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Abstract approved: _____
Dr. James E. Oldfield

The investigation of factors related to hairlessness in young BCX strain mice of the Small Animal Laboratory at Oregon State University is the subject of this thesis.

Many types of hairlessness in mice have been reported in the literature during the past fifty years. Only two reports have been found which describe a hairless condition similar to that found in the BCX mice at the O.S.U. Small Animal Laboratory. These involve Hypotrichosis juvenilis Loeffler (1934), Pinkus (1964), a hairless condition found in adolescent mice which affects only certain areas of the mouse's body and is not a permanent characteristic throughout the life of the individual. The loss of the hair coat can first be recognized at about 16 days of age, the hair loss

persisting for three weeks. By four to five weeks of age all affected mice have regained normal pelage.

Like the Hypotrichosis juvenilis mice described by Loeffler and Pinkus, the BCX strain mice developed adolescent hairlessness, however the incidence of hairlessness was related to the ration fed the mice. Only those mice receiving the commercial Rockland mouse ration had a high incidence of hair loss. Five sets of experiments were designed to probe this diet related hairlessness.

The first experiment involved the securing of an adequate breeding stock as well as a comparison of the hairlessness as it related to BCX mice fed the Rockland Diet and Purina Mouse Chow. Only those mice fed the Rockland ration had a high incidence of hairlessness. Changes were made in the experimental procedure to insure a more constant breeding environment through the regulation of the diurnal lighting and temperature.

In experiment two, trials were carried out using the Rockland ration* supplemented with minerals, vitamins or protein. A group fed the unsupplemented Rockland ration served as a control group. Both groups were maintained in the same breeding environment for several months during which time the incidence of hairlessness in the two groups was compared. In all groups except the protein supplemented

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*very widely used diet for laboratory animals - produced by Teklad Incorporated, Monmouth, Ill.

group, the incidence of hairlessness was not significantly affected by the supplementation. In the protein supplemented group the incidence of hairlessness declined by 42%. Vitamin supplementation substantially increased the number of litters born.

The favourable results achieved by the protein supplementation prompted a closer investigation of the interrelationship of hairlessness and the amino acid content of the diet. Three amino acid supplemented diets were tried. Diet one contained added arginine, cystine and lysine; diet two contained added methionine, phenylalanine, tryptrophan and valine. The group fed diet two exhibited only 12% hairlessness in the offspring as compared to 40% in diets one and three and the control group.

In experiment four conducted at the University of British Columbia Farm,* the mice were fed Rockland ration supplemented with cows' milk or pork fat. The incidence of hairlessness in the group fed milk supplementation was less than 4%. Furthermore, the breeding animals appeared in improved physical condition with shiny coats. Preweaning mortality was zero, and weaned body weight was significantly higher than that of the control group. The pork fat supplementation decreased the incidence of hairlessness, although the mice showed decreased fertility following initially increased reproduction.

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*Oyster River, British Columbia, Canada.

In experiment five, cottonseed oil, oleic, linolenic and linoleic acids were used as supplements to the Rockland ration. Oleic acid supplementation, although causing the reproductive rate to vary somewhat over the course of the study, did produce the lowest incidence of hairlessness in weaned offspring. The incidence of hairlessness was only 8% as compared to 28% in the linolenic group. In addition the mice fed the oleic acid supplement appeared in better general physical condition compared to the control group. Linolenic acid adversely affected the reproductive rate, halving the control group litter production.

The favorable results from dietary protein and fat supplementation in suppressing incidence of hairlessness, along with the known genetic involvement in this condition in BCX strain mice clearly identify this hairless condition as multicausal. These mice can serve further as useful animal models for investigating such conditions, and experimentation leading to identification of animal nutrient involvements (amino acids, minerals, etc.) would be in order. Most importantly, the implications of genetic - environmental interactions in the production processes of domestic animals should be recognized in future selection and breed improvement programs.

Hairlessness in the Young Mouse Resulting From an Interaction of Genetic, Nutritional and Environmental Factors

by

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HAIRLESSNESS IN THE YOUNG MOUSE RESULTING FROM AN INTERACTION OF GENETIC, NUTRITIONAL AND ENVIRONMENTAL FACTORS

INTRODUCTION

A high incidence of transitory hairlessness has been observed in young, BCX strain laboratory mice maintained at the small animal laboratory in the Department of Animal Science, Oregon State University. The hairlessness was only noted in the BCX strain,* with both hairless and normal animals appearing in the same litter. Other mouse strains, raised under similar conditions exhibited no abnormal hair loss.

Typically, the loss of hair occurred between the 14th and 18th day, following the growth of nascent hair at the 5th or 6th day. The mice remained hairless until 32 to 36 days of age, whereupon normal hair growth resumed. Subsequently the hair coats remained normal. In a few cases

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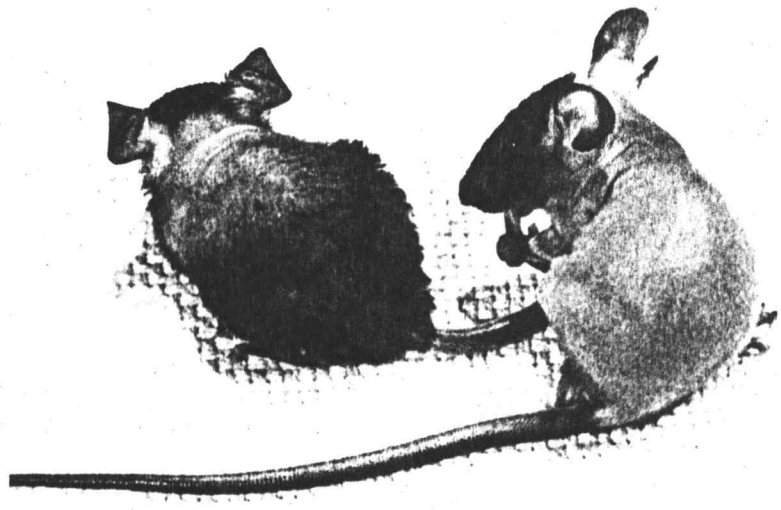
*The BCX strain of laboratory mice originated at the Animal Laboratory of the Faculty of Agriculture at the University of British Columbia in Vancouver, B.C.

The laboratory records (1950-52) mentioned the appearance of hairless young mice - probably as mutation in UBC BC (black) mouse strain. A small number of mice showing the hairless condition, was donated to the Small Animal Laboratory at the Oregon State University for experimental purposes - known as BCX strain of mice.

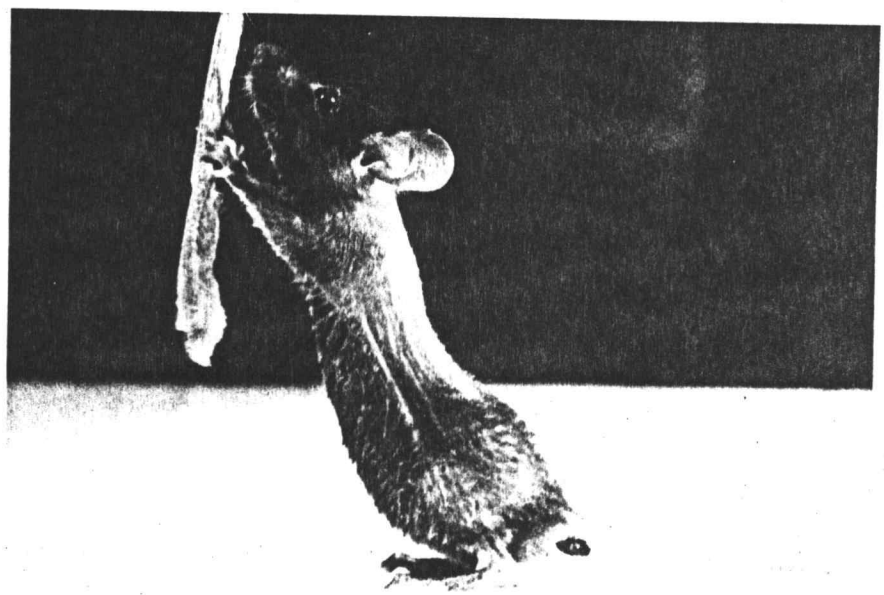
however, the hair loss continued throughout the life span of the animal. Furthermore, the degree of hairlessness was variable, generally affecting the head and upper trunk, with little involvement of the limbs and lower trunk. This type of hairlessness in young mice is similar to the Hypotrichosis juvenilis described by Loeffler (1934).

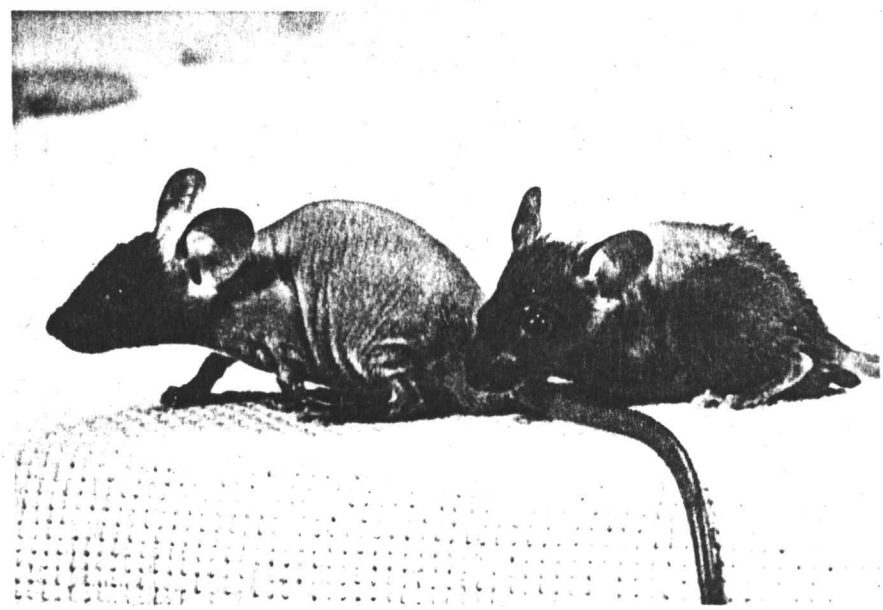
In preliminary attempts to isolate the etiology of the hairlessness, it was noted that the BCX strain mice developed hair loss only when fed the commercially available Rockland Mouse/Rat Diet. Substitution of this feed with Purina Mouse Chow alleviated the condition. However the Rockland feed did not cause hairlessness in other mice strains. In addition to this dietary aspect of the condition it was observed that changes in the room temperature affected the incidence of

Different Stages of Hairlessness in young BCX strain laboratory mice



Different Stages of Hairlessness in young BCX strain laboratory mice





hair loss and the fertility of the BCX strain. Consequently, the hair loss appears to result from a basic genetic susceptibility which can be either reinforced or decreased by nutritional and environmental factors.

Although a great deal of work has been done in the areas relating to mouse genetics, nutrition and environmental influences, no studies have been undertaken to investigate the interaction of these three factors as they relate to hairlessness in young mice. As this interaction is obviously of prime importance in the determination of hairlessness, a study was mounted from 1963 to 1970 to investigate the isolated and combined effects of genetics, nutrition and environment in the hairlessness of BCX strain mice.

REVIEW OF LITERATURE

Hairlessness in Mice

From a review of the literature it is apparent that hairlessness in mice is reasonably common. One of the first reports is Gordon's description of three hairless house mice in 1850. Following this came a series of reports, some concerned only with individual cases, by Gaskoin (1856), Marshall (1887), Bateson (1894), Campbell (1907), Year-Book of the Amateur Menagerie Club (1922), Sumner (1924), Lebedinsky and Dauvart (1927), and Snell (1931). While various types of hairlessness in mice have been described in these reports, all investigators observed that hairlessness in the mice was due to genetic, nutritional or environmental factors.

Genetic Factors

The outstanding characteristic of these mice, as described by the earlier workers was a complete loss of hair except for a few muzzle hairs with the possible retention of hair on the feet and tails of some animals. The deep folding of the skin suggested the name "Rhinoceros Mouse" to the earlier authors. The skin was also described as "corrugated". Gaskoin showed the hairless condition was probably an inherited trait.

