it was possible to obtain seven biopsies per animal.

Up to 23 days of age no essential histological differences were found between skin biopsies taken from twelve genetically hairless mice fed vitamin A from birth (at a dose level of 100 I.U. to 200 I.U./gm./day) and those of comparable skin specimens from seven control littermates (e.g., Figs. 20, 21). (Since part of the control skin was sliced tangentially, the epidermis in Fig. 21 appears to be thicker than that from the treated animal).

4. Biotin - Severe biotin deficiency has been shown to lead to alopecia in normal mice (Nielson and Black, 1944) by adversely affecting keratinization and thus normal hair growth (Montagna, 1950; Rauch, 1952). Preliminary observations on the effect of feeding biotin to young hairless mice have been made.

Three hairless animals (2 ♂ , 1 ♀), given daily oral doses of 0.5 mcg. crystalline biotin (dissolved in distilled water) per gram body weight from birth to sixteen days of age, showed no significant gross changes from the usual hairless pattern in rate of hair depilation. Neither 13-day nor 16-day biopsy specimens of dorsal skin from one treated male exhibited any essential histological differences from

those of a control litter-mate fed distilled water.

5. "Epidermal Warts " - From the age of four weeks, epidermal warts developed in the majority of control hairless mice. Histological examination of these cutaneous outgrowths revealed that, in addition to all epidermal layers of the skin, most of the corium is involved (Figs. 22, 23). The most obvious features are the overdevelopment of all Malpighian layers and the extensive overproduction of the stratum corneum. The stratum germinativum is fifteen cell-layers thick in some places. Many clusters of cells which stain like cells of the germinal layer infiltrate the corium; these may represent partially developing hair germs. The stratum spinosum is not clearly differentiated, but both the stratum granulosum and stratum lucidum are distinct. Distal to the stratum lucidum is the highly developed stratum corneum.

Warts also developed in vitamin A-treated hairless mice (Table VIII), but were fewer in number, and usually appeared one to two weeks later than in control litter-mates. With continued vitamin A administration, the warts became smaller in size and disappeared in most treated mice. In a few litters, very few warty outgrowths developed on the skin of either treated or control animals, and all warts disappeared about the same time. Among a total of 29 hairless mice fed vitamin A from birth or the first week of life, warts did not develop in eight animals (by 11 weeks of age), whereas in none of the controls did warts fail to develop at five or six weeks. The difference between the proportion of treated hairless mice in which warts developed

TABLE VIII

Distribution of Epidermal Warts
Among Young Treated Hairless Mice

Treatment (Vitamin A Dose level, in I.U./ gm./day)	Total Number of Mice	Number With Warts		Number Without Warts	
		♂♂	♀♀	♂♂	♀♀
50	13	4	4	2	3
75	1	-	1	-	-
100	11	6	3	2	-
200	4	1	2	-	1
Total Vitamin A	29	11	10	4	4
Oil (Control)	16	8	8	-	-
TOTALS	45	$\chi^2 = 3.681$ $P > 0.05$			

(21/29) with the proportion in oil-fed controls (16/16), however, was not significant ($\chi^2 = 3.681$; $P > 0.05$). There were no sex differences in the distribution of epidermal warts (Table VIII).

D. Effect of Sex and Pregnancy on Response to Vitamin A

1. Untreated Mutant Mice - Early in the present investigation, gross differences in the appearance and texture of the skin were observed between untreated male hairless mice and their female littermates, particularly in breeding cages. From the age of six to eight weeks, females had pinker, smoother and thinner skins than their brothers. Skin samples from three pairs of untreated littermates exhibit some consistent intra-litter histological differences in support of the gross differences (compare Figs. 24, 26, and 28, skin from untreated hairless females, with Figs. 25, 27, and 29, skin from their littermate brothers). Female skin appears to be thinner, contains fewer cysts, and more fat cells.

No gross or histological differences were found between male and female rhinos.

2. Treated Hairless Mice - The sex differences observed in the skin of untreated hairless littermates were also evident during treatment with vitamin A. For example, as noted above, both ♀ 1375 (fed vitamin A) and ♀ 1374 (a littermate control) manifested pinker and smoother skins than either their control or treated brothers, ♂ 1373 and ♂ 1372, from the beginning of treatment (at 16 weeks of age) through 26 weeks of treatment. Spleens of both females were

clearly visible for at least two and one-half weeks after the start of treatment (for an additional week in the vitamin A-fed female), whereas the spleens of both males could not be seen clearly through the skin at the end of the first week. An interesting observation was that the spleen of the oil-fed control, ♀ 1374, was visible through the skin clearly enough to be measured during, but not between, the first five of her six pregnancies.

Histological differences between skin samples from the treated littermates, ♂ 1372 and ♀ 1375, after six months of vitamin A at the same dose level clearly support the differences observed grossly. Compared to skin sections from ♀ 1375 (Fig. 10), those from ♂ 1372 (Fig. 7) show a denser corium (more fibrous, less cellular), a more pronounced hyperkeratinization, and larger and more numerous dermal cysts. While there are some fat cells in the skin from ♂ 1372, they are more profuse and stratified in the skin from the treated female.

Both control mice exhibited skin changes characteristic of untreated hairless animals, including sex differences described above, during the six months of observation; furthermore, as noted, the skin of the female, ♀ 1374, became very much thinner during pregnancy, as evident by the visualization of the spleen. Sections of the skin taken from ♀ 1374 two days after the birth of her last litter (Fig. 11), compared to those from the control male (Fig. 6), reveal obvious differences in the corium, with respect to its thickness, degree of fibrosity, and amount of adipose tissue.

Sex differences in the skin also developed between male and female hairless littermates despite treatment with vitamin A which was started before the age at which these differences appear in untreated animals (6 to 8 weeks). In addition, other skin differences between younger treated males and females were observed. For instance, among the seven animals in litter-389 fed vitamin A (50 or 100 I.U./gm./day) from two days of age, substantially fewer epidermal warts developed at four weeks of age in the three females than in three males (warts did not develop at any time in one male). These outgrowths disappeared within four to six days in all treated females and in one of the males given the higher dosage, whereas in the remaining two males, some warts were still manifest a week later. During the next 20 weeks of treatment, one treated female maintained a pinker and smoother skin than her three treated brothers (two females and one male of this litter had been sacrificed at eight weeks of age). Even the skin of the control female was pinker and smoother than that of treated males. In both treated and control females the spleen was clearly visible through the skin throughout the period of treatment, whereas in all treated males, it could not be seen clearly enough to be measured accurately at ten weeks of age, and became progressively harder to see through the skin during the next month; at fifteen weeks, the organ could not be visualized at all in males. Skin samples from all treated animals at the termination of treatment show, in histological section, fewer differences than would be expected on the basis of gross observations (Figs. 14-17). Sections

from the treated female (Fig. 17) are thinner and the corium is less fibrous than those from treated males (Figs. 15 and 16). The development of dermal sebaceous cysts apparently has been less affected in the treated female than in the male littermate treated at the same dose level (50 I.U./gm./day).

