THE EFFECT OF A VARIATION OF ZINC AND PROTEIN IN THE DIET ON HAIR FORMATION, ZINC CONTENT OF THE HAIR, AND BODY GROWTH OF THE ALBINO RAT

by

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INTRODUCTION

During the past century, the effects of zinc deficiency and zinc toxicity had been analyzed in a variety of organisms and many aspects of the metal's influence on normal animal and plant physiology revealed (Underwood, 1962). Early recognition of the essential nature of minor or "trace" elements, such as zinc, was impeded by the fact that they were present as impurities in the diets.

Since 1926, when Sommer and Lipman demonstrated the essential role of zinc in the nutrition of sunflower and barley (Bonner and Galston, 1952), the plant zinc deficiency syndrome has been studied extensively (DeKock, 1961). The results have been utilized by agronomists to redeem large areas of land and to improve the production of a variety of crops (Roach, 1959; Andurer, 1959).

The action of zinc in the normal metabolism of living things has been explored in zinc deficiency experiments in microorganisms, such as Rhizopus nigricans (Foster, 1950), Mycobacterium smegmatis (Winder and Denneny, 1959), Aspergillus niger (Vallee, 1959), and Neurospora crassa (Nason et al., 1951; Nason et al., 1953). Also, biochemical assays have revealed the presence of zinc in insulin, carbonic anhydrase, and other metabolically important substances (Karlson, 1963; Boyer et al., 1961; Harrison et al., 1962). Further biochemical investigations involving the

interactions of metabolites with zinc have further clarified the micronutrient's role *in vivo* (Fruton, 1958; Fujii, 1954; Fujii, *et al.*, 1955; Hofstee, 1955; Wacker and Vallee, 1959; Vallee, 1955).

The possible involvement of zinc in hair formation in the rat was first indicated by investigations which sought to determine the status of zinc in normal rat nutrition by studying the rodents under conditions of extreme zinc deficiency (Todd *et al.*, 1934; Stirn *et al.*, 1935). In 1934, Todd and co-workers from the University of Wisconsin reported that zinc deficient rats developed alopecia about the neck and shoulders which sometimes covered the entire venter (Todd *et al.*, 1934). The zinc deficiency syndrome in the rat was studied later by other workers using slightly different diets (Hove *et al.*, 1938; Day and McCallom, 1940). Using what seemed to be a decreased level of zinc, Hove and colleagues observed a retardation in body growth, but no alteration in hair formation and retention (Hove *et al.*, 1938).

In 1941, Follis and co-workers investigated the histopathological aspects of extreme zinc deficiency in the rat. They reported a variety of lesions in the skin of the dorsum, including hyperkeratinization, epidermal thickening, dermatitis, and degeneration of the follicles (Follis *et al.*, 1941). Later deficiency experiments with mice disclosed that alopecia developed on the shoulders, nape of the neck, and parts of the face (Day, 1942; Day and Skidmore, 1947).

Experimental zinc deficiency in chicks revealed a syndrome which included symptoms, such as retarded growth, skin lesions,

and feather and follicle atrophy (O'Dell et al., 1958). Other recent experiments have shown a gross alteration in the skeleton and integument of chick embryos when the mother hen was placed on a low-zinc diet (Kienholz, et al., 1961).

Parakeratosis in swine has been shown to be caused by low zinc intakes (Underwood, 1962; Kernkamp and Ferrin, 1953). In New Guinea, cattle, which were grazed on Trachypogon, plants with low zinc content, developed a syndrome which included extreme hair loss, hyperkeratinization, and poor body growth. The cattle returned to normal when treated with zinc sulphate or grazed on other plants (Legg and Sears, 1960).

In 1961, a team of NAMRU[1] workers in Southwest Iran, reported a syndrome in male human dwarfs which they attributed to zinc deficiency resulting from abnormal intestinal absorption. The characteristics of the syndrome included extremely underdeveloped sexual features, hepatosplenomegaly, and hypochromic anemia caused by iron deficiency (Prasad et al., 1961). Geophagia was prevalent among those suffering from the syndrome. Recent experiments, comparing the concentration of zinc in the hair of Iranian villagers with that found in the hair of city dwellers, have revealed significantly different lower values for the villagers (Reinhold et al., 1965).

In 1963, Prasad and co-workers reported the results of their investigations in certain villages near Cairo. The Egyptian

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1 U.S. Naval Medical Research Unit.

patients showed retarded growth, hypogonadism, anemia and the absence of pubic, axillary, and facial hair. However, they differed from the Iranian subjects in that they were not geophagic, but were afflicted with parasites. Clinical tests on these patients showed a low plasma zinc level, high plasma zinc turnover rate, small 24 hour exchangable zinc pool, and a low zinc content in the hair (Prasad, et al., 1963a).

More convincing implications of zinc deficiency as the cause of this syndrome in human male dwarfs was provided by investigations in the oasis village of Kharga, Egypt (Prasad et al., 1963b). Severe anemia, ancylostomiasis, schistosomiasis, and geophagia were absent. Low plasma level of zinc was found in all patients in whom it was determined. The zinc content of a sample of water from the artesian spring of the village revealed a concentration of 1.8 ug per 100 ml. In Cairo, the water supply was found to have a zinc content of 40 ug per 100 ml.

In both Iran and Egypt, the manifestations of the syndrome were found only in human males.

Scope of the Investigation

The present study was made to investigate the effects of a variation in the quantity of protein and zinc components of the diet on hair formation, hair zinc content, and body growth of albino rats.

In 1960, Forbes and Yohe varied the quantity of calcium and quality of protein in the diet "to elucidate the zinc requirement

of the rat and to study the excretory pattern of dietary zinc under varied conditions". However, no experiment has been reported which varied the quantity of protein with the quantity of zinc to study the effect on hair growth and zinc content of the hair in albino rats.

Also, although alopecia and malformation of the hair has been shown to result when rodents were placed on a diet extremely deficient in zinc content (Todd et al., 1934; Stirn et al., 1935; Day, 1942; Follis et al., 1941), in none of these experiments was hair regrowth on shorn areas studied.

In addition, the diet for the present study was only moderately deficient in zinc content. This situation is probably more comparable to the level to which humans may be exposed. Thus, any manifestations resulting from the diet may indicate what symptoms might be expected in humans.

Another possibility of this investigation would be to confirm the use of the zinc content of the hair as an index of low dietary intake of zinc or of suspected pathological effects of hypozincosis as was done in the investigations in Iran and Egypt (Prasad et al., 1963a; Prasad et al., 1963b; Reinhold et al., 1965).

The histological aspect of the study was undertaken in the hope of providing some information concerning any morphological alterations resulting from the different diets. Information concerning the existence of unusual alterations in the skin, involving abnormal hair formation, may prove to be a source of motivation to biochemists, histochemists, and dermatologists to discover the metabolic cause.

MATERIALS AND METHODS

Maintenance and Diet

Twenty-three albino rats (Rattus norvegicus Berkenhout 1769) of the Sprague-Dawley strain[1] were matched according to weight and sex to establish five sets of four animals each and one set of three animals. The rats were obtained from three litters born on January 10, 12, and 18, 1965, respectively.

On February 4, 1965, when the rats were 17-25 days old, four groups were made according to a variation in the dietary composition: Normal protein-normal zinc (PZ); normal protein-low zinc (Pz); low protein-normal zinc (pZ); and low protein-low zinc (pz). Table 1 describes initial weight, sex and treatment.

The diets were not conforming to the standard or common diet of the rats, since standard diets for maintaining domestic rats vary according to laboratory conditions (Lane-Petter, 1963). For example, at the Rowett Research Institute at Aberdeen, the protein composition of the two cubed diets now in use is 14.9% in Diet No. 1 (Thomson cube) and 20.0% in Diet 86 (Porter, 1957). Nevertheless, throughout this paper, the term "normal" will be used to connote "adequate", i.e., sufficient for the requirement of healthy growth.

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1 Obtained from Animal Suppliers (London) Ltd.

The normal protein diet was composed of 10% casein and 5% gelatin. The low protein diet contained 5% casein and 2.5% gelatin. All diets contained 60% dextrin, 9% corn oil, and 5% vitamin-glucose mixture.[1] In addition, the normal protein diet contained 6% sucrose whereas the low protein diet contained 14% sucrose. Table 2. describes the complete vitamin, mineral and organic composition of the diets.

The normal zinc diet contained 30 µg of zinc per gram of food, provided by supplementing the low zinc diets with zinc sulfate. The low zinc diets contained between 1.42-2.92 µg of zinc per gram of food. Zinc was removed from the casein by four washings with EDTA.

The rats were provided with demineralized water ad libitum. The daily gram-intake of the food-mixture varied throughout the experimental period from 5 to 11 grams per day. The quantity of food provided to all of the animals of a particular set was equalized to the smallest amount consumed by any rat in that set.

The animals were kept in zinc-free stainless steel cages which were cleaned frequently.

Macroscopic Observations Procedure

After two weeks on the diets, the rats were clipped on the ventral and lateral areas from shoulders to pelvic-leg joints. Electric clippers were used to obtain a close shear. The hair was

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1 Obtained from the Nutritional Biochemical Corporation.

stored in glass vials. After an interval of 39-40 days, the rats were shorn on the area of hair regrowth. A third clipping was obtained from 14 of the animals after a second hair regrowth period varying from 3-7 weeks.

Prior to each clipping, observations of the hair regrowth area were interpreted in relative terms by the use of the numbers 1-5 to indicate the amount of regrowth and 0 to indicate that there was no significant regrowth of hair on the shorn area. The numbers and their corresponding significance are:

5 Excellent: Complete coverage of shorn area.
4 Good: Coverage not absolutely complete.
3 Fair: Coverage on about half of shorn area.
2 Poor: Coverage on about a quarter of shorn area.
1 Very poor: A few small patches of hair evident.
0 No noteworthy regrowth.

The rats were weighed weekly.

Hair Washing Procedure

Ten ml. of 0.01% liquid Lux[1] detergent in demineralized water was added to each of the samples in a beaker. After 10 minutes with frequent stirring, the washing water was decanted into a funnel lined with Whatman No. 1 filter paper to catch the suspended hair. Each sample was then rinsed with demineralized water and the rinsing-decanting procedure repeated until the filtrate became clear and no foam resulted when agitated. At this stage, one additional washing was made.

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1 Obtained from Port Sunlight, Chesire, England.

The hair samples were then washed in 25 ml. of absolute ethanol and finally with 25 ml. of ethyl ether. The ether was removed by warming under a lamp.

This washing procedure was adopted after the following experiments were performed to eliminate the possibility of any effect of the action of liquid detergent on the accuracy of the quantitative determination of zinc in the hair samples.

In Experiment 1, a hair sample obtained from a single human source was divided into five parts designated by the letters A to E, respectively. Four different concentrations of washing solutions were prepared (0.01%, 0.1%, 1.0%, and 0.0% detergent) and each assigned the same code letter as the corresponding hair sample which it cleansed. Hair sample E was left unwashed. Liquid Lux detergent and demineralized water were used.

The samples were added to 20 ml. of the washing solution and mixed for about 3 minutes each in a beaker. They were permitted to stand for 30 minutes with frequent stirring during the interval. Each sample was then rinsed and the supernatant decanted into a funnel lined with Whatman No. 1 filter paper. The rinsing procedure was continued until the filtrate became clear and no foaming was observed upon agitation. The hair samples were then treated with absolute ethanol and ethyl ether, and dried under an artificial light source.

Further analysis showed that there was no significant difference in the determined zinc content of hair in the hair

digests of the samples washed by the various solutions.

In Experiment 2 (male rat) and 3 (female rat), hair samples were treated with 0.0%, 0.01%, and 0.1% Lux detergent, respectively, and subjected to the same washing and timing procedure as that which was finally adopted. In Experiment 2 the hair samples contained 149-154 µg Zn/g hair and in Experiment 3, they contained 316-320 µg Zn/g hair.

Since in each of these experiments, there was no significant variation in the microgram zinc/gram of hair determinations due to a difference in the detergent concentration, the procedure outlined at the beginning of this section, utilizing 0.01% Lux detergent, was adopted.

Analytical Procedure

After the individual samples were washed and dried, each was subdivided into two subsamples of about 50 mg., weighed to an accuracy of 0.01 mg.

Digestion of the hair was carried out in borosilicate micro-Kjeldahl flasks. Three glass beads were added to prevent bumping. After adding 0.8 ml. of concentrated sulfuric acid, the mixture was heated on a digestion rack under a hood for ten minutes and cooled. Three-tenths ml. of concentrated nitric acid was added and the heating continued for 5 minutes after clarification. Digestion was continued for 20 minutes following the addition of three drops of 70% perchloric acid. After cooling, the digests

were neutralized with NaOH (4N) and the volume uniformly brought to 15 ml. by the addition of demineralized water. Reagent blanks were also subjected to the above procedure.

