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THE CONTROL OF PIGMENT FORMATION
IN AMPHIBIAN LARVAE

BY
MYRTLE E. JOHNSON

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MYRTLE E. JOHNSON

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A. INTRODUCTION

The experiments considered in this paper grew out of a series of feeding experiments carried on with larvae of *Hyla regilla* and *Rana* sp. While size differences only were considered at first, color differences soon became so marked as to demand attention. The amount of black pigment in the different lots of tadpoles varied considerably and this variation was not correlated with the size of the tadpole. It was apparent that certain foods

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contain elements which govern the formation of pigment independently of the effect of the food upon the size of the organism.

It was found that most of the tadpoles showed a large or medium amount of pigment excepting those that were fed on yolk of egg, which showed a much smaller amount. Experiments showed that when lecithin was fed along with foods which ordinarily produced much black pigment that a much smaller amount of pigment was produced. Experiments with tyrosinase showed that the tyrosinase reaction could in a measure be inhibited by the addition of lecithin or the products of digestion of egg yolk. The experiments thus give an example of a chemical substance inhibiting the tyrosinase reaction and when fed to the tadpoles inhibiting to some extent the production of melanin pigment in the epidermis.

This investigation has been carried on in the zoological laboratory of the University of California under the direction of Professor Harry Beal Torrey, and I am greatly indebted to him for his continued help and encouragement. I also wish to express to Professors T. B. Robertson and H. C. Biddle of this University my thanks for their kind interest and assistance in questions of physiological and organic chemistry.

B. CURRENT THEORIES OF PIGMENT FORMATION

In relating these results to the current theories of color formation and inheritance it will be convenient to consider Weismann's germinal selection theory, aspects of the Mendelian theory of inheritance, and the results of recent biochemical investigation.

Weismann postulates determinants, aggregations of ultimate "vital units" capable of transmission through the germ cells and able to determine "hereditary characters" of the body. Their presence determines the specific development of a particular part of the body which may consist of a group of cells, a single cell or a part of a cell. A struggle for existence between the biophores, the smallest elements composing the determinants, continues throughout the development of the embryo, different biophores being able to appropriate different amounts or kinds of nourishment. These differences in nourishment cause inequalities in the

growth of the different biophores which produce differences in the constitution of the determinants and consequently qualitative variations in the organism. These variations may be spontaneous resulting from intra-germinal nutritive conditions and therefore not affecting all the ids at once or they may be brought about by extra-germinal influences affecting all the ids at the same time.

Among Mendelians the factor hypothesis plays an important role. Castle (1909) names the following color factors which he finds in the case of the gray rabbit:

- “Symbol C. A common color factor necessary to the production of all pigment, wanting only in albinos.
- “ B. A factor for black, some substance which acting upon C produces black pigmentation.
- “ Br. A factor for brown, some substance which acting upon C produces a chocolate-brown pigmentation.
- “ Y. A factor for yellow, some substance which acting upon C produces yellow pigmentation.
- “ I. An intensity factor, which determines whether the pigmentation shall be intense (as in black and in yellow), dilute (as in blue and in cream), or of some intermediate degree of intensity.
- “ A. A pattern factor which causes the black or brown pigments to be excluded from certain portions of the individual hairs, where yellow then shows. A “ticked” gray coat results. When this factor is present the under surfaces of the rabbit (tail, belly) are unpigmented (white). The symbol, A, stands for agouti, this factor having first been demonstrated in the “agouti” guinea-pig. (See Castle, 1907.)
- “ U. A factor for uniformity of pigmentation (in distinction from spotting with white, S).
- “ E. A factor governing the extension of black and of brown pigmentation, but not of yellow. When most restricted in distribution the black or brown pigments are found in the eye and in the skin of the extremities only, but not in the hair, when more extended they occur also in the hair generally.”

The various types of coloration seen in different rabbits are represented by means of various arrangements of these symbols—in fashion resembling the formulae for the constitution of molecules of organic compounds.

As to the form and composition of these Mendelian factors, Castle says we can at present give no satisfactory answer, but he adds (p. 68): "It is, however, we think, not necessary to suppose that there exist in the minute germ-cell as many complex organic substances as there are activities of the cell; neither is it necessary to suppose a different substance present for every independent factor identified. The various independent factors may have a basis no more complicated than that of so many atoms attached to a complex molecular structure. Experiment shows that the factors may be detached one by one from the organic complex. The discontinuity of their coming and going is entirely in harmony with the conception of them as components merely of complex molecular bodies." Such a view attempts to provide for segregation of characters without discussing vital units.

Among the chemists is Dewitz (1902) who experimented with fly larvae (*Lucilia Caesar*) and found that while there was no tyrosinase in very young larvae (one or two days old) older larvae contained a considerable quantity. When the pupae formed they rapidly became pigmented, but this pigmentation could not go on without oxygen.

Phisalix (1905) working with cockroach larvae, found that the larvae when hatched were colorless, but within three hours changed through grey and brown to black. Phisalix concludes that tyrosin and tyrosinase exist in the embryo long before the color develops and that "it is probable that they coexist in the egg or that they are deposited at the time of ovogenesis."

Roques (1909) found that during the metamorphosis of *Limnophilus flavicornis* Fabr., the amount of tyrosinase in the body was greatest just before pigmentation began and as the amount of pigment increased, the tyrosinase decreased, until it was entirely absent when the beetle was fully pigmented.

Riddle (1909, p. 329) reviews our knowledge of melanin color formation and urges the futility of piling up factors one for every color in the germ to explain the production of melaninic color since without them "in an animal that produces melaninic color, there exists all the machinery necessary to produce a series or scale of these colors." He continues (p. 336): "This means that the animal that transmits the enzyme

for black, i.e., produces black-colored offspring must *at the same time* transmit also the enzyme for *brown, chocolate, red, yellow, etc.* (more accurately, an enzyme for each step of oxidation from tyrosin to black melanin), *without the absence of a single one.*" In a footnote (p. 337) he adds: "The sort of specificity of enzymes that has thus far been assumed by the Mendelians has, however, been of a different sort; namely, that for the production of each color only one enzyme is necessary, but the enzyme which produces any particular color is specifically different from those which produce other colors;"

Riddle (1909, p. 336) maintains that with tyrosinase able to produce a series of colors, several of Castle's factors (A, B, E, I, and D) may be reduced to one, tyrosinase, while C, the chromogen, may be left out entirely as a factor since such a chromogen is "universal in protoplasm." In conclusion he asks: "Is it too much to expect that the further application of such tests as the one here presented in outline for the melanin colors will in the end remove *many* of the Mendelian 'factors' from the germ cells? That many of their 'characters' will come to rest on a more proximate basis; will be known to have their 'determination' and origin in very general germinal powers, and in somatic conditions obtaining previous to, or at the time of, their development?"