Although marked differences in the skin between male and female hairless mice treated with vitamin A from birth have been observed grossly in most litters treated for four months or longer, these differences are less marked histologically in dorsal skin samples taken from the same animals, as noted for example in litter-374 (Fig. 30, ♀ 374-1, treated at 50 I.U./gm./day for 27 weeks; Fig. 31, ♂ 374-5, a littermate treated at the same dosage for the same period of time). Marked thinning of the skin (as evidenced by direct observation and by the fact that the spleen became especially clear through the skin) was also observed in two treated females (littermates) during the course of three pregnancies; this change was not as striking, however, as that observed in a control female, in whom the spleen was barely visible after ten weeks of age, except during pregnancy.

3. Sex and Vitamin A-Induced Systemic Toxicity - In addition to sex differences with respect to the hyperkeratotic process in the skin of hairless mice there was also an indication that the prolonged administration of vitamin A induced less extreme systemic effects in adult females than in their treated littermate brothers. For example, after six months treatment at a dosage level of 100 I.U./gm./day, the

liver of hairless ♀ 1375 had a relatively normal appearance and weighed 1.52 grams, a per cent ratio to body weight of 6.08%, i.e., very similar to the control value (Table II), whereas the liver from a treated male littermate, ♂ 1372, exhibited large white infiltrations of most lobes (one lobe had a large cystic area) and weighed 2.93 grams, a ratio of 12.63% of body weight. There were no sex differences between spleens in either appearance or gross weights in this pair of mice (the percent ratios of both were roughly three times those from their respective controls). Similar post-mortem sex differences with respect to hepatic changes were found in one other pair of adult hairless littermates treated at the same dose level for 20 weeks; in addition, the spleen ratio of the treated male was three and one-half times that of the treated female.

In four litters of younger hairless mice treated up to six months at the same dosages, however, evidence of systemic toxicity did not occur. For example, among both treated males and females in litter-389, livers and spleens appeared to be normal in all respects, and the ratios to body weight were not different from control values (6.01-6.57 for livers; 0.40-0.58 for spleens).

Among five pairs of treated normal mice, females (especially those that became pregnant during treatment) exhibited less extreme changes than males in the pelage, and as well, less extreme evidence of systemic effects attributable to prolonged administration of vitamin A at dosages of the order of 100 I.U./gm./day. The sex differences

were very slight among the three pairs of younger animals on which post-mortem examination was completed. Except for occasional evidence of hemorrhage in the lungs of a few males, there were no gross differences in the internal organs between normal males and females at autopsy. Only in older animals (two pair of mice 16 and 22 weeks old when treatment was started), were more marked sex differences observed.

E. Congenital Defects Induced by Vitamin A

During the oral administration of vitamin A, nineteen females were allowed to become pregnant to see if prenatal treatment with vitamin A would enhance the effects of postnatal treatment on the skin (it did not). They gave birth to a total of 35 litters, four of which were produced by three hairless females, the remainder by sixteen normal females. Among the 162 offspring from treated animals, 31 exhibited gross abnormalities, whereas all 119 offspring from eight control females (two hairless and six normal) were grossly normal in all respects (Table IX). There were no offspring from rhino females fed vitamin A or corn oil.

Only one of the three treated hairless females produced abnormal young (Table X). Hairless ♀ 360-6, fed vitamin A at a daily dose level of 50 I.U./gm./day throughout pregnancy, gave birth to a litter of three, two of which had multiple gross congenital defects. One newborn had a monstrously deformed skull, with a wide median cleft bisecting the entire snout, and bilateral exophthalmia, which gave it a "bull-dog" appearance; the second defective newborn manifested

TABLE IX

Effect of Oral Vitamin A on Offspring
of Females Treated During Pregnancy.

Pheno- type	Treatment	No. of ♀♀	Total Litters	No. Born	Av. Litter Size	No. Born Dead (%)	No. With Congenital Defects (%)
hr	Vitamin A	3	4	17	4.3	9 (53)	2* (11.8)
	Oil	2	8	43	5.4	6 (14)	0 (0.0)
+	Vitamin A	16	31	165	5.3	28 (17)	29 (17.8)
	Oil	6	10	76	7.6	4 (5.3)	0 (0.0)

*All in one litter

exencephaly associated with left cleft lip and palate. The remaining newborn of this litter was grossly normal except for a subdermal hemorrhagic spot at the junction of the parietal and frontal bones of the calvarium.

Among the 31 litters from treated normal females, seventeen contained a total of 29 young with gross congenital defect (Table X). There were 20 offspring with median cleft palate (12 ♂♂ , 6 ♀♀ , 2?), four with unilateral microphthalmia (including one newborn with left microphthalmia and right anophthalmia), one with an acute tail kink (skeletal), and four with multiple malformations -- two with a "short-head" syndrome associated with bilateral anophthalmia and malformed palate (which bulged into the mouth), one with exencephaly and median cleft palate, and one with unilateral microphthalmia and median cleft palate. In addition five offspring from two treated normal females exhibited subdermal hemorrhages on the skull, but no detectable gross defects. All grossly abnormal offspring were from mothers fed vitamin A before the twelfth day of gestation. Among a total of 43 young in eight litters from two hairless females fed the corn oil vehicle throughout pregnancy, all appeared to be grossly normal.

One hairless and seven normal females produced more than one litter during treatment (Table X). The normals bore a total of 22 litters, containing 111 offspring, 27 of which (24.3%) had congenital defects. Of the 40 offspring from the first pregnancies of these multiparous normal dams, ten (25%) had gross defects (cleft palates,

TABLE X

Distribution of Gross Congenital Defects in Successive
Litters From Females Treated With Vitamin A
Orally During Pregnancy

Pheno- type	Dose (I.U./ gm./day)	Female	L i t t e r									
			1		2		3		4		5	
			No.*	Ab.**	No.	Ab.	No.	Ab.	No.	Ab.	No.	Ab.
hr	50	360-4	5	-	4	-						
		360-6	3	2#								
	100	9129	5	-								
	Total hr	(3 ♀♀; 4 litters)	13	2	4	-						
+	50	356-1	4	-	4	-	6	1	5	1	4	2#
		414-5	7	3	5	1						
		417-5	5	-								
	75	1293	4	-								
	100	1338	3	3	5	5	2	2	1	-		
		1356	6	-	6	2	6	-	2	1		
		1352	7	1	3	-	7	-				
		412-6	6	-	6	-						
		1275	6	1								
		1252	3	-								
		1354	7									
		1353	10									
		1355	5	-								
		414-6	9	1								
	150	1311	5	-								
	200	414-7	7	3#	9	2						
	Total +	(16 ♀♀; 31 litters)	94	17	38	10	21	3	8	2	4	2

* Number born

** Number with defects

Two with multiple defects in each case

eye defects, monstrous heads); in nine of the fifteen successive litters from the same females, seventeen of 71 offspring (23.9%) were grossly abnormal, indicating that continued treatment did not increase the frequency of gross congenital defect. Litter size was 5.7 in first litters and 4.7 in successive litters. The remaining nine treated normal females gave birth to 54 young, two of which were grossly abnormal (one had a median cleft palate, the other, left microphthalmia associated with right anophthalmia).

