Red Hair in Black Cats Is Reversed by Addition of Tyrosine to the Diet

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EXPANDED ABSTRACTS

KEY WORDS: • melanin • eumelanin • hair color • kitten • tyrosine

Hair growth and replacement in cats is seasonal (1). In the Northern Hemisphere primary and secondary hair bulbs decrease activity in December, are totally inactive in January and remain so until March, with activity recommencing in April. Although the inheritance of hair color in cats has received considerable attention (2), evidently there have been no studies on the influence of nutritional factors on hair color in cats. The effect of diet on hair color of dogs was dismissed as a myth by Case et al. (3).

In the course of experiments to determine the folic acid requirements of cats (4) the observation was made that black cats given a purified diet in which gelatin was a major source of the protein developed reddish-brown hair. This diet supplied all the essential amino acids at 1.5 times the requirement suggested by the National Research Council (5). However, when the source of protein in the purified diet was casein–lactalbumin, no change in hair color occurred. In this communication the nutritional basis of the change in hair color induced by the gelatin diet is investigated and the nutrient requirements for prevention of color change are broadly defined.

MATERIALS AND METHODS

Purified diets based either on gelatin (supplemented with essential amino acids), casein and lactalbumin or on isolated amino acids as nitrogen sources were used. The composition of the diets is given in Table 1.

All kittens and cats had black hair coats and were from the U. C. Davis Feline Nutrition and Pet Care Center’s specific-pathogen-free colony. Kittens were adapted to eat purified diets, and entered experiments only after their food intakes and body weight gains were satisfactory. Diets and water were offered ad libitum at all times, and the kittens were housed in stainless steel metabolism cages (0.6 × 0.6 × 0.9 m) in a temperature- (20 ± 2.5°C) and light-controlled (14 h light) room. The experimental protocol was in compliance with the NIH guidelines and was approved by the University of California, Davis, Animal Use and Care Administrative Advisory Committee.

Design of experiments

Five experiments were conducted.

Experiment 1. Four black kittens from the same litter were given the purified casein–lactalbumin diet (Table 1) from weaning to 12 wk of age. The kittens were then randomly divided into two groups, one group receiving the gelatin + amino acids (AA) diet and the other the casein–lactalbumin diet. Both diets exceeded the 1986 NRC (5) requirements for growth. Kittens received these diets for 4 mo.

Experiment 2. Four black kittens were divided into two groups: one group received the gelatin–AA diet (containing 3 g tyrosine/kg) and the other group received the same diet supplemented with L-tyrosine, to give a total concentration of 19 g tyrosine/kg diet.

Experiment 3. Four black kittens (10–12 wk of age) were given the complete amino acid basal diet supplying all essential AAs (except phenylalanine) at least 1.5 times the 1986 NRC requirement. To the basal diet the following amounts of phenylalanine/tyrosine (g/kg diet) were added: 12/0, 12/4.5, 24/0 and 24/4.5. The diets were rendered isonitrogenous by adjustment of the amount of glycine. Kittens received these diets for 15 wk.

Experiment 4. Two black-haired queens were given the gelatin–AA diet for 3 mo, which produced a change in hair color. They were then mated to a black tom and gave birth 8 wk later.

Experiment 5. A basal gelatin diet was prepared and supplemented with amino acids to bring the concentration of all essential amino acids (except phenylalanine + tyrosine) to 1.5 times the 1986 NRC recommended requirement. The total phenylalanine and tyrosine concentrations in the basal diet (g/kg) were 11 g phenylalanine (7.8 g from gelatin and 3.12 g from the AAs) and 2.8 g of tyrosine from gelatin. Twenty black-haired weaned kittens were divided into five groups each of four kittens and given the basal diet to which 0, 1, 2, 3 or 6 g of L-tyrosine had been added per kg diet at the expense of starch. The diet was given to the kittens for 4 mo.

