Nature of Pigments Derived from Tyrosine and Tryptophan in Animals

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IT is well known that the pigments present in the compound eyes and serosa of eggs in insects are produced from tryptophan via kynurenine and 3-hydroxykynurenine (*1*). But the nature of the pigment itself has not yet been clarified.

Recently, however, a clue to the solution of this problem has been found. We have concluded that the pigments are composed of metallic complex salts containing such metals as iron, copper, cobalt, and nickel, which are coordinated with 3-hydroxykynurenine derivatives (*2*). The concept that natural pigments are composed of metallic complex salts is by no means new and original. Many kinds of metallic complex salts, such as hemin, chlorophyll, cobalamin (vitamin B_{12}), and others are found in nature. Hence, it has been assumed that other unknown natural pigments may also be present *in vivo* as metallic complex salts.

Pigment that is assumed to be derived from tyrosine was considered. Fortunately, many kinds of mutants involving such pigments can easily be obtained. Mammalian hair, bird plumage, fish skin, and insect skins or wings were used as experimental materials. The paper chromatographic method of analysis (*2*) was used to establish a method of detection of various metals.

Pigments must be extracted by proper methods, but the procedures of extraction are not constant and vary according to the kinds of samples. Generally, it is not easy to obtain an animal pigment in pure form, and this difficulty retards progress in this field. As is shown later, however, it is not always necessary to obtain a pure pigment, except where the structure of a chelate compound is concerned. Thus, blackish, yellowish, and whitish pigments obtained from various sources were burned to ashes by dry oxidation. The temperature of ignition should not exceed 600°C, for such metals as copper and titanium are frequently lost by evaporation or by other means. After dissolving the ash in a small quantity of water or dilute hydrochloric acid, the solution was condensed to a constant volume, and a definite amount of the solution was spotted on a filter paper.

The supernatant layer of a mixture of n-butanol-acetic acid-water (4:1:5) or a mixture of acetone-n-butanol-HCl (10:4:2) was employed as the solvent. The former is adequate in detecting Fe and Cu, and the latter is convenient in separating Cu, Co, Mo, Ni, and Ti. A list of the methods for the detection of these metals is shown in Table 1 (*3*).

A simple available example is mammalian hair. About 300 to 500 mg of hair cut from a black, yellow, or white rabbit was washed thoroughly with ether, methanol, and water and was burned directly to ashes. The ashes were analyzed by the method described here, and the results shown in Fig. 1 were obtained; that is, an abundant quantity of copper, cobalt, and iron in black hairs; of titanium and molybdenum and nickel in yellowish or reddish hairs; and only of nickel in white hairs can be demonstrated. The results of one experiment are shown in Table 2.

Table 1. Methods of detection of metals.

Metal	Solvent		Color of spot		
	Acetone-butanol-HCl (Rf)	Butanol-acetic acid-water (Rf)	Rubeanic acid* in alkaline state	Pyrocatechin† in neutral or slightly acidic state	Potassium ethylxanthogenate‡ in acidic state
Cu	0.60	0.30	Dark green§	Colorless	Yellow
Fe	.75	.47	Light brown‖	Colorless	Brown
Co	.48	.24	Yellowish brown§	Colorless	Light green
Ni	.02	.23	Bluish purple§	Colorless	Orange
Ti	.17	.00	Colorless	Yellow§	Colorless
Mo	.73	Tails near .00	Colorless	Light yellow	Reddish purple§

* 0.1 to 0.5 percent rubeanic acid dissolved in 98 percent ethanol.
† 1 to 5 percent pyrocatechin dissolved in water.
‡ 1 percent potassium ethylxanthogenate disolved in water.
§ Best reaction.
‖ For Fe, yellow prussiate of potash is used in acidic state. It shows a greenish-blue color.

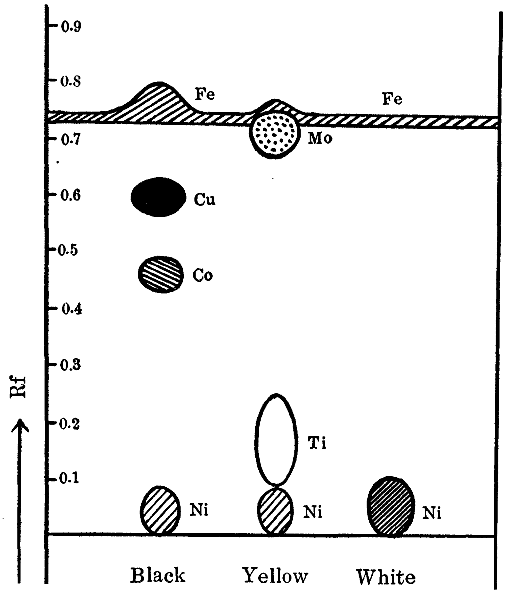


Fig. 1. A paper chromatogram showing metals involved in rabbit hairs. Solvent: acetone-butanol-HCl at 15°C.