In a preliminary report Lebedinsky and Dauvart (1927), described hairless mice which appeared as an incompletely dominant mutation in their laboratory stock of albino mice. Snell and Keeler (1931), regarded this mutation as being the same as that described by Gordon. Lebedinsky and Dauvart described the homozygous and heterozygous mice as "Naked" (Ganznackt), and "Half-naked" (Halbnackt), respectively. The "Naked" mice do not acquire a complete pelage, but only fine hairs in certain regions. The "Half-naked" mouse acquires a first pelage which it then begins to lose at the age of ten days to two weeks and subsequently undergoes repeated cycles of hair regeneration and loss, a cycle being complete in about one month. The hair loss in these heterozygous "Half-naked" animals, does not extend to the hair covering the nose, feet, tail, and ears.

David (1931), replaced the laboratory term "hairlessness" by the name "hypotrichosis" which has been generally accepted. In her extensive report three distinct types of hairlessness are described: Hypotrichosis hypokeratotica; hypotrichosis cystica; and hypotrichosis hypoplastica follicularis. The main changes within the skin which she suspected as being the causes of hairlessness are: abnormalities in keratinization or of hair structure; irregular direction of follicles and hair; resorption of unerupted hairs remaining in the corium or subcutis; and cyst formations and hyperkeratosis.

Grueneberg (1943), reviewed results of studies of hairlessness in mice and described the following three types:

a) Hypotrichosis Cystica;

b) Hypotrichosis Hypokeratotica; and

c) Hypotrichosis Juvenilis.

a) Hypotrichosis Cystica

This type of hairlessness can be first observed when animals are ten to fourteen days old and start to shed hair. It is characterized by the loss of a complete hair in contrast to the "half-naked" mice in which the hair breaks off. The hair loss starts above the eyes, on all four feet, and on the base of the tail, spreading to all other parts of the body. Shedding is complete in about ten days and only few sensory hairs remain. The skin is soft, smooth and pink. In all Hypotrichosis Cystica type hairless mice, a slight regeneration of thin, fuzzy, usually unpigmented hair occurs in different parts of the body, but this falls out in a very short time. The eyelids of adult hairless mice are swollen by a cystic enlargement of Meibom's tear glands, which in some cases covers the eyes.

This "Hairless" (hr) gene is a recessive characteristic that manifests itself in a regular manner. "Hairless" females are usually sterile and the fertility of males is often only of short duration. Occasionally they produce a litter, but because females are unable to nurse, the young

have to be fed by foster mothers. Crew and Mirskaia (1932), found that mammary glands of "hairless" mice are rudimentary and resemble those of infantile animals. They consist solely of nipple tissue without ducts and mammary tissue.

David (1932) carried out a thorough histological study and found that the skin and hairs of "hairless" mice are normal until the first hair generation has nearly been completed. After that, no proper hair clubs are formed and such hair, lacking skin support, falls out. Regeneration is incomplete and abnormal. Follicles undergo degenerative changes, mainly characterized by a separation of the upper and lower parts of the follicle, and by cyst formation. If the first of these changes occurs when a hair is beginning its formation, the result is a hair which ends blindly within the skin or forces an exit through the epidermis. Hairs, remaining within the skin undergo disintegration and resorption. Cell infiltrations are found around non-erupting hair. The sebaceous glands are hypertrophied and give rise to cystic formations. An excessive amount of stratum corneum is found in the cystically enlarged hair canals. This enlargement of the canal shows considerable variation both in different hairless animals and within the same individual. With age, "hairless" mice undergo a whitening and wrinkling of the skin and an elongation of the claws. The whitening and wrinkling of the skin is also found in old hairless rats.

Results of cross-breeding experiments carried out by different workers with "hairless" mice, are given in Table I.

Table 1. Genetic segregation of "hairless" (hr)

Investigator		Numbers Normal	Hairless	Ratio
Crew & Mirskaia (1932)	hr/hr x hr/hr	0	49*	0:1
Snell (1931)	+/hr x +/hr	115	40	2.9:1
Crew & Mirskaia (1932)	+/hr x +/hr	158	76	2.1:1
David (1932)	+/hr x +/hr	117	47	2.5:1
Snell (1931)	+/hr x hr/hr	92	101	0.9:1
Crew & Mirskaia (1932)	+/hr x hr/hr	31	25	1.2:1
David (1932)	+/hr x hr/hr	69	66	1:1

hr - hairless
\+ - normal
* - These animals have to be nursed by foster-mothers.

One allele of the "hairless" gene is called the "rhino" gene. This gene was first observed in the fifth generation of brother-sister matings following a cross between two highly inbred lines (Howard, 1940). Rhino ($\underline{hr}^{\underline{rh}}$) is a simple recessive characteristic; in crosses of "rhino" with "hairless", all F_1 and F_2 animals are devoid of hair. The loss of hair with "rhino" occurs at the same age as with "hairless" mice, but in different pattern. In "rhino" mice the hair falls out from all parts of the body at the same time, thinning the entire coat, while in "hairless" there is always a sharp line between areas of depilation and regions

not yet affected. In addition the "rhino" mouse differs from the nearly smooth-skinned "hairless" mouse by virtue of its pronounced skin folds which give rise to the name, "rhinoceros condition".

According to Steinberg and Fraser (1946) the pattern of hair loss in the "rhino" is variable, ranging from a condition resembling the "hairless" pattern, with hair loss progressing caudally leaving scattered hairs in the denuded area, to a generalized thinning of hair all over the body.

A second allele of the "hairless" gene called the "bald" ($\underline{hr}^{\underline{ba}}$) gene has been described by Garber (1952). Homozygous "bald" animals are normal in appearance until the sixteenth day at which time their hair starts to fall out. Heterozygous "bald" and normal mice could not be distinguished at any time. Homozygous "bald" mice were usually completely bald within twenty to twenty-eight days. Hair loss in "bald" mice resemble "rhino" with some minor differences. Depilation always proceeds in a caudal direction. Young "bald" mice have smooth, flexible, semi-transparent skin which later becomes coarser and thicker. Furthermore, "bald" mice are usually smaller than their normal littermates at weaning. Adult "bald" females have rudimentary nipples but completely lack mammary tissue. "Bald" males and females are fertile.

b) Hypotrichosis Hypokeratotica

In contrast to the "hairless" gene, "half-naked" (N) gene is semidominant. This gene was first observed as a spontaneous mutation in an albino mouse colony maintained at the Latvian University of Riga, (Lebedinsky, 1927). The loss of hair in "half-naked" animals occurs differently from that in the "hairless" gene mice as the feet and tail are not affected and retain an almost normal coat of hair. Furthermore unlike the hair of the "hairless" mice, the hair of the "half-naked" mice does not fall out but breaks off a little distance above the skin leaving the roots still embedded in the skin. Such shedding is usually finished within a week. Hair regeneration occurs throughout the mouse's life in cycles of about one month's duration. Sometimes a few intact hairs can be observed in the affected areas. This loss of hair in "half-naked" mice is due to incomplete keratinization of the hairs. The skin of these mice is completely normal.

Dominant hairlessness in mice is characterized in either homozygous or heterozygous animals by repeated cycles of hair growth at normal periods, (Fraser and Nay, 1955). The heterozygous animals (N/+) of both sexes are fully fertile and females are very good mothers. Homozygous animals (N/N) are of normal size at birth but within a few days they start to lose body weight and most die between 5-10 days of

age. In the survivors, growth is very slow with the animals seldom exceeding half-normal size.

c) Hypotrichosis Juvenilis

Loeffler (1934) observed another type of hairlessness, in which the adult pelage was entirely normal. "Hypotrichosis juvenilis" is a recessive character (hj) with irregular manifestation. However, the linkage relations have not yet been investigated, nor has crossing out with "hairless" or "naked" gene mice, (Grueneberg, 1943). Loeffler found that the expression of this characteristic depends on the temperature of the environment, being more prevalent at 24 than at 20 degrees centigrade. At the age of about 5 weeks, the hypotrichosis disappears, the second coat of hairs being normal. Histologically the skin of affected animals resembles that of the "hairless" mice described by David (1932), except that no cysts are formed.

A very similar type of hairlessness in weanling mice, termed Trichomalacia, has been observed and investigated by Pinkus (1964). The most interesting feature of this localized and temporary loss of hair involves its pathogenetic mechanism. Histologic examination revealed that the first growth of hair proceeded normally but the second generation hair shafts showed irregular accumulation of melanin and faulty arrangement of the keratin molecules. The hair did

not pierce the skin surface but doubled up and coiled in a sac-like follicle filled with loose keratin flakes. This hairless condition spread from venter to dorsum and in most cases the entire trunk of the animal was bare except for a tuft of hair at the root of the tail. Head, neck, and legs were not involved. The naked skin was smooth and pink. Pinkus did not rule out the possibility that club formation of the first generation was defective, causing the hair to fall out precipitating the formation of a second generation of abnormal hairs. Later generations of hair grew normally except that a few animals showed temporary alopecia when they reached an age near seven months.

An incidence of a new recessive hairless gene with pleiotropic effects in the mouse has been reported by Flanagan (1966). This hairless mutant differs phenotypically from previous cases and it has been named "nude" (nu). Affected mice never grow a first coat and adults are almost completely hairless. In addition to hairlessness, this gene (nu) gives rise to a variety of other abnormalities including reduced body growth rate, very low fertility, and fatal liver disease.

"Nude" mice may be classified at birth by the absence of vibrissae. The hair loss has been traced to an abnormal keratinization of hair in the follicles. The majority of "nude" mice die of general body weakness within two weeks of birth. The survivors grow slowly and usually die between

3 and 14 weeks of age. Low fertility of "nude" can be attributed to non-motile sperm, small ovaries and low egg counts. The cause of the liver disease has not been determined but the disease has been traced to its initial stage: i.e. necrosis of small areas of parenchymal tissue at various points throughout the liver.

A large amount of breeding data has been presented by Dickie (1955) in her studies of "Alopecia" (Al), a dominant mutation in mice. "Alopecia" affected mice regularly lose all but their guard hairs at each moult. The characteristic seems to be incompletely dominant since heterozygous and homozygous animals can be distinguished.

Flanagan & Isaacson (1967), investigated a hairless mutant found in the Clinical Endocrinology Research Unit, Edinburgh. This hairlessness is caused by the incompletely dominant gene called "Shaven" (Sha) which is closely linked to the "naked" gene. Newborn homozygous "Shaven" animals never grow a first hair coat and adults grow only a few short hairs. Heterozygotes grow a full coat with a characteristic greasy appearance. Absence of the coat "Shaven" homozygotes is caused by weak keratinization of the hairs which are thin and lack a rigid cortex. The "Shaven" hair fails to penetrate the epidermis and becomes entangled in the cells of the upper dermis. Hair follicles in this abnormality were found to be deficient in sulfhydryl groups. Greasiness of the coat of heterozygotes is due to presence

of a sudanophilic fluid in the hair medullae. Another unusual feature of the "Shaven" gene is its dominance over the normal allele. Previously it has been found that semi-dominant genes causing hairlessness exhibit an occurrence related effect with the lack of hair being more extreme in homozygotes than in heterozygotes. More investigation will be needed to explain how the "Shaven" gene in the homozygote (Sha/Sha) causes almost complete hairlessness, while in the heterozygote, it (Sha/+) has no effect on the growth of the coat but causes greasy hair. Another hairless mutant in the house mouse has been described by Van Pelt, Knorr and Cain (1969). The condition is the expression of a single recessive autosomal gene called "apampischo" (ao). The pattern of hair loss in the "ao" mice resembles that of hypotrichosis-juvenilis in which regrowth does occur and the adult fur is normal. However in hypotrichosis-juvenilis pregnant females do not lose their fur coat. "Apampischo" females on the other hand exhibit an extensive loss of hair during periods of pregnancy and nursing. Hair loss in these pregnant females becomes apparent about the beginning of the second week of pregnancy and continues, peaking just before the litter is born. The hair starts to regrow shortly after nursing is discontinued.

