XVI ON THE OCCURRENCE OF IRIDOCYTES IN THE LARVA OF *MICROHYLA ORNATA*, BOUL.

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(Plate XI).

INTRODUCTION.

There are some good observations recorded as regards the colour of batrachian larvae in life, but in most cases the descriptions refer to preserved specimens. Such descriptions must necessarily differ, for the material sent to leading authorities for examination generally arrives in a state in which the colour is somewhat different from what occurs in living forms, the usual methods of preservation, either in alcohol or formalin, greatly affecting the pigments. Moreover, the colouration of specimens of the same species of batrachian is not uniform as a rule, inasmuch as it depends in a great measure on the character of the surroundings from which they are taken and the conditions under which they live. For example, if the olive green tadpoles of the genus *Rana* or *Rhacophorus* should be transferred, from the green weeds amidst which they live, to a more exposed area of another pond, they turn grey; and if the same larvae should be retransferred to a third pond with a black clayey bottom, they become brown. Similarly dearth or abundance of food will greatly influence the colour. Starvation nearly causes the absorption of the yellow pigments with the consequence that the melanin chromatophores show through to some extent, the tadpole looking more or less darker. On the other hand, generous feeding favours the deposit of more than one kind of lipochrome pigment, and accordingly the larvae appear beautiful with a variety of colours.

The tadpole of *M. ornata* has been described by Capt. S. Flower (*Proc. Zool. Soc. London*, 1899, p. 902), and there is a short note on the same subject by Mr. H. S. Ferguson (*Journ. Bombay Nat. Hist. Soc.*, XV, p. 506). No allusion is made by either of these writers to the occurrence of the bright metallic dorsal band or the silver brilliancy on the sides of this beautiful tadpole. It is perhaps worthy of mention that this tadpole and its congener that of *M. rubra* are probably most singular in the possession at once of golden and silvery brilliance, of all the Anuran larvae that have been studied up till now.

The scope of this paper is to record the results of the investigations commenced with a view to discover the nature of the substance which produces this interesting phenomenon, which has also been observed in fishes, and further to trace the relation of it to the other histological elements. Incidentally, reference will be made to the colouring matter of other tadpoles, chiefly Indian forms known to me,—in all those particulars in which they approach the general scheme of pigmentation occurring in the two species of *Microhyla* which form the subject of this paper. The literature referring to this section of the paper is given below in a foot-note.[1]

The head of the tadpole of *M. ornata*, which is nearly two-thirds of the size of the body, is perfectly transparent, but this is not so in *M. rubra*. Behind the eyes, in the former species, there is a characteristic diamond-shaped black mark just above the cranium. Usually a yellow line runs fore and aft of this mark behind which is a glandular area. Over the vertebral column is the characteristic golden streak which may also extend in front over the diamond-shaped mark already referred to. Sometimes the lungs show through the transparent skin on either side of the vertebral band; more often, however, the skin may be yellowish green. The sides and ventral surface of the abdomen glitter with silvery brightness, while the throat is colourless. The ventral lobe of the tail is more or less pale copper coloured. When the dorsal metallic streak is absent, due to absorption, the underlying black band can then be seen.

The other Engystomatid larvae known to me are uninteresting in regard to their colouration. The tadpole of one species of *Kaloula* (*K. triangularis*) is absolutely transparent without any colour markings except on the head; and another (*K. variegata*) has a transparent head, but the body and tail are blotched, besides two blue spots in the region of the groin The tadpoles of *K. pulchra*, *K. obscura*, and *Cacopus systoma* are densely pigmented.

