PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 830–834 (1975)

Amelioration by Copper Supplementation of Mutant Gene Effects in the Crinkled Mouse (38908)

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In the course of investigations on genetic-nutritional interactions during prenatal development, we were impressed with phenotypic similarities, especially of hair growth, between mice homozygous for the autosomal recessive mutant gene *crinkled* (*cr*) and copper deficient animals. The crinkled mouse has a smooth-looking coat with thin skin, delayed pigmentation and early mortality. Hair-bulb development is retarded and abnormal; only one type of hair is formed (straight) rather than four types (some bent or crimped) as found in normal mice (1). Similarly, one characteristic of copper deficiency in sheep is loss of crimp in the wool (caused by defective keratinization). In severe cases, the fibers may emerge from the skin as almost straight (2). In rats, copper deficiency during pregnancy resulted in the birth of weak offspring with very thin skin (3). We therefore investigated the possibility of a relationship between the mutant gene *cr* and copper by examining the effect of copper supplementation during pregnancy and lactation on the expression of the crinkled gene in the homozygous mutant young.

Materials and Methods. Known heterozygous male and female carriers of *cr* in a hybrid background were obtained from the Jackson Laboratory, Bar Harbor, Maine, and mated. At the beginning of pregnancy, ascertained by vaginal plugs, the females were given one of four diets: (a) Purina Laboratory Chow, called stock diet, (b) a complete purified diet with all essential minerals and vitamins added, called control diet (4), c) the same complete purified diet to which was added 500 ppm of copper, called high copper diet, or (d) the same complete purified diet (b) to which was added 2000 ppm of manganese, called high manganese diet. The control diet contained 6 ppm of copper, while the stock diet contained 11 ppm, as measured by atomic absorption spectroscopy. Aliquots of the commercial pelleted diet and purified diets were ashed in acid washed crucibles at 450° for 48 hr and wet ashed in concentrated nitric acid. The nitric acid was allowed to evaporate and the crucibles were again heated to 450°. They were diluted with 1 *N* HCl for analysis in the Unicam SP 90. The female carriers were given these diets throughout pregnancy and lactation.

The females were kept three to a cage in stainless steel cages in a room with temperature, humidity, and light control (12 hr light and 12 hr dark). The females were allowed to deliver their young, separated and preliminary identification of the mutants was made at birth by noting the absence of the postorbital sensory hairs (1). Young were weighed at birth and on days 7, 14, 21, 28 and 30. Positive identification of the mutants was made on day 14.

In certain litters of each group one nonmutant and one crinkled mutant were killed at 6 days *postpartum*, and pieces of middorsal skin were fixed in buffered formalin, paraffin embedded, sectioned, and stained with haemotoxylin and eosin (H and E) for histological study. Ten photographs were taken of 10 separate sections (2 per slide of 20) from each sample and enlarged. Measurements of whole skin thickness (three per photo), epidermal thickness (three per photo), and hair-bulb number (one per photo) were made directly on the photomicrographs using a vinyl overlay with three permanent grid lines. Pieces of the same skin were dried using the critical point (liquid CO_2) technique, coated with a layer of silver and then gold, and were observed with the Cambridge Stereoscan Mark II scanning electron microscope (SEM). Hairs of adult crinkled and noncrinkled mice were plucked from the middorsal area, coated with silver

and gold and viewed and photographed in the SEM by a Polaroid camera back using Polaroid P/N Type 55 film.

Results. High copper intake during pregnancy and lactation had no significant effect on the growth of either mutants or nonmutants. In contrast, survival of the mutant mice to weaning at 30 days was clearly improved by copper supplementation (Table I). Although only 22–24% of crinkled young in the stock and control diet groups survived to weaning, 50% of those in the high copper group survived to this age. The improvement in survival to weaning appeared to be due to a lower mortality rate in the first 14 days of life, since survival from day 14 to day 30 was normal.