A hairless mutation in Asiatic tame mice has been described by Makino (1950). Results of the breeding experiments showed that the hairlessness was a simple recessive

gene. The loss of hair begins when the mice are between two and three weeks old. The hairless young are very delicate and difficult to rear. As the hairless females demonstrated a very low fertility this hairless stock could not be maintained. Sections of hairless skin show that it differs from that of normal mice in having a much thickened cutis. The epidermis is normal in appearance. The hair follicle of the skin has normal epithelial cells which have however no papillae in the follicles. Apparently the hair has been shed from the follicle and its papilla has then undergone degeneration.

Chlumecky (1967) described briefly the characteristics of a new strain of hairless mice bred from a noninbred haired albino "H" strain. After losing their primary coat at about twenty days, the animals remain completely hairless. Loss of hair in these mice is the result of changes in the skin. Atrophy of the hair follicles can be observed and in older individuals numerous epidermal cysts are present in the corium. Hairlessness in these animals is inherited as a simple recessive character.

Fraser (1946) reviewed cases of hereditary hypotrichosis in mice and other animal species that have been studied histologically and showed that the histological patterns of hairless mutants fall into these well defined classes:

1. Partial agenesis of hair follicles in which functional follicles do develop but are greatly reduced in number. This occurs in the rabbit, (David 1932) and in swine (David 1932, Roberts and Carroll 1931).

2. Delay in the development of an otherwise normal follicle. Such a condition is reported by Mohr and Wried (1927-28) in cattle.

3. Premature keratinization of hair follicles and sebaceous glands preventing eruption of all except the guard hairs as in the furless rabbit described by Castle (1933).

4. Imperfect keratinization of the hair shaft causing the hair to break off after its eruption, occuring in the house mouse as the mutant "naked" gene. (David 1932, Clark 1939).

5. Shedding of juvenile pelage at the time of the first moult, with occasional regeneration of the hair coat and varying degrees of cyst formation in later stages. The mutant gene forms of "hairless" and "rhino" in the mouse, fall into this group.

6. Characteristic follicular hyperkeratosis, combined with a premature keratinization of the hair bulb causes Hypotrichosis juvenilis in the house mouse, a condition described by Loeffler (1934), which presents characteristics of both classes three and five in the above classification.

Nutritional Factors

Numerous reports on specific nutritional experiments with mice have appeared in the literature, especially in the last twenty years. Many compounded mouse diets are on the market, but there is no presently available diet which is satisfactory for all strains of mice. For example it has been observed that the same diet which supports optimum growth, fertility and lactation in one strain of mice, causes hairlessness and low fertility in other strains. This specific dietary requirement of the individual mouse strains suggest a genetic-nutritional interaction.

Porter et al. (1963) found that the same diet, made by three different manufacturers, produced significantly different growth results. A given formula was not sufficient specification for a diet; more important is the composition of the diet as offered to the animals. They suggested that a suitable inbred strain would be used as a sensitive indicator in assessing a diet. Furthermore they suggested that measurements should be made in addition to weight gain.

Experiments with mice carried out by Krueger and Bogart (1962) brought out the fact that genetic, endocrine and environmental factors determined the individual nutritional requirements.

Morris (1944) and others have contributed considerably to the basic information concerning the nutritional

requirements of mice. This information has been utilized recently in the formulation of laboratory mouse diets.

Hoag & Dickie (1960) compared five different commercially-available laboratory rations with each of two strains of inbred mice for an eighteen week period. One diet was shown to be far superior, markedly increasing the number of young in one strain, and resulting in heavier, and more numerous young in the other strain. When tested on a strain of mouse with muscular dystrophy, the same diet resulted in increased longevity.

Fenton & Carr (1951) studied the utilization of high and low protein diets for growth in four strains of highly inbred mice and found a wide range of protein efficiency ratios:

$$\text{PER} = \frac{\text{Weight gain of growing mice}}{\text{Weight of protein consumed}}$$

Two strains grew equally well on either 10% or 30% protein levels. The other two strains grew distinctively better on the 30% level. Levels higher than 30% inhibited growth in proportion to the excess of dietary protein content. Furthermore, the results of Fenton & Carr's experiment (1951) showed that:

1. increasing the fat level of a synthetic diet increased the weight gain of two strains of mice, but that two other strains failed to show a similar weight

gain.

2. efficiency of food utilization for body weight gain increased with increasing dietary fat level.
3. transferring mature animals from a commercial stock ration to a highly purified diet caused least disturbance in body weight if the synthetic diet contained a high percentage of fat.

Protein levels in most commercial feeds range from about 17% to 25%. Therefore, these levels fall well within the range of the demonstrated mouse protein requirements.

Fats and fatty acids are recognized as being essential to the mouse. The fat content of commercial feeds ranges from 4% to 11%, the level usually being inversely proportional to the protein content of the feed. However differences have been observed in the response of various strains of mice to diets containing different levels of fat. Hoag & Dickie (1962) found that inbred strains differed in their response to the dietary protein levels (17%, 20%, 22% and 24% protein at a constant 11% fat), and to the dietary fat levels (6%, 11% fat at a constant 24% protein). They found that if the protein content was maintained at 24% while the fat level was varied from 6% to 11% that one mouse strain showed the best reproductive ability at the lower dietary

fat level whereas a second inbred strain functioned better with the higher fat diet.

Cerecedo et al. (1952) described fatty acid deficiencies in three strains of mice. Although the reaction of each strain of mouse to the fat deficiency differed, some common and characteristic symptoms were: dermatitis of the skin and extremities, scaliness of the ears, alopecia, a neck lesion, and retardation of growth. Prophylactive and curative experiments indicated that under the experimental conditions used, 5 miligrams of methyl linoleate per day satisfied the requirement of the mouse for linoleic acid.

Natural and processed fats vary greatly in the proportions of their essential and non-essential fatty acid content. Unfortunately there is little information available concerning the requirements of essential fatty acids (EFA), when these are diluted with dietary non-essential fatty acids. Non-essential fatty acids, when fed with EFA, appear to stimulate better growth than do supplements of EFA alone. Greenberg et al. (1950) found that EFA-deficient rats fed high levels of cottonseed oil in their diet grew better and had higher food efficiencies than those receiving only EFA supplements. Different nutritive responses have been observed with rats fed high-fat diets differing widely in their EFA content. Studies of Peifer and Holman (1961) demonstrated that essential fatty acids are required for the proper utilization of fat calories. Furthermore, high ratios

of saturated fat to EFA (SF/EFA) promote the onset of EFA-deficiency symptoms in the rat.

Although analyses of a number of commercial mouse diets show that the carbohydrate content ranges from about 53% to 65%, the quality of the carbohydrate content is more important than the quantity. The cereals, which comprise a large proportion of the diet vary in nutritional value according to kind, grade, processing methods, age, contaminants, etc. As commercial diets for mice usually contain a large number of cereal ingredients, if one of these should be inferior in some respect, the nutritional hazard is less than if only a few ingredients formed the diet.

The important role of vitamins in mouse nutrition was recognized at an early stage. Numerous studies have established the existence of interdependencies among single vitamins or between various groups. Mirone (1954) demonstrated that choline-deficient diets result in 66% lower fertility. The young were found to be smaller, usually dying within four days of birth. Lee et al. (1953) found critical differences in dietary requirements for reproduction between the two strains of mice (A, Z). A diet which was fully satisfactory for 'A' strain caused infertility in females of 'Z' strain. Addition of trace elements of Co, Cu, Zn, and Mn, with an increased amount of iodine to a basic diet, adversely affected reproduction; when either alpha-tocopherol or Vitamin B_{12} was added, the reproductive function was

partially restored.

Attempts to cure cases of hereditary hypotrichosis by treatment with various substances have met with little success. Results of an attempt to discover the interrelation of the "rhino" mutation and metabolism, by feeding "rhino" mice massive doses of Vitamin A, were inconclusive, (Fraser, 1946). Martin & Gardner (1935) fed hairless rats cystine and cysteine, and reported a regeneration of the hair coat. Roberts (1937), who repeated the experiment with cysteine, failed to stimulate any such growth. The feeding of potassium iodide or thyroid gland also failed to have any effect on the hair growth in hairless rats. Furthermore, no significant differences in weights of the pituitary, testes, spleen or thyroids, or structural differences in the pancreas, thyroids or adrenals were found for normal and hairless rats (Roberts et al., 1940).

Loewenthal (1956) studied the effects of Vitamin A deficiency on skin and hair growth in mice. Although the intake of the mice did not decrease during the experiment, Vitamin A deficient mice lost weight and became severely ill when they were 70 to 90 days old. In control mice, the hair growth cycle was completed in 21 days, but the cycle lasted 24 days in Vitamin A deficient mice. Histologically, the skin of Vitamin A deficient mice was normal even though the rate of hair growth was retarded. The lack of Vitamin A did not result in hyperkeratinization, such as that described for the

skin of Vitamin A deficient rats and humans.

Sauberlich (1959) studied the relationship of methionine, homocystine, choline, folic acid, and Vitamin B_{12} in the nutrition of the mouse. Weanling mice were used in the experiment and the basal diet was free of methionine and choline, but supplemented with homocystine. Results of the study are shown in Table II.

Table 2. Results of nutritional experiment (Sauberlich 1959).

Basal Diet	Additional Supplements	Results
Methionine free and choline free; supplemented with homocystine.	none	fatty livers, death.
	folic acid and Vitamin B_{12}	fatty livers - survival, but little growth.
	choline or betaine	liver fat reduced and slight growth.
	choline, folic acid and Vitamin B_{12}	normal livers.
	folic acid	died or failed to grow.
	Vitamin B_{12}	died or failed to grow.
	folic acid, Vitamin B_{12} glycine, serine, and threonine	50% of normal growth.
	folic acid plus choline or betaine	nearly normal growth.
	methionine	nearly normal growth.

Environmental Factors

The most important environmental factors affecting growth, fertility and breeding in mice appear to be temperature, humidity and light.

Cold appears to have little effect on breeding and fertility in mice, but heat is markedly injurious. Barnett et al. (1954) found that mice bred at -2° C reproduced satisfactorily. Several generations were successfully reared in the temperature range -2° C to -4° C. Bruce (1954) showed that temperatures of 90° F to 91° F significantly decreased the fertility rate in comparison with littermates kept at 70° F. At the higher temperature, sexual maturity was delayed, litter size was reduced and still-birth rate was increased. The second generation was sterile, but transfer to a cold environment restored fertility.

The influence of light on laboratory animals has been the subject of many studies. It is generally accepted that a regime of half-time light and half-time darkness is the most favorable for breeding. A seasonal decrease in the number of hours of daylight has been associated with a depressed fertility, but this can be largely overcome with artificial light supplementation. By increasing the period of light from 8 to 13 hours, Alexander and Fraser (1952) were able to improve mating in a rat colony during the

winter months. Conversely, Baker and Ranson (1932), by decreasing the light period from 15 hours to 9 hours a day, reduced reproduction to a minimum in a colony of field mice maintained under laboratory conditions.

EXPERIMENTAL

General Objectives

This research on hairlessness in young mice was undertaken to describe and assess the role of genetic, nutritional and environmental factors by: 1) investigating the mode of inheritance; 2) determining nutrients involved, 3) determining the influence of temperature and light, 4) resolving any interactions of these factors.

General Procedures

Prior to this present investigation, the BCX mouse strain originating at the University of British Columbia, had been perpetuated and expanded at the Oregon State University laboratory over several years. Very few hairless mice were observed however, in the year 1963. It was notable that a change in ration from a Rockland feed to a Purina product was implemented in the same period, suggesting a nutritional as well as a genetic basis for the hairless condition.

The BCX mouse strain was used in five experiments, with white mice (Swiss Webster) being used for the preliminary cross-breeding trial in experiment four. For classification, all BCX strain mice which demonstrated hairlessness in the pre-weaning period will be called "hairless", while

those which failed to develop hairlessness in the same period will be referred to as "normal".

