acid in the cells during regeneration. Determinations of the changes of ribonucleic acid during regeneration are already in progress and will be published soon.

I wish to express my best thanks to Dr. A. MONROY for his constant interest, advice, and encouragement.

F. GHIRETTI

Zoological Station of Naples, Department of Physiology, October 10, 1949.

Zusammenfassung

Die Aktivität der sauren und alkalischen Phosphatase wurde während der Schwanzregeneration bei *Triturus cristatus* untersucht. Der Höhepunkt der enzymatischen Aktivität fällt mit der Determination des Blastems zusammen.

Flavones in *Helix pomatia* L.

Various reports are found scattered in the literature concerning the occurrence of flavonoid pigments in insects, e. g. the papers of PALMER and KNIGHT[1], of MANUNTA[2], and of THOMPSON[3]. The presence of flavones in the tissues of Gastropods has not been recognized till now, as far as I know.

In some organs of the snail (*Helix pomatia* L.) a yellow substance was found, extractable with methyl alcohol, ethyl alcohol, acetone, and aqueous trichloroacetic acid, but not with chloroform or petrol ether. While extracts in organic solvents are yellow, and their colour deepens after the solution has been alcalized with sodium hydroxide or ammonia, the extract in trichloroacetic acid is quite colourless from some organs (foot, lungs) or brown from the digestive gland, but acquires, however, a deep yellow colour when alcalized. The pigment does not show the characteristic greenish yellow fluorescence of riboflavin in the p_H range 3–9. It is adsorbed readily on aluminium oxide from aqueous methyl alcohol or ethyl alcohol, forming a bright yellow zone with a green-yellow fluorescence. It is precipitated by lead hydroxide in the form of a yellow lead salt.

This salt was decomposed by alcoholic sulfuric acid, the excess of the acid was neutralized with calcium carbonate, and the alcohol was distilled off in vacuum. The residue was dissolved in anhydrous acetone. This solution was very slightly yellow itself, but the addition of a solution of boric acid and citric acid, both in anhydrous acetone, gave a distinctly yellow colour and green fluorescence in ultraviolet light.

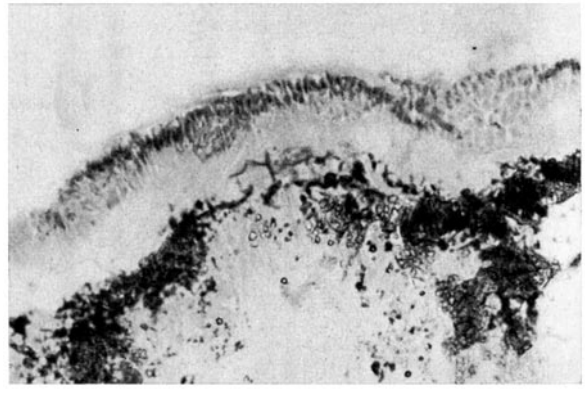
The adsorption on alumina[4], the properties of the lead salt[5], and the colour reaction with boric acid[6] all show that the yellow pigment found is of a flavonoid nature.

Only the digestive gland, foot and lungs, and, to a smaller extent, the collar and the kidney of *Helix* contain this pigment. No colour is found in the alcalized extracts from the reproductive organs, the crop and the columellar muscle.

In search of a method for an exact localization of the flavones in the organs use was made of the observation that in alçalized trichloroacetic acid extracts from the snail's foot a yellow precipitate is formed, the filtrate remaining colourless. This precipitate appeared to be the calcium salt of the flavone.

Therefore, the following method was used for histochemical demonstration of the localization of flavones:–

Frozen sections of the foot or lungs or the collar of the snail were transferred to a 2% alcoholic solution of calcium chloride, to which a drop of aqueous ammonia was added. After 15 minutes, the precipitation of the calcium flavone salt was completed by holding the sections some seconds in ammonia vapour (Figure).



A section of the edge of the mantle of the snail. The cells of the external epithelium appear yellow (histochemical reaction of flavones).

The sections were then thoroughly washed with alcohol and finally transferred to Cædax.

In the foot, the lungs and the collar, flavones appeared to be limited to the external epithelial layer, the cells of which show a bright yellow colour in sections 30μ thick. This colour slowly disappears, yet after six months it is still distinct.

I wish to express my sincere thanks to Dr. KAREL WENIG for the help he has given me and for his interest in my work.

VÁCLAV KUBIŠTA

Institute of Animal Physiology, Charles University, Prague, November 1, 1949.

Zusammenfassung

In verschiedenen Organen der Weinbergschnecke sind Flavonfarbstoffe chemisch nachgewiesen und histochemisch lokalisiert worden.

