So the principle mentioned here might be one of the most important causing the evolutionary increase in size which is so obvious in many groups of animals, including the invertebrates as shown by NEWELL[1].

RENSCH[2] has shown that large-sized species of different groups of cold-blooded vertebrates have more eggs than small species of the same group. This increases the possibility for bigger species to survive.

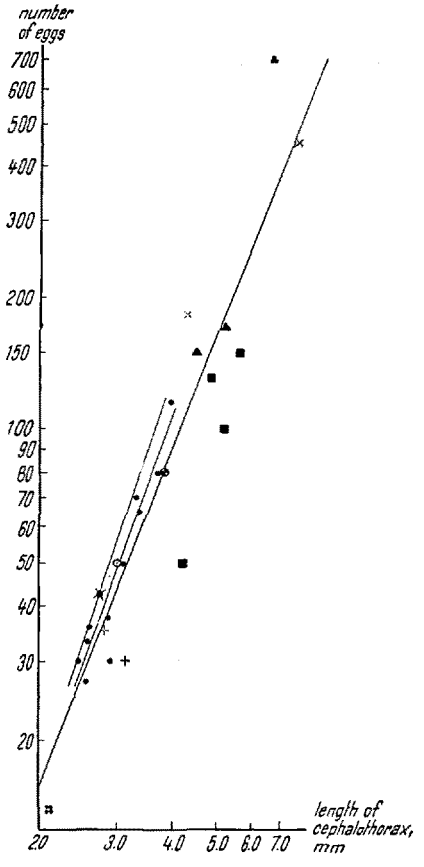

Fig. 2. – Covariation between length of cephalothorax and number of eggs in some species of the families *Lycosidæ* and *Pisauridæ*. The upper regression line represents *Lyc. palustris*, the middle line *Lycosa* ssp. ($\alpha = 2{\cdot}79$, $r = 0{\cdot}95$) and the lower line all species ($\alpha = 2{\cdot}53$, $r = 0{\cdot}94$). The genera are indicated by the following marks: *Lycosa* •, *Pirata* ⊙, *Trochosa* ▲, *Tarentula* ■, *Arctosa* ∴, *Aulonia* ::, *Xerolycosa* +, *Acantholycosa* ⓘ, *Dolomedes* and *Pisaura* ×.

Similar conditions are present in spiders (cf. Fig. 2, where the data given in this paper are added to those of HOLM[3]).

BJÖRN PETERSEN

Zoological Institute, University of Uppsala, November 11, 1949.

Zusammenfassung

Eine positive Korrelation zwischen Größe der Mutter und Zahl der Nachkommen wurde in drei Arten der Spinnenfamilie *Lycosidae* gefunden. Da die Größe der Mutter zum Teil erblich bedingt ist, führt diese Korrelation automatisch zu einem Selektionsdruck gegen gesteigerte Körpergröße. Die Korrelation ist wahrscheinlich bei vielen Tier- und Pflanzengruppen vorhanden und ist einer der bedeutendsten Faktoren, die die Körpergrößenzunahme während der Evolution bewirkt haben.

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[2] B. RENSCH, *Neuere Probleme der Abstammungslehre* (Stuttgart 1947).

[3] Å. HOLM, Svensk spindelfauna utgiven av entomologiska föreningen i Stockholm. 3. Egentliga spindlar. Aranceæ. Fam. 8–10. Oxyopidæ, Lycosidæ och Pisauridæ (Stockholm, 1947).

On the Activity of Acid- and Alkaline Phosphatase during Tail Regeneration in *Triturus cristatus* (Laur.)

Very little is known about chemical processes during regeneration. This is especially true as far as regeneration in amphibia is concerned. So far research has been centered mainly on autolytic processes and on proteolytic activity in the regenerating tissues (BROMLEY and ORECHOWITSCH, OREKOWISTCH *et al.*, RYVKINA, STRIGANOVA, VLADIMIROVA[1]). Only more recently attention has been focused on respiratory changes and on the activity of some enzyme systems involved in phosphorus and nucleoprotein metabolism (JAEGER and BARTH, BARTH, MILLERS, BODIAN, BODIAN and MELLORS, CLEMENT-NOEL[2]).