Three females (one hairless and two normals), under treatment with vitamin A in corn oil by intramuscular injection, became pregnant and gave birth to a total of 22 apparently normal live offspring in four litters. Two control females injected with corn oil produced four litters (three from the hairless control) which contained four dead and 26 live offspring; one live newborn had epidermal contusions about the head and neck (probably due to birth injury). In all females injected with the oily preparations, injection sites were swollen by depots of material which persisted throughout the courses of treatment.

Five hairless and seven normal females were given intramuscular injections of "aqueous" vitamin A (but only during pregnancy) at dose levels ranging from 50 to 200 I.U./gram body weight daily. Treatment was begun on the eighth or ninth days of gestation, and terminated about the nineteenth or twentieth days. (Gestation days were established by palpation, and also by counting back from the day of

TABLE XI

Effect of Aqueous Vitamin A by Intramuscular Injection
on Offspring of Treated Pregnant Females

Pheno- type	Female	Dose (I.U./ gm./day (In Succ. Litters)	L i t t e r						Av. litter size	% mor- tal- ity	% cong. def.
			1			2					
			No.*	D.#	Ab."	No.	D.	Ab.			
hr	1404	50	9	1	-						
	1316	50	5	3	-						
	1435	100	7	5	1 ^a						
	1438	100	6	-	-						
	1317	100	5	1	1 ^b						
	Total hr (5 ♀♀; 5 litters):		32	10	2				6.4	31.3	6.3
+	1353 ^o	50, 100	10	1	-	3	-	1 ^b			
	1395	75	5	-	1 ^c						
	1351	100, 200	8	-	-	5	1	-			
	1354 ^o	100	8	1	-			-			
	1431	100, 200	11	2	-	14	2				
	1425	100	4	-	-						
	1421	200	11	3	-						
Total + (7 ♀♀; 10 litters):		57	7	1	22	3	1	7.9	12.7	5.1	

* Number born

Number born dead

" Number with defects: a) short snout, associated with micro-
phthalmia

b) median cleft palate

c) short snout, minute nares, harelip

o Previously fed vitamin A in corn oil (cf - Table X) which was
discontinued one month previous.

birth, which was called "day-21"). In most of the treated females, depilation was observed around the sites of injection. Since the aqueous vehicle was not available, there were no controls.

Among the 111 offspring in fifteen litters from these twelve treated females (five litters from hairless, ten from normal, dams), four newborn had gross congenital defects (Table XI). The malformations induced by the parenteral administration of aqueous vitamin A were similar to those induced by the oral administration of vitamin A in corn oil, viz., median cleft palate, microphthalmia, and grossly malformed heads. There were two newborn with a "syndrome" of defects which consisted of short snout, minute nares, harelip and microphthalmia.

IV. DISCUSSION

The results of the present investigation demonstrated that the chronic administration of large doses of vitamin A modified the hyperkeratotic process in both rhino and hairless mice, and thus confirms and extends the results of previous experimental work reported from this laboratory (Fraser, 1949). The basic anomaly in the skin leading to such mutant phenotypes is, however, apparently not altered. Briefly it was found here that:

i) When treatment was begun immediately after depilation was completed, there was inhibition of dermal cyst formation and epidermal hyperkeratinization;

ii) High doses of vitamin A administered over a long period of time (up to seven months) produced systemic toxicity which appeared to be correlated with the degree of cyst inhibition in the skin; and,

iii) The response to treatment of hairless mice was qualitatively similar to that of rhino mice.

A. Effect of Vitamin A on the Skin

In normal mice, only one unit of vitamin A per day has been shown to be entirely adequate for maximal growth and the prevention of deficiency symptoms (McCarthy and Cerecedo, 1953). Attempts to induce a specific syndrome of effects in normal mice by dietary deficiency of vitamin A have been disappointing (Loewenthal, 1956). However, for

the purposes of the present investigation it was assumed that there was an intimate relationship between the action of vitamin A and the action of genes at the hairless locus in the mouse which produces hypotrichosis and follicular hyperkeratosis. In some way, mice carrying a double dose of the mutant alleles, hr or hr^{rh}, manifest cutaneous lesions similar to those found in otherwise normal individuals of other species deprived of dietary vitamin A (Frazier and Hu, 1936; Moulton, 1943; etc.). Rhino and hairless mice apparently cannot utilize the vitamin A provided in the normal diet to maintain a normal skin and pelage, either because it is deficient in the blood (due to faulty gastrointestinal absorption or liver dysfunction), or because the skin cells themselves cannot utilize it at the normal concentrations (Fraser, 1949). If the etiological factor is a blood deficiency (by whatever cause), however, one would expect other typical signs of vitamin A deficiency, viz., keratomalacia, squamous metaplasia, etc. (Wolbach, 1954). Since the mutants appear to be otherwise grossly normal except for the skin defects, the factors responsible for the phenotypic abnormalities more likely reside in the skin.

Vitamin A treatment did not retard the loss of the juvenile pelage (which begins at two weeks of age) in either hairless or rhino mice. It is noted that the mean age at onset of hair loss was younger in both mutant types when fed either vitamin A or corn oil than it was in untreated animals. One wonders if this was a result of the extra handling to which the fed mice were exposed. No effect of treatment

on hair loss was observed histologically in skin biopsies of hairless mice. In hairless mice, Chase and his colleagues (cf., Chase, 1954) demonstrated histologically a failure in the production of a portion of the connective tissue sheath surrounding the hair follicle (the "glassy membrane"). This is associated with abnormal club hair formation, failure of the entire follicle to shorten, and premature falling out of the hair. The continuity of the follicle base with the hair germ of the next generation is disrupted, and since there is no repair of the epithelial cords, subsequent hair formation does not occur. Following depilation of body hair in hairless mice, the generative layer of the epidermis hypertrophies, resulting in hyperplasia of the keratinizing epithelium. Stranded cell aggregates of the epithelial cord and hair germ undergo a sebaceous transformation and develop, as do also the sebaceous glands, into cysts which become cornified (Montagna et al, 1952). Whether the same sequence of events occurs in the skin of rhino mice has not been demonstrated. In general, hyperkeratosis with cyst formation develops more rapidly, and is more extreme in rhinos; in addition, hair canal cysts ("utriculi") develop early. Furthermore, in both mutants a follicular keratosis is evident histologically at about the time depilation begins (Fraser, 1946).

In mutant mice fed vitamin A from birth, the development of hyperkeratosis and sebaceous cysts is markedly inhibited, but there appears to be no alteration of the basic follicular defect leading to depilation. Once dermal cysts have formed, vitamin A treatment has

little if any effect on them; hyperkeratinization, however, is to some extent reversed.