The points of interest that call for remark, in regard to the colouration of the larvae of the Ranid family, are the occurrence of bright orange red in the posterior third of the tail in *R. breviceps* and *Rhacophorus maculatus*,[2] which as metamorphosis

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[1] For an account of the colouration of Indian tadpoles the following literature, though not complete, may be consulted:—

Annandale, *Rec. Ind. Mus.*, VIII, p. 21 (1912).
Boulenger, *Ann. Mus. Genova*, (2) V, p. 420 (1887-88); *Proc. Zool. Soc. London*, 1893, pp. 526-527.
Flower, *Proc. Zool. Soc. London*, 1896, p. 911 and 1899, pp. 892, 902.
Anderson, *Proc. Zool. Soc. London*, 1895, p. 660.
Butler, *Journ. Bombay Nat. Hist. Soc.*, XV, pp. 193, 387 (1903-04).
Ferguson, *Journ. Bombay Nat. Hist. Soc.*, XV, p. 499 (1903-04).
Narayan Rao, *Rec. Ind. Mus.*, X, p. 265 (1914); XI, pp. 31, 349 (1915).
Boulenger, *Proc. Zool. Soc. London*, 1891, pp. 606-607, mentions the occurrence of metallic dots in the larvae of *Rana arvalis* and *R. temporaria*.

[2] Flower, in his account of the tadpole of the Malay race of this species, describes the colour as pinkish (*Proc. Zool. Soc. London*, 1896, p. 906).

progresses is changed into intense black. The ventral surface of all these larvae is dead chalky white, more or less speckled on the throat and the sides. On the dorsal surface one meets with every shade of colouration, ranging from bright yellow to dark brown, with or without spots. A few exceptions may be cited to this prevailing scheme of colouration For instance, the perfectly grey larvae of *Rh. plurostictus*, which bear numerous round black spots, and are perhaps the biggest tadpoles yet discovered in India.[1] The yellow dorsal streak of the larvae of *R. breviceps* and *R. tigrina* is only a premature appearance of an adult character, and the round red spots on the back and thighs of the same tadpoles are mainly larval features. In a few cases like *R. alticola* and *R. liebigii* the tail may be diversified by ocelli or vertical bars.

The tadpoles of *Bufo* are all uniformally brown. Occasionally there are metallic dots on the dorsal surface, the ventral side being dirty white as in *B. microtympanum.*

After this brief survey, it need only be mentioned that the outstanding character of the larvae of *M. ornata* and *M rubra* is the possession of metallic bands and surfaces which make the colouration as a whole markedly striking.

BIOLOGICAL SIGNIFICANCE.

The larvae of *M ornata* and *M. rubra* float on the surface in comparatively large shoals chiefly in the middle of the pond. In the aquarium the same habits are exhibited by these tadpoles.

Other tadpoles of the Ranid group, which I have reared and kept under observation, are unable to remain at the surface for any length of time being without the provision of a special structure like a float, as in *Megalophrys montana.* The ability to remain at the surface throughout metamorphosis is a feature that has some structural bearing. When the animal remains stationary at the top it is really occupying a plane of least effort, which it can do only when the body is for the time being lighter than the water, bulk for bulk. The gill chambers of the larvae of the two species of *Microhyla* possess large cavities filled with air, which can be easily seen through the transparent skin. These air spaces account for the enormous size of the cephalic region. On pressing the bulging portion of the throat, large bubbles of air may be driven out through the spiracle. An examination of the transverse and longitudinal sections of the larvae reveal these air cavities, situated between the first and the second and the second and the third gill arches on each side of the pharynx. This structural peculiarity, absent in the Ranid group (as revealed by sections), which bears some resemblance to the secondary air sacs of *Clarias*, accounts

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[1] In my experience the dimensions quoted by Anderson for *R. cyanophlyctis* (*Proc. Zool. Soc. London*, 1895, p. 660) are not of the normal tadpole. If metamorphosis, however, is hindered through any cause the larvae attain such a size.

for the floating habit of the tadpole and like the fish it blows out a few bubbles of air through the mouth or the spiracle before sinking to the bottom.

While floating, the tadpole must be peculiarly exposed to attacks from enemies who may have some difficulty in hunting for other forms which lead a concealed mode of life. It is obvious that unless there is some special provision which to a greater or less extent secures immunity, the larvae of *Microhyla* will utterly perish.

