HOUSE FINCH PIGMENTATION: CAROTENOID METABOLISM AND THE EFFECT OF DIET

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THE carotenoids are among the major pigments in avian plumage and provide bright colors to species in a number of orders. Birds cannot synthesize carotenoids metabolically, but can modify extensively those obtained in the diet (Thommen 1971, Brush 1976). The chemical nature of the pigment molecules deposited in the plumage is at least partly under genetic control, as the genotype is initially responsible for the patterns and colors that distinguish each species. The genetic control of plumage colors is understood for some species on the basis of circumstantial evidence such as the pigments found in sexually dichromatic species, color mutants, and hybrid populations (Brush 1976). Presumably the biochemical processes and the organization of pigment deposition are under relatively simple genetic control. In spite of the enormous biological importance of these factors, the relationships among internal pigment deposits, feather pigments, and diet are only vaguely understood from feeding experiments (Kritzler 1943, Test 1969, Fox and McBeth 1970). Despite the relative degree of sophistication available about the chemistry of avian carotenoid pigments, much needs to be learned regarding the metabolic and biochemical basis of color variation in populations in the wild and in controlled feeding experiments. Such work can discriminate between genetic and dietary factors in coloration, illustrate the degree of phenotype plasticity in pigmentary systems, and give insight into both the control and evolution of the biochemical pathways associated with plumage coloration in birds. Information regarding the evolution of pathways also has potential value in systematics.

The cardueline House Finch (*Carpodacus mexicanus*) is well suited for studies on carotenoid pigmentation. Over much of the body the plumage pigment is melanin; contour feathers are typically brown and white, and flight feathers a more uniform brown. The species is sexually dichromatic. Adult males normally have bright red feathers on the head, throat, chest, and rump. "Red" is used to include the terms scarlet, crimson, rosy, raspberry, and others common in the literature. Fully adult females may have tinges of yellow, orange, or red on the throat or rump, but the overall appearance is one of cryptic brown and white. Color variation within populations is often extensive. A large series of males collected from a single locality will show birds ranging from pale yellow to deep red. The House Finch occurs widely in the western United States and

Mexico and a number of subspecies are recognized (A.O.U. 1957, Miller et al. 1957), some on the basis of distribution of carotenoid pigmentation in the male. Males show considerable geographic variation in the extent of ventral pigmentation (Moore 1939). For example, extensive red is found over most of the throat, chest, and belly in *C. m. ruberrimus* of the southern half of Baja California and parts of Sonora, Sinaloa, and Chihuahua, and in *C. m. rhodopnus* of central Sinaloa. On the other hand, the ventral red is a restricted and sharply defined throat patch in the subspecies *C. m. mexicanus* of central eastern Mexico.

For experimental purposes House Finches are easily kept in cages and do extremely well on a diet exclusively of seeds and water. These conditions allow for control of dietary carotenoids and with the use of known pigments allow the investigator to manipulate pigment intake. Besides the possibility of establishing the effect of diet on plumage pigment, feeding experiments with known precursors provide insight into the metabolic capacities of this species. The objectives of the work reported here were to document the nature of the pigments present in normal and variant individuals in natural populations, to compare the pigment composition of birds from California with those of the populations introduced in New England and the Hawaiian Islands, to establish the effect of feeding specific carotenoids on the coloration and composition of the plumage pigmentation, and to test hypotheses regarding the metabolic pathways and specificities of the plumage pigments.

The relationship of the pigments of *C. mexicanus* to those of other carduelines, the closely related passerine families, and their use in specific taxonomic problems are discussed elsewhere (Brush MS). The effects of hormonal treatment on plumage coloration is also currently under investigation (Power and Brush MS).