Gortner (1910a, 1911a, 1911c) has recently published results of similar investigations on the meal worm, cicada, and potato beetle. He finds that in the meal worm the chromogen is secreted only as needed for pigmentation and is present in exceedingly small amounts at any one time, that tyrosinase is present in both the pupa and beetle, but the chromogen is apparently lacking in the pupa stage, the only stage without pigmentation.

In the case of the periodical cicada, the tyrosinase is not found in the body of the pupa or the adult but is apparently secreted with the new cuticula since the oxidase is present in water in which the newly emerged adults have been washed. In his study of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) Gortner finds the pigmentation "produced by the interaction of an oxidizing enzyme of the tyrosinase type, and an oxidizable chromogen. The color pattern is caused by the

localized secretion of chromogen." Gortner (1911d) after further investigation of different melanins concludes that they are formed by the interaction of an oxidase and an oxidizable chromogen. He finds that there are at least two types of melanin which may be differentiated by their appearance and by their solubility in acids. He thinks it probable that the two types are formed by the oxidation of different chromogens.

Some of these later results, it seems to me, lead one to take a little different view of the situation from the one taken by Riddle. He (1909, p. 334) speaks of the different colors of the tyrosin series as obtainable by different degrees of oxidation, for instance he says: "At present the *biological* data are wanting to quantitatively seriate all of the several colors; but there is apparently enough data to warrant the definite statement that *yellow mice* are forms with the power to oxidize tyrosin compounds to an *intermediate* point." If we adopt this view we are under the necessity of postulating some chemical difference between the oxidase in yellow mice and that in brown mice that causes this difference in oxidation.

The results of Bertrand (1908) and of Abderhalden and Guggenheim (1907, 1908) show that the end result of the series of colors shown by melanin pigment is different when the tyrosin exists in different combinations, or when different chromogens are used. Gortner showed that the spots on the elytra of the potato beetle are due to a localized secretion of the chromogen rather than to a difference in the oxidase which is apparently present over the whole area. It would appear from these facts that slight differences in color between two individuals are due to slight differences in the combination in which the chromogen exists (or existed when the pigment was produced) and that variations in the marking of different individuals are due to slight variations in the character or diffusion of the chromogen in different localities.

The difference in color in the case of the tadpoles here described seems to be brought about by a reduction in the amount of black pigment rather than by a change in the color of the pigment. This reduction in the amount of black pigment allows

the epidermal pigments of other sorts and colors to come into greater prominence.

One must distinguish then between differences in coloration which involve actual differences in the color of one or more of the pigments and those which involve differences in the proportional amounts of the different pigments. Although the inhibitors here discussed merely reduce the amount of melanin, instances cited show that the tyrosinase reaction may be so affected by certain chemical substances that the color of the resulting pigment is different from that of ordinary melanin. The "factors" postulated by Mendelians may operate in this latter fashion, also the chemical substances cited below which are able to change the color of the plumage of birds undoubtedly act in this way.

C. THE RELATION OF AMOUNT OF NUTRITION TO PIGMENTATION

Weismann's view that color is profoundly influenced by nutritive conditions is supported by Tornier's experiments in pigment control. My results with similar experiments, however, do not support this view.

Tornier (1907, 1908), experimenting with *Pelobates* larvae, divided the tadpoles into lots for differential feeding. He found that tadpoles receiving a minimum amount of food (algae, together with varying amounts of fish) contained little or no pigment and that by feeding increased amounts of fish it was possible to change the epidermal coloring from white through yellow, red, and grey, to black. Comparable effects were produced by removing a portion of the yolk from the egg. This reduced the nutrition of the animals and produced albinism, erythrism, or blackness depending upon the state of nutrition. He also found that the experiments could be carried in the opposite direction and that through a diminution of feeding, well fed black larvae could be changed back through a series of colors in the order of black, brown, red, grey, white. He sought to show not only that increase in food supply caused a greater development of pigment in the chromatophores but also that the pigment

granules of the chromatophores acted as reserve material in cases of inanition.

Experiments similar to these of Tornier were carried on by me during two successive seasons with *Hyla* and *Rana* tadpoles. The results do not confirm those of Tornier, since the tadpoles receiving a small amount of food were no lighter than those receiving an abundance.

The tadpoles from a single egg mass were divided into three lots and each lot given a different amount of food. As it is impossible when feeding graduated amounts of food to be sure that the tadpoles in a given dish share the food alike, instead of placing different amounts of food in the different dishes, the food was withheld from the dishes for different lengths of time. On days when the tadpoles received food they were given as much as they could eat, and on other days all food was removed from the dishes. One set of tadpoles was fed every day, the food of the second set was withheld every third day and that of the third set was withheld every second day. The three sets of tadpoles thus received large, medium, and small amounts of food approximately in the ratio of $1 : \frac{2}{3} : \frac{1}{2}$.

The growth of the tadpoles was proportional to the amount of food received and the amount of pigment seemed also to be proportioned to size since the large, medium, and small tadpoles on the same diet were all of the same color, those that were half starved being *no lighter than those that were large and well fed*. In order that this might not be a prejudiced judgment the different sets of tadpoles were repeatedly submitted to other observers before explaining the object of the experiment. The verdict always was that the tadpoles were all the same color or that the smaller ones were a little darker. The experiments were performed for two successive years with the same result (table 1).

TABLE 1

Length measurements and color of tadpoles fed on different amounts of food. Tadpoles of lots A and B were all the same size when the experiment began Feb. 10, 1912; those of C and D were the same size when the experiment began, April 28, 1911. Length measurements for lots A and B represent average length of all tadpoles in the dish, those for C and D, the average of several typical tadpoles.

A. *Rana* tadpoles fed on liver

Amount of food	Length in mm.			Number of tadpoles	Color
	Feb. 13	Feb. 20	Mar. 6		
Full amount	16.1	21.4	27.4	8	Dark
$\frac{2}{3}$ full amount	15.4	20.2	24.0	8	Dark
$\frac{1}{2}$ full amount	15.6	19.1	22.5	8	Dark

B. *Rana* tadpoles fed on egg yolk

Amount of food	Length in mm.			Number of tadpoles	Color
	Feb. 13	Feb. 20	Mar. 6		
Full amount	15.3	21.3	28.1	8	Light
$\frac{2}{3}$ full amount	15.0	19.5	24.4	8	Light
$\frac{1}{2}$ full amount	15.4	19.1	23.2	8	Light

C. *Hyla* tadpoles fed on liver

Amount of food	Length in mm.		Number of tadpoles	Color
	May 10	June 3		
Full amount	16	30	6	Dark
$\frac{2}{3}$ full amount	13	23	6	Dark
$\frac{1}{2}$ full amount	12	20	7	Dark

D. *Hyla* tadpoles fed on egg yolk

Amount of food	Length in mm.		Number of tadpoles	Color
	May 10	June 3		
Full amount	20	32	4	Medium light
$\frac{2}{3}$ full amount	14	24	4	Medium light
$\frac{1}{2}$ full amount	13	18	5	Medium light

Owing to accidents and the small allowance of food, most of the tadpoles living on half the regular fare died before metamorphosing. I have one preserved specimen, however, which is about half the normal size and hardly more than skin and bones, but is as deeply pigmented as any of the larger specimens.

