Fall ist, die eine ganz besonders kurze Halbwertszeit besitzen[8]. Eine relativ langsam verlaufende Komplementproduktion ergab sich auch aus früheren Untersuchungen von BÜSING und ZUZAK[9] über den Zusammenhang zwischen Vitamin-K-Versorgung und Gesamtkomplement des Serums beim Küken. Die Autoren konnten zeigen, dass der bei Vitamin-K-Mangel erniedrigte Gehalt des Gesamtkomplementes erst eine Woche nach Verabreichung von 2-Methyl-1,4-disuccinyl-naphto-hydrochinon (2,5 µg täglich) normalisiert wurde.

Tabelle II. Messung der Blutgerinnung (Prothrombinzeit) bei den gleichen Küken, die zur Bestimmung des Serumkomplementes verwendet wurden

Tiergruppe	Anzahl Küken	Prothrombinzeit[a] in sec[b]
Kontrollküken	11	35,4 ± 2,5
Vitamin K-Mangelküken	9	>180
Gleiche Mangeltiere nach Vitamin K_1-Verabreichung[c]	8	33,0 ± 1,8

[a] Die Bestimmung der Prothrombinzeit erfolgte nach der Methode von DAM et al.[7].
[b] Mittelwert ± mittlerer Fehler (2σ-Grenze).
[c] An drei aufeinanderfolgenden Tagen je 50 µg Vitamin K_1 in 0,2 ml Arachisöl mit der Schlundsonde verabreicht.

Berichte[10], denen zufolge eine direkte Beziehung zwischen der Komponente C'1 und dem Prothrombin besteht, können auf Grund der vorliegenden Ergebnisse nicht gestützt werden.

Summary. In vitamin K deficient chicken the serum complement (C') level is significantly decreased, as compared with normal animals, due to a lower level of its component C'1. Administration of vitamin K_1 for three days did not restore its titer although the prolonged blood clotting time became normal. The results thus indicate a considerably slower resynthesis of the complement factor C'1 than of prothrombin.

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17. Dezember 1962.

[7] H. DAM, I. KRUSE und E. SØNDERGAARD, Acta physiol. scand. *22*, 238 (1951).
[8] C. MARTIUS, Dtsch. med. Wschr. *83*, 1701 (1958).
[9] K. H. BÜSING und H. ZUZAK, Z. Immun.-Forsch. *102*, 401 (1943).
[10] M. VON FALKENHAUSEN, Biochem. Z. *218*, 453 (1930).

Free Amino-Acids in the Snail *Limnaea* and their Changes with Morphogenesis

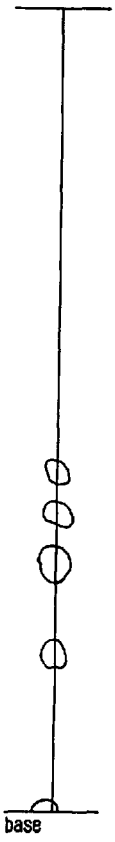
HADORN and MITCHELL[1] first described a method of crushing organic material like Drosophila eggs, larvae, pupae etc. directly on the filter paper for separating the fluorescent and ninhydrin positive substances by means of paper chromatography. This technique was later used by BUZZATI-TRAVERSO[2], and BUZZATI-TRAVERSO and RECHNITZER[3]. KIRK et al.[4] studied the chromatographic UV-absorption and fluorescence pattern of foot muscles from 7 species of land snails. The pattern is independent of the animal's size or age but is species specific. This, according to the authors, may be an aid towards understanding the biochemical basis of individuality. On the basis of their study on fish-tissues, BUZZATI-TRAVERSO and RECHNITZER[3] had also come to a similar conclusion.

Amino-acids, being the building blocks of proteins, are an interesting study. ROBERTS and SIMONSEN[5] have indeed compared the free amino-acid patterns of many marine organisms with those of certain malignant cells. KAVANAU[6] and CHEN[7] have studied in detail the amino-acid pattern of sea urchin and urodeles during embryonic stages. In a similar way we have studied the free amino-acid pattern of the snail *Limnaea sp.* at different stages of morphogenesis from egg to adult stage with the help of paper chromatography and electrophoresis. The solvent used for chromatography was *n*-Butanol-acetic acid-water :: 4:1:1. Three other solvent systems, namely (1) 70% ethanol, (2) *n*-Butanol-acetic acid-water :: 4:1:6, (3) *n*-Butanol-Pyridine-water :: 1:1:1, were also tried and found to be unsatisfactory. The following attempts were made. (1) Direct crushing of egg masses, young snails with shells and of shelled adult snails on Whatman paper No. 1 *never gave any resolution* but the intensity of ninhydrin staining with progressive age was very striking. (2) Whatman paper No. 4 gave clearer results with young snails crushed directly on it. (3) The supernatant, after centrifuging the product obtained from grinding the adult snail with sand in a glass mortar and then treating the mass with 70% ethanol, was further concentrated. This gave a comparatively faint but *excellent resolution* on Whatman No. 1. This method cannot be used with younger snails and egg masses with their low intrinsic contents of amino-acids. (4) The electropherograms with M/50 Borax buffer of adult snails crushed directly on Whatman No. 4 were fairly good ($1^1/_2$ h 250–300 V, 2–3 mA) but the intensity of ninhydrin-stain was low. They could not be used with young snails or egg masses.