Four ml. aliquots of the diluted digests, the diluted digested blanks, water, and the standard, respectively, were measured into test tubes. The standard contained 0.5 µg of zinc per ml. One ml. of borate buffer solution at pH of 9.0 was added to each solution.

The procedure for zinc analysis was adapted from that of Platte and Marcy (1959) and of Williams et al. (1962) in which cyanide complexes of the trace metals are formed and then zinc preferentially demasked by chloral hydrate. In the presence of zincon, the a blue zinc-zinconate complex is formed. A subsequent spectrophotometric reading within one minute at 630 mµ excludes interference by zincon complexes of other metals.

The three steps for zinc determination by this technique are:

(1) $Zn^{++} + 4\ CN^{-} \longrightarrow Zn(CN)_4^{--}$

(2) $Zn(CN)_4^{--} + 4\ CCl_3.CH(OH)_2 \longrightarrow$

(Zinc-cyanide complex) + (Chloral hydrate) $\longrightarrow$

$Zn^{++} + 4\ CCl_3.CH(OH)CN + 4\ OH^{-}$

(Zinc ion) + (3-trichloro-2 hydroxypropionitrile) + (hydroxyl ions)

(3) Zn^{++} + Zincon $\longrightarrow$ Zinc-zinconate complex

(2-carboxy-2'-hydroxy-5' sulfoformazylbenzene)

In the actual procedure used to determine the zinc content of the hair digests, 0.2 ml. of 0.75% sodium cyanide was added to each test tube and mixed with the solution, and 0.6 ml. of zincon solution was added and mixed. The zincon reagent contained 65 mg. in 50 ml. of water with 3 drops of NaOH (1N) to increase solubility.

The optical density scale of a Coleman Spectrophotometer was adjusted to zero reading at 630 mμ. One-tenths ml. of 60% chloral hydrate was added to the tube contents and mixed thoroughly. Optical density was determined within one minute. Since the absorbance of the zinc-zinconate complex follows Beer's Law (Rush and Yoe, 1954), calculations were made accordingly to determine the concentration of the unknown samples. The procedure for the calculations is shown in Appendix A.

All glassware was cleaned with detergent, treated with dilute nitric acid (1:1) and rinsed thoroughly with demineralized water.

Histological Procedures

Skin specimens were obtained from various parts of the body within one hour after the sacrifice of each rat. Samples were either fixed immediately in 10% neutral formalin or flash-frozen by immersion in liquid air. Tissues which were to be flash-frozen were wrapped in gauze, labeled, wrapped in aluminum foil, and externally labeled. After freezing, they were stored at a temperature below -15° C.

The tissues which were to be used for alkaline phosphatase localization were fixed in cold 10% formalin at 4° C. for 4 hours. The incubation mixture for the alkaline phosphatase method was prepared by dissolving 10 mg. of sodium alpha-naphthyl phosphate in 3 ml. of distilled water, adding 3 ml. of 4% borax solution, 40 ml. of distilled water, 5 drops of 10% magnesium sulfate solution, and 20 mg. of fast blue B. The substrate mixture was used immediately after preparation.

The procedure used was that adopted by the dermatology department of the American University of Beirut. Sections were cut on a freezing microtome at 50 microns. Slides were allowed to drain and dry for about 15 minutes. The tissues were then passed from absolute ethanol to distilled water and incubated for 20 minutes in the substrate mixture. After rinsing in distilled water, the tissues were dehydrated in two changes of acetone for 2 minutes each, and in an acetone-xylol mixture (1:1) for 3 minutes. After clearing in xylol, they were mounted in permount.

Tissues to be stained by hematoxylin and eosin were taken from the mid-ventral region, fixed in 10% formalin, and embedded in paraffin. The procedure used was that adopted by the pathology department of the American University Hospital. After deparrafinizing in two changes of xylol for 3 minutes each, the tissue slices were hydrated gradually from absolute to 70% ethanol, and washed for several minutes in running tap water. After staining in Harris' Hematoxylin for 10 minutes, the tissues were dipped

several times in 1% acid alcohol for differentiation. They were then washed for about 10 minutes in running tap water and immersed for 1 minute in 0.25% aqueous eosin. The tissues were washed quickly in tap water, dehydrated in 95% alcohol and absolute alcohol, and cleared in xylol. The stained specimens were mounted on Harleco's synthetic resin[1].

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1 Manufactured by Harleco Company, Woodlant Avenue, Philadelphia, Pennsylvania.

EXPERIMENTAL RESULTS

Macroscopic Results

Hair Regrowth. The regrowth of hair on the shorn ventral and lateral areas of the control animals receiving adequate protein and zinc was excellent. Hair was frequently observed to emerge as soon as two days after shearing. At 40 days after the initial shearing, complete coverage, comparable to the unshorn dorsal area, occurred in all of the rats receiving the adequate diet.

In the six rats receiving adequate protein coupled with a low zinc intake, the coverage of the shorn area was incomplete and usually occurred on a total of about half of the ventral and lateral area. Table 3 contains the observed relative hair regrowth in all of the animals receiving adequate protein. Observation B was made prior to the third shearing and the subsequent sacrifice of the rats, during a period from 1 to 7 weeks after the second shearing. Nevertheless, the control and experimental animals of each set were observed (and shorn) at the same time.

None of the twelve animals fed inadequate protein showed a complete coverage of the shorn area even after a renewal period of 7 weeks. However, the animals of the group which received adequate zinc usually possessed regrown hair on about a quarter of the shorn area. The animals on a low zinc diet, however, regrew only a few small patches of hair or showed no noteworthy regrowth

on the shorn area. Table 4 contains the observed relative hair regrowth for these animals.

In all of the animals showing poor regrowth, a phenomenon occurred in which the hair was renewed in waves or in patches. Often the hair would be renewed initially on either side of the mid-line of the venter and converge medially and also dorsally. At times, the hair pattern would seem to progress from the neck region down toward the mid-venter. Initial regrowth to the left and to the right of the abdomen was often observed. Animals on the low protein-low zinc diet sometimes formed small islands of growth. Figures 5-9 show the shorn area of five pairs of rats after about 5 weeks of regrowth.

Body Growth. In contrasting the gain in weight for a period of eight weeks of treatment, the rats receiving adequate zinc and protein gained more than three times as much weight (66.2 g)[1] as those receiving adequate zinc and low protein (21.5 g). The female rats receiving inadequate zinc and normal protein gained twenty-three times as much weight (52.6 g) as the females receiving inadequate zinc and protein (2.3 g). On the other hand, the male rats on the adequate protein-low zinc diet gained only 2.5 times (56 g) as much as the males on the low protein and zinc diet (22 g).

The data concerning the effect of variation of zinc intake on the general growth rate of the rats are listed in Tables 3 and 4. Also, Figures 1 and 2 show the weekly weight gain for the

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[1] Means of weight gain.

different sexes subjected to the treatments and Figure 3 shows the relative rates of growth between the sexes.

Analytical Results

Tables 3 and 4 show the microgram content of zinc in the hair of the rats subjected to the four treatments and also the relative increase or decrease in zinc content of the regrown hair as compared to the zinc content of the hair obtained from the original shearing. Figure 4 demonstrates the influence of the variation of zinc and protein in the diets on the zinc content in the hair obtained from each of the three shearings.

Analysis of variance was run on the data for the first and second shearings, to determine whether there existed any significant difference in the zinc content of the hair due to the four treatments. The data for the first shearing showed the zinc contents of the hair not to be significantly different at this point. However, the data for the second shearing is significantly different due to the four different dietary treatments. Analysis of variance on the change in zinc content of the hair, when the result from shearing 1 was subtracted from the result from shearing 2, was significantly different. Table 5 provides the analysis of variance of the four diets.

Analysis of variance for a two-way layout with six observations per cell was run on the data to determine any significant difference in the zinc content of the hair due to the zinc and

protein components of the diets, respectively. There was no significant difference due to either zinc or protein in the zinc content of the hair from the first shearing. However, the data for the second shearing was significantly different due to the different levels of protein and significantly different due to the different levels of zinc. Analysis of variance on the difference in zinc content of hair between the first two shearings proved significant for the zinc and for the protein. In addition, analysis showed that the zinc content of the hair was not determined by an interaction between the zinc and protein components of the diet. Table 6 contains the analysis of variance of the zinc and protein contents of the diets.

Statistical comparisons using Student's t test were made on the change in zinc content of the hair between the initial and the second shearings for each of the four diets. Also, comparisons between the initial and the third shearings data were made for the two adequate protein diets. The results of these analyses are listed in Table 7.

Histological Results

An interpretation of the results of the alkaline phosphatase slides is not included in this report for the following reasons:

(1) Adequate control slides were not made for the alkaline phosphatase slides from each of the rats.

(2) The wave-like phenomenon of hair renewal may have caused a gradient of enzyme localization in the different areas of the body (Chase, 1954).

(3) The reliability of the procedure for demonstrating sites of low enzyme activity is doubtful (Casselman, 1959).

Nevertheless, since the tissue used for preparing the alkaline phosphatase slides was from the same region as that used for the hematoxylin and eosin slides, the former were sometimes used to aid in the interpretation of the morphological appearance of the skin and its appendages.

The histological appearance of the skin and appendages of the animals on the adequate protein and zinc diet and low protein adequate zinc diet was generally good. The relative number of hair follicles under the high dry power of the microscope was 5-8. The follicles were located throughout the dermis. No hyperkeratosis was evident. The epidermis was 2-4 layers thick. One specimen from each group had most of the follicles in the upper dermis.

The appearance of the skin of two animals on the adequate protein-low zinc diet was generally good. Two specimens showed increased skin keratinization and three specimens had low density of the follicles.

Some unusual observations in the skin and appendages were observed in the specimens obtained from the animals on a low protein and zinc diet. In two animals, the hair shafts were absent or very small despite the mature and healthy appearance of the follicles.

Low density and atrophic follicles were found in two rats. Two specimens seemed comparable to the controls. Other abnormalities found in individual specimens of this group were increased skin keratinization, epidermal thickening, augmented stratum corneum, and an increase in the number of fibroblasts and inflammatory cells.

The results of the histological studies are summarized in Table 8. Figures 10-17 are microphotographs of some of the specimens.

DISCUSSION AND CONCLUSIONS

Effect of Zinc and Protein Deficiencies on Body Growth

The substantially slower rate of growth found in the animals on the low protein diet was anticipated, since not only were the rats actively growing, but also the "protein minimum"[1] for this species was not provided (Porter, 1963; Forbes and Yohe, 1960).

A retardation of body growth due to zinc deficiency was also anticipated from the results of earlier studies in the rat, in general, (Day and McCollum, 1940; Hove et al., 1938; Todd et al., 1934) and in male rats of the Sprague-Dawley strain, in particular, (Forbes and Yohe, 1960).

However, the exceptional retardation in the rate of growth found in the females on a low zinc and protein diet, as compared to the males on the same diet and to the controls of this group, was unexpected. Although the zinc deficiency syndrome in humans has only been noted in males, any sex influence on growth in animals of other species has not been reported (Day and Skidmore, 1947; O'Dell et al., 1958; Legg and Sears, 1960; Vallee, 1959). Nevertheless, consideration of this observation must be made, since the experimental animals in which it was noted were unique in that they suffered from both protein and zinc deficiency.

In the male dwarfs observed by Prasad and co-workers,

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[1] The minimal quantity of dietary nitrogen required for the maintenance of nitrogen equilibrium in a normal adult animal (Fruton, 1958, p. 724).

the growth retardation, hypogonadism and partial adrenal hypofunction was hypothesized to be due to malfunctioning of the anterior pituitary conditioned by zinc deficiency (Prasad et al., 1963a). In 1941, the hypopheses of zinc deficient rats was found to be smaller than the glands in the controls (Follis et al., 1941). However, no difference in the body growth rate between the sexes was recognized.

The high concentration of zinc in the epididymis, seminal vesicles, and prostate gland has been theorized to increase the requirement for zinc in the males (Prasad et al., 1963b). Although zinc has been found in high concentration in starfish oocytes (Fujii, 1954), no exceptional concentration of the metal has been determined in female organs of rat or man (Underwood, 1962). Therefore, the cause of this retardation in rate of growth in females on a low zinc and protein diet awaits further investigation.