On the Flight Reaction of Tadpoles of the Common Toad Caused by Chemical Substances

In Vol. 5 of this Journal I. EIBL-EIBESFELDT[1] described the flight reaction of tadpoles of *Bufo bufo* L. (= *Bufo vulgaris* LAUR.) caused by an unknown chemical substance contained in the epidermis of the common toad.

Independently I observed in June 1948 in a small pond that when a drop from a crushed tadpole is dropped among living tadpoles which were gathered in the warmed-through layer of water 1–2 cm deep, a chaotic flight of the tadpoles set in. In 20–40 cm deep water row formations, in which the tadpoles swim, are easily

[1] L. S. PALMER and H. H. KNIGHT, J. Biol. Chem. *59*, 443 (1924).
[2] C. MANUNTA, Arch. Zool. Ital. *23*, 273 (1936).
[3] D. L. THOMPSON, Bioch. J. *20*, 73 (1926).
[4] A. MAGER, Z. physiol. Chem. *247*, 109 (1942).
[5] A. SZENT-GYÖRGYI, Z. physiol. Chem. *235*, 126 (1938).
[6] C. W. WILSON, J. Amer. Chem. Soc. *61*, 2303 (1939).

[1] I. EIBL-EIBESFELDT, Exper. *5*, 236 (1949).

destroyed with a liquid from 5–10 crushed tadpoles. Under the influence of this liquid tadpoles suddenly change the direction and rapidly swim away, or they sink first to the bottom and after some seconds they swim very rapidly away.

In order to determine how many tadpoles have to be crushed in a liter of water for inducing the flight reaction, an aquarium 1 m long, 15 cm broad, and 15 cm high was used. In the middle of this aquarium two removable slide valves at a distance of 15 cm divided the aquarium into three compartments, two larger and one smaller. The liquid from crushed tadpoles was prepared so that one tadpole was crushed in 25 ml of water and either the whole volume or only a part transferred into the smaller compartment and thoroughly mixed. The depth of water during experiments was 4·5 or 9 cm. 60 to 70 specimens of the tadpoles were transferred into one of the larger compartments, and in the front wall of the opposite compartment a bulb was placed. The other walls of the aquarium were darkened. The aquarium thus prepared for experiment was left to stand for half an hour. Then the slide valves were removed, the tadpoles in consequence of their phototropism swam into the compartment filled with the test liquid. The flight reaction was called distinct when in the test compartment at least half the individually observed animals (30–40 in each experiment) turned or at least distinctly and suddenly changed direction or sank suddenly to the bottom. When only ¼ of the animals reacted, the reaction was called indistinct, when a still smaller number reacted it was called negative. To make sure that the results were comparable the crushed tadpoles were selected so that their weight was 0·12–0·14 g.

Under otherwise equal conditions the distinctness of the flight reaction is strongly influenced by nutrition. Tadpoles left 3 days without food did not react typically at all, instead up to $^2/_3$ of all tadpoles gathered in the compartment in which the liquid from the crushed tadpoles had been added; here the tadpoles went to the bottom and walls of the receptacle. Similar conditions can occur also in nature, for I observed in a small puddle, about 3 m^2 large, that these tadpoles did not show a flight reaction to the liquid from a crushed tadpole. Tadpoles fed only with vegetable food reacted indistinctly even to a liquid from 10 tadpoles in 1 l of water. Tadpoles fed with vegetable and fleshy food reacted always when one tadpole had been crushed in 1 l of water (56 tests). In two cases they reacted distinctly also to $^1/_{10}$ tadpole in 1 l of water, in 10 experiments the reaction was distinct to $^1/_5$ tadpole, in 25 cases to ½ tadpole in 1 l of water.

In tadpoles kept for more than a week in the laboratory with suitable feeding, even when no experiments were made, the sensitivity declined. At the end of the metamorphosis the sensitivity also generally declined.

In some experiments in nature, when pure water was dropped on tadpoles in shallow water, and they had thus been disquieted, the behaviour of the tadpoles indicated the possibility that the substance which causes the flight reaction was present in a very weak concentration. To prove this several tens of tadpoles were caught so that the possibility of a washing-off of any secretion was reduced. These tadpoles were then thoroughly washed in the compartment between the slide valves. It was found that the secretion of about 50 tadpoles is as effective as ½–$^1/_5$ crushed tadpole (5 tests).

The liquid from crushed, cut-off tails of the same weight as one whole tadpole was sometimes (4 tests) nearly as effective as the liquid from a whole crushed tadpole, sometimes far less effective (5 experiments).

Calculated for the same body weight the efficiency of the crushed tadpole was smaller by ½ in the tadpole of *Bufo viridis* LAUR (1 test) and by ½–$^1/_5$ in the tadpole of *Rana esculenta* L. (6 tests). Liquids from crushed tadpoles of the specimens *Rana temporaria* L. (3 tests), *Bombina bombina* L. = *Bombinator igneus* LAUR. (2 tests) and *Pelobates fuscus* LAUR. (4 tests) were ineffective.