The results based on (2) and (3) may be summed up thus: The egg mass at the stage of '2 days before hatching' has a significantly greater amino acid content than 6 days before hatching. 11 days before hatching, the amino acid content is very faint, in fact hardly distinguishable. Newly hatched snails (2–9 days) show three recognizable ninhydrin spots. One of these, starting from the origin itself,

[1] E. HADORN and H. K. MITCHELL, Proc. Nat. Acad. Sci. *37*, 650 (1951).
[2] A. A. BUZZATI-TRAVERSO, in *New Approaches in Cell Biology* (Academic Press, 1960), p. 95.
[3] A. A. BUZZATI-TRAVERSO and A.B. RECHNITZER, Science *117*, 58 (1953).
[4] R. L. KIRK et al., Biochem. J. *57*, 440 (1954).
[5] E. ROBERTS and S. SIMONSEN, in *Amino Acids, Proteins and Cancer Biochemistry* (Academic Press, 1960), p. 142.
[6] J. L. KAVANAU, Exp. Cell. Res. *7*, 530 (1954).
[7] P. S. CHEN, Exp. Cell. Res. *10*, 675 (1956).

is probably a peptide and the other two have Rf values 0.30 and 0.20. At a much later stage (0.75 cm in length), the snails show precisely these two spots with enormously enhanced intensity. Adult or big snails (above 1 cm) show four spots with Rf values 0.42, 0.37, 0.31, 0.21 (0.20) (see Figure). (The solvent for all these was *n*-Butanol-acetic acid-water :: 4:1:1.) As the Rf-values alter considerably with temperature, they were redetermined in a room of constant temperature (about 25°C) and they turned out to be 0.34, 0.28, 0.22, 0.14.



Thus, the biochemical pattern of free amino acids is almost established at practically the hatching time and it is only accentuated up to a comparatively late stage. In this case, the position is quite different from that seen by KAVANAU[6], CHEN[7], and BRAHMACHARY[8] who detected a large number of amino acids in sea-urchin embryo, urodele embryo and germinating seeds of Mung bean. However, a second pattern of faint spots (detected only with chromatography) with higher Rf values is evident in snails above the size 1.45 cm. It is interesting to note that, even after starvation of two weeks, the pattern (including the second, fainter set) remains unaltered. This has been verified also with another species, namely *Viviparous Sp.* Unless the catabolic rate itself slows down in the starving snails, this might mean that the free amino acid pattern is maintained by breaking down some of the proteins. This would be further evidence for the 'stubborn' gene determined biochemical pattern of individuality[9].

Résumé. Les auteurs étudient l'état des acides-aminés libres aux différents stades de développement de l'escargot (Gastéropode) *Limnéa.* Cet état est constant de l'éclosion jusqu'à un stade avancé; ce n'est qu'après celui-ci que l'on trouve de nouveaux acides-aminés libres. Par contre, le modèle-type se maintient inchangé dans les animaux affamés.

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[8] Unpublished data.

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Influence of Cu^{++} and Zn^{++} Ions on the Effects of Ethyl Methanesulfonate (EMS) on Chromosomes

The chromosome-breaking activity of ethyl methanesulfonate (EMS) is still somewhat controversial, partly due to differences in experimental conditions. Some workers[1] have reported this monofunctional alkylating agent as causing chromosomal rearrangements, whereas others have not found such an activity[2].

In our own experiments, variations in the effects were observed on chromosomes as well as on seed germination and on plant growth. It was demonstrated that these effects may be (or are) dependent on experimental conditions such as temperature, duration of exposure and some water contaminations. In order to test this last possibility, distilled waters from several origins were investigated. Sharp differences were found to occur, which was an incitement to further investigations.

Those water fractions in which EMS exhibited an increased activity, were regularly found to be contaminated by copper or zinc or both together.

On the basis of these observations, the ions mentioned were tested systematically. To about 10^{-3} m*M* solutions of $CuSO_4$ or $ZnSO_4$ in bi-distilled water, EMS was added at several concentrations. When needed, pH was controlled by means of 0.1 molar Sørensen buffers. These solutions were used for treatments of resting seeds of *Vicia faba* for 3 h.

The following points could be clarified, using an anaphase scoring technique in *Vicia faba*: (1) Solutions containing neither ion under investigation were found to be inefficient in inducing chromosome aberrations. (2) Solutions containing either Cu^{++} or Zn^{++} were found to be efficient in this respect, the synergistic effect of the copper ions being greater than that of zinc (Figure 1). (3) Copper and zinc containing control solutions showed some activity (Figure 1), much higher for Zn^{++} than for Cu^{++}. These results are in agreement with VON ROSEN'S

[1] R. RIEGER and A. MICHAELIS, *Die Kulturpflanze* (Akademie-Verlag, Berlin 1960), vol. 8, p. 230.

[2] E. A. FAVRET, cited in Hereditas *46*, 622 (1960).