MATERIALS AND METHODS

Animals.—Feathers were taken from study skins, from freshly collected birds, from individuals caught alive and released, and from experimental birds maintained in captivity. Specimens were obtained from native populations in Santa Barbara County, California, and from the introduced populations on Oahu, Hawaii, in Rockville, Connecticut, and in Duxbury, Massachusetts. Feeding experiments were performed on live birds captured at Santa Barbara. Birds in captivity were kept in outdoor flight cages ($6\frac{1}{2} \times 6\frac{1}{2} \times 3$ feet) on the grounds of the Santa Barbara Museum of Natural History. They were shaded from direct sunlight but otherwise exposed to local environmental conditions. The birds, held two to four per cage, remained healthy under these conditions and molted in synchrony with local wild birds. Seeds and water were provided *ad libitum*. The seeds were commercially available parakeet or finch mix.

As is well known to aviculturalists and as reported beyond, male House Finches fed only seed in plain water molt normally bright red feathers and replace them

with yellow feathers. A source of dietary carotenoids in sufficient quantity allows normal appearing plumage to be maintained. In our experiments we used two carotenoids, canthaxanthin and β-carotene. Canthaxanthin was the easiest to handle as it is water soluble. A stock solution of 1 g of 10% dry canthaxanthin beadlets (Hoffman-LaRoche) per 100 ml water was mixed and kept refrigerated. The stock solution was added to the drinking water supply in the ratio of 1 ml stock solution per 200 ml water. Beta-carotene is soluble only in certain organic solvents and had to be applied directly to the seed in powdered form. Carotene (Eastman, 100% β) was added in the ratio of 1 g per 500 g seed and shaken thoroughly. The pigment fades rapidly and must be kept refrigerated and in the dark.

Chemical analysis.—The feather pigments were extracted routinely in acetone, saponified by treatment with 10% KOH (Rothblat et al. 1964), and then transferred to *n*-hexane. Some samples were extracted in alkaline-ethanol but, because of the possibility of side reactions producing astacene, this procedure was not used extensively. The pigment in *n*-hexane was washed with saline. The spectrum of the crude extract was recorded in several solvents on a Bausch and Lomb 505 recording spectrophotometer. Each extract from feathers of individual birds was subject to thin-layer chromatography (T-LC) on silica gel or alumina sheets (Baker, ChromAR, or Eastman) in several solvent systems. Up to 15 samples could be compared conveniently on a standard 20 × 20 cm sheet. Smaller sheets were often used for various analyses and, because of the effect of sheet size and tank configuration, one or more known pigments was included in each run. Preparative T-LC on either Baker or ChromAR-500 sheets was used to isolate individual pigments for identification. In our hands this system gave better resolution than chromatographic columns, and recovery was adequate for our purposes. Each pigment was identified on the basis of its solubility and spectra in several solvents, partitioning coefficient (Petracek and Zechmeister 1956) and cochromatography with known pigments. The presence of the keto- group was verified by treatment with $NaBH_4$ (Krinsky and Goldsmith 1960), which reduced it to the corresponding hydroxy-form. Treatment with acid anhydride and methylation was used to detect and locate secondary hydroxyl groups (Gilchrist and Lee 1972). All feather samples and two different feeds and cornmeal were analyzed in this manner.

Results

Feeding Experiments

On 27 April 1973 three male House Finches were separated from a flock captured the previous winter. They were chosen for their range of coloration; one was naturally pigmented red, another orange, and the third yellow. These three constituted an experimental group fed solely on seed and water containing canthaxanthin. A second group of three males was placed in another cage. At the time of transfer these were classed as red-orange, orange, and pale red; "pale" in this last case indicates a reduced amount of pigment, not a different hue. These three constituted the control group and were fed seed and plain water. Following the complete prebasic (= postnuptial) molt of summer, all plumages were inspected. The canthaxanthin-fed birds were uniformly bright red in appropriate places, giving every apperaance of being normally pigmented males. The control birds were uniformly yellow, some with a reduced area of color. On 18 February 1974 the throat feathers of one bird from each group were plucked for chemical analysis. On 15 March the remaining two birds in each of the groups were similarly plucked.