Comparison of the body weight at the end of eight weeks on the diet showed a gap between the males on the adequate protein and zinc diet, as compared to the males on the adequate protein-low zinc diet (Figure 2). However, a histograph comparing the rates of growth between the sexes (Figure 3), showed the rate of growth of the males to be faster than the females on the respective diets. Also, the difference in the rates of growth between the males and females receiving adequate zinc was approximately the same as the difference between the males and females receiving low zinc.

Hooded Lister rats[1] were found to have an average weaning

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[1] Rowett Research Institute Strain, Aberdeen.

weight of 51.0 for the males and 47.6 for the females (Porter, 1957). The hooded rats are the same species as the Sprague-Dawley strain. Therefore, the difference in weight between the sexes may be a normal occurrence. However, before a conclusion may be reached concerning this matter, further investigation with less variables is necessary.

Effect of Zinc and Protein Deficiencies on the Wave Phenomenon of Hair Growth

The synchronous behavior of hair follicles, resulting in the wave phenomenon of hair growth, is a normal occurrence in rats (Emmel and Cowdry, 1964; Chase, 1954). A wave has been defined as "an orderly progression in time and space of follicles entering the growth phase, that is anagen[1] of their cycles" (Chase and Eaton, 1959, p. 365).

The ability to observe the wave phenomenon on the shorn areas of deficient rats for a prolonged period of time is indicative that, under conditions of zinc and protein deficiencies, a delay of growth occurred.

In sheep, the rate of wool growth has been found to be influenced by the amount of protein in the diet. However, under conditions of constant protein consumption, zinc deficiency did not affect wool growth (Ryder, 1958).

Delayed regrowth of hair in the rats on a low protein-

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1 "Anagen" is the stage of a growing hair follicle. Quiescent hair follicles are said to be in "telogen". The period of transition between the two is called "catagen" (Montagna and Van Scott, 1958).

adequate zinc diet indicates that hair formation is influenced by the amount of protein in the diet. Also, delayed hair formation in the rats on the adequate protein-low zinc diet and on the low protein and zinc diet, relative to the amount of regrowth hair found in the rats on the respective control diets, indicates that, under conditions of constant protein consumption, zinc deficiency did affect hair regrowth on the shorn areas.

A possible explanation for this retardation in hair formation, or prolongation of the span of time between waves, is that dietary deficiency results in (1) an increase in an inhibitor substance which is assumed to cause the normal wave phenomenon (Chase and Eaton, 1959), or (2) a decrease in a substance which leeches the inhibitor.

Effects of Zinc and Protein Deficiencies on Hair Formation

Interference in normal hair formation in the protein deficient animals may be explained simply by the fact that all of the essential amino acids needed for the formation of the hair keratin itself and/or the protein enzymes needed to elaborate the keratin directly or indirectly were not available to the organism. Also, such a deficiency could cause an upset of hormone balance which would affect the integument, as well as other systems and tissues.

Although aberrations in the skin of animals on a diet extremely deficient in zinc were quite marked (Follis et al., 1941), organisms on an adequate protein diet, only moderately deficient in zinc, were not as affected histomorphologically.

However, the variety of abnormalities found in the specimens from organisms which were on the low protein and zinc diet is worthy of note. One symptom is especially significant, i.e., the absence of hair shafts in fully developed and apparently morphologically healthy follicles. This symptom indicates that the abnormal hair formation was due to an interference in the hair keratinization process itself.

What phase or phases of the keratinization process were impeded is not known. As a matter of fact, how the hair follicle controls normal growth and differentiation of the hair is largely unknown (Montagna and Van Scott, 1958).

Nevertheless, the author would like to suggest two possible mechanisms concerning how zinc deficiency interferes with normal hair keratinization:

(1) Inhibition of RNA synthesis.

(2) Inhibition of alkaline phosphatase synthesis.

The lines of evidence, taken from the literature, which form the basis for these hypotheses are as follows:

Nucleic Acid Inhibition. Desoxyribose nucleoprotein from calf thymus and mouse sarcoma contain a high concentration of zinc (Heath, 1949). In 1954, Fujii reported the localization of zinc in chromosomes and in the nucleoli of starfish oocytes. Evidence for the possible roles of zinc as a structural agent in chromosomes and in the process of mitosis was introduced (Fujii, 1954).

Zinc deficiency experiments with Mycobacterium smegmatis showed a decrease in concentration of RNA, due to a halt in RNA synthesis (Winder and Denneny, 1959). Usually when growth ceases, DNA concentration increases. However, during zinc deficiency, the decreased rate of growth has been found to be accompanied by a decrease in the conentration of DNA (Underwood, 1962; Winder and Denneny, 1959).

In zinc deficiency experiments in the mouse, the lower catalase activity of the liver and kidney was not restored to normal by adding zinc salts to the tissue preparations (Day, 1942). This may indicate the involvement of zinc in the synthesis of the enzyme itself.

In Euglena gracilis, certain metals, including zinc, were found to link nucleic acid to the protein moiety of ribonucleoprotein. "Since ribonucleic acids are known to be nonhomogeneous, further purification may result in the isolation of ribonucleic acids which contain only one specific metal". (Wacker and Vallee, 1959, p. 3260).

In the formation of hard keratin of the hair and amorphous keratin of the hair cuticle, submicroscopic ribonucleoprotein particles (RNP) are closely involved (Rhodin and Reith, 1962). RNA decreases in the bulb of hair follicles at the same rate as keratin fibrils increase in these cells (Braun-Falco, 1958). Uric acid, a water-soluble constituent of the hair, is probably derived from the purines of degenerated nuclei and cytoplasm of

keratinized cells (Bolliger, 1951).

In 1959, Winder and Denneny suggested that the primary effect of zinc deficiency on the nucleic acids was on RNA synthesis, which caused a subsequent inhibition of protein and DNA synthesis (Winder and Denneny, 1959). In Rhizopus nigricans, zinc has been found to increase growth and substrate utilization (Wegener and Romano, 1963). In 1963, Wegener and Romano postulated that the primary effect of the stimulation was on RNA synthesis.

Thus, interference in keratin synthesis may be caused by a direct inhibition of RNA or RNP synthesis resulting in the abnormal hair formation found under conditions of zinc deficiency.

This theory is supported by the observation of the especially retarded regrowth of hair and also especially abnormal hair formation (absent hair shafts) in the rats on the zinc protein deficient diet.

Alkaline Phosphatase[1] Inhibition. About 0.15% firmly bound zinc was reported in preparations of kidney alkaline phosphatase (Stadtman, 1961). Also, alkaline phosphatase of E coli has been found to contain a stoichiometric quantity of zinc, essential to the enzyme's activity (Harrison, 1962). In 1955, Hofstee proposed that active alkaline phosphatase I consisted of an enzyme-zinc complex associated with a nondialyzable coenzyme (Hofstee, 1955). The precise role of the zinc ion is unknown (Stadtman, 1961).

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[1] Orthophosphoric monoester phosphohydrolase (Report of the Commission on Enzymes of the International Union of Biochemistry, 1961).

In zinc deficient experimental animals, failure of synthesis of the holoenzyme results in decreased alkaline phosphatase activity (Harrison, 1962). This change in alkaline phosphatase level may be influenced by a change in growth hormone level (Prasad, 1963).

Alkaline phosphatase is especially abundant in areas of active ossification (Karlson, 1963). In zinc deficiency experiments with the growing chick and the chick embryo interference in bone formation was marked (O'Dell et al., 1958; Kienholz et al., 1961). This symptom may further involve zinc in phosphatase activity.

The differentiated hair papilla contains a high concentration of alkaline phosphatase which permits the papilla to carry out the function of supplying the materials for keratinization of the hair (Balinsky, 1960).

In addition, alkaline phosphatase has been histochemically determined in the outer root sheath and in the lower part of the connective tissue sheath of the follicle (Braun-Falco, 1958). Zinc has also been localized in the outer root sheath of hair follicles (Braun-Falco, 1958).

Strong implications that an alteration of alkaline phosphatase activity in the hair follicle may have caused the aberrations in hair keratinization are: (1) The presence of zinc in the molecule of a alkaline phosphatase as an essential activator (Hofstee, 1955); (2) the effect of zinc deficiency on the synthesis

of the enzyme itself (Harrison, 1962); (3) the role of alkaline phosphatase in the formation of keratin (Balinsky, 1960); and (4) the common localization of alkaline phosphatase and zinc in the outer root sheath (Braun-Falco, 1958).

Effect of Zinc and Protein Deficiencies on the Zinc Content of the Hair

Statistical analysis of the data has shown that the zinc content of the hair was affected by the zinc content of the diet, as well as by the protein content of the diet, as independent factors. In otherwords, low zinc content of the hair of the rat may indicate a state of protein deficiency or it may indicate a state of zinc deficiency.

This finding implies that the use of the zinc content of the hair as an indication of zinc deficiency (Prasad et al., 1963a) or as an indication of low zinc intake (Reinhold et al., 1965) may not be entirely justified. In Egypt, the villagers were known to have a low intake of protein (Prasad et al., 1963a). The zinc content of the hair was compared to controls in the United States, who probably had a protein intake with higher biological value. The zinc content of the hair of villagers in Iran, who were reported to have a low protein-high carbohydrate diet (Reinhold et al., 1965) were compared to that of the city dwellers who were known to have a more substantial diet.

Although the low zinc content of the hair in both Egypt and Iran may be the result of zinc deficiency and/or low zinc

intake, the use of the zinc content of the hair as an index of these conditions should possibly be used along with other clinical symptoms before the diagnoses are completed. Also, full consideration should be given to the influence of a low level of protein in the diet on the manifestations of zinc deficiency.

SUMMARY

1. A study was made to determine the effect of a variation in the protein and zinc components of a diet on the hair formation, hair zinc content, and body growth of albino rats. The rats were subjected to four dietary treatments: Adequate protein and zinc, adequate protein-low zinc, low protein-adequate zinc, and low protein and zinc. The adequate protein diets contained 15 percent protein and the inadequate protein diets contained 7.5 percent protein in the form of casein and gelatin. The adequate zinc diets contained 30 p.p.m. zinc and the inadequate zinc diets contained 1.42-2.92 p.p.m. zinc. The rats were shorn at regular intervals. A special hair-washing procedure was devised. A modified zincon method for the micro-determination of zinc in digested hair samples was used. Macroscopic observations of the relative amount of hair regrowth on the shorn areas were made. Specimens of the skin from the mid-ventral region were stained with hematoxylin and eosin and diagnosed for any histopathological changes.

2. Statistical analysis showed that the zinc content of the hair of albino rats (1) was conditioned by the zinc content of the diet, (2) was conditioned by the protein content of the diet, and (3) was not conditioned by a synergistic interaction between the zinc and protein components of the diets.

3. The zinc content of the hair was found not to be proportional to the body weight. A definite sex influence on the

rate of growth was seen in animals on a low zinc and protein diet. For the initial eight weeks of treatment, females gained 0.29 grams per week, compared to the males which gained 2.75 grams per week. The average rate of growth for the rats on a low protein-adequate zinc diet was 2.7 grams per week.

4. Macroscopic observations showed that zinc deficiency and protein deficiency, respectively, caused a delay in the regrowth of hair. When both zinc and protein were decreased in the diet, regrowth of hair was retarded to a greater extent than that observed in either the zinc or protein deficient animals.

5. Microscopic observations showed that zinc deficiency may possibly cause abnormalities in the integument, such as increased skin keratinization and low follicle density. Keratinization of the hair shafts was found to be absent or retarded in two animals on a low protein and zinc diet. Other abnormalities, found in specimens taken from animals on a low zinc and protein diet, were augmented stratum corneum, an increased number of fibroblasts and inflammatory cells, and follicle atrophy.

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TABLES

Table 1. Grouping of rats according to initial weight, sex, and treatment. The rat code numbers are in parentheses.

Set Number	Normal Protein Normal Zinc	Normal Protein Low Zinc	Low Protein Normal Zinc	Low Protein Low Zinc
		FEMALES		
I	(1)	(2)	(3)	(4)
	44 g	52 g	54 g	58 g
III	(9)	(10)	(11)	(12)
	62 g	65 g	65 g	62 g
V	*	(18)	(19)	(20)
		68 g	63 g	68 g
		MALES		
II	(5)	(6)	(7)	(8)
	52 g	34 g	54 g	53 g
IV	(13)	(14)	(15)	(16)
	63 g	62 g	53 g	59 g
VI	(21)	(22)	(23)	(24)
	63 g	70 g	68 g	67 g

* Rat unavailable at beginning of experiment.