The precise role of vitamin A in cutaneous keratinization in normal animals is as yet not known. Flesch (1953) postulated that large amounts of vitamin A have a non-specific "drug effect" upon epidermis, possibly due to an interference with sulfhydryl metabolism. This could not be confirmed histologically or histochemically by Rademacher and Montagna (1956), who found a normal distribution of sulfhydryl groups throughout the skin of treated mice. Fell and Mellanby (1953) demonstrated in vitro the suppression of keratinization of embryonic chick ectoderm by vitamin A; parallel experiments with mouse skin were inconclusive (Fell, 1953). In treated hairless mice, and to a lesser degree in treated rhinos, it was found that hyperkeratinization was suppressed by vitamin A, when treatment was started early in life. This may or may not represent a direct effect of the vitamin A on skin cells. That vitamin A could not be demonstrated in the epidermis either chemically or by fluorescence microscopy, even after excessive dosing (Popper, 1944), argues against a direct effect, although a metabolite not demonstrable by such procedures may be involved (Flesch, 1953). Furthermore, it was observed in the present investigation that the most clear-cut effect of treatment on hyperkeratosis, as well as on the development of sebaceous cysts, was shown in those animals in whom systemic toxicity occurred. The association of systemic toxicity and histological evidence of an interference with

normal keratinization (and in addition, the production of alopecia) was also observed in treated normal littermates. Thus it would appear that the effects observed in the skin of treated mutants may represent non-specific changes secondary to the systemic toxicity induced by vitamin A excess. It would be informative to see if chronic intoxication by other means would produce similar effects on the skin.

B. Differences in Response Between Rhino and Hairless Mice to Treatment

In the present study the oral administration of vitamin A to hairless mice resulted in effects qualitatively similar to those observed in treated rhinos. Thus, in treated hairless mice, vitamin A: i) prevents the formation of sebaceous cysts in the dermis, but does not affect those already formed; ii) suppresses hyperkeratinization; and, iii) produces pathological lesions in liver, spleen, kidneys and lungs. Certain differences in the response to treatment between rhino and hairless mice, however, deserve comment.

Hairless mice rarely manifested signs of systemic toxicity at dosages which eventually kill rhinos (ca. 100 I.U./gm./day). This may reflect strain differences, since untreated hairless mice appear to be more vigorous than rhinos. Although the data are scanty, the weights of spleens and livers of treated normals of the RH line tended to be greater than those of treated HR normals (Tables II, III). If confirmed, these line differences would play a significant role in the differences in resistance to the systemic toxic effects of vitamin A treatment observed between the respective mutants. However, the "beneficial"

effects induced in the skin by vitamin A appeared in hairless mice at dosages which had little or no effect in rhinos (i.e., 50-75 I.U./gm./day). The fact that there is a wider range between therapeutic effect and toxic level in the hairless mutant than there is in the rhino mutant would be difficult to interpret simply on the basis of a non-specific greater vigour in the hairless strain. It would seem more likely that the greater response to treatment of the hairless strain is associated with the fact that it is a less extreme mutant (Steinberg and Fraser, 1946; Fraser, 1946). Hairless animals do not develop the extreme wrinkling characteristic of older rhinos. Histologically, only a few utriculi are found in hairless skin, and sebaceous cysts are usually smaller; consequently, the thickening of hairless skin does not approach that found in rhinos. Thus, the less extreme involvement of the skin in hairless mice, together with their greater resistance to systemic toxic effect induced by treatment, suggest that the postulated block in vitamin A metabolism is less complete in the hairless mutant than it is in the rhino mutant.

Although no histochemical tests were made, it appears that subdermal fat cells do not completely disappear from the skin of adult hairless mice, as they do from the skin of rhinos. Fluorescence microscopy has demonstrated the presence of vitamin A in cutaneous adipose tissue (but not in epidermis) in the normal rat (Fopper, 1944). One may argue that fat cells are necessary for the maintenance of a normal epithelium because they serve as natural depots for essential

fat-soluble substances, such as circulating vitamin A. Hence it is an attractive hypothesis that the less extreme involvement of the skin of untreated adult hairless animals compared to that in rhino adults is due to residual amounts of vitamin A, or its active metabolites, in these persistent fat cells. That such a natural depot for vitamin A persists would be consistent with the hypothesis that vitamin A or its active metabolites reach effective levels in hairless skin when fed at dosages that have little or no effect in rhinos (i.e., 50-75 I.U./gm./day). It may be relevant that Josephs (1942) found that the blood lipids in rats fed diets deficient in vitamin A decreased several weeks preceding overt signs of deficiency, and increased above normal levels when supplements of the vitamin A were fed to deficient animals. On this evidence Josephs advanced the hypothesis that one function of vitamin A was concerned with lipid metabolism, and thus, during deficiency, the body's stores of fat (including the subcutaneous fat stores) tended to disappear. Liver lipids also tended to fall slightly in deficient rats.

Despite the differences noted between hairless and rhino mice, it has not been proved that vitamin A, even at dosages producing systemic toxicity and death, has a specific effect on the skin of these mutants. In treated hairless mice, vitamin A apparently has no effect on the failure of the "glassy membrane" to function normally, since no gross or microscopical differences were noted between treated and control skin with respect to the loss of the juvenile pelage. Subsequent hair

generations in hairless mice are affected by treatment in that more than one-half of the vitamin A-treated animals did not develop secondary hair at five weeks of age (when it should have appeared), whereas only one control animal failed to do so.

C. Role of Vitamin A in Keratinization

Hyperkeratinization (as distinct from the production of cysts) seems to be modified by vitamin A in both hairless and rhino mice, regardless of the degree of cystic involvement of the skin. A satisfactory mechanism to explain the action of vitamin A on keratinization is lacking. The studies of Montagna (1954) in guinea pigs, and Rademacher and Montagna (1956) in normal mice, fail to support the contention that vitamin A affects sulfhydryl metabolism directly, as had been postulated by Flesch (1953) on the basis of in vitro experiments. Since compounds other than vitamin A inhibit sulfhydryl metabolism (Flesch, 1953), it is doubtful that vitamin A would be specific in this respect.

An alternative suggestion is that the suppression of keratinization in the skin of mutant mice is a secondary (and non-specific) effect of the systemic toxicity induced in hypervitaminotic animals. The results of treatment with vitamin A reported by Studer and Frey (1949) in rats, and Rodahl (1950) in rodents and other animals, as well as the clinical "success" of the systemic administration of vitamin A reported by many investigators, are explicable on this basis. Although Montagna (1954) could not demonstrate an "anti-keratinizing" effect of vitamin A when it was applied locally to the skin of guinea

pigs, both Sabella and his associates (1951) and Wolbach (1954) support such an action of vitamin A in rats and mice. Furthermore, in vitro studies of skin from various species has demonstrated that vitamin A can suppress keratinization directly. For instance, Fell and Mellanby (1953,1957) found that in embryonic ectoderm cultivated continuously in a medium containing vitamin A, keratinization was completely suppressed; the explants developed into a mucus-secreting, often ciliated epithelium resembling nasal mucosa. When transferred to a vitamin A-free medium, new squamous keratinizing epithelium differentiated beneath the metaplastic mucosa, which subsequently was sloughed off. These results were confirmed by Weiss and James (1955) who also used embryonic chick ectoderm explants, but employed only brief exposure to vitamin A, as well as by Fell (1953) in embryonic mouse ectoderm and by Lasnitski (1956) in skin from three to four month-old human fetuses. In the case of the mouse explants, however, there was only partial suppression of keratinization and no metaplasia, whereas explants of human fetal skin exhibited complete suppression of keratin, but only a partial tendency toward mucoid metaplasia.