The experiment with β-carotene was designed differently. In this case birds were plucked from an area about 1 cm square on the throat and upper chest and allowed to regenerate feathers, rather than allowed normal prebasic molt. On 30 May 1974 β-carotene-coated seeds and plain water were given the previous control group, while plain seeds and plain water were given the previous experimental group. On 31 May feathers were plucked from the throat as indicated. On 9 July the new feathers were inspected. The β-carotene-fed group had two birds classed as red-orange and one as orange. The control group had two birds classed as orange and one as yellow. Although not so definitive as the canthaxanthin experiment, the results suggest that birds with the carotenoid supplement to the diet assume a more nearly normal male plumage. On 23 July feathers were clipped and saved for chemical analysis.

A second β-carotene experiment was performed with a set of six normally pigmented birds captured in mid-September 1974. On 2 October all birds had throat patches plucked; three were fed β-carotene-coated seeds and plain water, and three received plain seed and water. On 8 November the new feathers were inspected, clipped, and analyzed chemically. Those birds receiving β-carotene grew back a female or juvenilelike plumage (a melanin central stripe paralleling the rachis of each feather) with a distinct pinkish cast. Those in the control group grew back a femalelike plumage with traces of yellow. Again, the results indicate the influence of dietary carotenoids for maintenance of male coloration.

CHEMICAL ANALYSIS

Intrapopulation variation.—Male birds from wild House Finch populations in Hawaii, California, and New England included yellow, orange, and red individuals. The extract from feathers of red or deep orange-red birds when separated by T-LC consistently produced four spots. They were identified as a yellow, rapidly migrating group that contained β-carotene and probably traces of α-carotene; a slower red spot identified as echinenone (4-keto-β-carotene); a still slower yellow spot identical to isocryptoxanthin (4-hydroxy-β-carotene); and finally a yellow spot of varying intensity and low mobility that consisted of unidentified mixed xanthophylls.

Wild yellow birds contained β-carotene, mixed xanthophylls, and isocryptoxanthin but lacked echinenone or any other red pigment. Those with the least intensely pigmented plumage also lacked detectable amounts of isocryptoxanthin. Wild orange birds had increased concentrations of isocryptoxanthin but no detectable echinenone. Thus the differences between orange and yellow birds was an apparent buildup of isocryptoxanthin, but did not involve echinenone. The step from yellow to red plumage presumably involved the production and deposition of a new metabolic derivative, echinenone. This was especially noticeable in the Oahu sample.

Interpopulation variation.—No differences in the types of pigments present or their relative distribution among the color types was detected in birds from New England, California, or Hawaii. The plumage pigments obtained from museum skins and wild birds of similar color showed no differences.

Feeds.—The major pigments of the parakeet mix were β-carotene and hypophasic xanthophylls. The seed of the finch mix, which looked more yellowish, contained mono- and dihydroxy-carotenes and relatively little β-carotene. Neither seed mix was adequate in pigment content to provide the normal plumage color. Both plucked birds and those undergoing natural molt replaced feathers with a light yellow

plumage and often deposited no pigment at all when on a simple seed diet. The yellow pigments in the plucked birds on low carotenoid diets were mostly hydroxyxanthophylls and carotenes in very small quantities. The pattern on T-LC resembled closely the pigments extracted from the feed. Finch mix was a better pigmenter than parakeet mix, but inadequate to provide enough pigment to color the plumage.