Tornier's experiments with yolk removal were also imitated with the *Hyla* and *Rana* tadpoles. Here again it was found that the tadpoles which had lost yolk, though smaller than the others,

were not necessarily lighter colored. A part of the yolk was removed from a number of tadpoles by sucking it through a capillary tube. Approximately half of the yolk was removed from the *Rana* tadpoles and a larger proportion from the *Hyla* tadpoles. The yolk was not removed until the embryos were quite large—nearly ready to hatch—so it is probable that in the case of *Hyla* at least, a portion of the alimentary tract was removed with the yolk.

The two lots of *Rana* tadpoles, those from which the yolk had been removed, and the control presented practically the same appearance: both were dark grey, but those which had suffered the loss of yolk were no lighter than the control (table 2).

TABLE 2

Length measurements of tadpoles from which yolk has been removed. Measurements represent average lengths of all tadpoles in the dish.

A. *Rana* tadpoles. Yolk removed Feb. 2, 1912

	Length measurements in mm. taken					Feb. 26	
	Feb. 6	Feb. 9	Feb. 13	Feb. 20	Feb. 26	Number of tadpoles	Color
Yolk removed	10.3	12.5	13.8	15.0	14.8	8	Medium
Control	9.9	12.2	14.5	14.9	14.9	5	Medium

B. *Hyla* tadpoles. Yolk removed Feb. 19

	Length measurements in mm. taken				Mar. 2	
	Feb. 20	Feb. 22	Feb. 26	Mar. 2	Color	Number of tadpoles
Yolk removed	7.87	9.08	9.55	9.4	A few somewhat lighter than control	3
			(10 tadpoles)			
Control	8.1	9.51	10.15	10.05	Medium	9
			(10 tadpoles)			

The two lots of *Hyla* tadpoles showed slight differences. Some of the individuals that had suffered the loss of yolk were as dark as the control tadpoles but others were a little lighter. Microscopical examination shows that these lighter individuals have about the same number of pigment spots, but that some of the spots are smaller and some are a little lighter than those of the control tadpoles.

This difference seems to me to be a direct result of the loss of nourishment—the amount of pigment is less just as the length and width measurements are less and the muscles and other

tissues appear more transparent. The yolk loss has meant to the tadpole a loss of substances from which directly or indirectly pigment is ultimately formed, as well as a loss of tissue forming substance. The very smallness of the differences indicates that they are due to a lack of material for tissues and pigment building rather than to a using up of pigment once formed.

This view is supported by Riddle's investigation of pigment formation in the feathers of young birds. Although there is little definite knowledge concerning the formation of the particular melanins present in this instance or the other cases cited, it is highly probable that they are formed similarly and that they are equally dependent upon or independent of nutrition.

After carrying on feeding experiments with young birds, Riddle (1908) concludes that pigment and barbule forming cells are both reduced in rate of production relative to growth in certain other parts of the feather because of the less favorable relations which the pigment producing cells bear to the nutriment carried by the blood. He says, "In just the same way that a lack of nutrition checks the production of barbule forming cells, it reduces the amount of pigment formed and taken up by the barbule cells."

There is in this instance as in the case of the tadpole, a loss of certain tissue building substances along with a loss of pigment forming substances. The "less favorable relations which the pigment producing cells bear to the nutriment carried by the blood" may make the difference between daily and nightly growth more marked here than in other places but the difference is probably present in other parts though from the nature of the structure it is less easily discovered.

Similarly, in reporting observations on *Amblystoma tigrinum*, Powers (1908, p. 38) says: "I have kept many adults, young and old, for three and even four years, and have subjected them to very varying conditions of nutrition, temperature, etc., and by means of photographs I have compared the appearance of many during successive seasons. Starvation does, of course, produce a marked effect on many organs and upon the animal's whole appearance, save color."

These instances and the results of the experiments all point to the same conclusion, that with reduced nutrition there is a reduced pigment production along with a reduction of tissue or tissue formation with the result in the case of the tadpole that the color of the animal appears neither darker nor lighter.

D. EFFECT OF VARIOUS KINDS OF FOOD UPON PIGMENTATION

Of especial interest in a discussion of the effect of food upon the color of animals are the following classical examples cited by Darwin (1868, vol. 2, p. 337) in "*The Variation of Animals and Plants under Domestication*." He says: "It is well known that hemp-seed causes bullfinches and certain other birds to become black. Mr. Wallace has communicated to me some much more remarkable facts of the same nature. The natives of the Amazonian region feed the common green parrot (*Chrysotis festiva*, Linn.) with the fat of large Siluroid fishes, and the birds thus treated become beautifully variegated with red and yellow feathers. In the Malayan archipelago, the natives of Gilolo alter in an analogous manner the colours of another parrot, namely the *Lorius garrulus* Linn., and thus produce the *Lori rajah* or King-Lory. These parrots in the Malay Islands and South America, when fed by the natives on natural vegetable food, such as rice and plantains, retain their proper colours. Mr. Wallace has, also, recorded (A. R. Wallace, *Travels on the Amazon and Rio Negro*, p. 294) a still more singular fact. 'The Indians (of S. America) have a curious art by which they change the colours of the feathers of many birds. They pluck out those from the part they wish to paint, and inoculate the fresh wound with the milky secretion from the skin of a small toad. The feathers grow of a brilliant yellow colour, and on being plucked out, it is said, grow again of the same colour without any fresh operation.'"

Romanes (1895, vol. 2, p. 218) cites the above instances of changes in the plumage of birds and the fact also noted by Darwin that canaries become red when fed on cayenne pepper, and adds: "Dr. Sauermann has recently investigated the subject experimentally; and finds that not only the finches, but likewise other birds, such as fowls, and pigeons, are subject to similar

variations of colour when fed on cayenne pepper; but in all cases the effect is produced only if the pepper is given to the young birds before their first moult. Moreover, he finds that a moist atmosphere facilitates the change of colour, and that the ruddy hue is discharged under the influence either of sunlight or of cold. Lastly, he has observed that sundry other materials such as glycerine and aniline dyes, produce the same results; so there can be no doubt that organic compounds probably occur in nature which are capable of directly affecting the colours of plumage when eaten by birds."

These facts indicate that significant variations in color are produced by different *kinds* of food and the following experiments with tadpoles bear out the suggestion.