Observation shows that in the aquarium these larvae are avoided by both fish and snakes, like *Clarias*, *Saccobranchus*, *Ophiocephalous* and *Tropidonotus*, and in ponds ducks and geese also do not touch them. They, however, greedily seize and devour other amphibian larvae. Reference has already been made to the occurrence of a cephalic gland and it is clear that the offensive matter, by which the larvae are protected, is situated in this gland. When a scraping from this gland was introduced into the conjunctiva of a dog, the eye was kept closed and at the same time it became blood-shot with a watery discharge. If an entire larva be placed in the mouth, nothing will induce the dog to swallow it; those of *Rana* are, however, swallowed. The fish *Opiocephalus* was tried. Forcible feeding of the fish was found to be futile, for as often as the larvae were introduced into the mouth, they were thrown out with considerable force. The secretion is acid in reaction, as may be tested with blue litmus paper. The colouration may have a warning effect.

THE COLOUR ELEMENTS.

For convenience of treatment, the colour elements of the larvae of *M. ornata* may be considered under the following heads:—

1. Black chromatophores of the melanin group.
2. Coloured pigments of the lipochrome group.
3. Iridocytes which are guanin crystals, occurring chiefly in the form of plates.
4. Argenteum or reflecting tissue, on the sides and the ventral regions of the abdomen.

The first two elements combine in various proportions or individually produce the several colours referred to already in the foregoing paragraphs, while the latter elements account for the metallic brilliance. The dead chalky white on the ventral surface of Ranid larvae is due to the argenteum being impregnated more or less with calcium, the compound thus produced being known as guaninkalk.

1. BLACK CHROMATOPHORES.

These elements occur in chiefly two forms, as mere dots and as dendritic structures. The former are confined to regions that are more or less transparent, such as the head and the caudal membranes, while the latter are aggregated on the dorsal surface. A third variety—the stellate type—accounts for the dark pigmentation of the peritoneum.

2. COLOURED PIGMENT.

The true chromatophores which give colour to the skin are either yellow or orange and the degree of colouration depends on two factors. Firstly the number of coloured chromatophores present and the manner of their distribution, and secondly the extent to which they are diluted by the black chromatophores. The lipochrome pigment occurs in the form of scales or minute granules, the latter when present give the effect of colour suffusion. Only very fine granules of chromatophores produce the blue and orange, such as occur in *K. variegata* and *R. breviceps*, and in the yellow and scarlet red spots found in *Rh. maculatus* and *R. tigrina* respectively only large scaly chromatophores are met with. Green is simply the effect of the fusion of yellow and black, while purple or brown is caused by mixing red and black in various proportions. As will be shown in subsequent paragraphs, orange red is simply intensified yellow and is not a separate pigment at all.

3. IRIDOCYTES.

As has been stated already, the bright metallic band in the mid-dorsal line is formed by an opaque plate of iridocytes. Each is scaly and is irregular in outline, and when numbers of them form a thick band, they acquire strongly reflecting powers. The band is sunk in a groove in the spinal region covered over by the dermal tissue and bounded laterally by the nine pairs of dorsal muscles. Where this band occurs, the melanin chromatophores are absent. Iridocytes occur in the iris, the peritoneum, the lungs and subcutaneous tissue of the tail membranes. They are absent from the skin.

In two important respects the iridocytes of the batrachian larvae differ from those of fishes. In the first place they are irregular in outline, and are formed of minute spherical granules. The chief characters of these bodies in fishes are that they are regular, laminated structures with a clear nuclear spot, with divisions showing common origin. In the second place the crystals are most unstable in the tadpoles, falling almost to powder on removal from the subcutaneous setting; whereas in fishes they occur in the majority of cases in the skin and the crystals can be easily examined.