Individuals given canthaxanthin in addition to the seed diet replaced plucked feathers with red or red-orange feathers that contained canthaxanthin plus the seed pigments. The original plumage color type did not affect the deposition of canthaxanthin. Birds with a seed diet supplemented with β-carotene deposited β-carotene, isocryptoxanthin, and echinenone in the replaced feathers, but were always red-orange, orange, or pinkish in color. Control birds in every case produced yellow feathers severely depleted in pigment, or lacked pigment entirely. Birds on a simple seed diet had precursor available (at least in amounts detectable by our methods) but consistently failed to produce red feathers. Presumably this was due to either inadequate precursor levels or a mechanism that prevented the transformed molecule from appearing in the replaced feather. The latter may not hold as canthaxanthin, when available, was deposited in quantity by birds of all color types, indicating that the follicular cells were capable of handling keto-carotenes. All birds fed β-carotene converted it to echinenone, indicating that the difference was not genetic or simply metabolic. In several birds fed canthaxanthin and then denied pigments other than those present in the seeds, canthaxanthin appeared in feathers replaced up to 3 months later. This implies the presence of stored quantities of this pigment.

The feeding experiments can be summarized briefly. First, when denied adequate dietary pigment birds were incapable of producing colored feathers. When β-carotene, a common dietary carotenoid, was present birds produced isocryptoxanthin and echinenone, the same pigments present in native birds. Beta-carotene was also present in the regenerated feathers. The color variation in wild birds is not simply the result of genetic differences. The fact that pigment intensities similar to the reddest native birds were not obtained in feeding experiments could be due to the function of other factors such as hormone levels. Dietary canthaxanthin was deposited by all birds.

We can now propose the following mechanism for the production of the normal red pigment echinenone in male finches from a dietary source of β-carotene.

(A)	β-carotene→	isocryptoxanthin→	echinenone
	in diet	(4-hydroxy-β-carotene)	(4-keto-β-carotene)
		normal orange	normal red

(B) canthaxanthin→canthaxanthin (4-4′-diketo-β-carotene)

in diet experimental red

A dietary source of β-carotene is available from many plant sources. The above scheme explains the normal coloration, and the result of feeding experiments with canthaxanthin, which is deposited in the feathers with no further modification and presumably is directly acceptable to the follicular cells. Under no conditions was echinenone converted to canthaxanthin, nor was canthaxanthin found in wild birds.

DISCUSSION

That the plumage coloration of House Finches will undergo intensification without a molt and that color changes occur with molt in the plumage of captive birds is well known (Keeler 1893). The first phenomenon is due to abrasion and wear in a single feather generation. In older, abraded feathers the unpigmented distal ends of the barbs are worn off, removing the grayish feather edges and exposing the brightly pigmented parts of the barbs (Grinnell 1911, Michener and Michener 1931). The second phenomenon reflects the effect of insufficient dietary pigments on subsequent plumage generations. Both aptosochromatic and diet-related changes undeniably occur in native populations of many species, but the extent of their occurrence is difficult to quantify and their biological significance even harder to assess.

The existence of color variation is widespread in avian populations. Variation ranges from well-defined subspecific differences, through sexual dichromatism and genetic based polymorphisms, to variation among individuals in the color intensity of plumage. The latter occurs frequently but is most difficult to document. The biological roles of the variability at each level have been given various degrees of significance despite the fact that the metabolic and control mechanisms responsible for their production and maintenance within populations are, at best, only vaguely understood (Table 1). In the House Finch variation in the distribution of carotenoid pigment over the body seems in large part under genetic control. For example, males of central eastern Mexico all have a restricted, sharply defined throat patch, whereas males of parts of northwestern Mexico have an extensive wash over much of the ventral surface (Moore 1939). Geographic variation in the extent of color exists elsewhere too, but variation in the color itself, rather than the distribution of pigment, is of interest here and is what is meant when we use the phrase or an equivalent to "variation in plumage color." A number of hypotheses exist that may account for the variation in plumage color in *Carpodacus mexicanus*.