In addition to early mortality, another effect of the gene *crinkled* is delayed pigmentation. At 6 days of age, mutant mice whose mothers were given the high copper diet during pregnancy and lactation were noticeably darker in color than those whose mothers were fed either stock diet or the control purified diet (Fig. 1).

Histological sections of skin at 6 days of age revealed other striking effects of copper supplementation (Fig. 2). Skin from crinkled mutants in the stock diet or control diet groups was markedly thinner than skin from their nonmutant littermates. Hair-bulb development was reduced and there were few if any visible hairs emerging from the surface of the skin. However, in the mutant animals whose mothers received the high copper diet during pregnancy and lactation, thickness of the skin and epidermis at 6 days of age was nearly normal, and the number of hair bulbs was significantly increased (Table I). The number of hair bulbs in skin from mutants in the purified control diet group was also higher than that of crinkled mice fed stock diet, possibly due to greater availability of copper from the purified diet. The effect of high copper supplementation on the heterozygote carrier was variable. It seemed to decrease the number of hair bulbs and skin thickness, with no change in epidermal thickness and a slight positive change, if any, in the survival. Perhaps variable heterozygote effects are involved, but it should be remembered that the number of animals and measurements was relatively small. Manganese supplementation during the same developmental period had no beneficial effect on mutant skin development as evidenced by histological examination.

Scanning electron micrographs of skin surface also showed similar differences

TABLE I. EFFECT OF DIETARY SUPPLEMENTATION WITH COPPER ON SURVIVAL AND SKIN DEVELOPMENT OF *crinkled* (*cr*) AND NONCRINKLED (+) LITTERMATE MICE.

Diet	Genotype	Survival					Skin development at 6 days			
		Litters	Mice	Day 0–30	Day 0–14	Day 14–30	Mice	Skin thickness[a]	Epidermal thickness[a]	Hairbulbs[b]
		No.	No.	%	%	%	No.	μ	μ	No. per photo
Stock	+	11	81	70	72	98	2	375 ± 3	102 ± 3	80 ± 5
	cr		25	24	32	75	2	197 ± 7	87 ± 4	14 ± 1
Control	+	5	32	69	69	100	1	542 ± 10	106 ± 4	93 ± 3
(purified)	cr		9	22	22	100	2	250 ± 7	95 ± 3	43 ± 1[e]
High	+	7	55	84	84	100	4	341 ± 8[d]	102 ± 2	57 ± 5[e]
Copper	cr		22	50	59	86	7	273 ± 6[f]	102 ± 2[g]	55 ± 3[h]

[a] 30 measurements per animal.
[b] 10 measurements per animal.
[c] Significantly different from stock fed and high copper fed mutants by students *t* test ($p < 0.01$)
[d] Significantly different from stock fed and purified control fed nonmutants ($p < 0.02$, $p < 0.001$).
[e] Significantly different from stock fed and purified control fed nonmutants ($p < 0.05$, $p < 0.02$)
[f] Significantly different from stock fed and control fed mutants ($p < 0.001$, $p < 0.05$).
[g] Significantly different from stock fed and purified control fed mutants ($p < 0.02$).
[h] Significantly different from stock fed and purified control fed mutants ($p < 0.001$, $p < 0.01$).