The iridescent phenomenon caused by this dorsal band in the *Microhyla* larva is simply the effect of light being reflected from the numerous surfaces and sides of these crystalline structures. But the golden colour is, however, due to a thin layer of yellow lipochrome spread over this band, and the occurrence of similar pigment produces the coppery hue of the tail lobes.

THE ARGENTEUM.

The argenteum is a thick, opaque, continuous layer of reflecting subcutaneous tissue in which the iridocytes no longer retain their individual character but are broken up so minutely that it is by no means possible to make out any definite structure with the microscope. This layer is covered over by the transparent epidermis which here is singularly free from all chromatophores. The silver brilliance of the argenteum is simply due to the powdering of the iridocytes which are thickly impregnated into the subcutaneous tissue; the same cause accounts for all absence of iridescence in this region. Thus the abdominal wall has a bright silver lustre on the outer surface and a spangle-like appearance on the peritoneal wall. As has been said already, the pericardium—the parietal layer—is also an argenteum and the visceral layer bears only chromatophores.

The only organs that contain iridocytes and black chromatophores are the lungs; all the other organs are perfectly devoid of them. The occurrence of argenteum in the air-bladder of fishes has been noticed, and the homology of the lungs of air-breathing Craniates with the air-bladder of fishes here receives fresh corroboration from the chemical side.

RELATION OF COLOUR AND HISTOLOGICAL ELEMENTS.

It is not possible to demonstrate the presence of connective tissue corpuscles in the dermis or epidermis of grown tadpoles, though gold chloride staining of the skin of very young tadpoles sometimes reveals the presence of a few corpuscles. The chromatophores occur in between the epidermal cells, and their cellular origin can be explained on the hypothesis that after formation in the deeper tissues they migrate bodily to the surface region. In sections of the skin two kinds of dots are noticeable, the smaller ones belong to the granular chromatophores and the larger ones represent the cut ends of the dendritic forms.

The coloured elements are absent from these sections and stained preparations, for they are most susceptible to the action of even mild solvents like rectified spirit. In the fresh specimens, the scale-like coloured chromatophores lie partly in the dermis and partly below, only a few occurring in the epidermis. Even in regard to them, their connective tissue origin can only be inferentially gathered.

The iridocytes are $\frac{1}{10}$ mm. *in situ*, while the coloured chromatophores are $\frac{3}{10}$ mm. and the granules of the former are less than $10\,\mu$. The spinal groove in which the metallic band lies is quite open in young specimens; the epidermal tissue growing over as metamorphosis advances. If the iridocytes from these young specimens are examined, under a high power of the microscope, their cellular origin can be made out. It is probable that when they leave their place of origin, they become lightly held together by some organic matrix, too feeble, however, to bind them together when mounted

The argenteum is so opaque and dense that the nature of the relation of the reflecting particles and histological elements of the subcutaneous tissue cannot be made out.

TIME OF APPEARANCE OF COLOUR ELEMENTS.

Such of the batrachian anuran larvae as are known to me are either dark or brown at the time of hatching, and the formation of coloured chromatophores is not complete till after the larvae come under the influence of sunlight. The time of appearance of colour varies in different families, mainly depending upon the environmental circumstances under which development progresses.

In *Microhyla ornata* the large cephalic region remains transparent throughout the metamorphosis, and the diamond-shaped mark appears as soon as the larvae adopt habits of floating on the surface of the water, when they measure about 10 to 12 mm. The other characters, such as the metallic band and the reflecting surfaces, gradually emerge into view as the tadpole increases in size (16 to 18 mm.). It may be mentioned that at this stage the peritoneum bears more numerous iridocytes than at later stages, so much so that they form a continuous metallic surface over a dark background formed by the melanin chromatophores. Perhaps the most important feature in the development of the argenteum at this stage is the fact that when a piece of fresh subcutaneous tissue is examined under the microscope, before it has become too opaque for such treatment, two kinds of metal elements can be noticed. The larger crystals are fairly regular in their outline, unlike those of the mid-dorsal band, and the smaller ones are irregular. As no broken pieces of these larger plates have yet been examined, the view that they contribute towards the formation of the argenteum is only tentatively put forward. When the tadpoles develop the front limbs, the dorsal golden streak and the argenteum are absorbed and the normal colouration of the adult begins to appear.