Old and infertile animals were removed at the beginning. The remaining mice were identified by a serial number. A record was kept on pertinent information about each animal, including breeding date, date and number of young born, number of hairless and normal mice, ear or toe mark, weight of litter at weaning time, and general condition of the litter.

Two commercial mouse rations - Rockland* and Purina** - were used in pelleted form, and fed in small wire containers, in individual breeding cages. Feeding and watering was done once a day, and bedding (shavings) was changed twice a week. All mice were kept in a large room in the Small Animal Laboratory. The room temperature was 74° F. No attempt was made to control the hours of daylight or the humidity.

Because of the very small number of hairless mice available, both hairless and normal animals were used in the first experiment.

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*Rockland Mouse/Rat Diet
by Teklad Incorporated, Monmouth, Ill. (Appendix I)

**Purina Mouse Chow
by Ralston Purina Co., St. Louis, Mo. (Appendix II)

Experiment 1

Objectives

The first experiment was designed to re-establish hairlessness, and to increase the numbers of the breeding stock to insure that there would be an adequate number of animals for subsequent nutritional and genetic trials and to assess the effect of two commercial mouse diets on occurrence of hairlessness and reproduction. This trial involved the feeding of Rockland and Purina rations to both hairless and normal mice of BCX strain.

Methods

Three months of pre-experimental period (November, December 1963 and January 1964) were used to increase the breeding stock, to determine the suitability of small mating cages, and to study the behavior of the mating pairs in the new environment. Previously a harem breeding system had been used, in which 30 females and 6 males were kept in a large stainless steel dishpan.

During the first three months of the pre-experimental period, fertility in entire breeding stock available was very low. Only a very few litters were born, and none showed the hairless condition. A similar reproductive pattern had been observed for several years before 1963. The fertility in BCX strain would decrease to a minimum during

the winter months and then would return to normal during the spring and summer months using other strains of laboratory mice as controls.

The experiment was begun as of February 1, 1964. All available hairless mice were randomly divided into two groups, consisting of 25 females and 25 males in group 1A, and 24 females and 24 males in group 1B. Each animal was identified by an ear - or toe - mark. Group 1A was fed the Rockland mouse ration, group 1B was fed the Purina ration. Two other groups, 1C and 1D, each consisting of 12 females and 12 males of normal mice of the BCX strain, were used as control groups. Group 1C was fed the Rockland, and group 1D, the Purina ration. Each female was put with one male in an individual mating cage. All cages were placed four feet above floor level in a large room. Feed and fresh tap water were supplied once a day. Bedding was changed once a week and when a litter was born, bedding was changed twice a week.

As the animal population increased - both hairless and normal - the experiment was terminated at the end of June. The infertile females and males were replaced in all four groups by young animals of breeding age. The total number of mating pairs was brought up to 30 in all groups during the month of July 1964. July was an interim period, during which no data were recorded. The second part of the experiment was carried out from August 1 to December 31, 1964 - under the same conditions and environment.

Results

The incidence of hairlessness and the breeding results during the first five months of the experimental period (February - June 1964) are shown in Table 3. The fertility increased and hairlessness reappeared.

More significant results were obtained during the second part of the experiment (August - December 1964) with increased numbers of mating pairs in all four groups. These results are summarized in Table 4. During these experimental periods, all hairless mice were kept as breeding stock for future experiments.

The results show that 84% of all hairless mice weaned (146 of 174) were born from hairless and normal parents fed the Rockland ration.

Discussion

Several important observations were made during this breeding and feeding experiment. It was noticed that all hairless mice were smaller than normal mice, at weaning time. Mortality of hairless mice, especially during the pre-weaning period, or when exposed to minor stress, such as a temperature change, was considerably higher than that of the normal mice. Furthermore, most of the hairless mice were born in Group 1A, which consisted of hairless parents fed the Rockland ration. Analysis of both rations, to the

Table 3. Incidence of hairlessness in offspring of hairless and normal groups of mice of the BCX strain, fed two rations for 150 days (February - June 1964).

Group	1A	1B	1C	1D
Hair condition	Hairless	Hairless	Normal	Normal
Mouse ration	Rockland	Purina	Rockland	Purina
Mating pairs: (No.)	25	24	12	12
Litters born: (No.)	34	48	27	31
Young born: (No.)	204	382	182	213
Young weaned: (No.)	171	311	167	186
Ave. offspring/litter born: (No.)	6.0	8.0	6.7	6.9
Ave. offspring/litter weaned: (No.)	5.0	6.5	6.2	6.0
Ave. wt. of young weaned: (grams)	7.1	8.4	10.1	11.5
Hairless weaned: (No.)	64^{ab}	17^{a}	22	3^{b}
Hairless at weaning: (%)	37.4^{abc}	5.5^{a}	13.2^{b}	1.6^{c}

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

Table 4. Incidence of hairlessness in offspring of hairless and normal mice groups of the BCX strain fed 2 rations for 152 days (August - December 1964).

Group	1A	1B	1C	1D
Hair condition	Hairless	Hairless	Normal	Normal
Mouse ration	Rockland	Purina	Rockland	Purina
Mating pairs: (No.)	30	30	30	30
Litters born: (No.)	18[a b]	23	31[a]	37[b]
Young born: (No.)	164[a]	191	285[a]	263
Young weaned: (No.)	129	177	211	234
Ave. offspring/litter born: (No.)	9.1	8.3	9.2	7.1
Ave. offspring/litter weaned: (No.)	7.2	7.4	6.8	6.3
Ave. wt. of young weaned: (grams)	7.0	8.4	9.1	10.8
Hairless weaned: (No.)	53[a b c]	8[a]	7.0[b]	0.0[c]
Hairless at weaning: (%)	35.5[a b c]	4.5[a]	3.4[b]	0.0[c]

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

Table 5. Analysis of the Rockland and Purina pelleted mouse feeds.*

		Rockland	Purina
Dry matter %		92.7	93.0
Crude protein	as fed %	26.1	24.6
	dry %	28.2	26.5
Calcium	as fed %	1.5	1.2
	dry %	1.6	1.2
Phosphorus	as fed %	0.6	0.9
	dry %	0.7	0.9
Copper	as fed ppm	4.7	10.2
	dry ppm	5.1	11.0
Iron	as fed ppm	243.0	237.0
	dry ppm	262.0	255.0
Magnesium	as fed %	0.2	0.2
	dry %	0.2	0.3
Manganese	as fed ppm	57.0	48.0
	dry ppm	61.0	52.0
Molybdenum	as fed ppm	1.0	1.0
	dry ppm	1.1	1.1
Zinc	as fed ppm	32.0	59.0
	dry ppm	35.0	63.0
Potassium	as fed %	1.0	1.0
	dry %	1.1	1.1
Crude fibre	as fed %	5.9	6.0
	dry %	6.4	6.4

*British Columbia Feed Analysis Service, 523 Columbia Street, Kamloops, B.C., Canada. Analyzed April 21, 1971.

extent performed, did not disclose any significant differences between the Rockland and Purina feeds although a few minor variations were noted: Purina feed has a slightly lower protein level, and the copper and zinc contents were approximately doubled as compared to the Rockland ration. The analytical data are shown in Table 5. It was also observed that the hairless condition developed between two and five weeks of age. In most cases, following the initial loss of hair, normal hair growth resumed. Taken together, these facts suggest the presence of genetic and nutritional factors in the manifestation of hairlessness in BCX mice.

Experiment 2

Introduction

Experiment 1 was carried out in a large room having ambient temperature, humidity, and lighting. The temperature averaged 74°F with periodic increases in the summer months. Both the humidity and the available light varied with seasonal fluctuations. In order to ascertain if the above factors could influence the expression of hairlessness, Experiment 2 was devised with the idea in mind to control temperature, diurnal light exposure and to some extent, humidity. To achieve this control, a small, well ventilated room was constructed. In this room the diurnal lighting corresponded to 12 hours of light per day with a constant temperature of 82 F. To stabilize the humidity, two large, water-filled containers were placed in the room.

Bearing in mind the low fertility and the decreased incidence of hairlessness in the winter months, Experiment 2 was postponed until October 1965. As the mice were initially placed in their new environment in February 1965, the interim between February and October was felt to be sufficient to allow for adaptation.

Objectives

The results of Experiment 1 showed clearly that the incidence of hairlessness in the BCX mouse strain is related

to diet. The objective of Experiment 2 was to study separately the effect of minerals, vitamins and protein supplementing the Rockland ration, on causation of the hairless condition of young mice of the BCX strain.

Methods

The breeding system was changed from paired to harem mating. Four groups of hairless mice of breeding age were randomly selected from the available breeding stock. Each group contained 25 females and 6 males. Each group was kept in a large stainless steel dishpan. Fresh tap water and feed were supplied once a day. Porcelain containers with metal lids were used for feeding. A small opening (½ inch in diameter) in the lid prevented an excessive wastage of feed. The bedding was changed twice a week. When females became pregnant, they were put into individual cages, with continuation of the experimental diet. All litters were weaned at the age of 21 days.

The following data were recorded: number of young born and weaned; date of birth; weaning weight; date of first hair appearance; date when hair was lost in hairless mice; and the number of hairless mice weaned. All young hairless females and a sufficient number of hairless males were kept as replacements for the breeding stock. Old and infertile females and males were continuously replaced by

young hairless animals of breeding age. All young mice raised as future breeding animals were fed Rockland pelleted feed until they went into an experimental breeding group.

Experimental design was as follows:

Group 2A: Rockland ration plus a general vitamin supplement (Diet 1)

Group 2B: Rockland ration plus a mineral salt mixture (Diet 2)

Group 2C: Rockland ration plus a protein supplement (Diet 3)

Group 2D: Rockland ration unsupplemented (Diet 4)

The basis for all experimental diets was the finely ground Rockland pelleted food. The various supplements were added with thoroughly mixing.

Diet 1: To fifty pounds of ground Rockland Mouse Feed was added 500 grams of Vitamin Fortification Mixture,[1] as recommended for supplemental use by NBC.

Diet 2: 1.14 kg. of Briggs Mineral Salt Mix[2] was

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[1]Source: Nutritional Biochemicals Corporation, Cleveland, Ohio.

[2]Source: Nutritional Biochemicals Corporation, Cleveland, Ohio.

added to 50 pounds of ground Rockland ration (approximately 4.8% added salt).

Diet 3: 1.95 kg. of Vitamin free casein[2] was added to 50 pounds of the basic Rockland feed to bring the dietary protein level to 30%. This protein level was kept constant during the first four months of the experiment. For the second half of the experiment (February - May 1966) the protein level was raised to 35% by mixing 3.84 kg. of the casein product with 50 pounds of ground Rockland Mouse feed.

Diet 4: Rockland Mouse pellets, finely ground, without supplement, were used as diet for the control group.

Ingredients of the supplements used in the first three diets are given in Appendix III, IV, and V.

Experiment 2 was carried out for 243 days, from October 1965 until the end of May 1966.

Results

Results from the four experimental groups of hairless mice fed the Rockland ration are given in Table 6. The percentage of hairless mice weaned during the eight month experimental period is shown in Figure 1. Figure 2 compares numbers of normal and hairless mice weaned during the 243-day

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[2]Source: Nutritional Biochemicals Corporation, Cleveland, Ohio.

Table 6. Experiment 2 - Summary

Incidence of hairlessness in four groups of hairless mice, fed Rockland mouse ration plus vitamin, mineral, and protein supplements for 243 days: (October, 1965 - May, 1966).

Group	2A	2B	2C	2D
Supplement:	vitamin	minerals	protein	none
Litters born: (No.)	66[a]	43	42	44[a]
Young born: (No.)	328	231[a]	236[b]	295[a b]
Young weaned: (No.)	275	197	207	248
Normal weaned: (No.)	140	112	142	114
Hairless weaned: (No.)	135	85	65[a]	134[a]
Hairless weaned: (%)	49.1	43.2	31.4[a]	54.0[a]
Ave. offspring/litter born: (No.)	5.0	5.4	5.6	6.7
Ave. offspring/litter weaned: (No.)	4.2	4.6	4.9	5.6
Ave. age when hair appears: (days)	6.3	6.5	6.1	6.3
Ave. age when hair lost: (days)	15.0	14.8	16.1	15.8
Pre-weaning mortality: (%)	16.2	14.7	12.3	15.9

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

Experiment 2.