Both *Rana* and *Hyla* tadpoles were used for these experiments. Different bunches of eggs were separated so that the larvae used for one set of experiments were from a single egg mass. As soon as the tadpoles hatched they were divided into different groups and each group was fed on one kind of food. The water in the dishes was changed every other day and the tadpoles given fresh food. In the later experiments the table and the sides of the dishes were covered with black paper. This allowed the light to enter only from above and insured equal illumination for all the dishes. The foods first used were beef liver, egg yolk, egg albumen, beef suet, brown and white beans, white bread, and fish. The foods were all cooked by boiling and finely divided by being put through a sieve or rubbed between the fingers. A few measurements given in the following tables will show the size relations fairly well. The intensity of the color is also noted (table 3).

TABLE 3

Length measurements and color of tadpoles fed on different kinds of food. Measurements represent average of several typical tadpoles from each dish.

A. *Hyla*. Experiment started Feb. 18, 1911

Food	Length in mm.		Color	Number of tadpoles	
	April 18			Feb. 18	Apr. 8
Liver	38.0 mm.		Dark	7	3
Yolk	40.0		Light	7	2
Bread	31.0		Medium	7	7
Fish	22.5		Medium	7	2
Albumen	26.0		Dark	7	3

B. *Hyla*. Experiment started Apr. 17, 1911

Food	Length in mm.		Color	Number of tadpoles	
	May 8	June 3		Apr. 17	June 3
Liver	25	37	Dark	4	3
White beans	22	35	Medium	5	4
Brown beans	21	40	Medium	5	5
Suet	14	16	Dark	5	4

C. *Hyla*. Experiment started Apr. 17, 1911

Food	Length in mm.		Color	Number of tadpoles
	Apr. 22	May 8		Apr. 17
Egg	13	22	Light	4
White beans	13	20	Medium	5
Brown beans	11	22	Medium	5
Suet	11	14	Dark	5

To see if it would be possible to lighten the color of tadpoles that were already strongly pigmented, some very dark tadpoles collected from a pond were fed upon yolk of egg. In nine days they were distinctly lighter than tadpoles from the same source that had been kept in the aquarium on a mixed diet. I do not regard this as a disappearance of pigment already present in the tadpole, but rather as a decrease in pigment production relative to growth. The proportion of pigment formed is less with egg yolk as food, so that the ratio of pigment to body size decreases, until within nine days, the difference in color is noticeable.

If we assume an oxidase reaction as the basis of pigment formation in the tadpoles we explain a decrease in the rate of

pigment formation by a decrease in the amount or power of the oxidase or by a decrease in the amount of the chromogen or by the presence of something which inhibits the action of the oxidase.

The oxidase in these experiments was certainly not administered to the animal directly, since all of the foods were boiled before being given, and oxidases are destroyed at this temperature. Therefore the oxidase was produced by the organism. If the difference in pigmentation was due to a greater amount of oxidase, the later could have been furnished only indirectly by the foods.

In considering the amount of chromogen contributed by the foods, one must consider their proteid content, since tyrosin, a common chromogen, is a decomposition product of the digested proteids. A study of the analyses of the various foods shows that the pigmentation is not correlated with the amount of proteid, since egg yolk which produced little pigment contains a large percentage of proteid, larger than beans, which produced more pigment. From the table it will be seen that while pigmentation may depend upon the kind of proteid fed, it cannot depend upon the gross amount of proteid (table 4). A few tabulations of the cleavage products of these foods are available (see table 10). It is notable that in these ov-albumen shows a larger percentage (2.4%) of tyrosin than does vitellin (1.6%). The differences are very slight, but it will be remembered that the differences in pigmentation are also small, and that tyrosin is so slightly soluble in water that artificial melanins are formed with very dilute solutions of the chromogen.

TABLE 4

Analyses of the foods used in experiments, from Hammarsten, "A Text Book of Physiological Chemistry," trans. by Mandel (1911, p.882-3).

	1000 parts contain						Relation of 1:2:3		
	1	2	3	4	5	6	1	:2	:3
	Proteids and ex- tractives	Fat	Carbo- hydrates	Ash	Water	Waste			
Beef liver	196	56	11	17	720		100	28	0
Egg yolk	160	307		13	520		100	192	0
Egg albumen	103	7	7	8	875		100	7	7
Beans	27	1	66	6	888	12	100	4	244

It is quite possible that the two causes, perhaps three, always operate to some degree, that a strong-pigment forming food such as liver may furnish in the digestive process a large proportion of tyrosin or some other chromogen, and at the same time furnish few inhibiting factors. The third factor, not so easily accessible to experiment, may be an increased formation of the oxidase by a strong pigment-forming food.

The large proportion of fat in egg yolk suggested that the lecithin of the yolk might be the element that in some way brought about a decrease in pigment production. The results of Danilewsky supported the suggestion that lecithin might be an inhibitor, possibly the only one.

Danilewsky (1895) placed tadpoles in a solution containing one part of lecithin to 15,000 parts of water. Other tadpoles were kept in water alone to serve as a control. The former became three times heavier and nearly twice as long as the corresponding controls. He thought the effect was due to the stimulating effect of the lecithin rather than to any great amount of nourishment contained in it. He adds: "Il faut encore noter que tous les têtards *lécithiniques* étaient beaucoup moins pigmentés que les larves de contrôle." He found also that when lecithin is injected into young rabbits or dogs there is a marked increase in their growth.

Miss King (1907) conducted a series of feeding experiments with large numbers of toad tadpoles. She refers to an apparent stimulating effect of lecithin in egg yolk and mentions meat as a good pigment producer.

Goldfarb (1910, p. 272) reviews the results of these and other experiments with lecithin and reports upon similar investigations of his own. He says, "Frog and toad tadpoles were placed in graded lecithin solutions ranging from one part of lecithin in 20,000 of water to toxic solutions of $\frac{1}{50}$ and kept therein throughout their period of metamorphosis (33 to 51 days). Other conditions, such as temperature, amount of solution, number of tadpoles in each dish, food, etc., were constant for each series. The control tadpoles showed at the end of the experiment a variation of 9 to 53 per cent in weight, 3 to 44 per cent in length. About 1,000 tadpoles kept in lecithin solutions showed a maxi-

imum variation of 23 to 64 per cent for the same period of time. There was no definite increase in size or weight in the increasing concentrations of lecithin, nor was there a definite gain among those in lecithin taken as a whole as contrasted with the controls."

These facts led to farther investigation of lecithin, its effect both upon the tyrosinase reaction and pigmentation in the larvae.

E. EFFECT OF LECITHIN AND VARIOUS FOODS USED IN EXPERIMENTATION, UPON THE TYROSINASE REACTION

The following tests *in vitro* of the effect of the various foods upon the tyrosinase reaction support the suggestion that the lecithin in egg yolk inhibits pigment formation.