Of course, similar results cannot be expected from all experiments. In general, in cases where a spotted individual with hair of all three colors—black, yellow, and white—was used, the black hairs contained a small quantity of Ti or Mo in addition to Cu, Fe, and Co, and the white hairs contained a small quantity of Cu or Co in addition to Ni. Although this paper is not a detailed account, some results on the amounts of these metals estimated by our quantitative method on samples similar to those shown in Table 2 are given in Table 3 for reference purposes.

Table 2. Relative values of the amount of metals present in hair of black, yellow, and white rabbits. Relative values were estimated for spots on a filter paper by A.K.A. photoelectric colorimeter with appropriate filters.

Metal	Black hair	Yellow hair	White hair
Cu	0.49	+ 0.0	± 0.0
Fe	+	–	–
Co	0.54	+ 0.0	0.05
Ni	.51	0.24	1.41
Ti	+ 0.0	.30	± 0.0
Mo	+ 0.0	.12	± 0.0

Table 3. Practical amounts of metals (given in micrograms per 10 g of fresh hair) in samples similar to those used in Table 2.

Metal	Black hair	Yellow hair	White hair
Cu	30	4	Trace
Fe	204	160	A little
Co	10	Trace	Trace
Ni	22	17	31
Ti	Trace	9	Trace
Mo	Trace	12	Trace

By consulting many experimental results, a certain relationship between coloration and type of metal was established. This is shown in Table 4. This relationship seems to be applicable to any pigment that is assumed to be derived from tyrosine, *o*-dihydroxyphenyl derivatives, or related substances.

Table 4. Relationship between coloration and type of metal. The words *leuconin* and *luteanin* are used to represent whitish and yellow-reddish pigments, respectively, in contrast with *melanin*, which refers to blackish pigments.

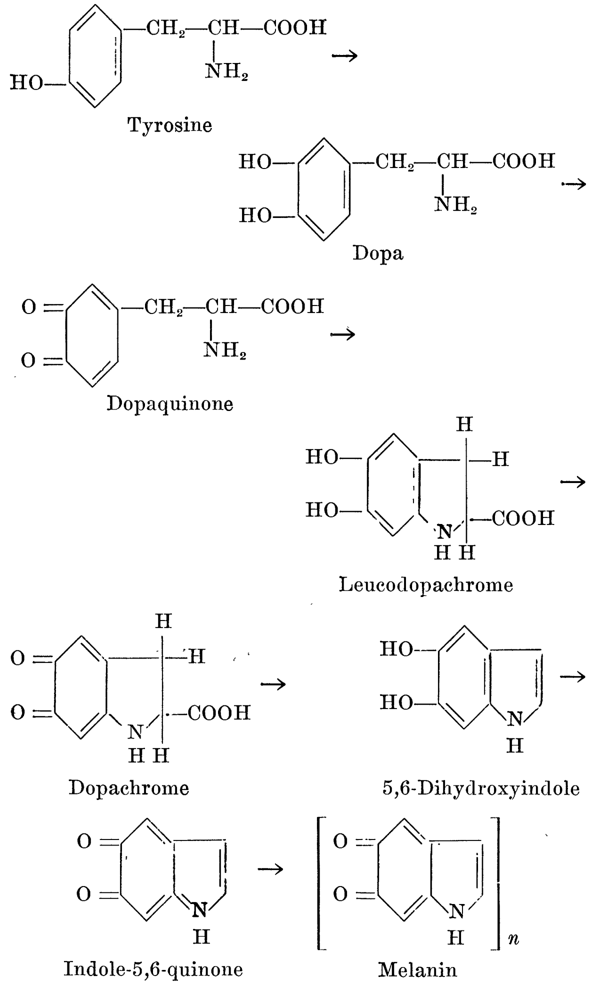
Name	Color of pigment	Related metals	Remarks
Leuconin	White	Ni	Extracted with ease
Luteanin	Yellow ~ red	Ti (yellow); Mo (red)	Extracted rather easily
Melanin	Blue ~ green ~ blackish	Cu, Co, Fe	Extracted with difficulty