Tadpoles show also the typical flight reaction to the water extracts of the skin from different body regions of mature toads (*Bufo bufo* (3 tests), *B. viridis* (2 tests), *B. calamita* (1 test).

The extract from bitterling—*Rhodeus sericeus* Pall. (= *amarus* BLOCH) produced only a quite indistinct reaction (3 tests). But the extract from tadpoles produced on the contrary on *Rhodeus sericeus* a distinct flight reaction (3 tests). These experiments were made under similar conditions to those described by v. FRISCH[1].

The substance causing the flight reaction does not on the whole lose its efficiency by 15 minutes boiling. From an acidic solution (p_H about 2) it can be shaken out for the larger part into ether. From an alcoholic extract after addition of ether and water it passes almost quantitatively into ether. After careful saponification of the etheric extract in alcohol and neutralization, the acting substance can be shaken out again into ether. After evaporation of the ether the substance is very difficult to dissolve in water and is almost ineffective. Only when emulgated by means of natrium glycocholate (which itself is ineffective in a concentration of 0·02 g in 1 l) an extract is obtained, the efficiency of which is about $^1/_{10}$ of the original water extract. In the alkaline solution after saponification no active substance could be found.

The glycerin-alcoholic extracts of *Digitalis purpurea* (*Folia digitalis*) as well as pure digitalin cause a typical flight reaction, whereas histamin and adrenalin in relatively high concentrations are ineffective. It is worth noting that the hungry toads reacted also to the extracts of *Digitalis* and to histamin solutions by gnawing the walls of the receptacle.

Comparing these experiments with the experiments of v. FRISCH[1] in fishes we find the following differences: In tadpoles there is a far smaller range of sensitivity (caused of course for the larger part methodologically, as in v. FRISCH's experiments the whole swarm behaves as one unit, whereas here only statistical results could be compared). The flight reaction in tadpoles is caused exclusively by the substance itself, and it is not increased and prolonged by psychological influence as in fish. Further, in fish a secretion of the active substance in the case of their being disturbed has up till now not been found (see also GÖZ[2]). Concerning the substance itself it is thermolabile in *Phoxinus phoxinus* (HÜTTEL[3]), whereas it is thermostabile in the tadpoles. In the case of tadpoles it is probable that it belongs to the group of steroid substances, and is very closely related or perhaps identical with some of the substances known as toad poisons, the lactone ring being very probably the most effective part of the molecule.

JAROSLAV HRBÁČEK

Department of Hydrobiology, Charles University, Prague, September 1, 1949.

[1] K. v. FRISCH, Z. vergl. Physiol. *29*, 46 (1942).
[2] H. GÖZ, Z. vergl. Physiol. *29*, 1 (1942).
[3] R. HÜTTEL, Naturwiss. *29*, 333 (1941).

Zusammenfassung

Der Körpersaft aus einer zerdrückten Erdkrötenquappe von *Bufo bufo* in 1 bis 10 l Wasser verdünnt löst eine Fluchtreaktion der lebenden Erdkrötenquappen aus. Die die Fluchtreaktion auslösende Substanz wird auch von lebenden Kaulquappen bei Beunruhigung sezerniert. Die Reaktionsfähigkeit wird in großem Maße vom Ernährungszustand beeinflußt. Die wässerigen Extrakte aus Kaulquappen von *Bufo viridis* und *Rana esculenta* wie auch die Extrakte aus der Haut der erwachsenen Erdkröten verursachen die gleiche Fluchtreaktion. Der Schreckstoff kann in Äther ausgeschüttelt werden. Extrakt aus den Blättern von *Digitalis purpurea* bewirkt die Reaktion in derselben Weise wie Extrakt aus den Erdkrötenquappen. Extrakt aus Erdkrötenquappen löst beim Bitterling (*Rhodeus sericeus* PALL.) die gleiche Fluchtreaktion aus wie der Extrakt aus diesem Fisch.

Sur les protéines de structure des muscles striés

D'après MATOLTSY et GERENDÁS[1], si l'on traite de la pulpe de muscle de Lapin tout d'abord par une solution de Weber (0,6 m KCl, 0,04 m $NaHCO_3$, 0,01 m Na_2CO_3) pour éliminer l'actomyosine et la myosine, puis par une solution 0,6 m NaI pour éliminer l'actine (sous une forme dépolymérisée), on peut alors préparer, au moyen de solutions de Weber contenant 30% d'urée, un extrait possédant *une biréfringence négative d'écoulement*. La protéine responsable de cette propriété serait la même que la protéine de structure signalée, en 1940, par BANGA et SZENT-GYÖRGYI[2] et qui est si abondamment représentée dans le tissu rénal; elle est riche en P[2] et possède dans l'U.V. le spectre particulier des nucléoprotéines[1]. Elle reçut, de ce fait, la dénomination: *N protéine*[1].