Tyrosinase for the tests was obtained from different sources as follows:

(1) Fly larvae from six to ten days old were washed, chloroformed, and then ground with distilled water in a glass mortar. The aqueous extract thus obtained was filtered through glass or cotton wool, and used immediately. This solution is a very powerful one and turns orange pink throughout before one can get it measured into the test tubes.

(2) Meal worms were extracted in the same way and the course of the reaction is much the same, but the solution does not color quite as rapidly as in the case of the fly larvae.

(3) Mushrooms were extracted with glycerine and water, after having been ground in a glass mortar. The extract was filtered through ordinary filter papers.

(4) Potatoes were scraped and extracted with water for about an hour. This extract was filtered through filter papers and used as soon as possible. The solution is a pale orange pink by the time it is measured out into the test tubes.

A saturated solution of tyrosin in water was usually added to these solutions for the experiments, but the extracts already contained a chromogen since all of them darken after standing for half an hour. Since solutions color up in the same way when tyrosin is added as when it is not added, it seems likely that tyrosin is the chromogen already present in the solution. The extract from fly larvae gives a positive result for tyrosin when

tested with Million's reagent. Owing to the fact that the tyrosinase when isolated by precipitation with alcohol or other agents, is much less powerful than the original extract, in most of these experiments no attempt was made to isolate the oxidase. Kastle (1910, p. 63) has experienced the same difficulty and says, "Inasmuch as we have no criterion for judging of the absolute purity of a ferment, it is very doubtful whether much is gained by the attempt to isolate laccase and the other oxidases in pure condition."

Two series of experiments were made. In the first, the various foods under investigation were cooked and ground and then added to solutions of tyrosin and tyrosinase; in the second the foods were digested in pancreatin and pepsin and the filtrates used.

SERIES A. UNDIGESTED FOODS

For these experiments 0.2 gm. of the food was weighed out after it had been thoroughly cooked by boiling. This portion was ground in a glass mortar with 10 cc. of distilled water and added to the tyrosinase solution with or without tyrosin. The results are more or less variable, but certain facts may be stated with assurance to hold for all sorts of tyrosinase used.

Albumen inhibits the action of the tyrosinase the least of any of the foods. Yolk and liver inhibit the reaction more than albumen—liver usually inhibiting more than yolk. Liver allowed to spoil and then cooked inhibits the reaction completely, giving a white precipitate and a clear, colorless liquid. Liver that has been kept for several days, but cooked from time to time to prevent its putrefaction, inhibits the reaction less than does the fresh liver. Fresh and stale eggs inhibit the reaction equally. Lecithin* inhibits the action of the tyrosinase markedly—the color appearing slowly and only toward the surface of the liquid.

* Lecithin was prepared as follows: Yolks of hen's eggs were washed in water without breaking the membrane. An equal volume of 10 per cent sodium chloride solution was added and the mixture extracted in a separatory funnel with two volumes of ether. To the ether fraction was added an equal volume of acetone. The precipitated lecithin was dried in a sulphuric acid dessicator.

Pepsin and pancreatin (Merck in both cases) were tested in the same way, 0.2 grams being ground in 10 cubic centimeters of water and the tyrosinase solution added. Pepsin inhibits the reaction markedly, sometimes entirely. The pancreatin tubes, on the other hand, are usually as dark as the control.

Although, in these tests, liver inhibits the reaction more than egg yolk, the result is reversed when the foods are digested, digestion apparently lessening the inhibitory action of the liver. As one would expect, whatever effects the foods may have on the reaction before digestion, these effects are more or less changed after digestion.

SERIES B. DIGESTED FOODS

In these experiments the foods were digested with pepsin and pancreatin and the filtrates added to the tyrosinase solution. In each case five grams of the food were ground in a glass mortar with ten cubic centimeters of distilled water, and digested with 0.6 gram of pepsin or pancreatin. A few drops of toluol were added and the tubes were kept in a warm place. After digesting for forty-eight hours, the masses were filtered. The tyrosin and tyrosinase were mixed and the solution measured out from a burette into test tubes containing a few drops of toluol and seven drops of filtrate or, in the case of the control, seven drops of distilled water.

By the time the solutions have been introduced into the tubes containing the filtrates and toluol and the tubes arranged for inspection they are all a delicate orange pink. The yolk tubes soon become paler, passing into a pinkish white and the pancreatins soon lose the pink tinge entirely, passing into a light greenish brown, at the same time turning a smoky black at the surface. This black color gradually deepens until the solution is jet black throughout. If after the reaction has gone on for twelve hours, the tubes are shaken, the three are all very dark, but the yolk tube is brown or greenish brown while the liver and albumen tubes are jet black.

The pepsin tubes go through the reaction more slowly, showing much of the orange pink color after the pancreatin tubes have lost their pink color and have become black at the surface.

The darkening toward the surface proceeds more slowly also showing more of the red and mahogany tints before reaching black. The solutions become as dark as the others if left long enough, and when shaken show the same slight difference between the yolk and the liver and albumen. Liver and albumen tubes are often the same shade, but if there is a difference, the albumen tube is usually darker. The control tube acts more like pepsin than pancreatin, retaining its pink color for some time as the pepsin filtrates do.

The more rapid formation of color in the pancreatin tubes as compared with the pepsin or control tubes is, I think, due in part to the fact that the filtrates contain products of digestion, among which are probably some which serve as chromogens. Tyrosin is present among the cleavage products of most foods; and there may be other chromogens present. Their presence would account for the fact that the test tubes containing pancreatin filtrates color more quickly than the control tubes do. The tubes containing pepsin filtrates, on the other hand, probably contain enough pepsin to slow down the reaction, since we found that pepsin alone inhibits the reaction markedly. It is also possible that they contain less chromogen than the pancreatin filtrates. Probably both factors operate to produce the result, a color reaction which generally runs parallel with that of the control tubes.

Gessard (1901) obtained a similar result when he found that forty drops of blood serum from a calf retarded the tyrosinase reaction nine days, while fifty drops of water retarded it for a month. It is highly probable that some chromogen was present in the blood serum which would at least contribute more toward pigment formation than would any elements contained in an equal amount of water.

Though the results of the experiments are not altogether constant, the variability in the reaction is probably to be accounted for partly by the fact that different eggs and livers were used in different experiments, partly by the fact that the foods may have reached different stages of digestion in the different sets of experiments, and perhaps by variability of other and unknown factors. In spite of these differences, however, the experiments

show plainly that lecithin and egg yolk inhibit to some extent the action of certain tyrosinases of vegetable and animal origin. This being so, what will be the effect when lecithin is fed to the tadpoles along with a food which produces marked pigmentation?

F. EFFECT OF LECITHIN UPON PIGMENTATION

Experiments were accordingly made to determine the effect of lecithin when it is fed along with a food that ordinarily produces considerable pigment. When lecithin was mixed with the food it was placed in the dishes every other day after the dishes had been cleaned and the food and water renewed. The amount of lecithin used was not weighed each time, but it probably varied between fifteen and twenty milligrams in weight. It dissolved after eight or ten hours.