D. Relation of Vitamin A to Mutation at the Hairless Locus in the Mouse

It would appear, then, that there may be at least two actions of mutant genes at the hairless locus in the mouse, separable on the basis of the response to high doses of vitamin A. One action concerns the abnormal development of the hair follicle at the time of normal club formation (demonstrated so far only in hairless mice), with subsequent

cystic transformation of portions of the broken follicle. The second action is concerned with the process of epidermal keratinization, differing in degree, but not in character, in rhino and hairless mice. In an attempt to delineate a unitary action of mutant genes on the skin of these mutants, we may ask the following: 1) Are cystic development and hyperkeratinization both secondary effects of the loss of hair? Follicular keratosis at the time depilation begins (ca. 2 weeks) argues against this; also, utriculus formation in rhinos and epithelial hypertrophy in both rhinos and hairless mice are well advanced before cysts develop in follicle-ends, sebaceous glands and from stranded cell clumps of the broken epithelial cord (Fraser, 1946; Montagna et al., 1952). 2) Does, then, hyperkeratosis of the surface layers and follicle neck plus the widening of the hair canal account for depilation and the subsequent development of cysts? At first, this appears to be a satisfactory alternate hypothesis, since the normal resistance to the outward movement of hair during formation of the faulty hair club is reduced in mutants, and the hair fall out prematurely (Fraser, 1946). This hypothesis, however, does not explain the failure of subsequent hair generations in mutant mice (although sparse regeneration does occur in hairless mice). Furthermore, the provision of an increased concentration of circulating vitamin A to the skin partially resolves the hyperkeratinization, but yet further hair growth fails to occur. In fact, in hairless mice which appear to be still capable of producing some hair after the juvenile pelage falls out, treatment inhibits it. Hence,

assuming that vitamin A, on systemic administration, has a specific anti-keratinizing action on the skin, we still must account for the failure of subsequent normal hair regeneration in both mutants. In mutant mice, it has been suggested that the development of hair canal cysts is associated with lack of secondary hair growth (Fraser, 1946), for, in rhinos, most if not all follicles contain utriculi and no secondary hair develops, whereas in hairless mice the failure of hair regeneration apparently occurs only in those follicles which contain utriculi (far fewer in number than in rhinos).

E. Sex Differences in Response to Vitamin A

The differences in the appearance and texture of the skin between male and female hairless mice described in this investigation have not been mentioned in previous studies of these mutants (Crew and Mirskaia, 1931-32; David, 1932, 1934; Chase, 1954; Steinberg and Fraser, 1946; Montagna et al, 1952, 1954). It was found that female hairless mice had pinker, oilier and thinner skin than hairless males, starting from the age of weaning on. Although histological examination of the skin of untreated animals did not reveal marked differences in support of the differences observed grossly, skin from females did exhibit a less dense corium and fewer dermal cysts, but more fat cells (Figs. 24-29). These sex differences persisted during treatment with vitamin A, even when treatment was begun prior to the age these differences first became apparent. No sex differences were observed in untreated rhino mice, and the response to treatment was similar in males and females.

Sex differences in the response of the skin to vitamin A deficiency have been reported in normal mice by Loewenthal (1956). She reported that a new growth cycle was initiated in hair follicles of all vitamin A-deficient females (new hair growth was initiated by plucking), regardless of the stage of deficiency, and that the few mice that did not respond were all males. Sex differences in response to vitamin A-deficient diets have also been reported in earlier investigations in normal mice (e. g., McCarthy and Cerecedo, 1952), and also in normal rats (Moult, 1943; Mayer and Krehl, 1948).

Differences between males and females in the systemic toxic effects induced by hypervitaminosis A were also observed in the present investigation. There was a tendency for females to be less affected by treatment; for example, fewer pathological lesions were found in the livers of treated female mutant mice. At very high doses, however, sex differences with respect to induced systemic toxicity were obliterated. No clear-cut differences were noted in normal mice fed toxic dose levels of vitamin A, nor have sex differences in the manifestations of systemic toxicity been reported in other normal animals (Rodahl, 1950; Wolbach, 1954; Nieman and Klein Obbink, 1954).

The influence of sex on the response to both deficiency and excess of vitamin A presumably stems from sex differences in the requirements, distribution and storage of this vitamin. In normal rats, Moore and Sharman (1951) found that liver stores were higher in females; previously, Brenner and her associates (1942) had reported that females

also accumulated higher liver stores after massive dosing, but had lower blood levels.

The protective effect of femaleness on vitamin A-induced systemic toxicity is difficult to explain. The fact that vitamin A reserves are higher in females may mean that the greater resistance to the pathological changes induced by treatment result from a higher "saturation potential". Estrogen, the "classical" antagonist to vitamin A, may also play a role in conferring such systemic protection. This would be consistent with the view that the skin changes in hairless mice given vitamin A systemically are a consequence of secondary, non-specific toxicity. The cause of the cutaneous skin differences in untreated hairless animals, however, cannot be accounted for on this premise. On the other hand, Selye (1943), on the basis of a limited number of observations, claimed that the local application of estradiol to the skin of adult rhino mice exacerbated the hyperkeratosis, particularly cystic development. Sabella and his associates (1951), however, found that estradiol had no effect on normal rat skin, nor did it counteract the effect of topically applied vitamin A, which was reported to decrease the rate of keratin formation.

F. Pregnancy and Skin Changes

A further feature of interest with respect to skin changes in hyperkeratotic mutant mice is the peculiar effect of pregnancy. In both treated and control female hairless mice, the skin became noticeably thinner during the last three to four days of pregnancy. Similar

observations were reported by Fraser and May (1953), who described the skin of pregnant naked females as "paper-thin" just before term. In addition, these authors noted that pelage hairs were shed at parturition, and the resumption of hair growth was suppressed during lactation. It was suggested that the high titre of endogenous estrogen (which occurs toward the end of pregnancy in the mouse) may have been responsible for the skin effects noted. In contrast, little or no sex differences in the skin were observed between non-pregnant females and littermate males (Fraser and May, 1953).