Group 2A: --------- Fed Rockland ration supplemented with vitamin mix.

Group 2B: ..... Fed Rockland ration supplemented with mineral mix.

Group 2C: _____ Fed Rockland ration supplemented with protein.

Group 2D: ++++++++++ Fed unsupplemented Rockland ration.

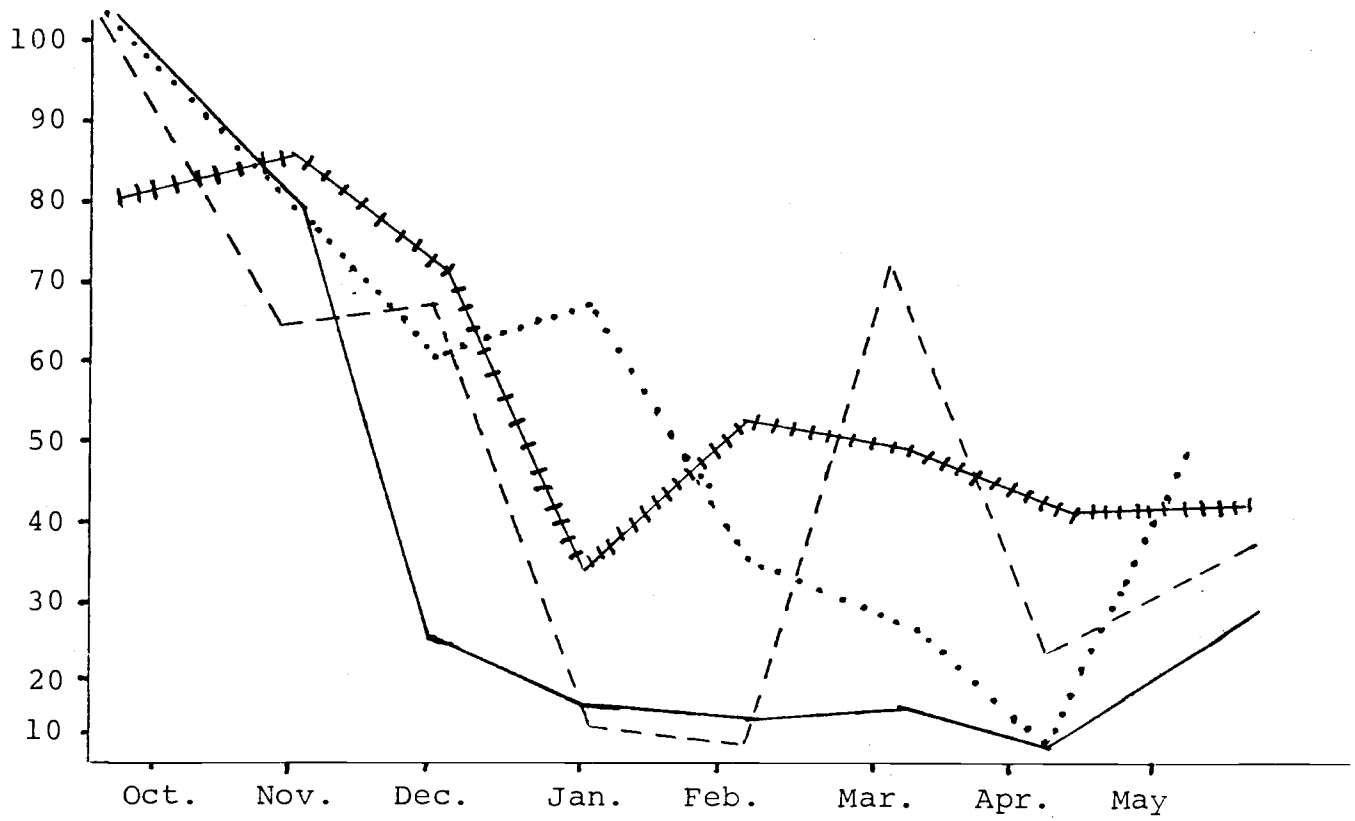

Figure 1: The Percentage of Hairless mice weaned during a 243-day period. (October, 1965 - May, 1966).

Experiment 2.

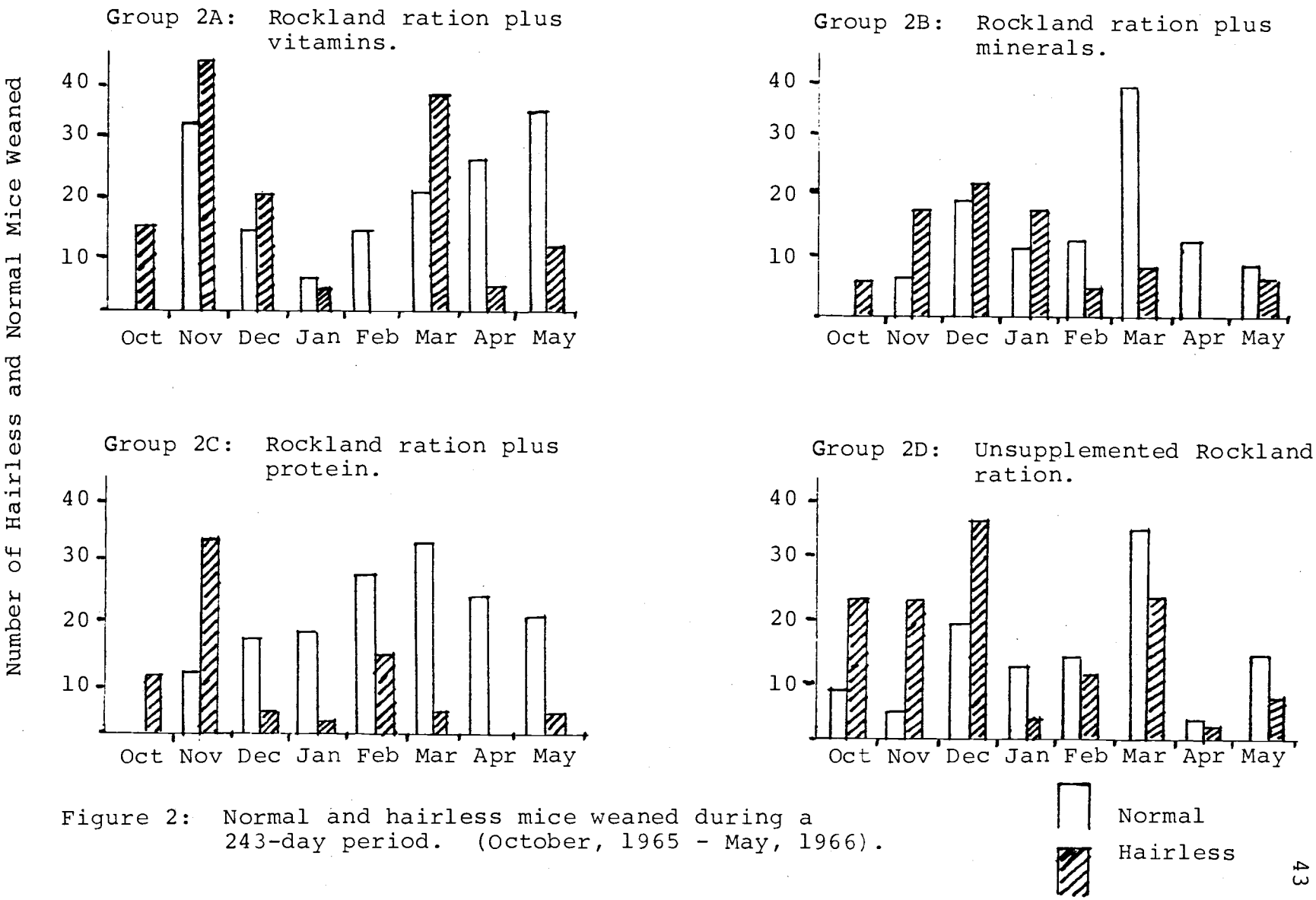

Figure 2: Normal and hairless mice weaned during a 243-day period. (October, 1965 - May, 1966).

period in a histogram form.

Discussion

Results of Experiment 2 showed clearly that each diet affected the experimental group of hairless mice in very different ways. Groups 2A, fed a vitamin supplement, showed an immediate response in fertility with the number of young born exceeding all other groups. The incidence of hairlessness was not affected: 135 young hairless mice were weaned during the vitamin trial, compared with 134 in control group 2D. The fertility in Group 2A decreased during January and February, and increased again in March, April and May. The number of litters born was significantly higher ($P < .05$) as compared to Group 2D (control group).

Mineral salts supplements in Diet 2 had a particularly adverse effect on reproduction in Group 2B. The number of young born in Group 2B was significantly lower than that in Group 2D ($P < .05$). The incidence of hairlessness was not affected by Diet 2 during the first four months of the experimental period. During the second four month period only 21 hairless mice were weaned, compared to 64 during the first four months of this experiment.

A steady increase in fertility was demonstrated in Group 2C fed the Rockland ration supplemented with protein. The number of hairless mice weaned was significantly lower ($P < .05$) than that of the control group. Pre-weaning

mortality was the lowest in Group 2C.

Figure 1 shows that the percentage of hairless mice weaned was declining in all four groups during the experimental period; statistically significant difference was found between Group 2C and Group 2D (31.4% in Group 2C and 54% in Group 2D).

Experiment 3

Objectives

Since the previous experiment showed that the Rockland mouse ration supplemented with casein resulted in a significant reduction in the percentage of hairless offspring produced, a decision was made to test the effect of the addition of amino acids to the basal Rockland ration on the incidence of hairlessness in young mice, and on the fertility of the hairless mice of the BCX strain.

Methods

General procedures of this experiment were similar to those of the previous experiment. Hairless mice of breeding age were randomly selected and divided into four groups. Each group was composed of 20 females and 5 males. The groups were fed the following diets: 50 pounds of ground Rockland pelleted mouse feed was mixed with the following quantities of amino acids:

Diet 1 for Group 3A:

L - arginine: 146.4 grams

L - cystine: 11.9 grams

L - lysine: 317.8 grams

Diet 2 for Group 3B:

L - histidine:	71.5 grams
DL - isoleucine:	214.5 grams
DL - leucine:	330.3 grams

Diet 3 for Group 3C:

DL - methionine:	115.8 grams
DL - phenylalanine:	170.2 grams
DL - tryptophan:	40.9 grams
DL - valine:	221.3 grams

Diet 4 for Group 3D (Control group):

Rockland mouse feed without supplement.

The amounts of amino acids added were calculated from the percentage of amino acids in casein, as listed in Hawk, Oser and Summerson, 1950 Edition.

	% AA in casein	AA lbs/ 100 lbs Feed*	Grams of AA/ 50 lbs of Feed
Arginine	4.3	0.645	146.4
Cystine	0.35	0.053	11.9
Lysine	7.6	1.140	317.8
Histidine	2.1	0.315	71.5
Isoleucine	6.3	0.945	214.5
Leucine	9.7	1.445	330.3
Methionine	3.4	0.510	115.8
Phenylalanine	5.0	0.750	170.2
Tryptophan	1.2	0.180	40.9
Valine	6.5	0.975	221.3

* If casein would supply 15% of the diet.

Experiment 3 was carried out for a period of 9 months - from December 1966 to October 1967. Two months (April, May) were excluded from the experiment for the following reasons: A new shipment of Rockland pelleted mouse feed, received at the beginning of April, was noticeably darker in color, and resulted in a lower consumption of the feed in all four groups. Although analysis of the feed did not show any difference, it was decided to allow a 60 day period for the mice to become adjusted to the new feed in case there was any undetected difference from the previous feed.