Of two lots of *Rana* tadpoles, one was fed albumen and the other albumen and lecithin. Following are the notes on the experiments. Table 5 shows the length measurements on different dates.

TABLE 5

Length measurements of *Rana* tadpoles fed on albumen and albumen plus lecithin. Length measurements represent average length of all the tadpoles. Eight tadpoles in each lot.

Food	Length in mm.			
	Feb. 19	Feb. 26	Mar. 5	Mar. 25
Albumen	17.6	18.6	19.3	21.5
Albumen + lecithin	18.2	21.1	23.0	26.5

Feb. 21. Tadpoles fed on albumen plus lecithin are lighter than those fed on albumen alone. The difference is small but distinct.

Mar. 5. Tadpoles fed on albumen plus lecithin are very plainly lighter and larger than tadpoles fed on albumen.

Mar. 14. (Same as Mar. 5.)

Mar. 25. Color difference seen before is now scarcely noticeable. Size difference very plain.

Apr. 2. Color difference plain once more.

Apr. 10. On account of the size difference it is difficult to compare the two sets of tadpoles with accuracy. One tadpole fed on albumen plus lecithin is darker than the others but the rest are lighter than the albumen-fed tadpoles, showing a yellowish brown tinge rather than black.

The same experiment was carried out with *Hyla* tadpoles with the same results (table 6).

TABLE 6

Length measurements of *Hyla* tadpoles fed on albumen and albumen plus lecithin. Length measurements represent average length of all the tadpoles in the dish.

Hyla. Experiment started Feb. 21

Food	Length in mm.		
	Feb. 26	Mar. 5	Mar. 25
Albumen	12.5	17.1	22.0
Albumen + lecithin	12.3	16.9	25.6

Mar. 5. Tadpoles fed on lecithin plus albumen are distinctly lighter than the others. The tadpoles fed on albumen alone have slender, pinched bodies, while the others are the normal shape and size.

Mar. 25. The color difference is scarcely noticeable.

Apr. 10. Some tadpoles on lecithin plus albumen are as dark as those on albumen alone, but most of them are lighter.

Liver plus lecithin, and liver alone, were fed to two different sets of *Rana* tadpoles with the following results (table 7) :

TABLE 7

Length measurements of *Rana* tadpoles fed on liver and liver plus lecithin. Length measurements represent average length of all the tadpoles. Eight tadpoles in each lot.

Rana. Experiment started Feb. 6, 1912

Food	Length in mm.			
	Feb. 20	Feb. 26	Mar. 6	Mar. 14
Liver	21.4	24.2	27.4	30.25
Liver + lecithin	20.6	24.05	28.2	30.29

Feb. 21. Tadpoles fed on liver alone are darker than the others.

Feb. 26. Tadpoles fed on liver alone are darker than the others.

Mar. 14. Some about the same color, others darker in dish containing liver alone.

The same experiments with *Hyla* gave the following results (table 8) :

TABLE 8

Length measurements of *Hyla* tadpoles fed on liver and liver plus lecithin.
Length measurement represents average length of all the tadpoles.
Six tadpoles in each lot.

Hyla. 1912

Food	Length in mm.	
	Mar. 5	Mar. 14
Liver	19.5	24.4
Liver + lecithin	19.6	26.2

Mar. 14. Tadpoles fed on liver plus lecithin are lightest—have a greenish tinge while others are black.

Mar. 25. Tadpoles fed on liver are all dead.

Two sets of *Hyla* tadpoles were fed on egg yolk and egg yolk plus lecithin (table 9):

TABLE 9

Length measurements of tadpoles fed on yolk and yolk plus lecithin.
Length measurements represent average length of all the tadpoles.
Eight tadpoles in each lot.

Hyla. 1912

Food	Length in mm.		
	Mar. 5	Mar. 14	Mar. 25
Yolk	15.7	18.2	22.6
Yolk + lecithin	15.6	17.6	22.1

Mar. 14. Sizes are uneven. No marked color differences. A few lighter individuals among the tadpoles fed on yolk.

Apr. 1. Tadpoles fed on yolk average slightly darker than the others—pigmentation very uneven.

(Tadpoles accidentally mixed, experiment discontinued.)

The experiments plainly show that lecithin lightens the color of the tadpoles. It is not clear that lecithin plus yolk of egg produces lighter colored tadpoles than yolk alone does. It would appear that for tadpoles of the size reached by these at least, additional amounts of lecithin do not lighten the tadpoles noticeably.

Miss King (1907) has suggested that the tadpoles that are fed with lecithin are not so thrifty as the others. Although there has been a slightly larger percentage of deaths among the tad-

poles fed on yolk of egg than among those fed on liver, the tadpoles on lecithin mixed with other foods show a smaller percentage of deaths than the control lots receiving no lecithin.

It has been noticed in the experiments with lecithin in connection with other foods, that the color differences vary from time to time. Such differences have been noticed in all the experiments to a greater or less extent. In general the difference between the color of tadpoles fed on yolk and those fed on liver is very noticeable at the end of the first week and continues to be very marked until the larvae are about half grown, when it becomes less distinct. From about the time the hind limbs appear until the metamorphosis is complete, the color difference is again marked.

These differences are never great enough to suggest an actual disappearance of pigment already formed, but rather a slight difference in the rate of pigment production at different stages of development. That is, the pigment production in tadpoles fed on egg yolk or lecithin though at all times less than in the tadpoles fed on liver may be greater at one time than at another. It is possible that the tadpole at different stages of development is differently influenced by the lecithin. The differences may be more or less periodic, but more observations and histological data are necessary for an intelligent discussion of this phase of the problem.

G. EFFECT OF CERTAIN OTHER ORGANIC SUBSTANCES UPON THE TYROSINASE REACTION AND UPON PIGMENTATION

A consideration of the effect of various other chemicals upon the tyrosinase reaction is important if we grant that chemical substances thus profoundly influence pigment formation.

Abderhalden and Guggenheim (1907) found that $n/100$ hydrochloric acid inhibits the action of tyrosinase and $n/100$ sodium hydroxide retards it considerably. Neutralization of acid or alkali fail to restore the original activity of the oxidase.

Chodat and Staub (1907) observe that the oxidation of tyrosin by tyrosinase is diminished by glycine, leucine, and alanine. They find that tyrosin acts upon certain peptids such as tyrosin

anhydride and glycyl-tyrosin anhydride, giving rise to yellow substances which do not become black as does tyrosin itself. However, a mixture a glycyl-tyrosin anhydride with glycine gives with tyrosinase a rose color changing to bluish green, with alanin it gives a deep red, and with leucin a deep brown color.