It has been reported that estrogen markedly affects the skin and hair growth of normal adult animals. For example, Houssay and Higgins (1949) reported that gonadectomy greatly accelerated hair regrowth after close clipping of the pelage in normal female mice (and strangely enough, also in males!), but had little or no stimulatory effect in rats (Houssay, 1953). Emmens (1942), on the other hand, found that growth of the hair after shaving was increased slightly in ovariectomized female rats, but was unaffected in castrated males. Emmens further reported that in intact normal animals given estrone, the rate of regrowth was decreased to a marked degree in males, but there was little effect in females, when the hormone was applied topically to the shaved skin or administered systemically; androgens (testosterone) had no effect in either sex. Emmens attributed the negligible suppression of hair growth in females to the high level of endogenous estrogen which had already induced the maximal inhibition possible. The

gonadectomized mouse appears to be more sensitive than the gonadectomized rat to the effect of estrogens, since Houssay (1953) found that the rate of hair growth was reduced by a considerably smaller dose of estradiol benzoate than was required for the gonadectomized rat.

Hooker and Pfeiffer (1943) reported that parenterally-administered estrogens produced alopecia and marked thinning of the skin in both male and female rats. Histologically, a reduction in the number of epidermal layers, thinning of the dermis, and an almost complete disappearance of adipose tissue were found. A reduction in size and number of sebaceous glands was reported, and hair follicles and hair shafts were described as "atrophic". The concurrent administration of androgens completely protected estrogen-treated animals against these effects.

The effect of estrogen in producing alopecia and in reducing skin thickness described by Hooker and Pfeiffer are in accord with the idea that the effect of pregnancy on the skin of Naked mice (Fraser and Nay, 1953), and in hairless mice in this study, was due to an increased level of estrogen during pregnancy.

G. Congenital Defects

Gross congenital defects have been reported in the offspring of vitamin A-deficient pregnant females of various species (Andersen, 1949; Hale, 1935; Lamming et al, 1954; Wilson et al, 1953). Typical anomalies included anophthalmia and other ocular defects (Hale, 1935; Wilson et al, 1953), hydrocephalus (Lamming et al, 1954), cleft palate

(Hale,1935; Warkany,1945), dental abnormalities (Warkany,1945), aortic arch defects (Wilson et al,1953), diaphragmic hernia (Andersen,1949; Wilson et al,1953), and abnormalities in heart, lungs and urogenital system (Wilson et al,1953).

Although congenital defects have been described in embryos from hypervitaminotic A rats (Cohlan,1953; Giroud and Martinet,1955; Woollam and Millen,1957; Millen and Woollam,1957), no studies have been reported thus far in mice. In the present investigation, gross malformations, similar to those reported in rat embryos, were found in newborn mice from mothers that had received vitamin A continuously before and during pregnancy, as well as in the offspring of females treated during pregnancy only (Tables IX - XI). Typical defects found included exencephaly, microphthalmia and anophthalmia, cleft palate, as well as embryos with monstrous heads; in a number of newborn, the only anomaly observed was subdermal petechial hemorrhages on the skull. The most frequent malformation was cleft palate (without cleft lip); among 29 grossly abnormal newborn from non-mutant pregnant females fed vitamin A continuously, 22 exhibited median clefts of the posterior palate, including two offspring with other cranial defects (exencephaly and microphthalmia). These 22 defective embryos represented 13.3% of all newborn from treated females fed vitamin A. In the same series, seven newborn (4.3% of all newborn from treated mothers) exhibited diminution of the eyeballs, usually unilateral, and five had subdermal hemorrhages not associated with gross abnormalities. Newborn mice with

exencephaly alone, skeletal defects and/or monstrous head were found less frequently.

Cohlan (1953) was the first investigator to describe congenital defects in embryos of pregnant mammals fed excessive amounts of vitamin A. Among over 50 litters obtained at or near term from rats fed an aqueous preparation of vitamin A, exencephaly was the most frequent gross defect found, followed by eye malformations, cleft palate, and a "syndrome" which included foreshortening of both maxilla and mandible associated with exencephaly (1954). In the litters of those females that were allowed to deliver spontaneously, there were many newborn which did not exhibit the major cranial defect, but did have small hematoma-like discolorations in the region of the anterior fontanelle (the junction of the frontal and parietal bones of the skull). Subsequent examination of the denuded skulls of these offspring at 30 days of age revealed defects of the bony continuity of the cranium. This is of particular interest since subdermal cranial hemorrhages were found at similar sites in the offspring of mice with hypervitaminosis A. Cohlan also found occult spina bifida in seven of fifteen late-stage fetuses taken from females that had received massive single doses of vitamin A (75,000 I.U.) on the tenth day of gestation.

Giroud and Martinet (1955), who used the same strain of rats (Wistar), induced similar frequencies of cleft palate and eye anomalies (microphthalmia, anophthalmia, cataracts) in embryos of pregnant females fed an oily preparation of vitamin A esters. Embryos with

"anencephaly", often associated with hemorrhage, were found in lesser frequency.

Woollam and Millen (1957) have recently reported that the oral administration of vitamin A acetate dissolved in arachis oil to Wistar female rats produced cleft palate and defects of the brain and skull in their embryos; the same dosage of vitamin A injected subcutaneously, however, was without apparent effect.

A comparison of the results of these three investigations in the same strain of rats and with those obtained in normal mice, has been compiled in Table XII. Although no firm conclusions can be drawn (since, for one thing, the experimental conditions of the four studies are not strictly comparable), the differences in results found in different laboratories where the same strain of rats was used are instructive. For example, Cohlman (1954) found that "aqueous" vitamin A fed at a dosage of 15,000 I.U./day (approximately 75-85 I.U./gram body weight/day on the basis of an average weight of 175-200 grams) from the third to the sixteenth days of gestation did not produce gross congenital defects, but the daily administration of 25,000 I.U. or more (approx. 140 I.U./gm., or more) was teratogenic. The overall incidence of defect found by Cohlman among litters from females fed vitamin A at doses of 25,000 I.U. or more was 39%. Giroud and Martinet (1955), who employed an oily preparation of vitamin A administered orally during the same period of gestation, obtained only abortion and resorption at doses of 35,000 I.U.. When the daily dose was dropped to 20,000 I.U., however

TABLE XII

Frequency of Gross Congenital Defects in Offspring of Normal Females Treated with Vitamin A

Species	Route	Daily dose* (I.U.)	Litter Data			% Minor. Embryos	% Defects			Ref.
			No.	Embryos	Av. Size		Calv.	Eye	CP	
Normal rats (Wistar)	Oral	35,000	25	158	6.3	48.7	48.7	?	** (38)	1
		25,000	26	222	8.6	16.7	?	?	?	
		15,000	9	76	8.4	0.0	-	-	-	
Normal rats (Wistar)	Oral	35,000	(Abortions with resorptions only)							2
		20,000	?	?	?	59.0	6	25	28	
Normal rats (Wistar)	Oral	60,000	12	77	6.4	29.7	7.8	?	29.7	3
	S.C.	60,000	12	69	5.8	0.0	-	-	-	
Normal mice (+ /hr, + /hrh)	Oral ***	50-75	9	44	4.9	18.1	4.6	2.3	15.9	4
		100	19	100	5.3	16.0	-	1.0	15.0	
		150-200	3	21	7.0	23.8	4.8	23.8	4.8	
		(Total oral	31	165	5.3	17.6	1.8	4.8	13.3)	
	I.M. ****	50-100	3	18	6.0	0.0	-	-	-	
	I.M. ****	50-200	10	79	7.9	2.3	1.2	-	1.2	