Table 2. Basal Diet Composition

Ingredient	Normal Protein	Low Protein
	Grams	Grams
Casein (zinc-free)	450	225
Gelatin	225	112
Dextrin	2700	2700
Corn oil	420	420
Mineral mixture*	180	180
Vitamin-glucose mixture**	225	225
Sucrose	280	618
Total Weight	4480	4480

* Composition of mineral-mixture in parts: $CaHPO_4$, 708; NaCl, 101; K_2CO_3, 136.3; $MgCO_3$, 37.4; $FeSO_4.7H_2O$, 10.8; $MnSO_4.H_2O$, 3.266; $CoCl_2.6H_2O$, 1.080; $CuSO_4.5H_2O$, 1.633; NaF, 0.216; KI, 0.108.

** Composition of vitamin-glucose mixture, mg/kg of mix: Thiamine-HCl, 200; riboflavin, 120; pyridoxine. HCl, 80; Ca pantothenate, 320; biotin, 4; nicotinic acid, 500; folic acid, 10; choline tartrate, 30,000; menadione, 6.6. Additional vitamin supplement consisted of 750 I.U. vitamin D, 6000 I.U. vitamin A, and 0.5 g α-tocopherol of vitamin E.

Table 3. Influence of dietary zinc levels on body weight, concentration of zinc in hair, and hair regrowth in rats receiving adequate protein.

Rat No. and Sex	Weight Gain* (g/week)	Zinc Content of Hair (µg/g hair)			Difference in Zinc Content between shearings		Relative Hair Regrowth**	
		Shearing Number			2-1	3-1	Observation	
		1	2	3			A	B
Normal Zinc								
1 ♀	8.12	169	194		+25		5	5
9 ♀	7.40	193	207	206	+14	+13	5	5
5 ♂	9.5	182	205	185	+23	+3	5	5
13 ♂	7.62	181	189	171	+8	-10	5	5
21 ♂	8.75	205	211	184	+6	-21	5	5
Means	8.27	186 (±13.6)	201 (±13.6)	186.5 (±14.4)	+15	-4	5	5
Low Zinc								
2 ♀	7.5	174	182		+8		3	3
10 ♀	5.5	207	194	196	-13	-13	2	3
18 ♀	6.75	186	193	135	+7	-51	4	4
6 ♂	7.25	196	141	141	-55	-55	4	4
14 ♂	8.25	161	141	108	-20	-53	3	4
22 ♂	5.5	198	194	144	-4	-54	3	3
Means	6.79	187 (±17.0)	174 (±27.4)	145 (±30.8)	-13	-45	3.2	3.4

* Rate for initial eight weeks of treatment.

** Interpretation in materials and methods section.

Table 4. Influence of dietary zinc levels on body weight, concentration of zinc in hair, and hair regrowth in rats receiving inadequate protein.

Rat No. and sex	Weight Gain* (g/week)	Zinc Content of Hair (μg/g hair)			Difference in Zinc Content between shearings		Relative Hair Regrowth**	
		Shearing Number 1	2	3	2-1	3-1	Observation A	B
Normal Zinc								
3 ♀	2.0	188	197		+9		3	3
11 ♀	2.8	189	194	197	+5	+8	2	2
19 ♀	2.9	200	186	181	-14	-19	1	3
7 ♂	3.0	188	193		+5		4	2
15 ♂	3.1	176	178		+2		2	2.5
23 ♂	2.5	171	177	201	+5	+30	2	2.5
Means	2.7	185 (±15.9)	187.5 (±8.5)	193	+2	+6	2.3	2.5
Low Zinc								
4 ♀	0.38	183	164		-19		1	1
12 ♀	0.25	193	150		-43		1	0
20 ♀	0.25	209	148	172	-61	-37	1	1.5
8 ♂	3.4	204	169		-35		2	0
16 ♂	2.6	182	159		-23		1	0
24 ♂	2.25	194	147	147	-47	-47	1	1
Means	1.52	194 (±14.0)	156 (±12.1)	159.5	-38	-42	1.16	0.58

* Rate for initial eight weeks of treatment.

** Interpretation in materials and methods section.

Table 5. Analysis of variance of the four diets

	d.f.	S.S.	M.S.	F*	Significance P≤
Zinc Content of the Hair (µg Zn/g hair)					
SHEARING 1					
Between classes	3	301	100		
Within classes	19	3314	174		
Total	22**	3615	274	0.575	N.S.
SHEARING 2					
Between classes	3	6636	2212		
Within classes	19	4536	239		
Total	22**	11172	2451	9.255	0.5%
Change in the Zinc Content of the Hair (µg Zn/g hair difference)					
SHEARING 2 minus SHEARING 1					
Between classes	3	9356	3119		
Within classes	19	4592	242		
Total	22**	13948	3361	12.889	0.5%

* Variance-ratio factor.

** A degree of freedom was lost due to the absence of one observation.

Table 6. Analysis of variance due to the zinc and protein content of the diets, respectively

Zinc Content of the Hair
(µg Zn/g hair)

Source	d.f.	S.S.	M.S.	F	Significance
SHEARING 1**					
Zinc	1	145	145	0.8333	N.S.
Protein	1	64	64	0.368	N.S.
Interaction	1	92	92		
Cells	3	301	100		
Error	19	3314	174		
Total	22*	3615			
SHEARING 2***					
Zinc	1	5104	5104	21.356	0.5%
Protein	1	1504	1504	6.293	2.5%
Interaction	1	28	28		
Cells	3	6636	2212		
Error	19	4536	239		
Total	22*	11172			

Change in Zinc Content of the Hair
(µg Zn/g hair difference)

Source	d.f.	S.S.	M.S.	F	Significance
SHEARING 2 minus SHEARING 1****					
Zinc	1	6936	6936	28.66	0.5%
Protein	1	2204	2204	9.107	1.0%
Interaction	1	216	216		
Cells	3	9356	3119		
Error	19	4592	242		
Total	22*	13948			

Table 6. Continued

* A degree of freedom was lost due to the absence of one observation.

** Contingency table for the analysis of variance for the zinc content of the hair from shearing one.

	P	p	
	169	188	
	182	188	
Z	193	189	
	181	176	
	(186)	200	
	205	171	
Total	1116	1112	2228
	174	183	
	196	204	
z	207	193	
	161	182	
	186	209	
	198	194	
Total	1122	1165	2287
Sum total	2238	2277	4515

*** Contingency table for the analysis of variance for the zinc content of the hair from shearing two.

	P	p	
	194	197	
	205	193	
Z	207	194	
	189	178	
	211	186	
	(201)	177	
Total	1207	1125	2332
	182	164	
	141	169	
z	194	150	
	141	159	
	193	148	
	194	147	
Total	1045	937	1982
Sum total	2252	2062	4314

Table 6. Continued

**** Contingency table for the difference in zinc content of the hair between shearing 1 and shearing 2.

	P	p	
Z	25	9	
	23	5	
	14	5	
	8	2	
	(15)	-14	
	6	5	
Total	91	12	103
z	8	-19	
	-55	-35	
	-13	-43	
	-20	-23	
	7	-61	
	-4	-47	
Total	-77	-228	-305
Sum total	14	-216	-202

Table 7. Statistical Comparisons*

Change in Zinc Content of the Hair (μg Zn/g hair difference)	N**	t	P
SHEARING 2 minus SHEARING 1			
Adequate protein and zinc	5	1.975	10%
Adequate protein-low zinc	6	0.549	N.S.
Low protein-adequate zinc	6	0.245	N.S.
Low protein and zinc	6	2.424	5%
SHEARING 3 minus SHEARING 1			
Adequate protein and zinc	4	0.252	N.S.
Adequate protein-low zinc	5	2.503	5%

* Using Student's t Test

** Number of observations

Table 8. Histological appearance of the skin and appendages from the mid-ventral region.

DIETARY TREATMENTS	Adequate Protein Adequate Zinc					Adequate Protein Low Zinc						Low Protein Adequate Zinc						Low Protein Low Zinc					
RAT NUMBER	1	5	9	13	21	2	6	10	14	18	22	3	7	11	15	19	23	4	8	12	16	20	24
Generally good appearance*	+	+	+	+	+	+		+			+	+	+	+	+	+	+		+				+
Increased skin keratinization							+		+									+					
Most follicles in upper dermis		+										+						+		+			
Follicle appearance normal	+	+	+	+	+	+	+				+	+	+	+	+	+	+		+				+
Follicles abnormal																							
Low density								+	+	+								+		+			
Atrophied																		+		+			
Shafts absent																					+		
Shafts minute																					+	+	
Other symptoms				d				a	d									b d e f		c			

* Epidermis 2-4 layers thick. Stratum corneum almost as thick as epidermis. No hyperkeratosis. Relative number of hair follicles is 5-8 (h.p.). Follicles located throughout the dermis.

a. Skin normal on one side, abnormal on other.

b. Thickening of epidermis.

c. Increase in stratum corneum

d. Most follicles in catagen.

e. Increased fibroblasts and inflammatory cells.

f. Septa of hair shafts absent.

FIGURES

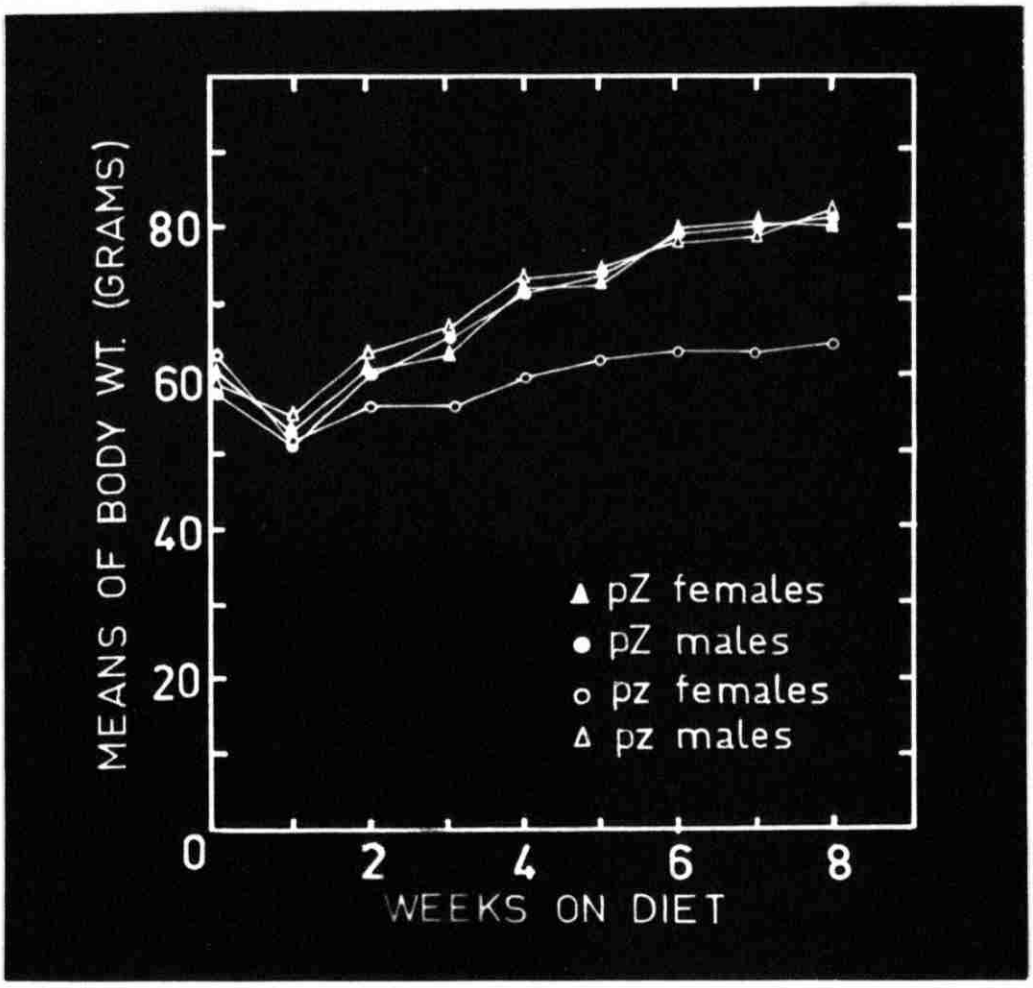


Figure 1. Sex influence on the body growth of rats fed inadequate protein with a variation in zinc content. Females fed a low zinc diet showed a retardation in growth relative to the males subjected to the same diet. The male experimentals were not exceptionally different from the controls.