L'intérêt de cette substance réside dans ce fait qu'elle serait localisée *in vivo* dans les disques I où sa biréfringence négative d'orientation y serait exactement compensée par la biréfringence positive d'orientation des filaments d'actomyosine[1].

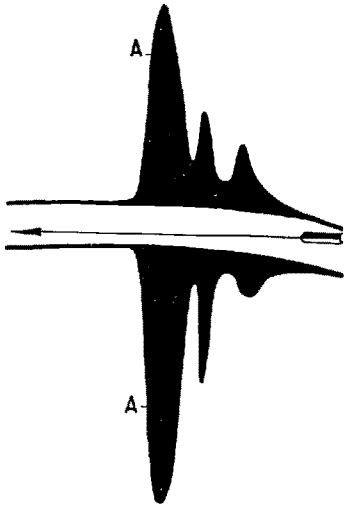


Fig. 1. – Tracé électrophorétique d'un extrait musculaire préparé par l'action de 1,5 vol. de KI 0,6 m sur de la pulpe musculaire de Lapin, débitée au microtome à congélation et de laquelle la myosine avait été enlevée par trois lavages successifs avec trois volumes de la solution de Weber. Au-dessus, frontières ascendantes; en dessous, frontières descendantes, après 7 heures d'électrophorèse (1,5 volt/cm) à μ 0,40 et p_H 7,40. *A* gradient de l'actine.

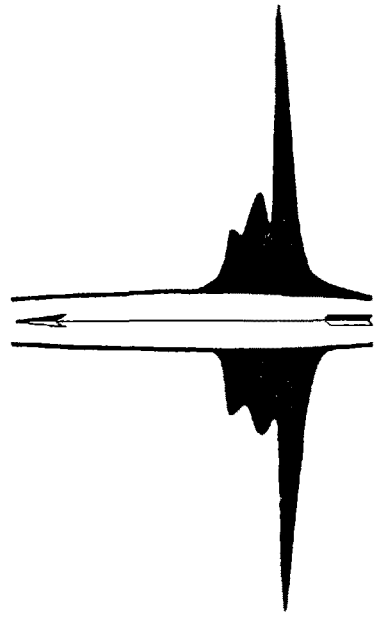


Fig. 2. – Même pulpe musculaire que la fig. 1, mais après action de la solution de Weber et de la solution de KI, la pulpe est extraite maintenant avec la solution de Weber contenant 30% d'urée. 5 h. 15 min. d'électrophorèse (1,5 volt/cm) à μ 0,40 et p_H 7,40.

Procédant dans des conditions de préparation aussi semblables que possible à celles signalées par ces auteurs, nous avons constaté ce qui suit:

Lorsque l'on a extrait, de la pulpe musculaire, la totalité de la myosine β et de l'actomyosine extractibles, par trois lavages consécutifs au moyen d'une solution de Weber (KCl 0,6 m, $NaHCO_3$ 0,04 m, Na_2CO_3 0,01 m, chaque fois trois volumes de solution), on peut, en utilisant 1,5 volume d'une solution de KI à 9,96% (0,6 m), enlever de nouvelles protéines qui présentent l'aspect électrophorétique de la fig. 1. On y voit trois composantes dont la plus rapide est l'actine dépolymérisée[2] (*A*, vitesse à μ 0,40 et p_H 7,40: $-4{,}55 \cdot 10^{-5}$ cm/sec (asc.) et $-4{,}6 \cdot 10^{-5}$ cm/sec (desc.). Les deux autres, de vitesses $-3{,}4$ et $-2{,}45$ (asc.) ou $-3{,}3$ et $-2{,}2$ (desc.) correspondent à des composantes inconnues).

Si l'on traite ensuite la pulpe par la solution de Weber additionnée de 30% d'urée, on extrait encore d'autres protéines (25 cm³ contenant de 1,1 à 1,4 mg N/cm³, pour 14 g de muscle) dont les caractéristiques électrophorétiques sont représentées fig. 2. Il y a trois composantes dont les vitesses (à μ 0,40 et p_H 7,4) et les concentrations sont:

asc.	desc.	Conc.
–2,6	–2,05	64,9%
–3,25	–2,95	26,2%
–4,10	–3,75	8,8%

[1] A.G. MATOLTSY et M. GERENDÁS, Nature *159*, 502 (1947); Hung. acta physiol. *1*, 116 et 128 (1928).

[2] I. BANGA et A. SZENT-GYÖRGYI, Enzymologia *9*, 111 (1940).

[1] A.G. MATOLTSY et M. GERENDAS, Nature *159*, 502 (1947); Hung. acta physiol. *1*, 116 et 128 (1948).

[2] M. DUBUISSON, Biochim. et biophys. acta, sous presse.