THE CHEMISTRY OF IRIDOCYTES AND ARGENTEUM.

In the sixties, Barreswil[1] and Voit[2] demonstrated the presence of guanin in the reflecting tissues and the air-bladder of fishes, and about 1845 this substance was isolated by Bodo Unger from guano. Some time later, Ewald and Krükenberg[3] found the occurrence of this substance in reptiles and amphibians as well, and their investigations go to show that the dead chalky white found on the ventral surface of the adult members of the Ranid family is really caused by a lime compound of guanin, which

1 *Comptes Rendus*, LIII, p. 246; 1861 (*Phil. Trans. Roy. Soc. London*, CLXXXIV B, p. 781; 1893).

2 *Zeitsch. f. wiss. Zool.*, XV (*Phil. Trans.*, p. 782).

3 *Zeitschr. f. Biologie*, XIX, p. 1: 1883 (*Phil. Trans.*, p. 785).

they called "Guaninkalk." About 1893 Cunningham and MacMunn,[1] as a result of extensive observations on fishes, established the fact that the iridescent effect produced in the skin of all fishes is due to the presence of iridocytes, and the silver brilliancy is caused by the reflecting tissue,—the argenteum. Guanin is the chemical substance present in both these structures.

It may be mentioned that guanin in the tissues of the body is the end product of the metabolic activity of the organism, and the utilisation of this waste matter for certain physiological ends is a feature of wide-spread occurrence in animals. As has been said in the foregoing paragraphs, the presence of guaninkalk and its chemical nature have already been worked out in the skin of adult batrachians, but so far the occurrence of the iridocytes and argenteum has not been determined either in the adult or the larvae. On chemical analysis of these substances it is established that they are guanin compounds identical with those worked out in the fishes, and the course of the chemical enquiry adopted for such a determination may now be proceeded with. I must mention here that in all stages of the work I have received considerable help from my colleague Mr. A. Subba Rao.

The tissues were thoroughly washed in distilled water till all albuminous matter was removed and then solutions of iridocytes and argenteum were obtained in nitric and hydrochloric acids.

I. A quantity of nitric acid solution was evaporated in a watch glass over a hot air bath. The residue formed is a yellow substance (guanin nitrate) which turned red on the addition of caustic potash. This is Barreswil's reaction.

II. A quantity of hydrochloric acid solution was evaporated similarly and the residue was treated with strong nitric acid. A yellow compound is obtained by reheating the solution to dryness which on the addition of caustic soda turned red, and purple on heating. This is Cunningham and MacMunn's test.

III. If to the yellow compound (nitrate of guanin), obtained in the first two cases, ammonia is added and heated the same colour changes are noticed. This test is given in Watt's chemical dictionary (p. 656).

It may be mentioned here that there is essentially no difference between Barreswil, Cunningham and MacMunn and Watt's reactions. In all the three cases the neutralising agent, a base, is added to the nitrate of guanin which on heating becomes purple.

IV. If silver nitrate is added to the nitrate of guanin a reddish-brown precipitate results, which on heating turns purple.

V. Potassium chromate gives an orange red precipitate on the addition of the nitrate of guanin (Watt).

VI. Potassium ferricyanide yields a brown precipitate with the same substance (Watt).

VII. Concentrated picric acid gives a bright red solution when treated with nitrate of guanin (Watt).

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[1] *Phil. Trans. Roy. Soc. London*, CLXXXIV B, p. 765 (1893).

VIII. Hydrochloric acid solution of guanin on heating turns red and the guanin hydrochloride—the ash obtained after boiling —is slightly reddish, which treated as in experiments V, VI and VII gives similar reactions.