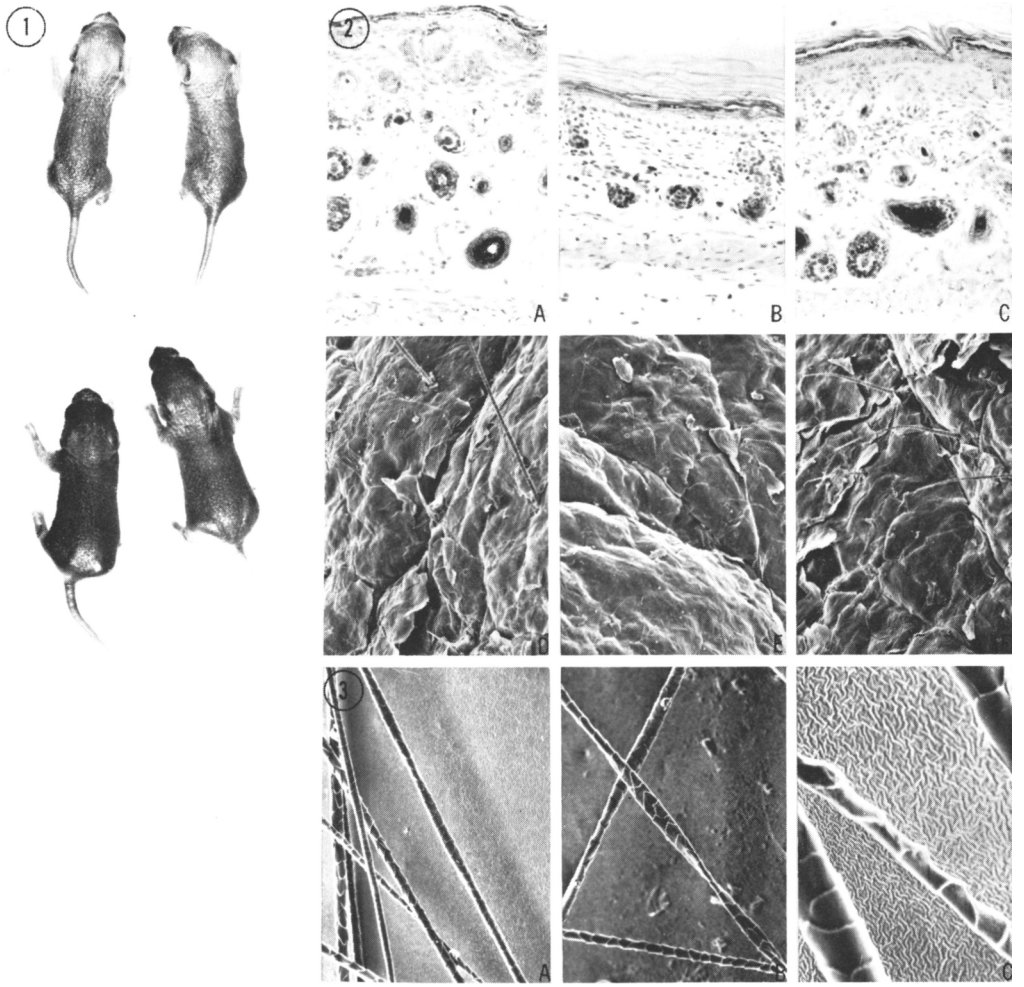

FIG. 1. Mutant mice homozygous for gene *crinkled* (*cr*) at 6 days of age fed the control purified diet (top) and the high copper diet (bottom) show an increase in pigmentation when fed copper and also early development of tail kinks not normally evident in crinkled mice until 10 days of age. The high copper diet also appears to have increased the body weight of the mutants of this litter.

FIG. 2. Photomicrographs of dorsal skin from 6-day-old mice. A) nonmutant from the stock diet group, B) crinkled mutant from stock or control diet groups and C) crinkled mutant from the high copper diet, show the increases in pigmentation, hair bulb development, and skin thickness of mutants after dietary copper treatment. Pieces of the same skin (D, E, F); dried using the critical point (liquid CO_2 technique) metal coated and viewed with the scanning electron microscope (SEM) show the increase in hair growth of crinkled mice from the high copper diet group. ×124.

FIG. 3. Hairs of adult crinkled and noncrinkled mice were plucked from the middorsal area, coated with silver and gold and viewed in the SEM. Scanning electron micrographs of plucked hairs from adult crinkled mice show, a) monilethrix (alternate narrowing and thickening of the shaft) ×95, b) pili torti (twisting of the hair along its longitudinal axis) ×134, and c) short nodular ends which have been described as leading to trichorrhexis nodosa (broken, frayed ends). ×480.

among the groups (Fig. 2). Skin from 6-day-old crinkled mutants in the control diet or stock diet groups had smooth surfaces and showed no sign of hair growth. In contrast, crinkled mice in the high copper group exhibited the hair growth and rough epidermal surface of nonmutant stock fed animals.