Human hair also follows this rule in principle; that is, white hair of any race contains Ni in quantity, golden or brown hair of the Caucasian race contains Ti or Mo in quantity, and black hair of the Mongolian race is rich in Cu, Co, and Fe in addition to a small amount of Mo and Ti, while black hair of the Negro race contains only Cu and Fe abundantly. Although investigations have not yet been conducted on human skin, it is expected that similar results may be obtained. Thus, in a literal sense, the descriptions white, yellow, and black races may be replaced by the descriptions nickel, titanium, and copper races, respectively.

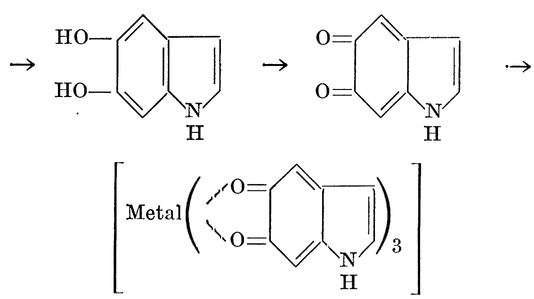
Another clear case is found in the pigment present in fish skin, although it is premature to conclude that the pigments are derived from tyrosine. Black, red, yellow, or white scales and skin from dorsal and ventral or spotted areas of such fishes as *Carassius, Cyprinus,* or *Aulacocephalus* were cut off. After being washed thoroughly with water and 80 percent methanol, the samples were dried and extracted with ether. The pigments may gradually transfer to ether. After evaporation of the ether, the pigments were burned to ashes, and the types of metal were examined. In general, copper was detected from black scales and skin, molybdenum from red, titanium from yellow, and nickel from white.

Investigations on bird plumages have not been thorough, but as far as our experiments show, white is associated with Ni, yellow with Ti, red with Mo, blue with Cu, green with Co, and brown with Fe. Combinations of Cu and Mo give purplish plumages. These relationships are generally applicable to the pigments present in insect cuticles.

In order to explain these phenomena, what sorts of assumptions can be considered? According to the well-known scheme proposed by Raper (*4*), Mason (*5*), and others, it is assumed that melanin pigments are derived from the following chemical processes.



According to our assumption based on the metallic complex salts, various pigments may be produced from a common precursor (probably indole-5,6-quinone or the related substance), as is shown in the following scheme. In other words, a fundamental coloration may be determined by a type of metal. But it is also probable that a slight difference in the structure of chelate compound (ligand) may be involved, together with the difference in metal.



Additional evidence to support the assumption that the natural pigments related to tyrosine are composed of metallic complex salts is provided by the following model experiment. To 4 ml of dihydroxyphenylalanine (dopa) solution (0.5 mg/ml) in which contaminated metals have been removed by diphenylthiocarbazone (dithizone), there is added 1 ml of a solution containing 50 to 100 μg of the metal in question. A small quantity of metal-free ammonia water is added, and the *p*H is adjusted to 10–11. The solution is permitted to stand for 15 to 60 min to undergo nonenzymic oxidation. By this procedure, metallic complex salts may be produced. Ultraviolet radiation induces the reaction to proceed more rapidly. Although the coloration changes gradually with the lapse of time, the solution containing Cu or Co becomes more blackish than the control, the Ti- or Mo-containing solution becomes yellow or reddish-yellow, and the Ni-containing solution shows a light yellow or pinkish color. These results conform well to those of extractions of natural pigments. Furthermore, when the dopa solution is added to the ashes of hair of different coloration, color solutions similar to extractions of natural pigments are easily obtained. An analogous phenomenon may be found also in cases where adrenalin (epinephrine) or pyrocatechin is used instead of the dopa solution.

Here the cumbrous behavior of iron should be noted. It is evident that Fe^{++} gives a yellow-brownish color, and Fe^{+++} a red-purplish color in the model experiment. But it is very difficult to remove traces of iron completely from various chemicals and reagents, and this often causes misjudgments in the results, not only in the model experiment, but also in the analysis of natural pigments. Thus, in Table 4, iron is assigned provisionally to the melanin group, but, strictly speaking, it may be proper to assign iron to a new group altogether. This question should be considered in future studies.