Results

The incidence of hairlessness, and the breeding results for the four groups are summarized in Table 7. Figure 3 compares the number of normal animals weaned with the number of hairless mice weaned.

Discussion

Experimental results obtained for Group 3A, in which arginine, cystine and lysine were added to the basal Rockland feed, do not suggest any improvement in reducing the frequency of hairlessness. In fact, reproduction was suppressed by Diet 1 during the first four months of the trial although recovery was achieved during the following five months of the experiment. Pre-weaning mortality was significantly higher

Table 7. Experiment 3 - Summary.

Effect of amino acid supplementation of Rockland mouse ration on incidence of hairlessness in the BCX mouse strain during a 274-day experimental period. (December, 1966 - October, 1967).

Group	3A	3B	3C	3D
AA Supplement	arginine cystine lysine	histidine isoleucine leucine	methionine phenylalanine tryptophan valine	none (Control)
Litters born (No.)	26	33	14	30
Young born (No.)	164	207	102[a]	201[a]
Young weaned (No.)	123	179	82	175
Normal weaned (No.)	80	157	50	106
Hairless weaned (No.)	43	22[a]	32	63[a]
Hairless weaned (%)	35.0	12.3[a]	39.0	39.4[a]
Ave. young/litter born (No.)	6.3	6.3	7.3	6.7
Ave. young/litter weaned (No.)	4.7	5.4	5.9	5.8
Age hair appears (days)	6.0	5.9	5.7	6.1
Age hair lost (days)	15.8	17.7	15.3	15.8
Pre-weaning mortality (%)	25.0[a]	13.5	19.6	12.9[a]

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

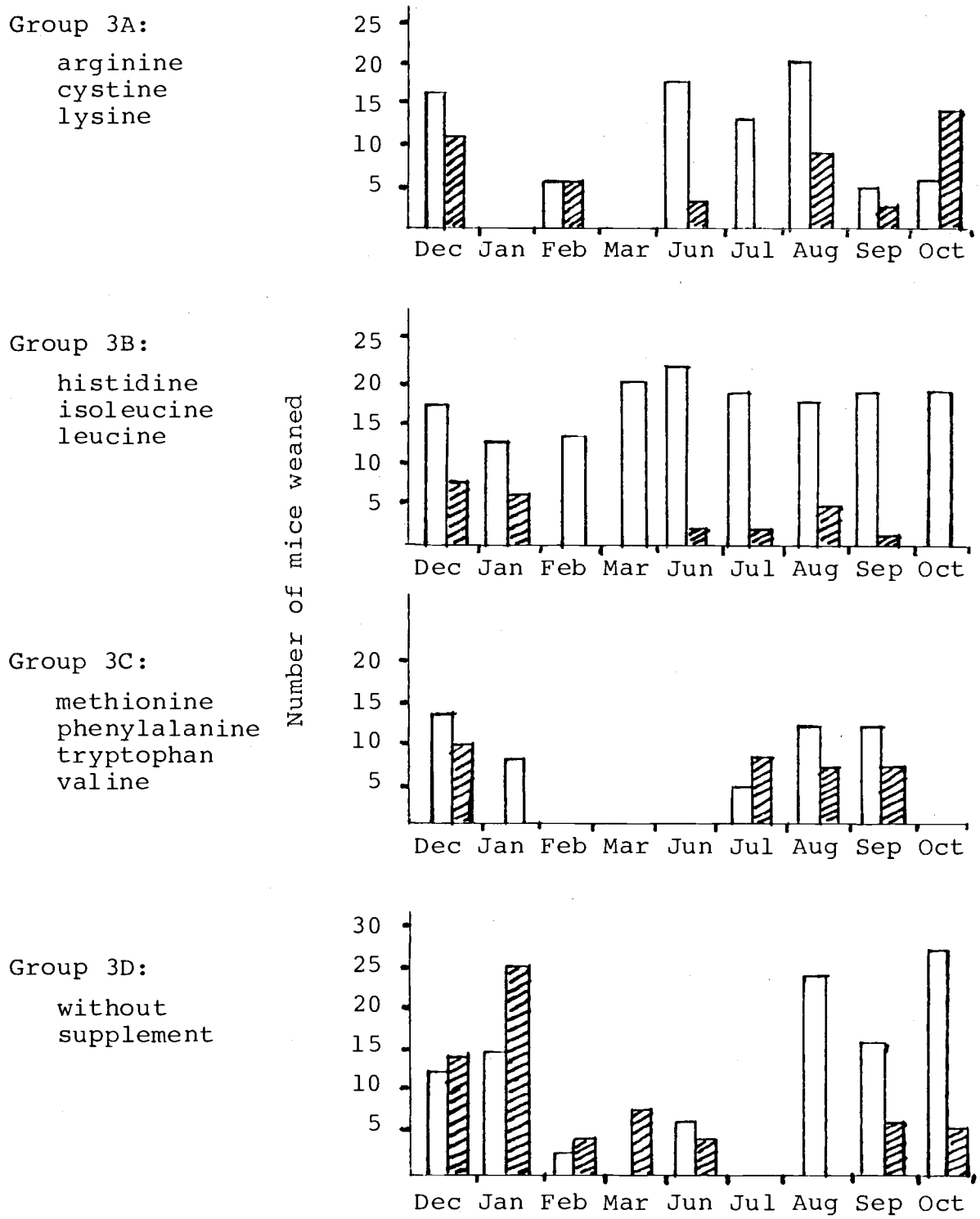

Figure 3: Monthly distribution of normal and hairless mice weaned on Rockland ration supplemented with groups of amino acids during a 274-day period. (December, 1966 - October, 1967). Experiment 3.

($P < .05$) as compared to the control group.

Diet 2 of Group 3B, with histidine, isoleucine and leucine added to the basal Rockland feed, resulted in increased fertility, even during the winter months. It proved to be the most efficient in the elimination of hairlessness. The number of hairless mice weaned in Group 3B was significantly lower ($P < .05$) than in Group 3D (control group). Pre-weaning mortality among the groups with supplemental feeding was lowest in Group 3B. Good physical condition as exhibited by shiny hair and healthy appearance of young mice exceeded that of all other groups.

A sharp decrease in reproduction was noted for Group 3C which was fed a diet consisting of the basal Rockland mouse ration, supplemented with the amino acids, methionine, phenylalanine, tryptophan and valine. No litters were born in the period between February and June. Only 14 litters were born during the entire experimental period between December 1966 and October 1967. The number of young mice was significantly lower ($P < .05$) compared to the control group. The percentage of hairless mice weaned was similar to that of the control group.

Experiment 4

Introduction

Experiment 4 was conducted at the University of British Columbia Research Farm on Vancouver Island, where the candidate held the position of farm manager.

Sixty females and 20 males of the BCX mouse strain were transported from Oregon State University Small Animal Laboratory to the University of B.C. Farm in March 1966.

Experiment 4 consisted of three different trials:

1. Fresh cow's milk was used to replace drinking water of the breeding mice.
2. Pork fat was added to pelleted Rockland mouse ration, to obtain a high energy diet.
3. A group of white Swiss Webster female mice was bred to hairless males of BCX strain.

Pelleted Rockland mouse ration was fed to all experimental animals.

Trial 1

Objectives

The objective of this trial was to investigate the effect of substituting fresh cow's milk for drinking water on hairlessness in young mice of BCX strain, introducing a high quality protein in the diet.

Methods

Two groups of hairless mice were randomly selected from the breeding stock, each group containing 30 females and 5 males. Group 4A was fed Rockland ration with fresh cow's milk replacing the drinking water. To insure freshness, milk was changed every four hours. Group 4B served as the Control and was fed the Rockland ration plus fresh tap drinking water.

General methods of feeding and breeding were similar to those described earlier. Both groups of mice were kept in a well-insulated room with room temperature maintained at 76°F. An automatically controlled ventilation fan was in operation every hour, for 10 minutes. The animals were subjected to natural lighting conditions from a large window.

Trial 1 was carried out for 60 days, from May 1 to June 30, 1966.

Results and Discussion

Trial 1 was discontinued after 60 days. Replacing fresh milk in Group 4A every four hours was not practical, as well as being time consuming. Although the experimental time period was brief, the following observations were made during this period. Firstly, of the 77 young mice born in Group 4A only three mice showed the hairless condition. Group 4B produced 93 young mice, with 21 mice showing

hairlessness. The breeding females and males in Group 4A were in better physical condition than the control group. They showed more activity and their coats were longer and shinier. Pre-weaning mortality was 0 in Group 4A. Average weaning weight of young mice in Group 4A was four grams higher than of the young in the control group.

Trial 2

Objectives

Trial 2 was designed to study the influence of energy, added in the form of pork fat, on the incidence of hairlessness and on the breeding performance of BCX strain mice.

Methods

General methods were similar to those described for Trial 1. Two groups of breeding animals were used in this trial. Each group consisted of 30 females and 5 males. Group 4C was fed 1 gram of pork fat per animal per day in addition to the Rockland mouse feed. Group 4D was a control group and was fed unsupplemented pelleted Rockland mouse feed. Feed and water were supplied once a day. Trial 2 was carried out for 8 months, from August 1966 to March 1967. Thirty-five grams of pork fat were daily placed in small pieces on top of a screen which served as

lid of the large stainless steel dishpan.

Results and Discussion

The results, compared with the control group, are summarized in Table 8. Litter distribution, and incidence of hairlessness with respect to time, are given in Figure 4.

A very significant decrease in manifestation of hairlessness was observed in Group 4C. The high fat ration, combined with a seasonal effect, showed a significant contrast within the supplemental and control group. Fertility of the control group was much higher during the 8 month period, with little seasonal dependence. An initial increase in reproduction of Group 4C was followed by its sharp decrease. However, although the high fat diet decreased fertility, at the same time the incidence of hairless mice was significantly decreased (15.8% in the group receiving pork fat, and 30% in the control group). Average weaning weight of young was 2 grams higher in Group 4C. Pre-weaning mortality was only 12.7% in Group 4C as compared to 17.3% in the control group.

Different environmental conditions in the Oyster River Small Animal Laboratory could have been partially responsible for the general increased fertility in Group 4D. The temperature was lower, 76° F. compared to 82° F. in the Small Animal Laboratory at the Oregon State University. Furthermore, the

Table 8. Effect of pork fat supplementation of Rockland mouse ration on incidence of hairlessness in BCX strain of mice during a 240-day experimental period. (August, 1966 - March, 1967).