In scanning electron micrographs of hairs

from crinkled mice, alternate narrowing and thickening of a hair or monilethrix, pili torti (twisting around the longitudinal axis of the hair), and hairs with rough nodular shafts (which may lead to frayed broken ends or trichorrhexis nodosa) were all found (Fig. 3). None of these abnormalities were seen in the hairs of nonmutant animals.

Discussion. The increased survival of mutant crinkled mice in the first 14 days of life which was brought about by a high copper intake during pregnancy and lactation demonstrates that copper supplementation ameliorated the effect of the gene *cr* on early mortality. Another effect of the mutant gene which was influenced by copper was delayed pigmentation. The lag in pigment development characteristic of crinkled mutants was prevented by dietary copper supplementation. Furthermore, skin and epidermal thickness and hair bulb development were nearly normal in the high copper group. These findings show that increased availability of copper favorably altered the expression of the mutant gene. The lack of such effects with manganese supplementation suggests some specificity for the role of copper in relation to the gene.

These results demonstrate the interaction of a gene and a nutrient in prenatal or perinatal development. We earlier reported a similar interaction in the case of the pallid mutant mouse, in which abnormal development of the inner ear (which could also be produced in nonmutant animals by prenatal manganese deficiency) was prevented by prenatal manganese supplementation (1, 5, 6). Recently, the occurrence of a mutant gene analogous to *pallid* has been reported in mink (7). Again, abnormal development of the inner ear could be prevented by manganese supplementation. Similarly, in the present findings concerning the gene *crinkled*, some of the phenotypic characteristics of the mutant also appear to be produced by copper deficiency in the wild genotype, while supplementation with the element modified the expression of the mutant gene.

An especially interesting finding in this study was the presence of pili torti, monilethrix and what appears to be trichorrhexis nodosa in crinkled mutant mice. A genetic disorder in young children, Menkes disease, is thought to be the only human disorder where these three hair abnormalities are seen together (8). Similar to *crinkled* in some ways, this syndrome is characterized by early mortality, thin skin, lack of pigmentation, abnormal growth and development of the hair (9–12) and has recently been found to exhibit abnormal copper metabolism (13, 14). Therefore, in addition to apparently analogous genes interacting with manganese in at least two species, as well as the copper-*crinkled* relationship reported here, it is suggested that similar interactions during development may occur in other species including the human. Animal models exemplifying genetic and nutritional interactions may be of value in determining prenatal nutrient therapy for genetic diseases.

Summary. The possibility of a relationship between the autosomal recessive mutant gene crinkled in mice and copper metabolism was investigated by examining the effect of copper supplementation during pregnancy and lactation on the expression of the gene in homozygous mutant young. Survival of mutant mice to 30 days of age was doubled by feeding their mothers a high copper diet (500 ppm copper) during pregnancy and lactation, as compared with controls (6–11 ppm dietary copper). High dietary copper also prevented the lag in pigment development characteristic of the mutants. Furthermore, skin and epidermal thickness and hair bulb development were nearly normal in the high copper group, in contrast to thin skin and paucity of hairs in controls. Supplementation with manganese did not have these effects. Scanning electron micrographs showed the presence of three types of hair abnormalities in crinkled mutants, monilethrix, pili torti, and possibly trichorrhexis nodosa. The results show that increased availability of copper favorably altered the expression of the mutant gene, and demonstrate the interaction of a gene and a trace metal in development.

We thank Donna Dungan for valuable assistance with animals and with histological preparations. This work was supported in part by NIH Grant No. HD-02355.

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Received February 10, 1975. P.S.E.B.M. 1975, Vol. 149.