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4 Abbreviations used: AA, amino acids; DHI, dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; DOPA, L-3,4-dihydroxyphenylalanine; PTCA, pyrrole tricarboxylic acid; TAA, total aromatic amino acids.
TABLE 1

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gelatin–amino acid diet</th>
<th>Casein–lactalbumin diet</th>
<th>Amino acid diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common ingredients¹</td>
<td>484.7</td>
<td>484.7</td>
<td>484.7</td>
</tr>
<tr>
<td>Gelatin</td>
<td>380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td>222.5</td>
<td></td>
</tr>
<tr>
<td>Lactalbumin</td>
<td></td>
<td></td>
<td>222.5</td>
</tr>
<tr>
<td>Amino acid mixture A²</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Amino acid mixture B³</td>
<td></td>
<td></td>
<td>297.5</td>
</tr>
<tr>
<td>Phe/tyr/gly⁴</td>
<td></td>
<td></td>
<td>83.5</td>
</tr>
<tr>
<td>Taurine</td>
<td>2.5</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Starch</td>
<td>92.8</td>
<td>67.8</td>
<td>131.3</td>
</tr>
</tbody>
</table>

¹ Common ingredients were g/kg diet: animal fat (Florin Tallow, Dixon, CA), 300; sucrose, 100; mineral mixture ([Williams et al. (6)], 50; cellulose, 20; vitamin mixture [Yu & Morris (7)], 10; and choline chloride, 4.7.
² Amino acid (Ajinomoto U.S.A., Raleigh, NC) mixture A contained (g): L-isoleucine, 4.64; L-leucine, 7.32; L-methionine, 7.04; L-phenylalanine, 3.12; L-threonine, 3.72; L-tyrosine, 2.32; L-tryptophan, 2.32; L-valine, 4.24; L-histidine, 3.20; L-lysine, 3.40; L-proline, 3.40.
³ Amino acid (Ajinomoto U.S.A.) mixture B contained (g): L-aspartic acid, 20; L-threonine, 20; L-serine, 15; L-glutamic acid, 38; L-alanine, 38; L-valine, 15; L-methionine, 10; L-isoleucine, 12.5; L-leucine, 30; L-lysine, 20; L-histidine, 7.5; L-arginine, 25; L-proline, 34; L-tryptophan, 3.75 and L-cystine, 8.75.
⁴ In experiment 3, the following amino acid mixtures were added/kg diet. For the 12/0 and 12/4.5 diets, 12 g L-phenylalanine and 4.5 g L-tyrosine, respectively. The difference in these diets was the protein source, this experiment demonstrated that the protein source was responsible for the change in hair color, and other dietary ingredients including minerals and vitamins were not involved. Microscopic examination of hair samples from the kittens given the gelatin–AA diet at 4 mo revealed incomplete melanin deposition in hairs, whereas the hair shafts from the same kittens before treatment were a solid black color.

RESULTS

No adverse clinical signs were observed in the kittens given the various diets other than changes in hair coat color. In experiment 1, the two kittens that received the gelatin–AA diet had a progressive change of the hair color from black to reddish-brown. The change first became apparent in the hair around the mouth and head and progressed to the forelimbs, shoulders and, eventually, by 4 mo, over the whole body. In contrast the two kittens given the casein–lactalbumin diet maintained their black hair color. Because the only difference in these diets was the protein source, this experiment demonstrated that the protein source was responsible for the change in hair color, and other dietary ingredients including minerals and vitamins were not involved. Microscopic examination of hair samples from the kittens given the gelatin–AA diet at 4 mo revealed incomplete melanin deposition in hairs, whereas the hair shafts from the same kittens before treatment were a solid black color.

The two kittens in experiment 2 given the gelatin–AA diet developed reddish-black hair, whereas the other two kittens receiving the same diet supplemented with L-tyrosine (total dietary concentration of 19 g/kg diet) had no change in hair color from their pretreatment black hair. This experiment demonstrated that supplemental tyrosine would prevent the development of the hair color change from black to reddish-brown.