Genetic, dietary, metabolic, and physiological factors are involved in the determination of carotenoid composition and concentration in plumage. Satisfactory resolution of the individual and collective influence of these factors has been difficult to obtain, particularly in regard to the origin and metabolism of keto-carotenoids. A major question pertains to whether birds are capable of transforming β-carotene obtained in the diet to keto-carotenoids or whether all carotenoids found in birds are simply ingested in the food. Thommen (1971) is the strongest proponent of the latter hypothesis. An alternative source of carotenoids, especially at trace levels,

TABLE 1

COMPARISONS OF CONTROL MECHANISMS FOR FEATHER COLOR IN SOME CLOSELY RELATED BIRDS

Species[1]	Nature of difference	Proposed mechanism	Reference
1	Sexual dichromatism, season change in males.	Seasonally active, single enzyme converts diOH→diKeto, possible hormonal control.	Brush 1967
2	Specific difference in rump patch color; intermediate in hybrids; individual variants.	Quantitative differences in pigment combined with modified feather. Morphology produces differences in hue and brightness, but not color purity.	Brush 1970
3	Color polymorphism.	Gray form selectively omits carotenoids from certain feather tracts. Possible follicular selectively.	Johnson and Brush 1972
4	Plumage color polymorphism.	Colors in similar plumage patterns produced by simple metabolic changes. Suggests genetic differences in morphs.	Brush and Siefried 1968
5	Sex, species and hybrid differences.	Specific differences susceptible to dietary modification but influenced by hormones. Genetic differences in metabolism.	Test 1940
6	White species does not produce red feathers on carotenoid diet.	Differences observed in concentration of carotenoid carrier protein. Presumed genetic differences in control of metabolism. Possible differences in follicular selectivity.	Trams 1969
7	Specific color differences.	Quantitative and qualitative difference in distribution of three pigments; major differences in canthaxanthin concentration.	Thommen 1971
8	Species specific plumage color.	Differences in patterns of fractionation, deposition and metabolism. Genetic control with dietary modification.	Fox 1962
9	Color difference in adult plumage.	Metabolic pigments not deposited unchanged from diet. Specific difference in concentration and composition.	Kritzler 1943
10	Intraspecific variation in males.	Dietary differences influenced by hormonal levels.	This study

[1] Species listed are: 1 = Scarlet Tanager (*Piranga olivacea*), 2 = tanager hybrids (*Ramphocelus* sp.), 3 = Sooty-capped Bush-Tanager (*Chlorospingus pileatus*), 4 = Gouldian Finches (*Poephila gouldiae*), 5 = flickers (*Colaptes* sp.), 6 = Scarlet and White Ibises (*Eudocimus ruber, E. albus*), 7 = Cock-of-the-Rock (*Rupicola peruviana, R. rupicola*), 8 = flamingos (*Phoenicopterus* sp.), 9 = bishop weavers (*Euplectes* sp.), 10 = House Finch.

may be the result of bacterial production in the gut. This hypothesis has not been tested directly for any avian species. The possibility of certain pigments being artifacts of the extraction or analytical procedure must be considered as well. Many invertebrates convert β-carotene to one or more keto-carotenoids. When pigmented invertebrates or plants form a major portion of the food complement of a particular bird species, the interpretation of questions regarding the presence of pathways in the bird must be made with caution (Fox and McBeth 1970, Fox et al. 1970). We believe that adequate evidence from a variety of sources indicates that a strong genetic and biochemical component influences plumage pigmentation in most avian species, and many aspects of coloration are not simply a passive function of diet (Table 1). These data indicate that small differences in chemical structure or distribution have great biological significance. This implies control at some level other than simply dietary or feeding differences. Further, the presence of similar derived compounds in unrelated birds on different diets indicates a commonality in metabolic pathways and cellular processes such as membrane selectivity.