Abderhalden and Guggenheim (1907) studied the action of tyrosinase from *Russula delica* on tyrosin and various tyrosin-containing polypeptids. Aspartic and glutaminic acids and other amino acids inhibited the action, especially if they were present in strong solution. Polypeptids containing tyrosin residues were colored by tyrosinase, the color being somewhat modified by the nature of the amino acid combined with the tyrosin in the polypeptid. They conclude that the character of the pigment resulting from the action of tyrosinase on tyrosin is dependent upon the combination in which the tyrosin exists.

TABLE 10

Cleavage products of certain animal and vegetable proteids; ov-albumin, vitellin, and gliadin taken from Hammarsten (1911), gluten from Plimmer (1908).

	Ov-albumin ¹	Vitellin ⁴	Gliadin		Gluten, from wheat ⁵
			a ⁵	b ⁶	
Glycocoll	0.0	1.1	0.02	0.9	0.4
Alanine	2.1	+	2.0	2.66	0.3
Valine		2.4	0.21	0.33	
Leucine	6.1	11.0	5.61	6.00	4.1
Serine	1.1		0.13	0.12	
Aspartic Acid	1.5	0.5	0.5	1.24	0.7
Glutamic Acid	8.0	12.2	37.33	31.5	24.0
Cystin	0.3 ³		0.45		
Phenylalanine	4.4	2.8	2.35	2.6	1.0
Tyrosine	2.4 ³	1.6	1.20	2.37	1.9
Proline	2.25	3.3	7.06	2.4	4.0
Oxyproline					
Tryptophane			+	1.0	+
Histidine			0.61	1.7	1.2
Arginine			3.16	3.4	4.4
Lysine			0.0	0.0	2.2
Ammonia			5.11		2.5

¹ Abderhalden and Pregl (1905).

² Levene and Beatty (1907).

³ K. Mörner (1901).

⁴ Abderhalden and Hunter (1906).

⁵ Osborne and Clapp (1906).

⁶ Abderhalden and Samuely (1905), and Abderhalden (1909).

⁷ Abderhalden and Malengreau; Kossel and Kutscher.

In the analyses of various cleavage products, table 10, gliadin and gluten are notable for their high percentage of glutamic acid. Glutamic acid is noted by Abderhalden and Guggenheim as an inhibitor of the tyrosinase reaction when tyrosinase from *Russula delica* is used. Accordingly gliadin and gluten (made from white flour, dried, ground, and fed in powdered form) were fed to different sets of tadpoles. These foods produce so little growth that the results are very inconclusive.

The results with liver, yolk and gliadin are as follows (table 11):

TABLE 11

Length measurements of *Hyla* tadpoles fed on liver, yolk, and gliadin. Length measurements represent average length of all the tadpoles in a dish.

Hyla. Experiment started Feb. 24, 1912

Food	Mar. 5		Mar. 25	
	Length in mm.	Number of tadpoles	Length in mm.	Number of tadpoles
Liver	15.8	18		0
Yolk	15.3	18	28.0	14
Gliadin	12.5	18	15.0	15

Mar. 5. Liver-fed tadpoles black. Gliadin-fed tadpoles dark, but not so dark as liver, yolk-fed tadpoles lighter with a few very light ones.

Mar. 14. Liver-fed tadpoles all black; yolk-fed tadpoles—four very light, the rest medium; gliadin, one light, the rest medium.

Of three sets of *Rana* tadpoles one was fed on raw flour, one on gluten, and the third on gliadin. Here again the growth was so slight that the experiment was unsatisfactory (table 12):

TABLE 12

Length measurements of *Rana* tadpoles fed on flour, gluten, and gliadin. Length measurements represent the average length of all the tadpoles. Equal number of tadpoles at the beginning of the experiment.

Rana. Experiment started Feb. 24, 1912

Feed	Length in mm.		Number of tadpoles May 6
	Mar. 6	Mar. 25	
Flour	15.7	19.1	9
Gluten	15.8	18.5	1
Gliadin	15.7		

Mar. 25. No noticeable color difference.

Apr. 10. Only one gluten-fed tadpole left. This one is larger and slightly darker than the average of the flour-fed tadpoles.

The same experiment with *Hyla* tadpoles gave the following data (table 13):

TABLE 13

Length measurements of *Hyla* tadpoles fed on flour, gluten, and gliadin. Length measurements represent the average length of all the tadpoles in a dish.

Hyla. Experiment started Feb. 24, 1912

Food	Length in mm.	
	Mar. 6	Mar. 24
Flour	13.0	18.3
Gluten	13.3	19.6
Gliadin	12.2	13.7

Mar. 24. Tadpoles all nearly the same color,—if any difference gluten-fed are darkest, gliadin-fed lightest.

Gortner (1911b) finds that orcin, resorcin, and phloroglucin inhibit the action of tyrosinase extracted from potatoes, meal worms, and the periodical cicada. He says: "It would appear from these data, that aromatic compounds which carry two hydroxyl groups in meta position to each other may act as chemical anti-oxidases on tyrosinase, and completely inhibit its action. Other oxidases are not inhibited, but are able to oxidize these same m-dihydroxyl compounds, forming colored bodies of an unknown nature."

Phloroglucin and resorcin were mixed with liver and fed to *Hyla* tadpoles. The amounts were not weighed, but about fifteen milligrams was rubbed up with the liver and put into the dish. The tadpoles on resorcin did not thrive, most of them died very soon (table 14):

TABLE 14

Length measurements of tadpoles fed on liver, liver + resorcin, and liver + phloroglucin. Measurements represent the average length of all the tadpoles in the dish.

Hyla. Experiment started Mar. 18, 1912

Food	Mar. 25	Apr. 11
	Length in mm.	Number of tadpoles
Liver	14.1	6
Liver + resorcin	15.4	0
Liver + phloroglucin	14.3	4

Mar. 25. All tadpoles the same color.

Apr. 1. All tadpoles the same color.

Apr. 11. Of the four remaining tadpoles on liver plus phloroglucin, two are as dark as the six tadpoles fed on liver alone, and two are slightly lighter.

The same experiment with albumen as food gives the following results (table 15):

TABLE 15

Length measurements of tadpoles fed on albumen, albumen + resorcin, and albumen + phloroglucin. Measurements represent the average length of all the tadpoles in the dish.

Hyla. Experiment started Mar. 18, 1912

Food	Length in mm. Mar. 25
Albumen	13.6
Albumen + resorcin	13.1
Albumen + phloroglucin	12.8

Mar. 25. Tadpoles fed on albumen plus phloroglucin are very slightly lighter than the other two sets, which are both the same color.

Apr. 11. Tadpoles fed on albumen plus phloroglucin are very slightly lighter than those on albumen, but the difference is scarcely noticeable.

This group of experiments, as will be noted, was tried but once and with a small number of tadpoles, so they can be regarded only as a beginning in this direction. The results indicate that the various substances may affect the color of the tadpoles to some extent.