There is no *a priori* ground that only the types of metals shown in Table 4 are concerned in pigment formation. Presumably, a number of new metals may be assigned to leuconin, luteanin, melanin, or to other new groups in the future.

Furthermore, some may view differently the mechanism of pigment formation, even if the hypothesis of metallic complex salts be accepted. For example, as is believed generally, the chain reaction tyrosine→leuconin→luteanin→melanin, or its modification, may be considered in lieu of our assumption described in preceding paragraphs. It is also possible to assume that these pigments are derived from quite different sources. The validity of these possible assumptions needs to be discussed thoroughly in the future.

Next, the question why certain metals are accumulated in particular parts of animals to form pigments should be taken up. This difficult question cannot be answered fully at present. However, a hypothesis is proposed here, based on the following experimental facts. As we have shown (*2*), an adult fly of *bw* (brown) or *cn* (cinnabar) type of *Drosophila melanogaster* contains Co abundantly in its body, whereas the fly of t^3 (tan^3) or *w* (white) type contains Ni instead of Co, despite its being reared on the same medium. A more crucial case has been found in *Bom-*

byx mori. As is shown by Tanaka (*6*), the newly hatched larva of a mutant called dominant-chocolate (*I-a*) has reddish-brown pigments in its skin, in contrast to black in a normal type. As is pointed out by Harizuka (*7*), these pigments are mainly composed of fuscanine derived from tryptophan. All the eggs produced by a female moth from a cross *I-a*/+ ♀ × +/+ ♂ show a uniform egg color and contain the same kinds of metals. But, in the offspring, two types of larvae segregated out, namely, black and chocolate, in the ratio of 1 to 1. If these two types of larvae are divided and left without food (mulberry leaves) for a few days, they evacuate their feces and die shortly. The results of an examination of the types of metals involved in the larvae and their feces, respectively, are shown in Table 5.

Table 5. Types of metals involved in the bodies of larvae and feces of *Bombyx mori.*

Metal	Black (+)		Dominant-chocolate (*I-a*)	
	Body of larva	Feces	Body of larva	Feces
Cu	+	±	±	+
Fe	++	±	±	++
Co	±	+	+	±
Ni	++	±	±	++

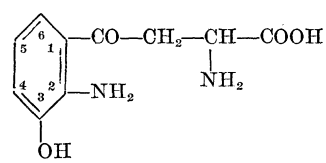
It is apparent that, even though these two types of embryonic larvae were reared under the same conditions, one type (normal) is able to preserve Fe, Cu, and Ni in its body, whereas the other type (*I-a*) is unable to do so. But, with regard to Co, this relationship is reversed. Biochemical and genetic studies show that a fundamental function of the *I-a* gene inhibits the formation of Fe-fuscanine in the skin of a newly hatched larva. It cannot be stated conclusively whether, in Table 5, variations in the amount of metals other than Fe have been caused by pleiotropic functions of the *I-a* gene itself or by other genes linked with the *I-a* gene. Yet this fact indicates clearly that the selection of metals occurs according to the genetic constitution of a larva.

Although the details will be described elsewhere, the assumption has been made that a particular protein or a nucleoprotein that is controlled by a special gene may have the potentiality of combining with a particular type of metal. When the metal protein has an enzymatic function, the metabolite produced by the enzyme may remain coordinated with the metal which operates as an active center of the enzyme in a certain condition or stage, in those cases where the product has the property of chelation. Thus, according to the foregoing assumption, the mechanism of pigment formation may be diagramed as shown at the bottom of this page. Of course, this is only one possible case.

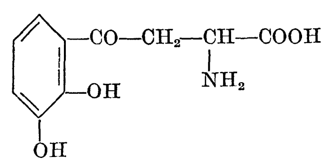
The coloration of an animal may be determined by various combinations of these pigment types, and it is obvious that various gene functions are concerned with expression of the coloration (*8*).

A similar concept may be applied to other cases in the formation of natural pigments. As is stated in a previous paper (*2*), tryptophan pigments may be produced from 3-hydroxykynurenine (I) as a common precursor. In this case, it is clear that an amino group of the 2-position and a hydroxy group of the 3-position of hydroxykynurenine are essential portions of chelation, because kynurenine or 3-methoxykynurenine is quite ineffective for producing the pigment in either the biological or the model experiment.