Carotenoids are absorbed efficiently from the guts of birds (Ziswiler and Farner 1972, Fisher 1972). Absorption is associated with the presence of small amounts of fats. The selectivity of assimilation may be species-specific (Fox and McBeth 1970). Some species, for example, do not exclude pigments other than those normally found in their plumage, while others assimilate only certain types. Ingested carotenoids are transported to various organs in the plasma. They may occur in free solution but are more commonly associated with plasma lipoproteins (Cheeseman et al. 1967, Trams 1969). Lush (1963) demonstrated the presence of a carotenoid-binding protein in the blood of laying hens. Carotenoids may be stored in the liver, fat bodies, or integument and often undergo rather extensive chemical modification. The kinetics of the carotenoids in various deposits in the Phoenicopteridae have been described but the time course of depletion experiments are not yet completely in agreement (Trams 1969, Fox and McBeth 1970, Fox et al. 1970). Except for the steps leading to vitamin A production, little is known about the enzymology or kinetics of the metabolic transformations in most vertebrates.

In carefully controlled feeding experiments, Rodriguez et al. (1973) identified the intermediates in the conversion of β-carotene to astaxanthin in the goldfish (*Carassius auratus*). The evidence for the metabolic conversion is convincing. Evidence for such conversion in birds is still relatively indirect and relies on the detection of sequential intermediate compounds both in feeding experiments and in the tissues of native birds.

For example Kritzler (1943) found that different species in the genus *Euplectes* responded quite specifically in feeding experiments. Species with red plumage (*E. franciscanus*) consistently responded differently from those species with yellow or orange feathers (*E. afra, E. nigroventis*) in tests of plucking birds on carotenoid-free diets and hormone injections or in specific carotenoid feeding experiments. In *Phoenicopterus ruber* β-carotene was assimilated but not found in the blood; the liver accumulated β-carotene, echinenone, and canthaxanthin (Fox et al. 1967, Fox and McBeth 1970). The oxy-carotenes were also the major pigments of the plasma and the plumage. Lutein, zeaxanthin, lyocopene, and γ-carotene were not assimilated (Fox et al. 1970). Dietary canthaxanthin was assimilated and appeared in the blood but at lower levels than in animals fed β-carotene. Normally red *Euplectes*, on the other hand, assimilated lyocopene (from tomato) and deposited it in the plumage. These data indicate differences among species in the selectivity of the gut and in the direction and degree to which metabolic modifications of dietary carotenoids occur.

Integumentary structures often have quite well-defined pigment composition and concentrations. Both may be under genetic control. Simple examples are the polymorphism in the Sooty-capped Bush-Tanager (Johnson and Brush 1972) and the species differences in *Ramphocelus* tanagers (Brush 1970). It is not clear whether the site of specificity and selectivity is in the metabolism of a given internal organ, in a transport mechanism such as a carotenoid binding protein, or in the follicle itself. It is important to distinguish among these possibilities. Such information would contribute significantly to our understanding of the mechanisms and control for the production of tract-specific coloration in birds.

Any theory regarding the metabolism of carotenoids in birds must be adequate to account for all the data from feeding experiments, the known plumage specificity and complexities, and the variation at all biological levels (e.g. individual, sexual, and differences within and between taxa). The variability and consistencies in plumage color must be evaluated in view of the widely diverse food sources, close control of variation in intensity with the same chemical end-products (i.e. effect of concentration and feather structure), and control of metabolic uniformity in closely related forms from different habitats. The implications of these factors make simple dietary control in most cases unlikely.

The data reported here for the House Finch bear on these problems in a number of ways. In caged birds the dietary composition of carotenoids influences directly the plumage color and composition. With no keto-carotenoids available, replacement feathers either in natural molt or following plucking had a greatly reduced pigment content and consisted

exclusively of the dietary β-carotenes and hydrophilic xanthophylls. This confirms previous observations that inadequate dietary precursor is a prime cause of color loss in captive birds (Wakernagel 1963, Brush 1974). Supplemental feeding of canthaxanthin produced intensely red plumages regardless of the color of the plumage prior to feeding but had essentially no effect on females. Canthaxanthin was never found in the plumage of native birds. Several conclusions follow. *Carpodacus* will deposit pigments not normally found in their food. Thus the selectivity of neither the gut nor the follicle is absolute in reference to carotenoid structure. Further, in view of the data on carotenoid turnover kinetics in the flamingos (Fox and McBeth 1970), the dietary composition just prior to and during the molt must be the most influential factor in the final determination of color. In the absence of the normal pigment precursors, probably β-carotene, the plumage pigmentation cannot be maintained.