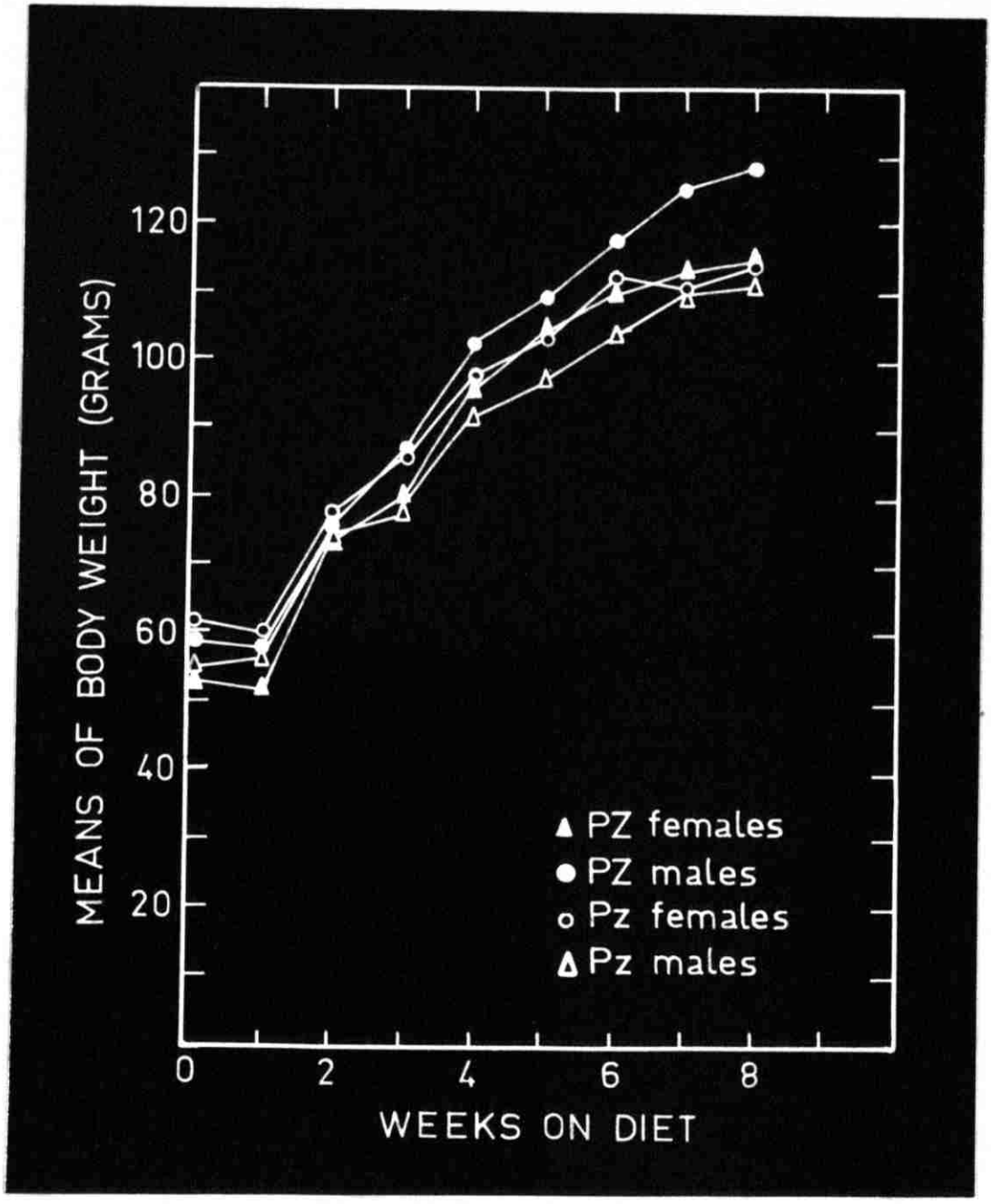


Figure 2. Sex influence on the body growth of rats fed adequate protein with a variation in zinc content. Males fed inadequate zinc showed an apparent retardation in growth relative to the males fed adequate zinc. The female experimentals were not exceptionally different from the controls.

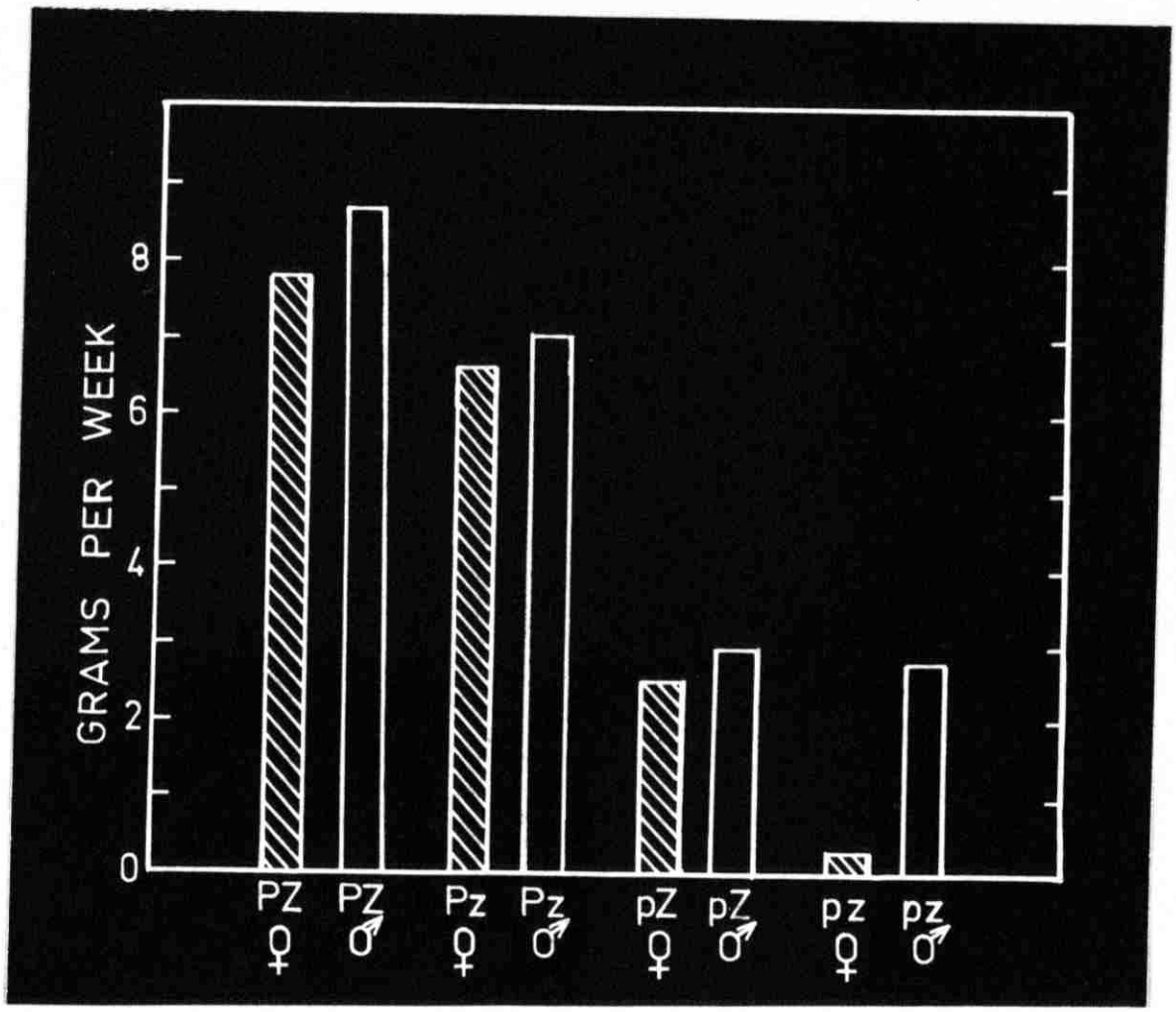


Figure 3. Average growth rates for both sexes for the initial eight weeks of treatment. Obvious decrease in growth rate due to low protein intake. Gross decrease in growth rate of female rats fed low protein and zinc is evident. Females on adequate protein diet showed a slower growth rate relative to the males, irregardless of the zinc content of the diet. Rats on adequate protein-low zinc grew slower than the controls.

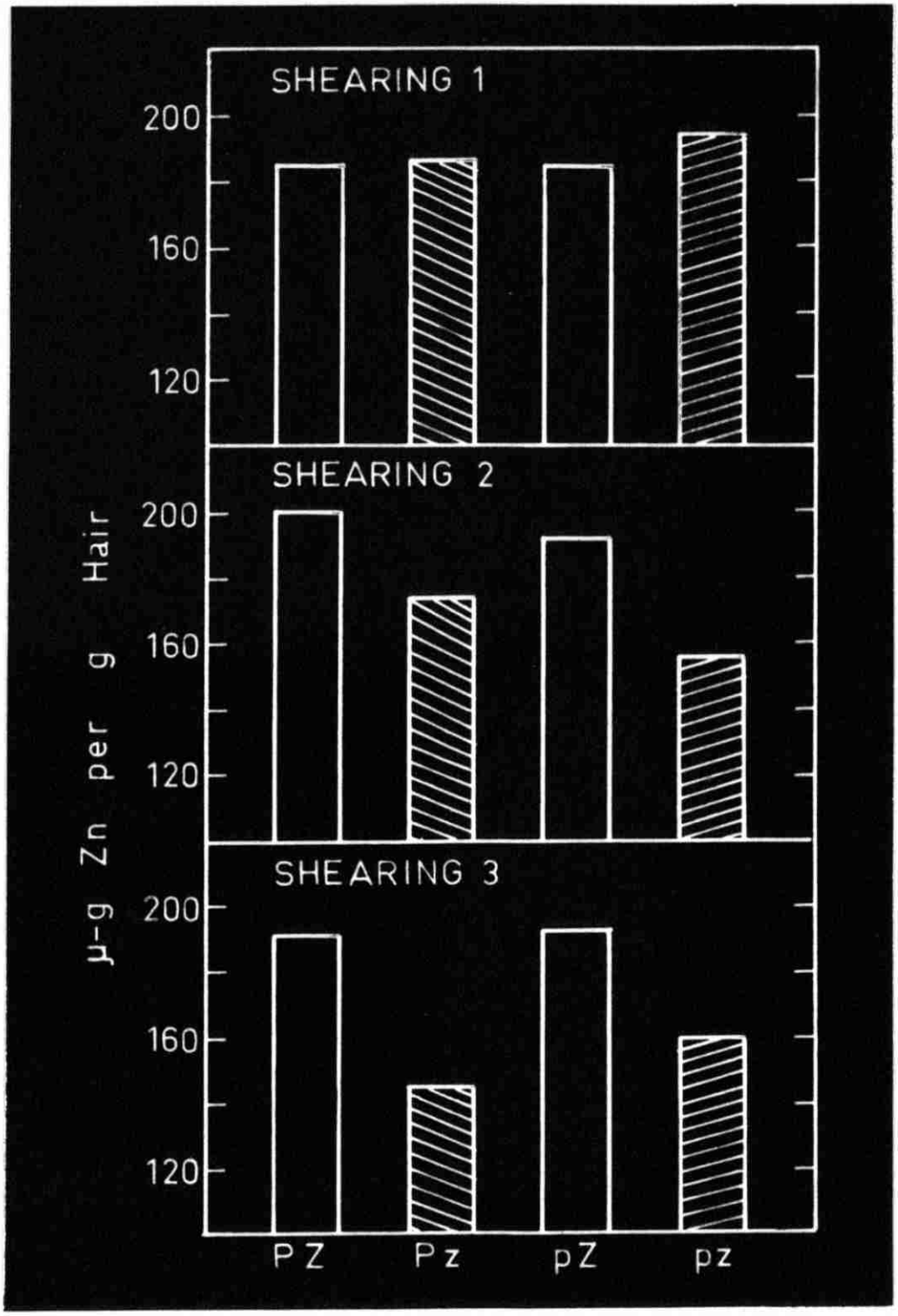


Figure 4. Influence of a variation of zinc and protein in the diets on the zinc content of the hair. Shearing 1 was made after two weeks prefing. Shearing 2 was made after a hair regrowth period of about six weeks. Shearing 3 was made after a second regrowth period varying from three to seven weeks. The zinc contents for each shearing are the means.