In experiment 3 the kittens receiving the isolated amino acids containing 12/0 and 12/4.5 g/kg developed reddish-brown hair, whereas the kittens receiving the 24/0 and 24/4.5 diets maintained their black hair color. This study indicated that 12 g phenylalanine + 4.5 g tyrosine or 16.5 g of total aromatic amino acids (TAAA) was insufficient to maintain black hair color but that 24 g of TAAA was adequate.

Kittens born to the queens that had coat color changes from consuming the gelatin–AA mixture A diet were also abnormal in color, and had gray hair at birth instead of black. By about 8 wk of age the coat color had changed to black. These changes of coat color were interpreted to be a result of queen’s milk containing adequate concentrations of TAAA, whereas the kittens in utero were exposed to an AA profile similar to that of the hair follicles of the queen.

In experiment 4 the color of the hair of the kittens was assessed after they had consumed the respective diets for 4 mo. In none of the five groups of kittens was the hair of all four kittens completely black.

DISCUSSION

The color of the skin and hair of mammals is primarily determined by the type and amount of melanin present (8). Melanin in hair is composed of a mixture of two pigments: eumelanins (brown and black pigments) and pheomelanin (a yellow to reddish-brown pigment) (9). The precursor of both pigments is dopaquinone, which is derived from tyrosine by two sequential oxidation steps (Fig. 1). The first step is the oxidation of tyrosine to L-DOPA (3,4-dihydroxyphenylalanine). In melanosomes this step is catalyzed by the copper-containing enzyme tyrosinase (EC 1.14.18.1), whereas in catecholaminergic neurons it is catalyzed by tyrosine hydroxylase (EC 1.14.16.2) (11). The oxidation of DOPA to dopaquinone is catalyzed in both tissues by tyrosinase. The Km of tyrosine hydroxylase for tyrosine is reported as 8.3 × 10⁻³ M (12) and is regarded as the rate-limiting step for DOPA synthesis in neuronal cells. Tyrosine hydroxylase requires the same cofactor (tetrahydropteridine) as phenylalanine hydroxylase (13).

In the production of eumelanins, dopaquinone cyclizes to form dopachrome, which can either be decarboxylated to dihydroxyindole (DHI) and produce the DHI melanin (Mason–Raper pathway) or, alternatively, the dopachrome may form 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and result in DHICA melanin. These two melanins are, respectively, black and brown colored melanins and are collectively known as eumelanin. The first committed step in the pathway of pheomelanin synthesis involves the combination of DOPA with cysteine and the sulfur donated by cysteine is retained in pheomelanin synthesis than about eumelanin synthesis.

Phenylalanine is an essential amino acid for kittens, but tyrosine is a dispensable amino acid (14). The normal pathway of phenylalanine metabolism is hydroxylation to tyrosine, catalyzed by phenylalanine hydroxylase. Because tyrosine is the precursor of melanin, the sum of both AAs may be used to calculate the TAAA in the diet for melanin synthesis. These studies have shown that a dietary concentration of 16.5 g of TAAA/kg diet supplied as isolated AA is inadequate to maintain black coat color, whereas 24 g/kg diet is adequate. In experiment 5, when the gelatin diet was supplemented with tyrosine and given to the cats, even the highest level of supplementation was inadequate. If the small intestinal digestibility of phenylalanine and tyrosine in gelatin is given a value of 0.8, then the concentrations of TAAA derived from gelatin would be: 7.8 × 0.8 = 6.24 g of phenylalanine, and 2.8 × 0.8 = 2.24 g of tyrosine or 8.48 g/kg diet. To this 3.12 g of phenylalanine had been added to all diets, to give a TAAA concentration of 11.6 g/kg from the basal diet. A further 6 g tyrosine was added at the highest level of supplemental tyrosine, making a total concentration of 17.6 g of TAAA/kg.
The pathways of synthesis of eumelanins and pheomelanin from phenylalanine and tyrosine. Note the multiple roles of tyrosinase in the pathways. Tyrosine hydroxylase, rather than tyrosinase, catalyzed the conversion of tyrosine to DOPA in catecholaminergic neurons. [Modified from Pawelek & Chakraborty (10).]