Group	4C	4D
Females - Males in group: (No.)	30 - 5	30 - 5
Litters born: (No.)	39	64
Young born: (No.)	268	399
Young weaned (No.) - (Total)	234	330
Normal young weaned: (No.)	197	231
Hairless young weaned: (No.)	37[a]	99[a]
Hairless weaned: (%)	15.8[a]	30.0[a]
Pre-weaning mortality: (No.)	34[a]	69[a]
Pre-weaning mortality: (%)	12.7	17.3
Age hair appears: (days)	5.9	6.2
Age hair lost: (days)	17.4	18.6
Ave. weaning wt. of young: (gr.)	11.7	9.1
Ave. young/litter born: (No.)	6.9	6.2
Ave. young/litter weaned: (No.)	6.0	5.1

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

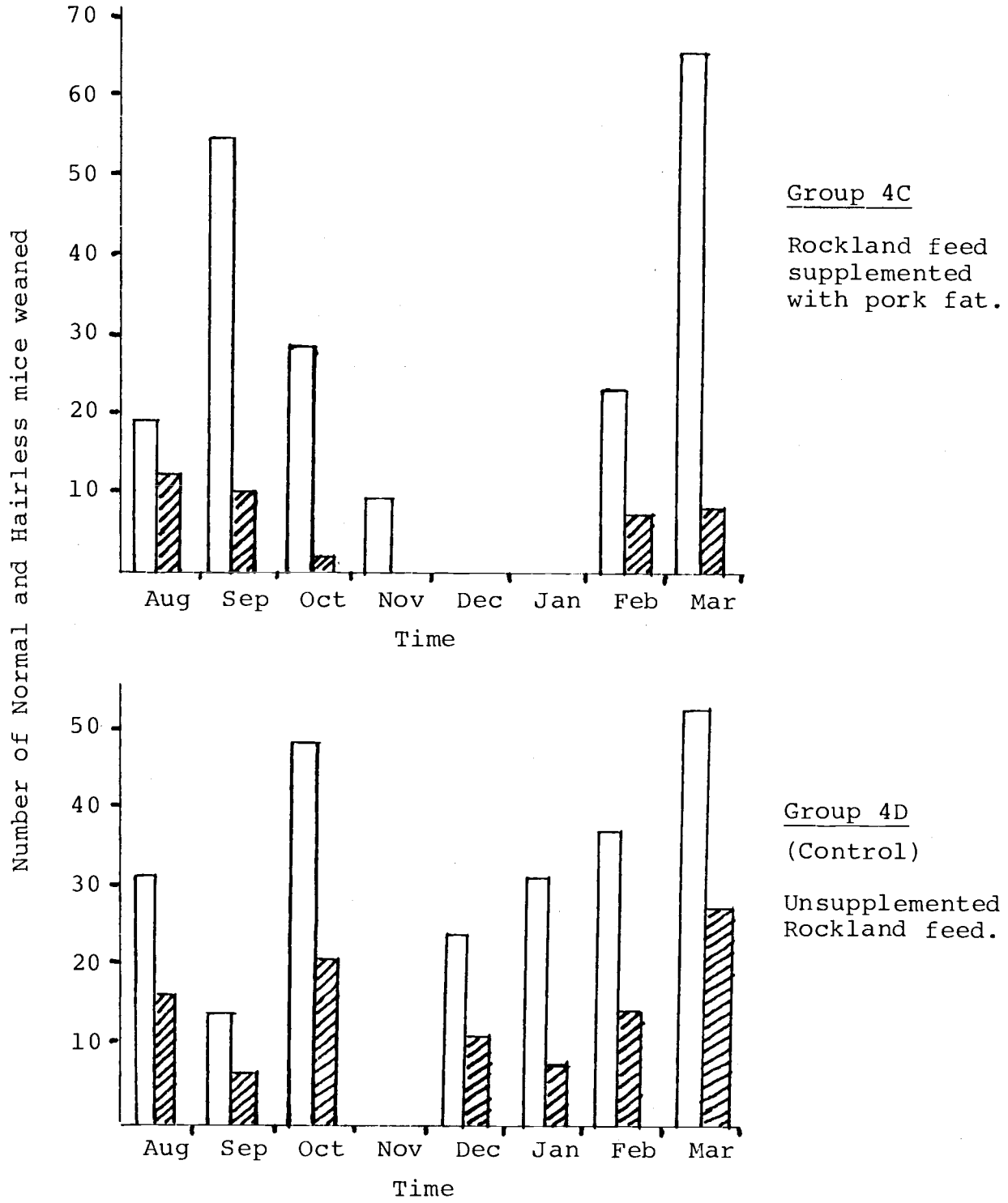

Figure 4: Normal and hairless mice weaned in a 240-day experimental period. (August, 1966 - March, 1967). Experiment 4.

mice received direct sunlight and fresh well drinking water at Oyster River University Farm.

Trial 3

Based on the results of all previous experiences showing very irregular manifestation of hairlessness in young mice of BCX strain, Trial 3 was carried out simultaneously with Trial 2.

Objective

The main objective of this trial was to study the mode of inheritance of hairlessness in young mice of the BCX strain.

Methods

Twenty Swiss-Webster white females and 5 hairless males of the BCX strain were used for a small-scale cross-breeding experiment.

In Trial 3 a harem mating system was used. The experimental animals were fed the Rockland mouse ration in pelleted form.

Results and Discussion

Offspring of the first generation did not show any hairlessness, but when the F1 females were bred again to

hairless males, the F2 generation showed a 14% incidence of hairlessness in those mice which were white in color. Hairless individuals of the susceptible BCX strain are all black. These results indicated a recombination between the color gene and the gene or genes affecting hairlessness.

The number of breeding animals used in this trial was not large enough to produce conclusive results warranting statistical analysis, but it suggested that the hairlessness in young mice (Hypotrichosis juvenilis) is caused by a simple recessive gene, or genes, with very irregular penetrance.

A more complex genetic experiment, using a larger number of animals, should be carried out in order to gain conclusive information on the mode of inheritance of this form of hairlessness.

Experiment 5

Objectives

A very significant decrease in manifestation of hairlessness in Group 4C, fed the Rockland ration supplemented with pork fat, was the basis for a further investigation of the effect of essential and non essential fatty acids on hairlessness and reproduction in the BCX strain of mouse.

Methods

Five groups of young hairless females and males of breeding age were randomly selected from the breeding pool. Each group contained 16 females and 4 males. Five diets were prepared as follows:

Group 5A: 2.85 kg of ground Rockland ration mixed with 150 grams cotton seed oil.

Group 5B: 2.85 kg of ground Rockland ration plus 150 grams oleic acid.

Group 5C: 2.85 kg of ground Rockland ration plus 200 grams linoleic acid.

Group 5D: 2.85 kg of ground Rockland ration plus 200 grams linolenic acid.

Group 5E (Control group): 3 kg of ground Rockland ration unsupplemented.

Each diet was mixed in small quantities (approx. 3 kg) every 4 to 5 weeks and kept in plastic bags, under refrigeration (40°F).

As in previous experiments, pregnant females were transferred to individual cages. After the litter had been weaned, the female was put back in the experimental group. Hairless mice were kept in a common breeding pool and normal mice were removed from the study. Nonfertile females and males were replaced by young hairless mice of breeding age.

Midway through the experiment the lighting conditions were changed from a 12 hour light, 12 hour dark diurnal pattern, to a period of daylight reflecting local conditions (Pacific-Northwest). It was hoped that this alteration would improve the reproductive capacity of the test animals.

Results

The effect of each diet on the incidence of hairlessness, and on reproduction of the five experimental groups is presented in Table 9. The number of normal and hairless mice weaned in 320-day experimental period (January - November, 1967) is shown in Figure 5.

The percentage of fatty acids in pork fat and cotton seed oil is given in Table 10.

Table 9. Experiment 5 - Summary

Effect of essential and non essential fatty acids and cotton seed oil supplementation of Rockland mouse ration on incidence of hairlessness in BCX strain of mice during a 320-day experimental period. (January, 1967 - November, 1967).

Group	5A	5B	5C	5D	5E
Supplement	cotton-seed oil	oleic acid	linoleic acid	linolenic acid	none (Control)
Litters born: (No.)	30	32	38	14[a]	33[a]
Young born: (No.)	180	200	287[b]	95[a b]	230[a]
Young weaned: (No.)	141	175	227[a]	85[a]	185
Normal weaned: (No.)	98	161	155	61[a]	113[a]
Hairless weaned: (No.)	43	14[a]	72	24	69[a]
Hairless weaned: (%)	30.5	8.0[a]	31.7	28.2	37.9[a]
Ave. young/litter born: (No.)	6	6.3	7.6	7.9	6.9
Ave. young/litter weaned: (No.)	4.7	5.5	6.0	6.1	5.5
Age hair appears: (days)	5.6	5.5	5.8	5.8	5.9
Age hair lost: (days)	16.5	17.6	19.9	18.5	19.3
Pre-weaning mortality: (%)	21.7	12.5	20.9	10.5	20.9

Values in the same row bearing the same superscripts are significantly different ($P < .05$).

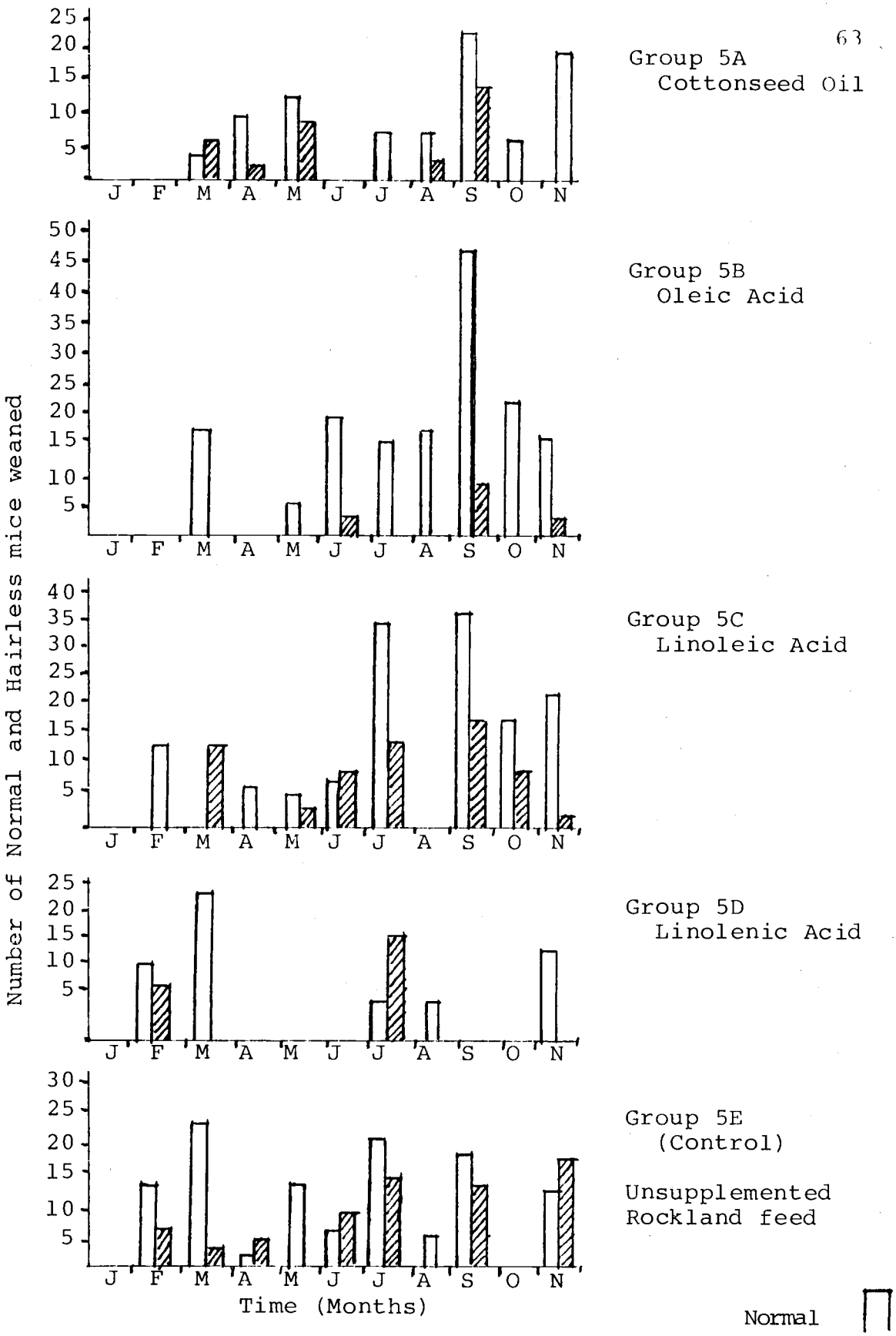

Figure 5: Normal and hairless mice weaned in a 320-day experimental period. (January, 1967 - November, 1967). Experiment 5.

Table 10. Fatty acids in pork fat and in cottonseed oil (%).*

A. Pork fat:	Fatty acids	%
	myristic	1.0
	palmitic	30.1
	stearic	16.2
	tetradecanoic	0.3
	hexadecanoic	2.7
	oleic	40.9
	octadecanoic	7.1
	C_{20-22} unsaturated	1.7

B. Cottonseed oil:	Fatty acids	%
	myristic	3.3
	palmitic	19.9
	stearic	1.3
	arachidic	0.6
	oleic	29.6
	linoleic	45.3

*The values are from Bull. 1170, Composition and Constants, Natural Fats and Oils, Ashland Chemical Company.