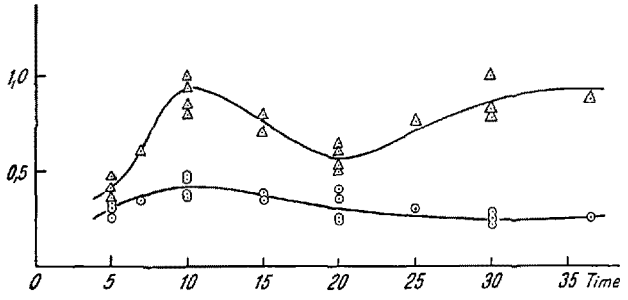

Fig. 1. – Activity of alkaline phosphatase during tail regeneration in *Triturus cristatus*. Ordinate: mg P split/mg N. Abscissa: days after amputation. Triangles: activity in the blastema; circles: activity in the underlying tissues.

The present paper brings the first results of a systematic investigation undertaken to study some chemical events during tail and limb regeneration in *Triturus cristatus*. The activity of acid and alkaline phosphatase has been investigated in various stages of tail regeneration. After amputation, the animals were kept in lots of ten or fifteen specimens at 16–18° C. At regular intervals the blastema were dissected and frozen at –50° C in dry ice. A small part of the underlying tissue was also collected after removal of the bones. Specimens were also prepared for histological control. Tissues were homogenized and extracted at + 3° C for 24 hours in Na-veronal buffer 0·15 M (p_H 9·5 and 5·0). After centrifugation, the phosphatase activity was tested on Na-β-

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[2] L. JAEGER and L.G. BARTH, J. Cell. and Comp. Physiol. *32*, 319 (1948). – L.G. BARTH, Physiol. Zool. *11*, 179 (1938); Biol. Bull. *74*, 155 (1938); ib. *78*, 366 (1940). – J.A. MILLER, Biol. Bull. *73*, 369 (1937). – D. BODIAN, Symp. Soc. Exp. Biol. *1*, 163 (1947). – De BODIAN and R.C. MELLORS, Proc. Soc. Exp. Biol. and Med. *55*, 243 (1944). – H. CLEMENT-NOEL, Ann. Soc. Roy. Zool. Belg. *75*, 25 (1944).

glycerophosphate in glycine (p_H 9·5) or acetate (p_H 5·0) buffer as substrate. $MgCl_2$ 0·02 M was added as an activator. Owing to the low enzymic activity of the material, incubation was prolonged for 24 hours at 38° C. At the end of the incubation, trichloroacetic acid 10% was added and inorganic phosphorus determined according to FISKE and SUBBAROW[1].

Table I

Days after amputation	Blastema	Under. Tis.
5	0·47	0·25
	0·40	0·32
	0·35	0·30
7	0·61	0·34
10	1·00	0·38
	0·80	0·36
	0·85	0·47
	0·94	0·48
15	0·80	0·38
	0·71	0·33
20	0·59	0·35
	0·53	0·24
	0·64	0·40
	0·50	0·25
25	0·75	0·29
30	1·00	0·25
	0·78	0·21
	0·83	0·28
38	0·88	0·27

Activity of the alkaline phosphatase during tail regeneration in *Triturus cristatus*. mg P split/mg N.

The experiments were carried out in two different periods, namely between the end of winter and the beginning of spring (December–March), during which time the regeneration rate is highest (first period) and then between the end of spring and the beginning of summer, when the regeneration rate begins to decrease (second period). The results concerning the alkaline phosphatase during the first period are given in Table I and Fig. 1. It is clear that the activity of the alkaline phosphatase is low in the very first stages after amputation. About the 5th day it begins to rise and attains its highest point approximately on the 10th day. Later on, it decreases again until the 20th day. Then increase sets in again very slowly. The process was examined until the 38th day after amputation. The phosphatase activity in the underlying tissues shows only a very small increase around the 10th day, remaining almost constant later on. Activity measurements of the alkaline phosphatase during the second period show that its highest point is reached between the 20th and 25th day after amputation.

As to the acid phosphatase, the only available data are those concerning its activity during the second period, i. e. when the regeneration rate is lower. Table II and Fig. 2 show indeed that a maximum is attained between the 15th and 20th day after amputation. No data are available concerning the later behaviour. Its activity on the whole is higher than that of the alkaline phosphatase during the same period.