* In the present investigation, dosages were based on body weight. ** Only 19/50 embryos examined grossly. *** Administered prior to, during and after pregnancy. **** Administered only during pregnancy. Ref.: 1. Cohan, 1954 - 2. Giroud & Martinet, 1955 - 3. Woollam and Millen, 1957 - 4. Present investigation

(approx. 100 I.U./gm. ??), they found equal incidences of cleft palate and eye abnormalities (respectively 28% and 25%), but a smaller incidence of brain and skull defects (approx. 6%). A similar ratio of palate abnormality to calvarial defect was found by Woollam and Millen (1957) with daily doses of 60,000 I.U. vitamin A in arachis oil, begun on the eighth day of gestation. In the present investigation, the oral administration of vitamin A in corn oil at daily doses of 50 to 200 I.U./gm. body weight/day (in most cases begun prior to and throughout pregnancy), resulted in a greater incidence of cleft palate than of skull defects. The intramuscular administration of aqueous vitamin A also produced grossly abnormal newborn from treated mice. In keeping with the experience of Woollam and Millen (1957), who observed no gross defects in embryos of pregnant rats given oily vitamin A by subcutaneous injection, all offspring from mice given intramuscular injections of vitamin A dissolved in corn oil were grossly normal.

Since genetic differences probably play an important role in conditioning the embryonic outcome of environmental insult during pregnancy (Andersen, 1949; Fraser, 1953), it was of interest that treated hairless female mice produced defective young. Although the series are small, it is suggestive that the incidence and type of malformation observed in litters of hypervitaminotic hairless females were similar to those found in litters from treated non-mutant females. Average litter size among litters from mice fed vitamin A continuously was 4.3 for hairless and 5.3 for normals (not significant, $d/S.E.d = 1.65$). The ratios

of the incidence of offspring born dead in experimental groups to those in the respective control groups are similar, viz., 3.8:1 for hairless and 3.2:1 for normals (cf., Table IX), indicating that intrauterine death was due primarily to treatment and not to the genetic difference.

The reproductive performance of female mice given vitamin A parenterally, but only during pregnancy, was better than among groups treated continuously by oral administration. Litter size was 6.4 for injected hairless mice, and 7.9 for injected normals (not significant, $d/S.E._d = 0.36$); the ratios of neonatal deaths to the respectively comparable orally-treated groups (there were no aqueous vehicle controls) were similar, viz., 2.2:1 for hairless and 2.4:1 for normals.

A meaningful comparison between the incidence of gross congenital defects between hairless and normal groups cannot be made at this time, since the total series are small, and many variables were not controlled. Stage of gestation is obviously important, as are the dose level of the agent and the route of administration; when these are taken into account, the groups become even smaller. The general impression gained from this preliminary investigation, however, is that embryos of hairless mothers are no more sensitive than embryos of non-hairless mothers to the teratogenic action of vitamin A.

Finally, the question must be considered whether the excess vitamin A administered to pregnant females affects the embryo directly or results in changes in maternal physiology with secondary effects on embryonic development. The types of malformation produced by vitamin

A excess are not specific, since similar defects have been reported when other agents were employed (including vitamin A deficiency (Wilson et al,1953)). Hypertrophy of the adrenals is a common effect of excess vitamin A (e.g., Rodahl,1950). Both these facts favour the view that the effects of hypervitaminosis A are secondary to changes in maternal physiology, perhaps as a consequence of stress reactions mediated through the maternal adrenals. On the other hand, ACTH does not produce this pattern of malformations (Heiberg et al,1959).

Furthermore, the administration of massive doses of vitamin A to pregnant female rats results in increases in the vitamin A content of fetal livers and whole embryos (Henry et al,1949). Although these increases in fetal reserves of the vitamin were found to be small compared to the huge increases found in maternal tissues (especially in those of the rat), embryonic tissues are probably very sensitive to even minor alterations in the immediate environmental milieu at critical stages of development. For example, in fifteen-day embryos from pregnant rats fed 60,000 I.U. vitamin A from the twelfth to the fourteenth days of gestation, Giroud and his colleagues (1957) found only a two-fold increase in total embryonic values for vitamin A over values in embryos from control mothers (10.08 I.U. vs. 4.15 I.U., per embryo); however, 92% of the embryos from vitamin A-treated females displayed cleft palates and/or other gross defects, whereas no congenital defects were reported in embryos from the controls. Furthermore, it has been shown that vitamin A has a direct and reproducible action on growing bone

and epithelia, both in in vitro studies (Barnicot,1948; Fell,1953), and in vivo (Wolbach,1954). These findings suggest that vitamin A crosses the placental barrier but does not prove that increased embryonic concentrations of the vitamin itself are responsible for the congenital defects found.

V. SUMMARY AND CONCLUSIONS

The hypothesis was tested that the skin of rhino and hairless mice manifest an inability to utilize amounts of vitamin A supplied by the normal diet, resulting in changes leading to epidermal hyperkeratinization and loss of body hair, with subsequent cyst formation of epithelial derivatives. When massive doses of vitamin A were fed to mutant mice, the beneficial effects on the hyperkeratotic process were inseparable from the pathological systemic effects induced in hypervitaminotic mice. At comparable dosage schedules, rhino mice were much more sensitive than hairless mice or normal littermates to the systemic effects of the continuous administration of vitamin A; fatty infiltration of the liver and spleen, with hepatosplenomegaly, were prominent pathological findings. These lesions may be interpreted as an inability of rhinos to utilize vitamin A, or as a result of a non-specific decrease in vigour caused by the homozygous rhino condition. Other systemic effects found in treated rhinos (and normal mice) were hyperemia of various internal organs (lungs, kidneys, gastrointestinal tract, etc.) with hemorrhage in severely affected animals. Bone fractures described in hypervitaminotic rats (cf., Wolbach, 1954; Nieman and Klein Obbink, 1954) were not observed in hypervitaminotic mice. Hairless were less affected systemically than rhinos, but did exhibit similar skin responses. Both the observation that the skin of male and female hairless mice differ grossly, and the sex differences found in response to treatment cannot as yet be directly

related to the action of vitamin A. In addition, the marked effect of pregnancy on the skin of hairless females implicates hormonal factors as important influences on skin function.

The assumption that a local lack of, or dysfunction in, the ability of skin cells to utilize vitamin A or its metabolites is responsible for the defect in hyperkeratotic mice is an attractive hypothesis. The particular metabolic block(s) involved must be rigidly specific for skin cells, since among epithelial tissues, only the skin and its appendages (including the nails) appear to be involved in the pathological process leading to hyperkeratosis. In mutant females, the vaginal epithelium for example (a sensitive indicator of vitamin A deficiency in rodents) apparently is unaffected, since female hairless animals were found to be fertile, and even rhino females produced litters, although the latter's fertility is much more reduced in comparison to that of normal mice. The differences between rhino and hairless patterns of hyperkeratosis (and hair loss) deserve further experimental investigation.