Discussion

Oleic acid mixed with ground Rockland ration significantly decreased the incidence of hairlessness ($P < .05$). Only 14 hairless animals out of a total of 175 weaned mice were noted in Group 5B. The oleic acid diet resulted in an initial decrease in reproduction in Group 5B. However after four months on the diet the reproductive capacity showed a marked improvement. It was also observed that the young mice in Group 5B grew well and their weaning weight was the highest of all the groups.

A partial suppression of reproduction in Group 5A during the first eight months of the experimental period was followed by an increase in reproduction during the last three months of the experiment. Group 5A was fed the Rockland ration supplemented with cottonseed oil. The incidence of hairlessness was not affected.

The highest number of hairless mice weaned was recorded in Group 5C. This group was fed the Rockland ration supplemented with linoleic acid. The reproductive pattern of Group 5C is very similar to that of Group 5A on the cottonseed oil diet. Group 5C exhibited low reproduction during the first six months followed by high reproduction during the last five months of the experiment.

Linolenic acid supplemented with the Rockland ration had an adverse effect on fertility of Group 5D. The

reproduction was reduced to less than half as compared to the results of the other four groups. The number of litters and of young mice born showed a significant decrease between the groups 5D and 5E ($P < .05$).

The detrimental effects of linolenic acid in these studies may relate to the highly oxidizable nature of this compound and to the lack of antioxidant protection in the diet.

SUMMARY AND CONCLUSIONS

This study was directed toward the explanation of a complex hairless condition in laboratory mice that appeared to involve genetic, nutritional and environmental causes. It was conducted over a period of eight years, and included about eighteen hundred mice of two separate strains. Both the nature of the problem and the method of its investigation have implications for conditions in other animals, including domestic species, which increasingly appear to be multicausal in origin.

The BCX-strain of mouse developed at the University of British Columbia exhibits the condition of transitory hairlessness. Typically, hair loss begins about the 14th-18th day after birth, affecting primarily the head and upper trunk. At approximately 32-36 days of age, hair growth resumes and in most cases the normal growth of hair is maintained throughout the adult lifespan.

Although this loss of hair is linked to the genetic make-up of the animal, the expression of the genes is moderated by nutritional and to a lesser extent by environmental factors. Full expression of this condition was noted only if the animals were fed the commercial Rockland mouse diet. Substitution of this diet with Purina Mouse Chow lowered the incidence of hairless offspring from 36% to 5% for BCX-strain hairless parents and from 3% to zero % for BCX non-hairless parents.

In an attempt to isolate the nutritional factor(s) which operates to either suppress or to elicit this hairless condition, five experiments were carried out, supplementing the Rockland mouse diet with protein, vitamins, minerals, fat and milk. The effect of each of these supplements on the incidence of hairlessness was followed by analyzing the frequency of occurrence of hairless condition at weaning for two groups of mice: one group was fed the supplemented diet over the experimental period while the control group was fed the unsupplemented Rockland feed. Hairless parents were used throughout the study. To obtain an adequate reproductive rate, temperature and light were adjusted to provide optimum breeding conditions. Nevertheless, it was noted that the breeding was subject to a marked seasonal variation. Typically, the largest number of mice weaned occurred in November and March-May, with very poor reproduction results in January and February.

The analysis of the Rockland and Purina diets did not show any meaningful differences in the levels of certain essential nutrients. Both diets compare favorably to NRC[1] recommendations of levels and intakes of these nutrients in meeting the nutritional requirements of the mouse.

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[1]National Academy of Sciences - National Research Council. 1966. Nutrient Requirements of Domestic Animals. Publication 990, Number 10:47.

To test the effect of vitamin, mineral and protein supplements, experiment 2 was conducted over an 8 month period with three breeding groups and one control group. In all groups, except the protein supplement group, the incidence of hairlessness was not significantly reduced from that of the control group. With the protein supplement (casein), the incidence of hairlessness in the offspring was significantly reduced by 42%, compared to control group ($P < .05$). Vitamin supplementation increased the number of litters born, whereas both the protein and mineral supplements reduced the average litter size. In general, the percentage of hairless mice weaned decreased throughout the course of the experiment for all groups, with protein supplementation demonstrating almost complete suppression of hairlessness.

The favourable results achieved by the protein supplementation of the Rockland mouse feed prompted a closer investigation of the interrelationship of hairlessness and the amino acid content of the diet. Three groups of amino acids were chosen for investigation: (1), arginine, cystine, lysine; (2), histidine, isoleucine, leucine; and (3), methionine, phenylalanine, tryptophan and valine. A positive effect of specific amino acid supplementation was evident only for group (2) (histidine, isoleucine, leucine). The BCX-mice fed this ration demonstrated only 12% hairlessness in weaned offspring as compared to 40% in groups (1), (3), and the control group.

In experiment 4 the BCX mice were fed fresh cow's milk in trial 1 and pork fat in trial 2, in addition to the regular Rockland ration. Although the experiment with milk supplementation was only carried on for two months, the incidence of hairlessness was substantially reduced. With the milk diet, only three of seventy-seven weaned mice were hairless compared with 21 out of a total of 93 in the control group. As well, the general physical condition of both the breeding mice and their offspring was notably improved under the milk regime, as evidenced by better growth of hair, low pre-weaning mortality and higher weaning weight. The reduction of hairlessness effected by the fresh milk supplementation was even greater than that caused by casein addition, suggesting that other nutrient factors in the milk beside protein were involved. Similar results were recorded for fat supplementation of the Rockland diet. The incidence of hairlessness was reduced by 50% if pork fat was added to the diet. In addition, there was an improved pre-weaning mortality number with the added fat diet, although the reproduction decreased considerably compared to the control group.

As fat supplementation was effective in the reduction of hairlessness, experiment 5 was carried out in which the Rockland diet was supplemented with cotton-seed oil, linoleic acid, oleic acid, and linolenic acid. Only oleic acid supplementation proved useful for the suppression of hair loss. While the control group showed a percentage of hairless mice weaned of 38, the group with oleic acid supplementation had only an 8% incidence of hairlessness. It was also

observed that the young mice in group 5B grew well and their weaning weight was the highest of all the groups.

Cotton-seed oil supplement to the Rockland ration did not affect the incidence of hairlessness in group 5A. A partial suppression of fertility was observed during the first eight months of the experimental period followed by an increased reproduction during the last three months of the experiment. The high content of oleic acid in cotton-seed oil could have caused a dilution of the essential fatty acids, important for growth and reproduction in mice.

Linolenic acid supplement produced only one half the number of litters as did the other experimental and control groups. With the linoleic acid supplement the incidence of hairless mice did not decrease and was approaching the control group.

In conclusion it is apparent from the results of this study that specific nutrients added to the Rockland mouse ration caused the reduction of incidence of hairlessness in the BCX strain mouse. Not only nutritional but also genetic and environmental factors were included in this study and an attempt was made to describe and to analyse their effect on incidence of hairless mice and on their reproduction.

The results tend to suggest that highly inbred mice of BCX strain could be extremely sensitive to various nutrients of the ration which may not be in the optimal balance.

Many cases of hairlessness in other animals have been reported during the last fifty years. Some cases are proven

to be hereditary while others are reported without evidence from which it could be determined whether they were hereditary or the result of environmental factors.

Hereditary hypotrichosis in swine has been reported by Roberts and Carroll (1931). The difference between the normal and hairless was due to a single gene, the normal condition being incompletely dominant. Cole (1919) and Mohr and Wriedt (1928) reported inherited hairlessness in cattle. In most cases the affected individuals were either dead at birth or died shortly after birth. Hairless dogs have been reported in many regions of Europe, Asia, Africa, and America. The hairless condition is hereditary and according to David (1931), the character is a dominant. Kislovsky (1928) reported a case of a recessive hypotrichosis in the rabbit. The condition was lethal and all animals died before the age of one month.

More recent reports on hairlessness in domestic animals recognize that coat or hair condition can indicate various metabolic problems, such as Cu deficiency, Se toxicity, EFA deficiency etc.

The favorable results from dietary protein and fat supplementation in suppressing incidence of hairlessness, along with the known genetic involvement in this condition in BCX strain mice clearly identify this hairless condition as multicausal. These mice can serve further as useful animal models for investigating such conditions, and experimentation leading to identification of individual nutrient involvements

(amino acids, minerals, etc.) would be in order. Most importantly, the implications of genetic-environmental interactions in the production processes of domestic animals should be recognized in future selection and breed improvement programs.

The demonstrated effectiveness of protein from animal sources (casein, whole milk) in reducing incidence of hairlessness in the genetically-susceptible strains of mice may be taken as broad evidence of the contributions that animal products may make to a mixed diet.

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APPENDICES

APPENDIX I

Ingredients and Analysis of Rockland Ration*

Ingredients:

Soybean meal, ground yellow corn, fish meal, pulverized barley, wheat, middlings, ground wheat, dehydrated alfalfa meal, pulverized oats, feeding oat meal, dried skimmed milk, 1% animal fat (preserved with propylene glycol, butylated hydroxytoluene, citric acid), vitamin A palmitate, irradiated dried yeast, niacin, calcium pantothenate, riboflavin supplement, menadione, vitamin B_{12} supplement, 1% calcium carbonate, 0.5% dicalcium phosphate, 1% salt, and traces of manganese oxide, copper oxide, cobalt carbonate, iron carbonate, zinc oxide, calcium iodate.

Guaranteed Analysis:

Crude Protein	24.0% Min.
Crude Fat	4.0% Min.
Crude Fiber	6.0% Max.

*Taken from feed tag: Rockland Mouse/Rat Diet by Teklad Incorporated, Monmouth, Ill.

APPENDIX II

Ingredients and Analysis of Purina Ration*

Ingredients:

Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, ground extruded corn, oat middlings, soybean meal, dehydrated alfalfa meal, cane molasses, animal fat preserved with BHA, vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewer's dried yeast, thiamin, niacin, vitamin A supplement, 0.5% iodized salt, 0.075% ferric ammonium citrate, 0.02% manganese sulphate and a trace of zine oxide.

Guaranteed Analysis:

Crude Protein not less than.....	23.0%
Crude Fat not less than.....	4.5%
Crude Fiber not more than.....	6.0%
Ash not more than.....	9.0%

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*Taken from feed tag: Purina Mouse Chow by Ralston Purina Co., St. Louis, Mo.

APPENDIX III

Vitamin Diet Fortification Mixture*

	grams/100 lbs. diet
Vitamin A Concentrate (200,000 units per gram)	4.5
Vitamin D Concentrate (400,000 units per gram)	0.25
Alpha Tocopherol	5.0
Ascorbic Acid	45.0
Inositol	5.0
Choline Chloride	75.0
Menadione	2.25
p Aminobenzoic Acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine Hydrochloride	1.0
Thiamine Hydrochloride	1.0
Biotin	20
Folic Acid	90
Vitamin B-12	1.35
Calcium Pantothenate	0.03

*Nutritional Biochemicals Corporation
Cleveland, Ohio.

APPENDIX IV

Salt Mixture Briggs*

COMPOSITION:

Calcium Carbonate	16.6%
Calcium Phosphate	47.3%
Copper Sulfate	.017%
Ferric Citrate	.333%
Magnesium Sulfate	5.0%
Manganese Sulfate	.417%
Potassium Chloride	11.6%
Potassium Iodide	.017%
Sodium Chloride	6.6%
Sodium Phosphate Dibasic	11.6%
Zinc Carbonate	.217%

*Nutritional Biochemicals Corporation
Cleveland, Ohio.

APPENDIX V

"Vitamin Free" Casein*

Total Nitrogen (Dry) not less than	14.5 %
Total Moisture-maximum	6.0 %
Total Ash-maximum	2.3 %
Fat (ether extractables)-maximum	.01%
pH	5.85
Lactic Acid does not exceed	0.1 %

MICROGRAMS PER GRAM

Thiamine HCl	0.15
Riboflavin	0.50
Niacin	0.30
Pyridoxine HCl	0.65
Pantothenic Acid	0.15
Biotin	0.0013
Folic Acid	0.030
Vitamin B-12	0.003

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