It is not necessary that echinenone, the major red pigment in the male plumage, be present in the food. If adequate β-carotene precursor is available, *C. mexicanus* produced this keto-carotene but no other. Excessive amounts of precursor apparently will not lead to the conversion of echinenone to canthaxanthin. That is, the steps of the carotenoid pathway include no mass action effect. On a diet of β-carotene the pigments produced metabolically in cage birds are identical to those found in the plumage of native individuals. The ability to metabolize β-carotene to echinenone was present in all naturally occurring color variants. Echinenone is also a major carotenoid in feathers of other cardueline finches (Brush MS).

In populations of wild birds from all three localities individuals with similar plumage color always contained the same pigment complement. Thus when adequate precursor levels were present, geographically different food sources had no specific effect on plumage color. The qualitative differences of the color variants were of metabolic origin. Large dietary differences in individual wild birds are difficult to imagine because of the varied diet and the propensity for this species to feed in flocks. Further, individuals in widely separated localities all deposited identical pigments indicating either a wide distribution of pigments in different foods or specific metabolic processes in closely related birds. Thus the quality and quantities of dietary carotenoids are necessary, but not completely sufficient to explain the color variation in native birds, nor can they account for all the results of the feeding experiments.

Additional observations on the nature of the controls involved in plumage pattern and color were generated by these investigations, but are based largely on negative data. For example female birds, regardless

of the types or level of dietary carotenoids, never produced a plumage approaching that of males in color intensity. Under optimal laboratory conditions or in wild individuals only tinges of yellow, orange, or red were apparent. Therefore sexual specificity must exist and presumably functions at some level other than intestinal absorption. Males showed some variation in the intensity of the pigment in replacement feathers that suggested a hormonal effect at the follicle. It is known that castrated or estrogen-treated males replace red feathers with "henny plumage" while controls (presumably on identical diets) regenerate normally pigmented plumage (Tewary and Farner 1973). The effect of testosterone on the coloration of male plumage is currently under study (Power and Brush MS). Finally, regardless of the availability of dietary carotenoids, pigments never appeared in areas of the plumage not normally pigmented. Thus there are follicular differences regarding specificity of activity. These may take the functional form of absolute thresholds.

It is possible that carotenoids are metabolized and stored in the liver and that the selectivity of deposition in the feathers is controlled by plasma proteins or transport mechanisms characteristic of the follicular cell membrane. The involvement of the avian liver in carotenoid metabolism needs additional study. In several species the liver and feather pigments are either identical or bear a precursor-derivative relationship to each other. At least one species, the Ring-necked Pheasant (*Phasianus colchicus*), does not store carotenoids in the liver (Thommen 1971). If the liver has the enzymatic capacity to convert all the precursor to a single end product, then it is unnecessary to postulate selectivity at the level of either plasma transport or follicular cells. The canthaxanthin feeding experiment indicated that the follicular cells are capable of handling members of the carotenoid pathway not normally found in the feather. The uptake of dietary canthaxanthin, the most polar molecule used, implies that no selective mechanism is absolute. If transport across cellular membranes was dependent on the polarity of the carotenoid molecule, then one would expect a sequence where keto-carotenoids would be transported preferentially to hydroxy-carotenes and β-carotenes. A chemical selectivity of this type explains the results of the canthaxanthin feeding experiments. Presumably native birds rarely ingest diketo-carotenes.