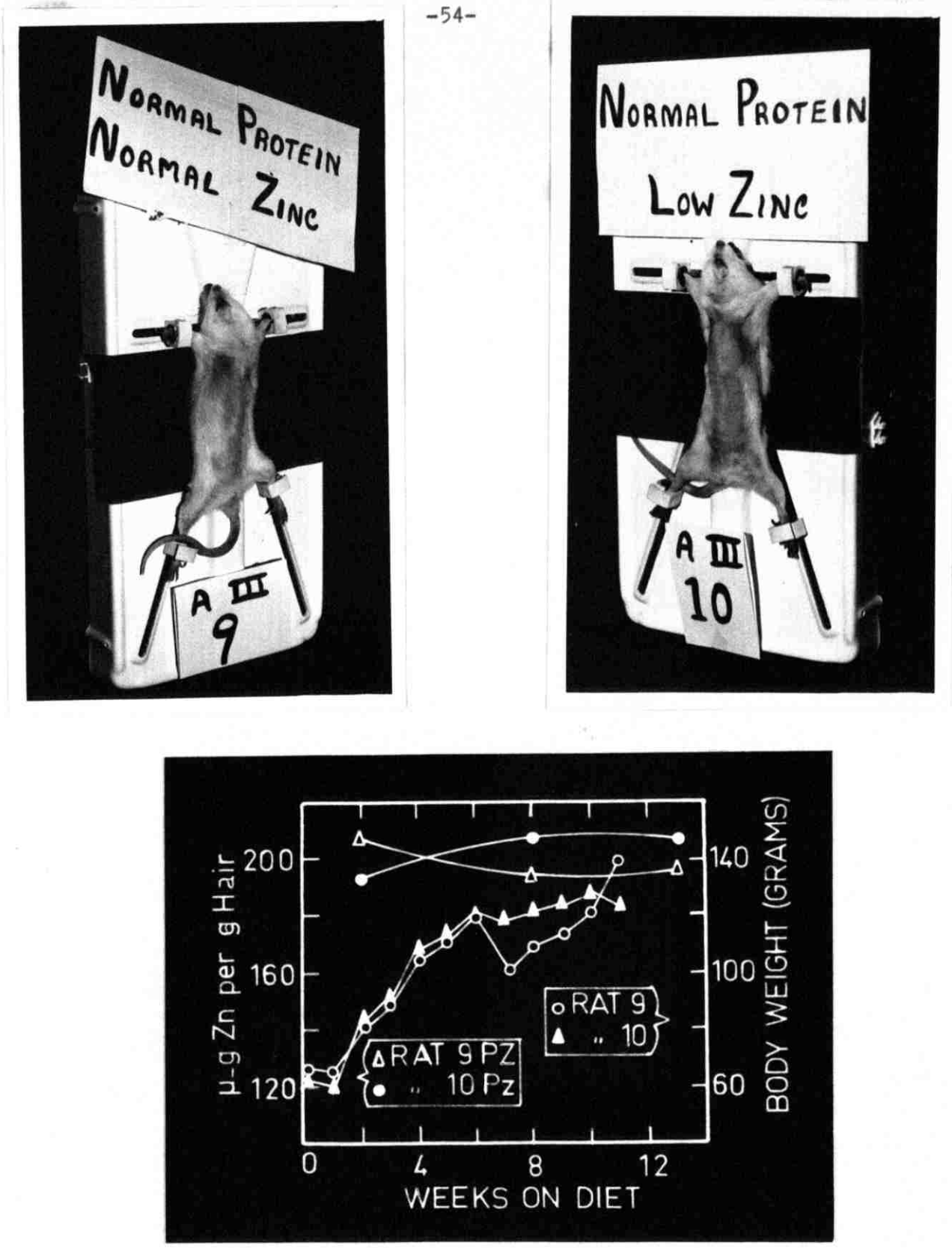


Figure 5. Female rats No. 9 (PZ) and No. 10 (Pz) showing hair regrowth 4.5 weeks after shearing and after 12 weeks on diet.

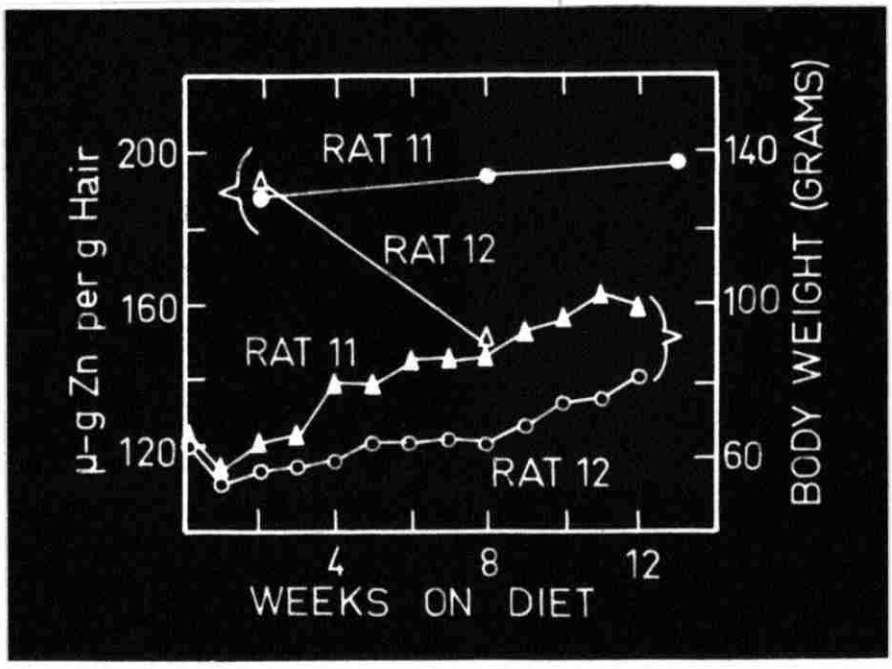
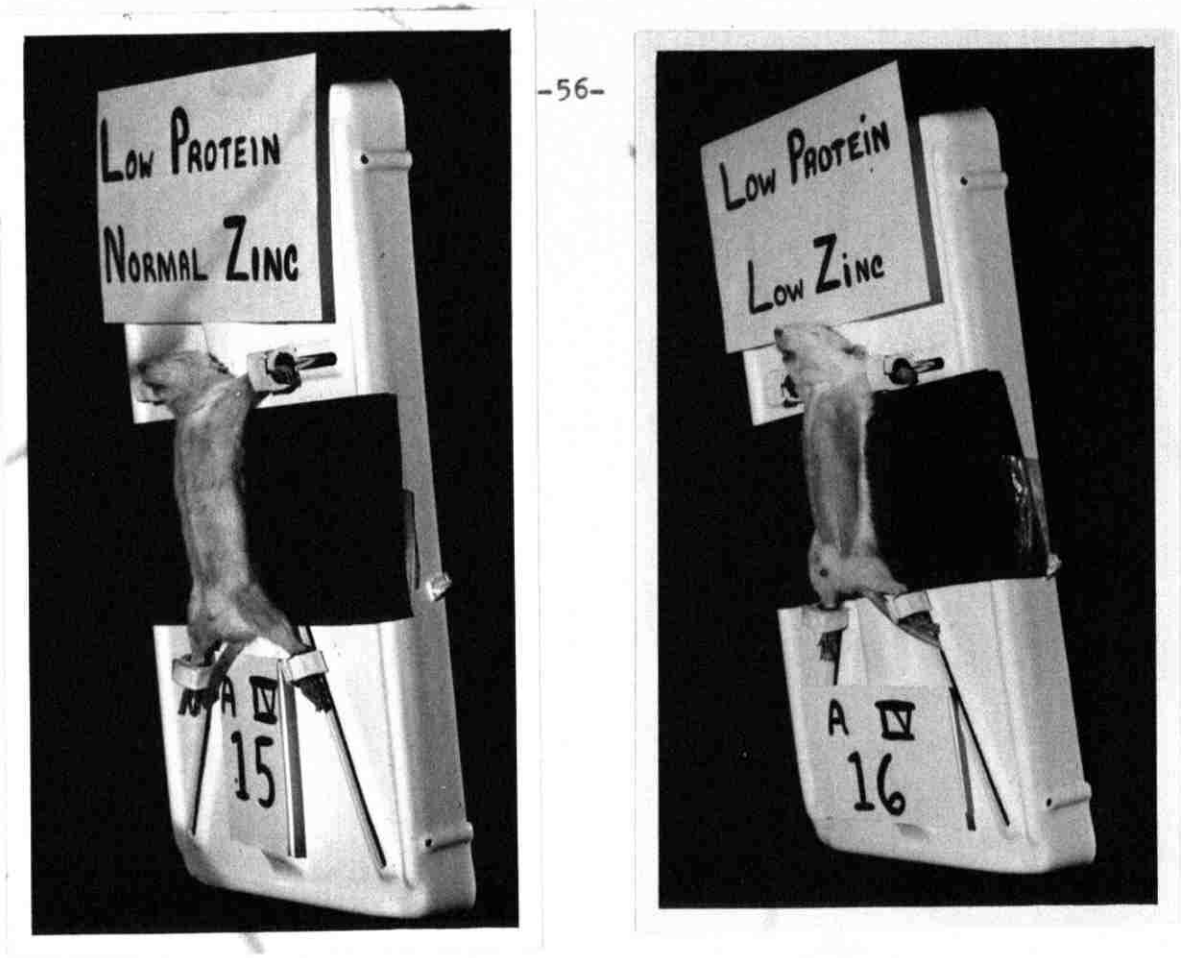


Figure 6. Female rats No. 11 (pZ) and No. 12 (pz) showing hair regrowth 5.5 weeks after shearing and after 13 weeks treatment.



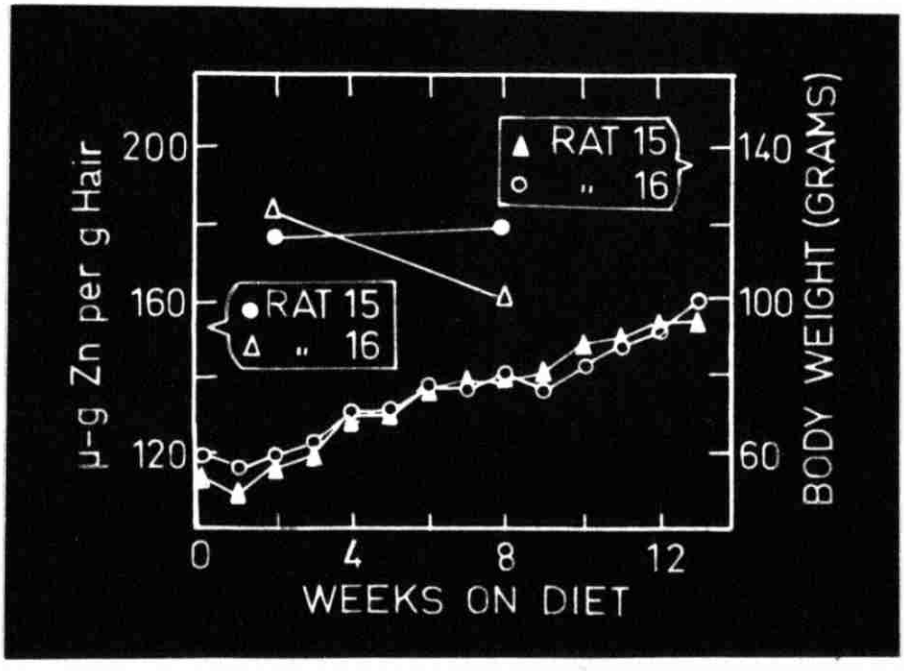
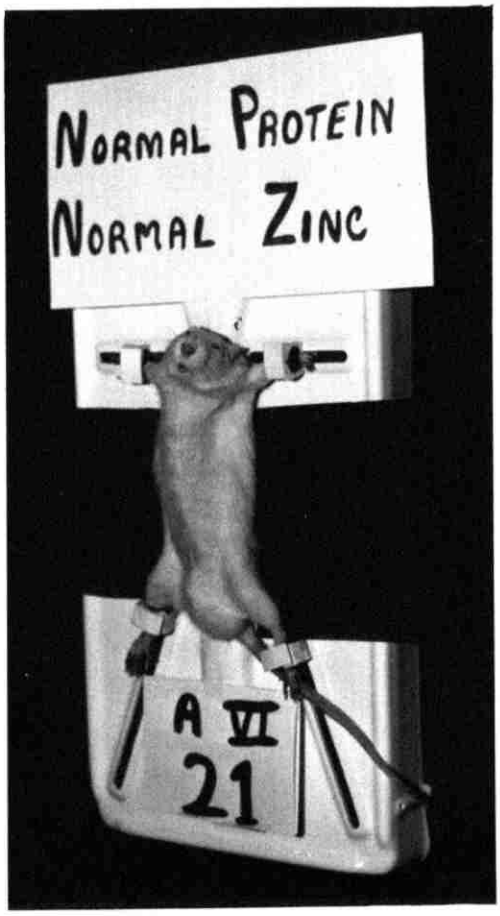
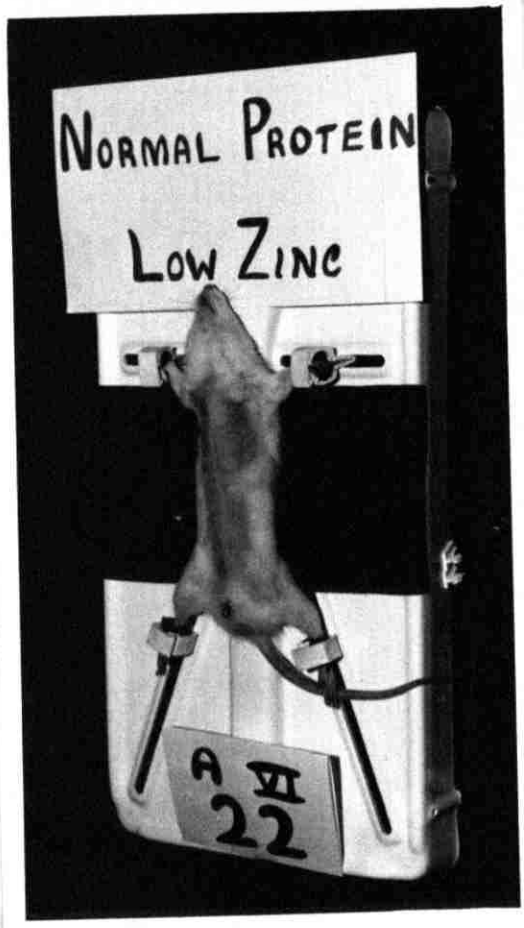


Figure 7. Male rats No. 15 (pZ) and No. 16 (pz) showing hair regrowth 5.5 weeks after shearing and 13 weeks treatment.