H. HISTOLOGICAL DIFFERENCES BETWEEN CHROMATOPHORES OF LARVAE FED UPON DIFFERENT FOODS

The statements as to the color differences in the tadpoles have been made thus far on the basis of their appearance to the unaided eye. The differences as shown by the microscope are no less marked and show plainly that there is actually less pigment in the egg-fed tadpoles (plate 1).

With the low powers of the microscope the black epidermal chromatophores of the dark *Rana* tadpoles appear to be greatly branched, and so filled with pigment that they come very close

together, while in the light egg-fed tadpoles, the chromatophores, though perhaps not less numerous are more slender and delicate and very little branched. In very light specimens the pigment is in spots that are scarcely elongated at all (pl. 1, figs. 5-8).

The form of the chromatophores in the *Hyla* is somewhat different. The body of the chromatophore is not so long, but the branching processes are longer and finer and in dark individuals form a close network of fine interlacing branches (pl. 1, figs. 3 and 4). Between the two extremes in both species are various grades of difference, so that the chromatophores of an unusually dark egg-fed tadpole may not differ greatly from those of a lighter colored liver-fed individual.

A study of sections shows that the chromatophores of the dark tadpoles are larger because they contain many more of the brown melanin granules. These granules are so numerous that the pigment forms a continuous network in the intercellular spaces, while in the light tadpoles the amount of melanin is so much less that the pigment masses appear as small spots with few or no processes. Camera drawings of the typical chromatophores of the two sorts make this difference clear.

Fig. 1

Fig. 2

Sections of epidermis of larvae of *Rana* sp. $\times 450$. (Drawn with aid of camera lucida.)

Fig. A. Tadpole fed on yolk of egg.

Fig. B. Tadpole fed on liver.

I. EFFECT OF CHANGES IN LIGHT, HEAT, AND FOOD UPON PIGMENTATION

To determine the influence of light and heat upon the color differences produced in the tadpoles by different foods, the following experiment was carried out. Eight dishes of tadpoles, all from the same bunch of eggs, were arranged as shown below.

Warm:

In constant temperature, box (of glass) kept at about 26.5 C.

In dark. (in black paste-board box)	a. fed on liver b. fed on yolk
In light.	c. fed on liver d. fed on yolk

Cold:

In north basement room. (The temperature of this room varied very little and was always considerably below ordinary room temperature.)

In dark. (in black paste-board box)	e. fed on liver f. fed on yolk
In light.	g. fed on liver h. fed on yolk

TABLE 16

Length measurements of *Rana* tadpoles under different conditions of light, temperature, and food. Measurements are average length of all the tadpoles in a dish. Experiment began with eight tadpoles in each lot.

Rana. Experiment started Feb. 10, 1912

Conditions of the experiment			March 5	
Temperature	Lighting	Food	Length in mm.	Number living
Warm	Dark	Liver	28.0	3
Warm	Dark	Egg yolk	27.5	5
Warm	Light	Liver	31.4	4
Warm	Light	Egg yolk	26.5	2
Cold	Dark	Liver	19.3	8
Cold	Dark	Egg yolk	20.8	8
Cold	Light	Liver	21.2	8
Cold	Light	Egg yolk	20.3	8

AVERAGES

Cold	20.4	32
Warm	28.6	14
Light	23.2	22
Dark	23.0	24
Egg yolk	22.6	23
Liver	23.2	23

The series of colors is the same under the different conditions of temperature. In both cases the series from darkest to lightest coloring is as follows:

- Darkest tadpoles—Those fed upon liver and kept in the light.
- Those fed upon egg and kept in the light.
- Those fed upon liver and kept in the dark.
- Lightest tadpoles—Those fed upon egg and kept in the dark.

It is plain from this experiment that tadpoles raised in the light are darker than those raised in the dark and that tadpoles fed upon liver and kept either in the light or in the dark are darker than those fed upon egg under the same conditions. The difference in size and development between the tadpoles kept at a high temperature and those in the low temperature are so great that the two series cannot well be compared, but no marked difference in color between the two series can be distinguished. It is evident that changes in both food and light influence the rate of pigment formation markedly and that light is a somewhat more potent factor than liver in increasing the rate of pigment production.

These conclusions as to increase of pigmentation in the light and increase in the rate of growth at a higher temperature confirm results already reported (table 16). The average length measurements of all tadpoles kept in the dark compared with those kept in the light are interesting in view of the conflicting statements that have been made by various observers as to the comparative rate of growth in the dark and in the light. These figures support the contention that there is very little if any difference between the amount of growth in the light and in the dark.

J. SUMMARY

A consideration of Weismann's theory, the factor hypothesis, and the results of chemical research, leads one, it seems to me, to see in the last two named the greatest hope for the solution of problems of color differentiation. The difficulties and complications are great, but the field for research is correspondingly large and fruitful.

The present research is by no means complete, the results so far attained suggesting numerous very pertinent points that should be investigated farther. It does, however, furnish certain definite facts which it is hoped will add something to current conceptions of color differentiation.

The research shows that pigment in *Rana* and *Hyla* tadpoles is not correlated with amount of nutrition, as claimed by Tornier for *Pelobates* larvae, but, as suggested by instances cited by Darwin and Wallace, is dependent rather upon substances specific for color in the nutritive material. These substances may bring about a change in the amount of pigment-forming substances produced or slightly alter the character or combination of these substances and thus change the amount or color of the pigment.

The fact is made clear that lecithin acts as a partial inhibitor of the tyrosinase reaction in the test tube, and when it is fed to tadpoles, pigment formation is checked to a noticeable degree, indicating that inhibitors or modifiers of the pigment formation may be introduced into the organism with the food.

The similarity of the effect of lecithin in the test tube and in the body of the tadpole makes it probable that the tyrosinase reaction or a similar oxidase reaction is the basis of pigment formation in the tadpole.

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EXPLANATION OF PLATE 1

Fig. 1. *Rana* sp. The lighter tadpole was fed upon yolk of egg, the darker one was fed upon liver. From photograph, natural size.

Fig. 2. *Hyla regilla*. The lighter tadpole was fed upon yolk of egg, the darker one was fed upon liver. From photograph, natural size.

Figs. 3-8. Epidermal chromatophores of amphibian larvae. From photographs, magnified about forty-five diameters.

Fig. 3. *Hyla regilla*. Tadpole fed on liver.

Fig. 4. *Hyla regilla*. Tadpole fed on yolk of egg.

Fig. 5. *Rana* sp. Chromatophores of tail region, tadpole fed on liver.

Fig. 6. *Rana* sp. Chromatophores of tail region, tadpole fed on yolk of egg.

Fig. 7. *Rana* sp. Chromatophores of body of larva, tadpole fed on liver.

Fig. 8. *Rana* sp. Chromatophores of body of larva, tadpole fed on yolk of egg.

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