Based on the available evidence we feel that individual color differences in *C. mexicanus* males are essentially dietary in origin for birds of the same hormonal conditions, e.g. during the same season or at similar stages of maturity. It seems apparent that hormonal concentrations can alter the manifestation of plumage color, even when significant amounts

of suitable carotenoid precursors are ingested. An alternative explanation, that individual color variation results from follicular cell damage, was rejected. Conceivably wild birds that produce yellow or orange plumage may have inefficient, ineffective, or damaged follicular cells, but the plucking experiments disproved this. Plucking the mature feather is the ultimate mechanical insult and if the "damage" hypothesis held, one would expect a large amount of color variation in subsequent feather generations. None was found; in fact a high degree of uniformity was typical of experimental populations.

Control by a combination of follicular cell selectivity determined by hormonal activity and changes in metabolic capacity seem to be the best explanation for the production of plumage color, given suitable levels of dietary carotenoids. However the interactions between these two elements and the individual steps in metabolism, transport, and precursor availability that lead to the final product are still unknown.

We do not know the relative importance of dietary factors in wild populations. If diet is sufficient to explain the range of color variation found in most populations, then an interesting evolutionary dilemma appears. The red of a male is presumed to function in sexual recognition. If the intensity of red is related to the success of a male in obtaining a mate or in driving other males from a perch, nest, or feeding site, then a trait that is in large measure under environmental (i.e. diet) rather than genetic control may have a selective advantage. It would help to know if it is predominantly the first-year or physiologically immature birds that are not the normal red. To this point we refer to Michener and Michener (1931) who banded House Finches from one area over many years and kept records of color change. These authors concluded that a large number of lighter colored birds were in their first adult plumage. Of 337 birds sampled over more than one year, only 21, or about 6%, failed to acquire red coloration in a 2nd, 3rd, 4th, or 5th year of recapture. And only nine, or less than 3%, showed a retrograde color change from red to orange in a later year (none was found to go from red or orange to yellow). It is likely, then, that yellow or orange birds are younger, but it is also apparent that a great many yearlings obtain normal adult red coloration with the first basic molt. Coloration of first year males may vary geographically (van Rossem 1936). Clearly laboratory experiments on the effect of hormone levels are important. The issue of the evolution and role of male coloration can be resolved by controlled tests. Comparisons of feeding and breeding success of normal red with orange and yellow males would indicate the role of plumage color in male-male and male-female encounters.

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SUMMARY

The extensive variation in coloration of male House Finches is of interest in the study of avian carotenoid uptake, metabolism, and deposition in feathers. By feeding experiments during normal molt and following plucking we verified under controlled conditions the often noted phenomenon that House Finches without an adequate source of suitable carotenoids in the diet will regenerate feathers that are abnormal (usually yellow). A diet of seed and water supplemented with canthaxanthin or β-carotene produced feathers that appeared normal, or nearly so. Cases that were not clear-cut seem due to hormone levels, and this and other evidence indicates interaction between diet and hormone concentration at a time when feather follicles are active.

Chemical analysis shows that feathers of wild or laboratory birds that were yellow contain mixed xanthophylls and small amounts of carotenes and sometimes isocryptoxanthin (4-hydroxy-β-carotene). Orange birds show a buildup of isocryptoxanthin. The step to normal red coloration, found both in wild and in β-carotene fed experimental male birds, involved the production of a new derivative, echinenone (4-keto-β-carotene). Captive birds fed canthaxanthin (4-4′-diketo-β-carotene) deposited this pigment directly in the replacement feathers. The feathers of wild birds never contained canthaxanthin, and this pigment seems not to be a normal feather pigment of House Finches. Echinenone was found in normal wild birds from New England, California, and Hawaii, indicating no genetic geographic variation in pigment molecule metabolism or deposition. The presumed normal pathway is from dietary β-carotene to isocryptoxanthin and then to echinenone, when sufficient precursor is available.

Although the effect of hormonal interaction is not entirely clear, our results suggest that a dietary source of β-carotene and a certain level of

maturity (i.e. concentration of hormone(s)) are necessary for the acquisition of normal adult red plumage. Our results for House Finches are shown to bear on a number of problems in the metabolism of carotenoids in birds.

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