

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 KNIGHT¹, of MANUNTA², and of THOMPSON³. 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 alumina⁴, the properties of the lead salt⁵, and the colour reaction with boric acid⁶ all show that the yellow pigment found is of a flavonoid nature.

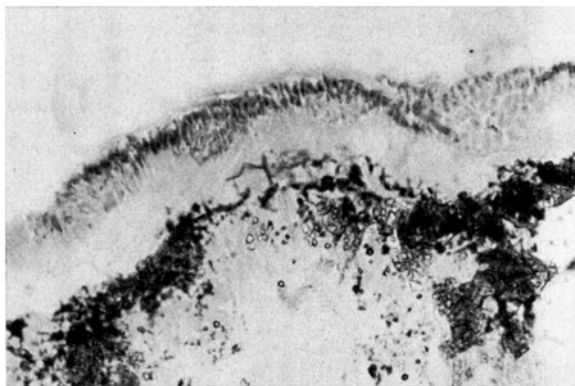
Only the digestive gland, foot and lungs, and, to a smaller extent, the collar and the kidney of *Helix* contain this pigment. No colour is found in the alcalized extracts from the reproductive organs, the crop and the columellar muscle.

In search of a method for an exact localization of the flavones in the organs use was made of the observation that in alcalized 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 30 μ 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-EIBESFELDT¹ 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

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⁶ C. W. WILSON, J. Amer. Chem. Soc. 61, 2303 (1939).

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