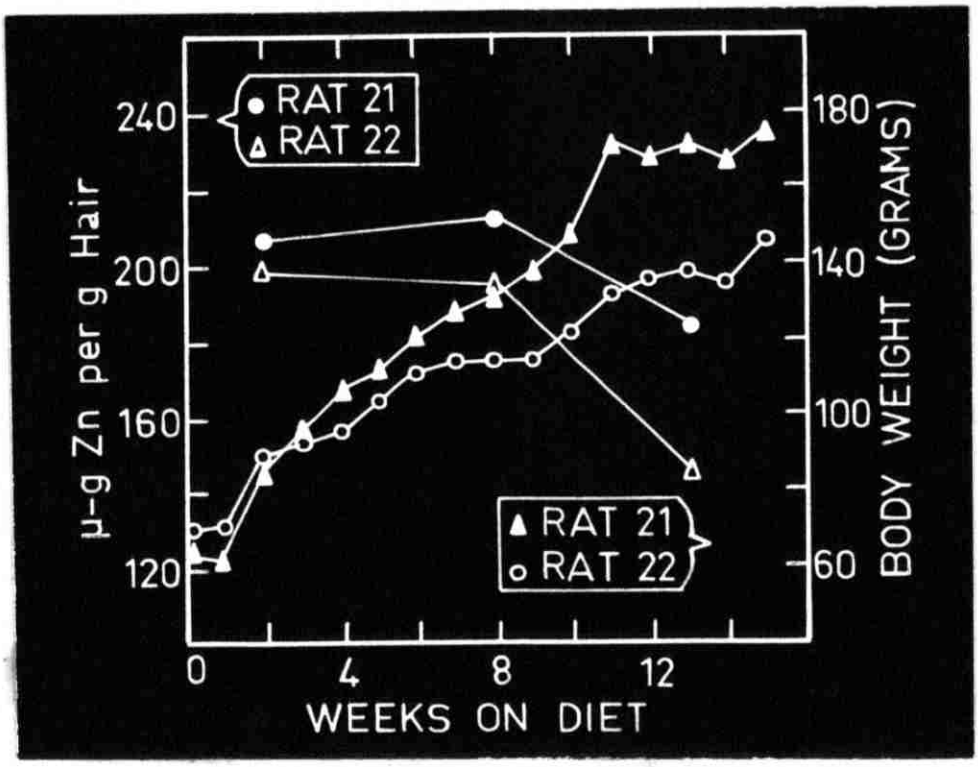
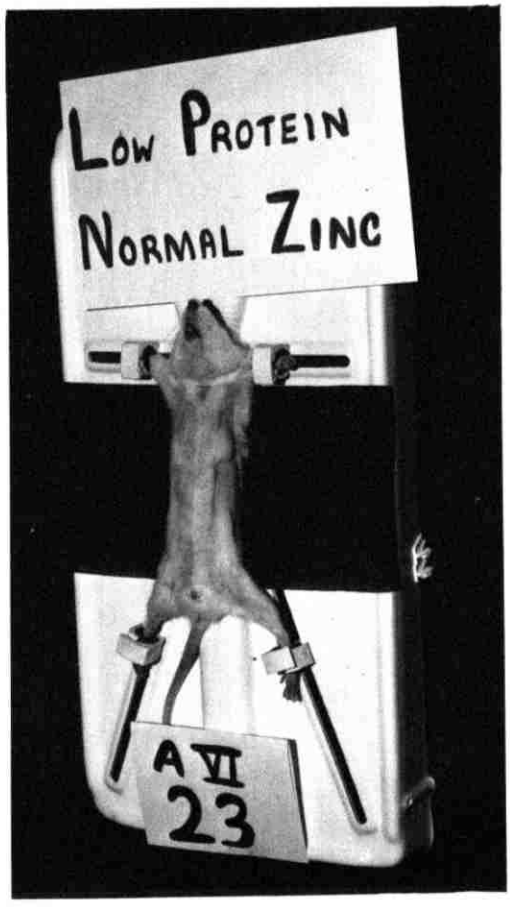
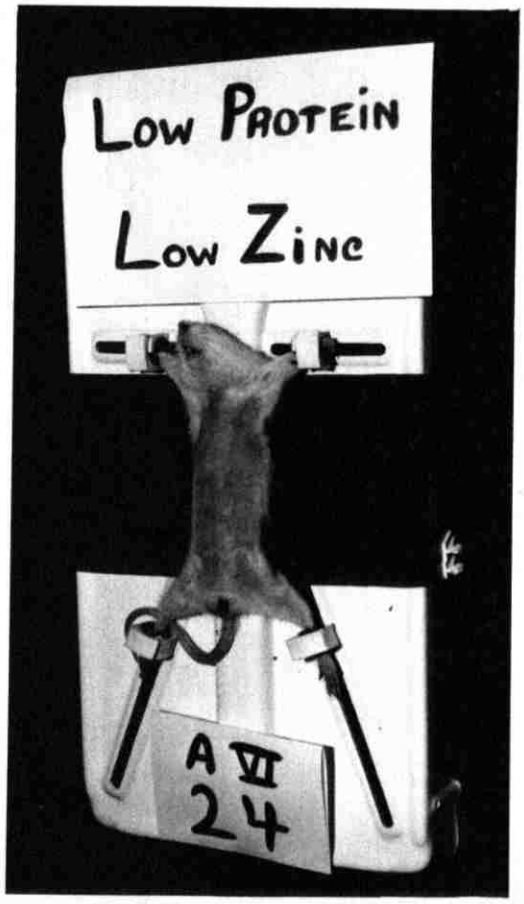


Figure 8. Male rats No. 21 (PZ) and No. 22 (Pz) showing hair regrowth 4.5 weeks after shearing and after 12 weeks treatment.





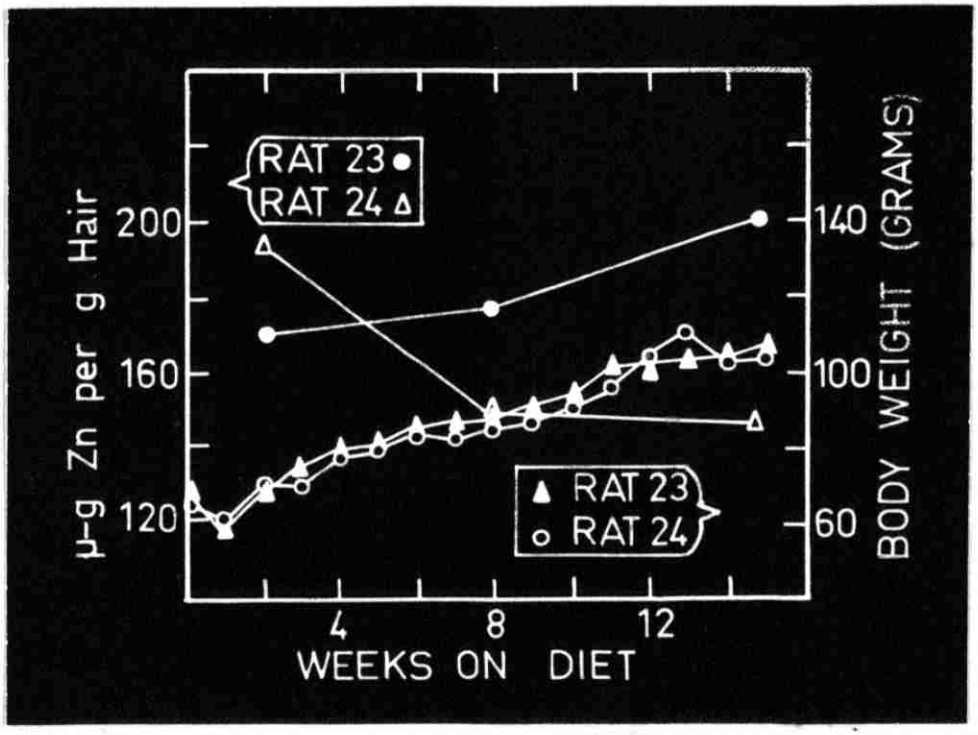


Figure 9. Male rats No. 23 (pZ) and No. 24 (pz) showing hair regrowth 4.5 weeks after shearing and after 12 weeks treatment.

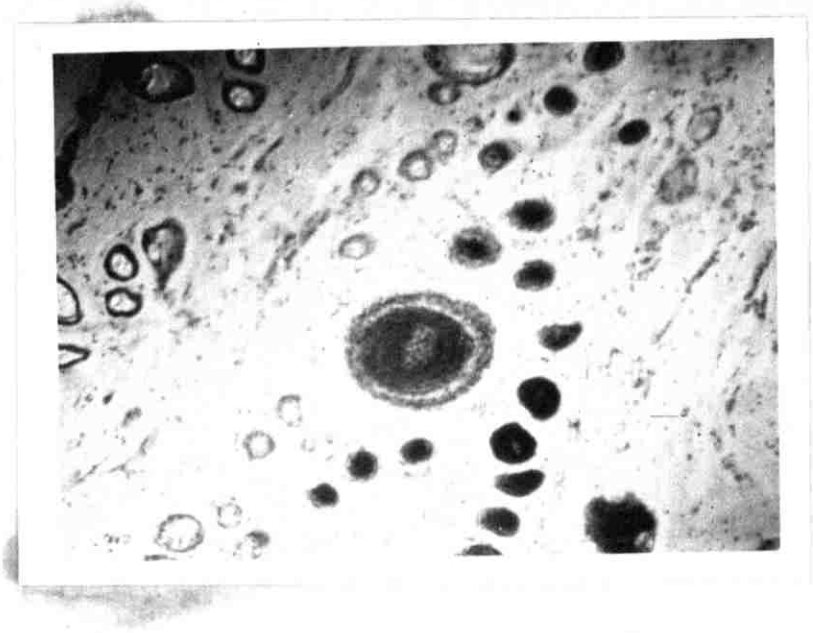


Figure 10. Skin specimen (l.p.)* from rat No. 5 treated with adequate protein and zinc diet (PZ). Epidermis in upper left part of microphotograph. Hematoxylin-eosin. Generally good appearance.

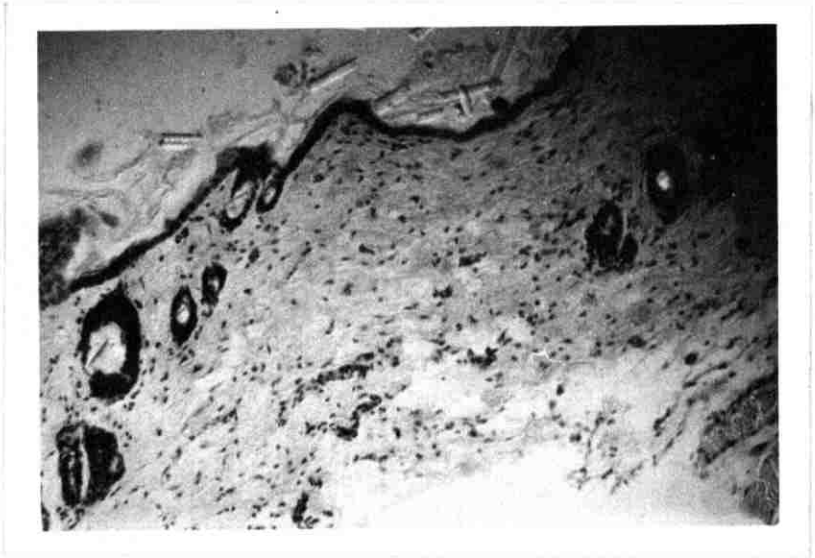


Figure 11. Skin specimen (l.p.)* from rat No. 10 treated with adequate protein-low zinc diet (Pz). Low density of follicles evident. Hematoxylin-eosin.