Suggestions for Further Study

The existence of two mutations at the hairless locus in the mouse that produce hypotrichosis and hyperkeratosis provide a fertile field for the investigation of factors influencing hair growth and epidermal differentiation. The differences in expressivity between the rhino and hairless patterns of hair loss and skin changes appear to be quantitative, and may be assumed to involve the same "reaction chain"

biochemically. This view is supported by the observation that the skin of hairless/rhino hybrids exhibit intermediate characteristics (Steinberg and Fraser, 1946; Fraser, 1946). The response of hairless animals to high doses of vitamin A is complicated by systemic toxicity at high dosage levels, but to a lesser degree than in treated rhinos. Since the primary mechanism precipitating hair loss and hyperkeratosis appears to reside in the skin itself, in vitro culture of mutant skin in vitamin A-enriched media, along the lines of investigations initiated by Fell and Mellanby (1953, 1957), would contribute to the determination of the role, if any, of vitamin A in the skin of these mutants. Many variations suggest themselves in this experimental system.

The unexpected finding of a sex differential in the skin of hairless animals requires extension. The possible role of gonadal hormones as modifiers of the skin responses, and the sex differences observed systemically to vitamin A, also need confirmation.

One of the most prominent pathological changes in hypervitaminosis A is liver dysfunction and cirrhotic changes. Following the suggestion of Nieman and Klein Obbink (1954) that excess vitamin A may precipitate "displacement" deficiencies of other vitamins, the simultaneous administration of massive doses of the B-complex may act as a protection against liver damage induced by hypervitaminosis A, especially in rhinos which are more sensitive than normal littermates (or hairless mice) to hepatic damage. The recent finding of Millen

and Woollam (1958) that concurrent administration of large doses of B-vitamins (thiamine, riboflavin, pyridoxine, niacinamide) to pregnant rats fed massive doses of vitamin A (60,000 I.U./day) significantly decreased the incidence of gross congenital defects in their embryos lends support to this concept. With respect to congenital defects induced in embryos of hypervitaminotic A mice, the preliminary findings of the present study that hairless mice do not produce more malformed mice than normal mice need confirmation, since it would suggest that the systemic metabolism of vitamin A is not abnormal in the hairless mutant. Larger series are required, with special importance paid to dose level, gestation day, length of prenatal treatment, and especially the frequency of missed abortion and resorption.

Finally, further post-natal developmental analysis and comparison of mutants are required, with a view to ascertaining whether the dysfunction of the connective tissue sheath leading to disturbance in the hair follicle demonstrated in hairless skin by Chase and his co-workers also occurs in rhinos. The significance of the loss or reduction of the fat layer in the skin of these mutants also bears investigation. Transferring the rhino and hairless alleles into lines carrying the yellow gene (which predisposes to the storage of large quantities of body fat), for instance, might prove interesting in the light of Joseph's suggestion of the significance of the response of serum lipids to the vitamin A status in the rat (1942).

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VIII. PLATES

PLATE I, Figs. 1-6. Dorsal skin specimens from rhino mice treated for varying periods of time (all approx. $\times 90$):

Fig. 1. ♂ 1359, 22 weeks of age, fed corn oil from 10 weeks of age.

Fig. 2. ♂ 1358, same age, fed 100-200 I.U. vitamin A/gm. body weight/day from 10 weeks of age.

Fig. 3. ♂ 417-3, 13 1/2 weeks of age, fed 100 I.U. vitamin A/gm./day from 2 weeks of age.

Fig. 4. ♂ 398-1, 15 weeks of age, fed corn oil from 2 1/2 weeks of age.

Fig. 5. ♀ 377-2, 13 weeks of age, fed 100 I.U. vitamin A/gm./day from 2 weeks of age.

Fig. 6. ♀ 396-6, 20 weeks of age, fed 50 I.U. vitamin A/gm./day from birth.

PLATE II, Figs. 7-11. Dorsal skin specimens from hairless mice treated for varying periods of time (all approx. x 90):

Fig. 7. ♂ 1372, 45 weeks of age, fed 100 I.U. vitamin A/gm. body weight/day from 18 weeks of age.

Fig. 8. ♂ 1373, same age, fed corn oil from 18 weeks of age.

Fig. 9. ♂ 1369, 42 weeks of age, fed 100 I.U. vitamin A/gm./day from 22 weeks of age.

Fig. 10. ♀ 1375, 46 1/2 weeks of age, fed 100 I.U. vitamin A/gm./day from 18 weeks of age.

Fig. 11. ♀ 1374, same age, fed corn oil from 18 weeks of age.

PLATE III, Figs. 12-17. Dorsal skin specimens from hairless litter-
mates treated for varying periods of time (all approx.
x 90):

Fig. 12. ♂ 389-4 (biopsy), 8 weeks of age, fed corn oil
from birth.

Fig. 13. ♂ 389-5 (post-mortem specimen), same age, fed
100 I.U. vitamin A/gm./day from birth.

Fig. 14. ♂ 389-4 (post-mortem specimen), 25 weeks of age,
fed corn oil from birth.

Fig. 15. ♂ 389-1, same age, fed 100 I.U. vitamin A/gm./day
from birth.

Fig. 16. ♂ 389-2, same age, fed 50 I.U. vitamin A/gm./day
from birth.

Fig. 17. ♀ 389-9, same age, fed 50 I.U. vitamin A/gm./day
from birth.

PLATE IV, Figs. 18-23. Dorsal skin specimens from young hairless mice treated for varying periods of time (Figs. 18-22, approx. x 90; Fig. 23, approx. x 360):

Fig. 18. ♂ 460-8, 5 weeks of age, fed 200 I.U. vitamin A/gm./day from 1 week of age.

Fig. 19. ♂ 460-5, same age, fed corn oil from 1 week of age.

Fig. 20. 21-day biopsy from ♂ 460-2, fed 100 I.U. vitamin A/gm./day from birth.

Fig. 21. 21-day biopsy from ♂ 460-1, fed corn oil from birth.

Fig. 22. Epidermal wart from ♂ 460-5, 5 weeks of age, fed corn oil.

Fig. 23. Same; detail on the right.

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PLATE V, Figs. 24-29. Dorsal skin specimens from untreated hairless mice (all approx. x 90):

Fig. 24. ♀ 1269, untreated, 58 weeks of age.

Fig. 25. ♂ 1268, untreated littermate, same age.

Fig. 26. ♀ 1184, untreated, 66 weeks of age.

Fig. 27. ♂ 1190, untreated littermate, same age.

Fig. 28. ♀ 1139, untreated, 75 weeks of age.

Fig. 29. ♂ 1135, untreated littermate, same age.

PLATE VI, Figs. 30, 31. Dorsal skin specimens of treated hairless
littermates (approx. x 90):

Fig. 30. ♀ 374-1, 27 weeks of age, fed 50 I.U. vitamin A/
gm./day from birth.

Fig. 31. ♂ 374-5, same age, fed 50 I.U. vitamin A/gm./day
from birth.