IX. Potassium permanganate solution treated with nitrate of guanin, with a touch of caustic soda. The green solution (green being due to the formation of K_2Mno_4) on heating gives an albuminous flocculent red precipitate (oxyguanin) which is insoluble in water, rectified spirit and weak acids (Watt).

X. The same reactions are obtained with the hydrochloric acid solution of guanin.

If any of these precipitates obtained with silver nitrate in the above experiments should be treated with oxyguanin obtained in experiment X, the silver chloride is precipitated in the form of a white stuff.

The iridocytes are insoluble in water, ether, chloroform, glycerine[1] and acetic acid, but soluble both in acids (Nitric, Hydrochloric and Sulphuric) and bases like caustic potash, soda and ammonia. Formalin and alcohol are also solvents.

With the alkaline (NaOH) solution of iridocytes and argenteum the following additional reactions and properties were obtained.

XI. The solution was treated with strong picric acid and boiled for a few minutes. The whole turned into orange red on being allowed to stand for 18 hours.

XII. With potassium permanganate solution the usual green reaction results. On boiling, the red flocculent precipitate is obtained even without the addition of any acid.

XIII. To the alkaline solution of iridocytes, potassium ferricyanide ($K_3fe(CN)_6$) was added and boiled for about 15 minutes. Silver nitrate being added gives a white precipitate. Reboiled the precipitate is transformed into bright red.

XIV. NaOH solution of iridocytes ($C_5H_5N_5O$) on boiling slowly turns reddish and the addition of another base like NH_3 and reboiling turns the red into purple.

XV A white ash is deposited on the sides of the test tube when the above solution (KOH or NaOH $C_5H_5N_5O$) is boiled to dryness. The calcified substance is refractory to concentrated acids and aqua regia; it dissolves, however, on heating, setting up a vigorous chemical action.

The next point which is worthy of notice is the fact that calcium in any form is absent from the argenteum of the larvae of *M. ornata* and *M. rubra* which has therefore nothing to do with the guaninkalk of Ewald and Krükenberg. Solutions of the subcutaneous tissue from the abdominal surface of the Ranid larvae react to the calcium tests and the dead white of the skin is due to guaninkalk in these cases. Guanin is silvery white, and the golden

[1] Iridocytes mounted in glycerine broke up and crystals were found at the end of a fortnight.

brightness of the mid-dorsal streak is caused by a layer of yellow lipochrome superimposed over the crystals. On the removal of the colouring matter by alcohol or glycerine, the band is transformed into a silver brilliancy. The following crystals were obtained and examined:

1. Strong solution of iridocytes and argenteum of the tadpole in hydrochloric acid, on being boiled, is precipitated in the form of delicate pointed needles, which on standing unite to form irregular plates. These are not hygroscopic (fig. 3).

2. Nitric acid solution gives broad plate-like crystals, somewhat prismatic, truncated at both ends. They arrange themselves in pectinate groups while hot, and break into spherical granules on cooling (fig. 4).

3. Sulphuric acid solution, which chars on boiling, produce blunt delicate needles that are bent in parallel rows. They straighten on cooling (fig. 2).

4. In caustic soda iridocytes crystallise in the form of pyramidal needles, often aggregated in wisps. The crystals are hygroscopic (fig. 1).

5. Hydrochloric solution of argenteum treated with $MGCL_2$ and precipitated gives three forms of crystals:—(1) Long silky fibres; long spindle-shaped pointed or blunt needles (aggregations of the first); (2) small delicate needles arranged in the form of brushes, and (3) smaller needles either isolated or forming rounded plates. The crystals are not hygroscopic (fig. 5).

6. If $ZNCL_2$ should be substituted for $MGCL_2$ in the above, the crystalline forms are rounded with jagged edges. They decompose into very delicate yellow needles (fig. 6).

7. Hydrochloric acid solution of iridocytes treated with strong picric acid will yield tall cylindrical coloured crystals truncated at both ends. They are hygroscopic (fig. 7).