* Low-power magnification (100x).

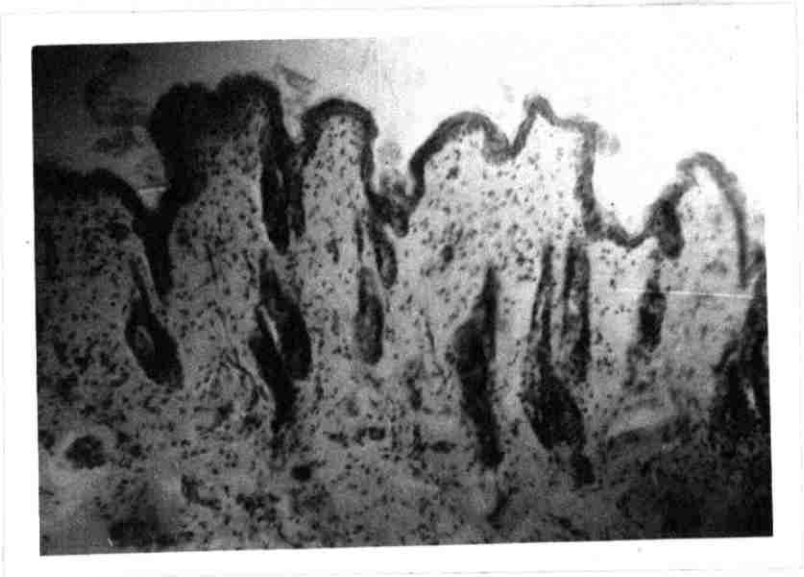


Figure 12. Skin specimen (l.p.) from rat No. 7 treated with low protein adequate zinc diet (pZ). Generally good appearance of follicles and epidermis. Hematoxylin-eosin.

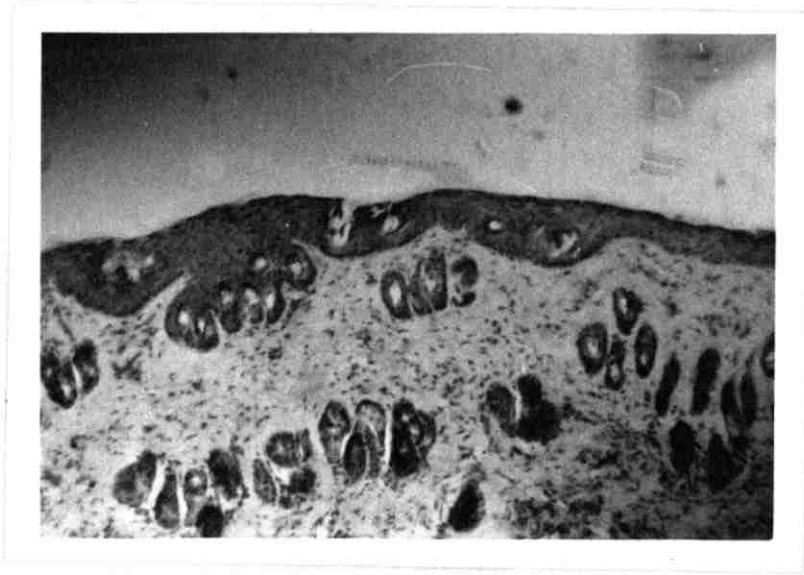


Figure 13. Skin specimen (l.p.) from rat No. 4 treated with low protein and zinc diet (pz). Epidermal thickening. Follicles atrophied and mostly in upper dermis. Hematoxylin-eosin.

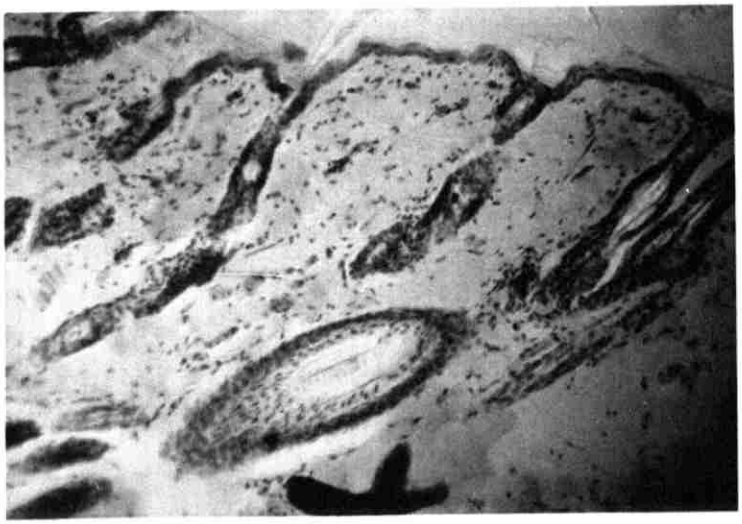


Figure 14. Skin specimen (l.p.) from rat No. 9 treated with adequate protein and zinc diet (PZ). Hair shafts evident.

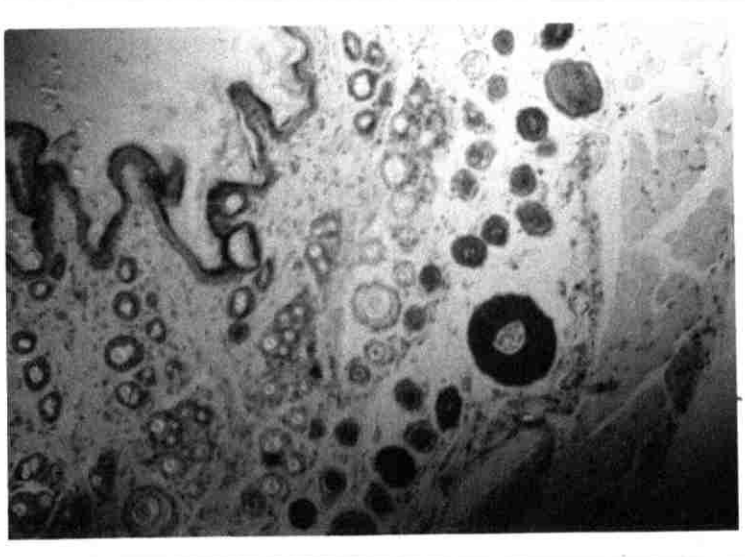


Figure 15. Skin specimen (l.p.) from rat No. 16 treated with low protein and zinc diet (pz). Mature follicles evident with hair shafts absent or minute.

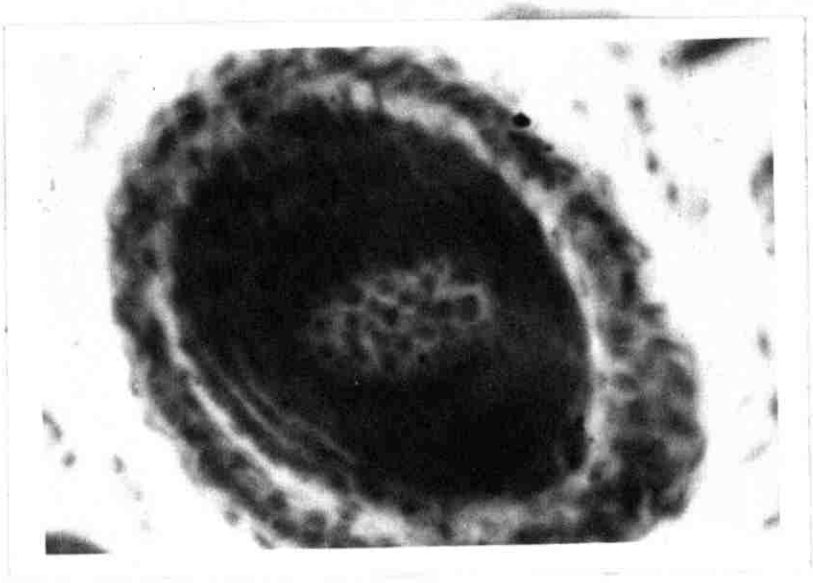


Figure 16. Mature hair follicle (h.p.) of rat No. 5 treated with adequate protein and zinc diet (PZ). Microphotograph is obscured by numerous nuclei. Hair shaft present. Hematoxylin-eosin.

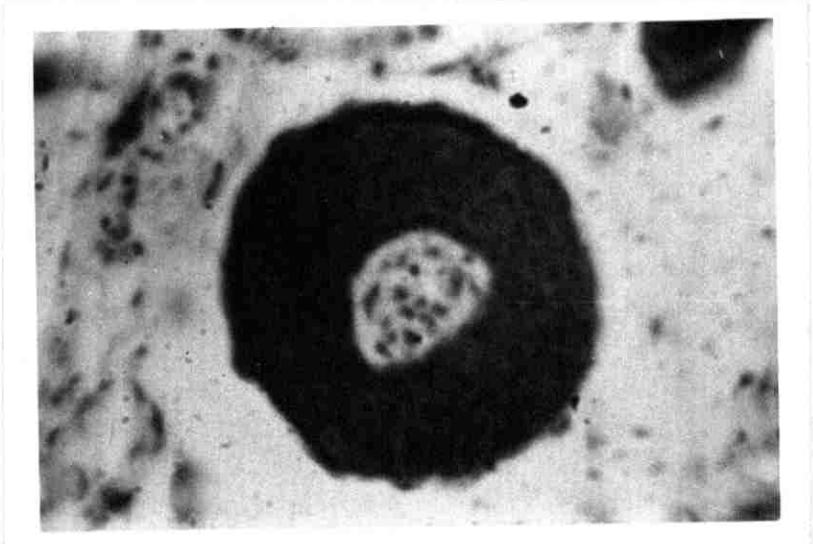


Figure 17. Mature hair follicle (h.p.) or rat No. 16 treated with low protein and zinc diet (pz). Hair shaft absent. Hematoxylin-eosin.

APPENDIX

APPENDIX A. Calculations for determination of µg. Zn/g. hair

OD_u = Optical density of unknown.

OD_s = Optical density of standard.

C_u = Concentration of unknown.

C_s = Concentration of standard.

A = Number of mg. of hair.

B = OD_u = OD of unknown in 4 ml. - OD of digested blank.

C = OD_s = OD of standard in 4 ml. - OD of water blank = OD for $\frac{2\ \mu g.\ Zn}{g.\ hair}$

$$C_u^{o} \text{ in 4 ml.} = A \times \frac{2\ \mu g.}{B} = OD_u \times \frac{C_s}{OD_s}$$

$$C_u \text{ in 15 ml.} = C_u^{o} \times \frac{15}{4} \times \frac{1000}{A} = \frac{\#\ \text{of } \mu g.\ \text{of zinc}}{g.\ \text{of hair}}$$

Formula: $B \times \frac{2\ \mu g.}{C} \times \frac{15}{4} \times \frac{1000}{A} = \mu g.\ Zn/g.\ hair$

***** ***** **** **** **** **** **** **** **** **** **** ****

Example: Calculations to determine ug. of zinc/g. of hair in the hair obtained from the first shearing of rat 20 (pz).

A = 52.50 mg.

Duplicate readings of the unknown in 4 ml. were 0.242 and 0.240.

Therefore, OD_u in 4 ml. is 0.241.

The average of the OD readings for the diluted solutions of the digested blanks was 0.043.

B = 0.241 - 0.043 = 0.198.

Average OD_s was 0.150.

Average OD of water was 0.015.

C = $OD_s - OD_{water}$ = 0.150 - 0.015 = 0.135.

Therefore, $0.198 \times \frac{2\ \mu g.}{0.135} \times \frac{15}{4} \times \frac{1000}{52.5} = \frac{193.9\ \mu g\ \text{of zinc}}{g\ \text{hair}}$

APPENDIX B. Binomial nomenclature of organisms referred to in text.

Aspergillus niger van Tiegh.

Escherichia coli Castellani and Chalmers, 1919.

Euglena gracilis Klebs.

Mycobacterium smegmatis Lehmann and Neumann, 1899.

Neurospora crassa Shear and Dodge.

Rattus norvegicus Berkenhout, 1769.

Rhizopus nigricans Ehrenberg.