It is of interest to note here that 2,3-dihydroxybenzoylalanine (II) gives rise to a bright yellow color in cases where the substance is fed to the *cn bw* larvae of *D. melanogaster*. When the substance is coordinated with cobalt, a similar bright yellow color solution can be obtained. In fact, the *cn bw* fly of *D. melanogaster* contains a large amount of cobalt in its compound eyes. This fact seems to indicate another evidence that the natural pigment is composed of a metallic complex salt.



(I)



(II)

It is well known that various kinds of pterins are present in wings of butterflies, together with other

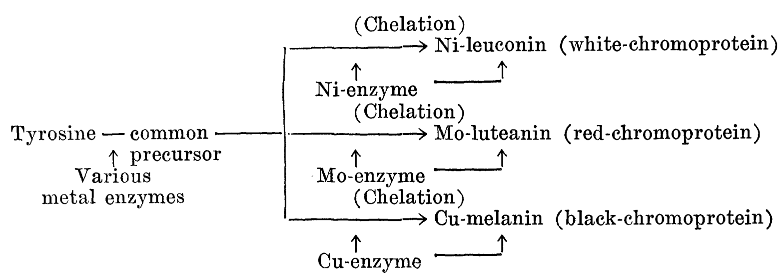


Table 6. Metals in mammalian hairs (μg/10 g fresh hair). Blanks indicate undetermined.

Animal	Color of hair	Cu	Fe	Co	Ni	Ti	Mo
Guinea pig*	Dominant white	23		0	47		0
	Yellow	52		0	17		3
	Black	80		3	25		0
Human being	Blonde (Caucasian race)	60	11	0	7		3
	Median brown (Caucasian race)	80	68	11	19	6	9
	Black (Mongolian race)	190	290	6	13		3
	Black (Negro)	310	200	0	3	0	0

* Samples obtained from the same individual.

different types of pigments. Our preliminary experiments seem to show that a particular pterin, which is extracted from one source as pure as possible, contains a particular metal. It is of interest especially that xanthopterin fractions always contain titanium. This fact suggests that Ti may be concerned with an active center of folic acid.

Carotenoids also have properties of chelation. If our assumption is applicable in this case, it may be that a more appropriate explanation can be given to the nature and function of photochemical substances present in the retina of animals.

Summary. Based on the concept that the pigments derived from tryptophan in insects are composed of metallic complex salts, a hypothesis that other unknown natural pigments may be present *in vivo* as metallic complex salts is proposed. A certain relationship between coloration and the type of metal is found especially in pigments assumed to be derived from tyrosine: namely, white is associated with Ni, yellow with Ti, red with Mo; blackish color involving blue, green, and brown is associated with Cu, Co, and Fe.

In order to account for these phenomena, it is suggested that each pigment is produced by a function of an enzyme having a particular metal as a prosthetic group, and that this enzyme function is controlled by a particular gene.

Addendum. After the manuscript was submitted, some additional data favorable to our hypotheses were accumulated: (i) Table 6 shows the results of quantitative analyses of metals in mammalian hairs. (ii) According to some authors (for example, D. L. Fox, *Animal Biochromes and Structural Colours,* Cambridge Univ. Press, New York, 1953), the pigment involved in fish skin and scale may be astacin, a kind of carotenoid. But this carotenoid has an *o*-quinone structure in its ionone nucleus. Hence, a relationship similar to that shown in Table 4 may be formed. (iii) Our recent studies indicate that there are analogous relationships between kinds of metals and anthocyanin or carotenoid pigments in petals or pericarps of plants, namely:

Anthocyanins: Red — Fe; blue or purple — Mo; white — Ni or Cu.
Carotenoids: Red — Fe; yellow — Ti; white — probably Cu or Ni.

It is of great interest that the corresponding metals are present in quantity in seeds or bulbs, even though they have no visible pigments in endosperm and embryo. Thus, it is possible to guess which kinds of flower-colors or pericarp colors the seeds or bulbs were derived from. Furthermore, predictions of colors may also be possible by only ashing the seeds or bulbs in cases where they are pure strains.

References

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It is the great beauty of our science, that advancement in it, whether in a degree great and small, instead of exhausting the subject of research, opens the doors to further and more abundant knowledge, overflowing with beauty and utility.—M. FARADAY.