8. If nitric acid solution is used instead of hydrochloric acid in the above, radiating coloured plates more or less oblong are obtained. They are also hygroscopic (fig. 8).

9. The silver derivative of oxyguanin also crystallises in the form of short delicate needles. They arrange themselves like wheels with a number of spoke-like structures radiating from the centre and very minute concentric circles. Between any two of such aggregations the silver oxide, which is also formed, is deposited. The crystals are hygroscopic and unstable (fig. 9).

There is only one primary form of crystals of iridocytes and argenteum obtained from all these sources which, under the influence of different substances, assumes widely divergent shapes.

The lipochromes are easily soluble in alcohol and solutions of yellow and red pigments were employed for wave length measurements of the absorption band with negative results. It is possible that the red in the tail of the forms mentioned above and other larvae is only a concentrated form of the yellow pigment. As a solution of any degree of concentration could not be obtained of the blue of *K. variegata* nothing can be said about the absorption band of this pigment.

SYNTHESIS OF IRIDOCYTES AND ARGENTEUM.

The iridocytes are most unstable and are easily affected and a few observations made in the aquarium may be here set forth.

1. If light should be absolutely cut off from the specimens, the iridocytes are absorbed in about 4 or 5 days but not the argenteum.

2. Starvation produces the same effect, and the time (4 or 5 days) depends on the condition of the larva previous to the commencement of the experiment.

3. Exposure to sunlight and liberal feeding produce two effects. The larvae become absolutely transparent, head and body included, and the metallic dorsal band extends over the cranium, at the same time becoming most brilliant.

4. If specimens used in experiments (1) and (2) are restored to normal conditions, the water (preferably tank water being used) in the aquarium being renewed every day, nearly 70 per cent of them acquire the dorsal band, appearing first in the anterior region of the vertebral column.

SUMMARY.

The leading facts discussed in this paper may be now summarised.

The first set of facts relate to the floating habits of the larvae of *M. ornata* and *M rubra* co-related with the presence of air-chambers between the branchial plates, which function more or less as hydrostatic organs. The danger of exposure to the attacks of enemies incidental to such habits is warded off by the presence of an acid offensive matter in the cephalic gland. This circumstance is probably advertised by the bright colouration.

The second set of facts deal with the unique occurrence of iridocytes and argenteum in the same larvae. Both from the morphological and evolutionary points of view the presence of iridocytes and black chromatophores on the lungs and peritoneum is full of significance, for their occurrence in the air-bladder of fishes and the peritoneum of embryonic fishes has been reported. It is established that the iridocytes of the mid-dorsal band, and the ventral argenteum of the subcutaneous tissue on the sides and the ventral surface of the abdomen of these tadpoles, are entirely free from calcium in any form, and, while both are in some respects identical with those of fishes, are entirely different from the *Guaninkalk* of Ewald and Krükenberg. The substance composing the iridocytes occurs in the form of irregular plates consisting of spherical granules, identical with those obtained by the breaking down of the guanin nitrate crystals, while the argenteum is a dense opaque reflecting subcutaneous tissue in which no structure can be made out. The dead chalky white on the ventral surface of the larvae of the genus *Rana* is due to guaninkalk.

The yellow and red lipochromes occurring in the tadpoles are not essentially different, though alcoholic solutions of them may appear quite separate.

Like the black chromatophores, the coloured ones are modifications of special connective tissue cells in which the pigments are deposited. Cells which have undergone such a change appear scaly and if the scale should break, as sometimes happens in the course of preparing tissues for mounting, the pigment occurs in the form of granules. Such a process must naturally take place in the subcutaneous tissues. Similarly the cells which develop guanin granules in the protoplasmic contents become transformed into iridocytes. They are easily marked off from the other tissue cells by their shape,—more or less flask-like, and the fact that they are not stained. As metamorphosis progresses, large amoebacytes make their appearance wherever iridocytes and argenteum occur (fig. 13).