It is interesting to note that, in the experiments carried out during the first period, the maximum of the alkaline phosphatase activity coincides with the time when the determination of the blastema is known to occur (MILOJEVIC, WEISS, POLEZHAYEV)[1]. No data are available in the literature concerning the time of determination of the blastema when the regeneration rate is in its decreasing period. Histological controls show that

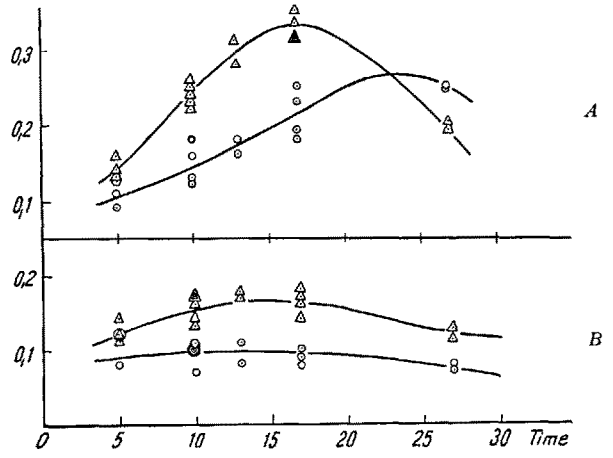


Fig. 2. – Activity of acid and alkaline phosphatase during tail regeneration in *Triturus cristatus*. Ordinate: mg P split/mg N. Abscissa: days after amputation. *A* activity in the blastema; *B* activity in the underlying tissues. Triangles: acid phosphatase; circles: alkaline phosphatase.

when the phosphatase activity is highest strong dedifferentiation and regressive processes go on in the bud. The second increase may depend on the beginning of differentiation. BODIAN and MELLORS[2] found an increase of acid phosphatase activity in the cytoplasm of chromatolytic cells in the advanced stages of the regressive period and considered it to be in connection with the recovery of Nissl bodies. According to BODIAN[3] this increase is correlated with nucleoprotein synthesis. Also CLEMENT-NOEL[4] found an increase of ribonucleic

Table II

Days after amputation	Blastema		Under. Tiss.	
	Acid	Alkal.	Acid	Alkal.
5	0·16	0·13	0·14	0·12
	0·14	0·11	0·11	0·12
	0·13	0·09	0·11	0·08
10	0·26	0·13	0·17	0·11
	0·22	0·12	0·17	0·10
	0·24	0·18	0·16	0·10
	0·23	0·16	0·14	0·10
	0·25	0·18	0·13	0·07
13	0·31	0·18	0·17	0·11
	0·28	0·16	0·18	0·08
17	0·35	0·25	0·18	0·10
	0·32	0·18	0·17	0·14
	0·34	0·19	0·16	0·09
	0·32	0·23	0·14	0·08
27	0·20	0·25	0·12	0·08
	0·19	0·24	0·11	0·07

Activity of acid and alkaline phosphatase during tail regeneration in *Triturus cristatus*. mg P split/mg N.

[1] C.H. FISKE and Y. SUBBAROW, J. Biol. Chem. *66*, 375 (1925).

[1] B.D. MILOJEVIC, Roux' Arch. Entw. Mech. *103*, 80 (1924). – P. WEISS, ib. *107*, 1 (1926); ib. *111*, 317 (1927). – L.W. POLEZHAYEV, Bull. Biol. France et Belg. *70*, 54 (1936).

[2] Loc. cit.

[3] Loc. cit.

[4] Loc. cit.

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.

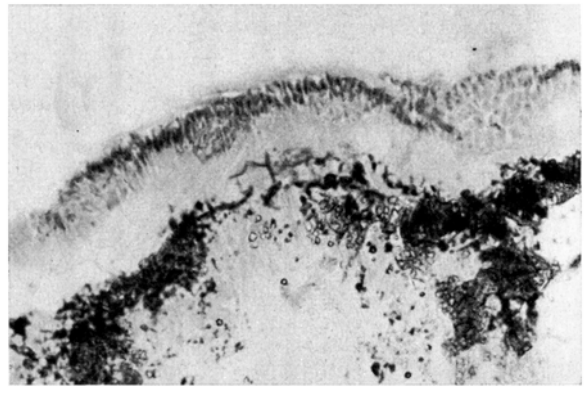
The adsorption on alumina[4], the properties of the lead salt[5], and the colour reaction with boric acid[6] all show that the yellow pigment found is of a flavonoid nature.

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).
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