Comparison of the inhibitory action of fraction IV on hemagglutination and mitosis produced by Con A.

Agglutinin	Peptide fraction	Agglutination[a] titer	Cpm[b]	Incorporation (%)[c]
Con A	None	4	9.981	100
None	I (from native Con A)	4	7.993	80
None	I (from denatured Con A)	0	180	2
None	II (from native Con A)	0	220	2
None	III (from native Con A)	0	230	2
None	IV (from native Con A)	0	243	2
Con A	I (II or III)	4	9.700	97
Con A	IV (from native Con A)	0	2.240	22
Con A	IV (from denatured Con A)	0	3.230	32
Con A	IV Pronase-treated	4	9.043	91
Con A	IV Trypsin-treated 48 h	4	—[d]	—
Bean PHA	IV (from native Con A)	4	16.850	91
Bean PHA	None	4	18.500	100

[a] Measured as described in text. [b] Counts per min incorporated ^{3}H-thymidine in cultured human lymphocytes. [c] Percent incorporation of ^{3}H-thymidine as compared to the one produced by Con A. [d] Not measured.

The inhibitory fragments from Con A are promizing tools for the further investigation of the role played by Con A receptor sites of normal and transformed cells. Probably, similar inhibitory peptides can be obtained from other phytohemagglutinins and can be used to study the different aspects of their biological activities[8].

Zusammenfassung. Durch Trypsinverdauung von nativem und denaturiertem Concanavalin A wurde eine Peptidfraktion erhalten, die sowohl die hämagglutinierende als auch die mitogene Wirkung von Concanavalin A hemmt. Die Fraktion zeigte jedoch keine Wirkung auf die Aktivität von Bohnenagglutinin.

DINAH S. SEIDL, A. PALOZZO, A. LEVY, V. AZAVACHE, M. JAFFÉ and W. G. JAFFÉ

Escuela de Biología, Facultad de Ciencias, P.O. Box 10098 Caracas (Venezuela), 20 June 1974.

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Electrophoretic Study of Carboxylesterases During the Ontogenesis of *Medicago scutellata*

During differentiation, it is known for a number of enzymes that ontogenesis is associated with alteration in the isoenzyme content of the organisms. The differential synthesis of isoenzymes in cells may be considered as an elementary process in cell differentiation[1]. Support for the concept of differential gene activation during cellular differentiation has been derived from studies of the ontogenesis of isoenzymes. Such studies in the case of carboxylesterases have been carried out mainly in animals[2–9], while no complete study has yet been made on the ontogenesis of carboxylesterases in plants[10–13]. The purpose of this study was to investigate whether multiple molecular forms of carboxylesterases occured in a wild species of Alphalpha (*Medicago scutellata*), and whether these forms were changed during the development of the plant.

Material and methods. Seeds of *Medicago scutellata* (Miller) were collected from Patras fields, disinfected with a 25% solution of commercial liquid bleach ('Klinex') and part of the seed-coat was removed in order to facilitate germination. The seeds were allowed to germinate at 25 °C in petri dishes lined with filter paper wetted with water. After the germination, the seedlings were grown in pots. Samples from different organs were taken at various developmental stages, homogenized with distilled water (1:5 W/V), filtered through cheese-cloth, centrifuged for 30 min at 30,000 *g* at 4 °C and the supernatant was collected. Horizontal starch gel electrophoresis was carried out using the discontinuous buffer of ASHTON and BRADEN[14] with some modifications (the gel buffer was diluted to $^1/_3$ with distilled water), and a voltage gradient of 20 V/cm for $2^1/_2$–3 h. After electrophoresis, the gels were sliced horizontally and the carboxylesterase

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bands were visualized by incubation in a 0.1 *M* phosphate buffer pH 6.0 containing α-naphthyl acetate as substrate and fast red T.R. salt as a coupling agent. For exclusion of the other forms of nonspecific esterases, EDTA-Nα_2 and eserine sulfate were used.

Results. Cotyledons. The pattern of carboxylesterases from cotyledons of fruits (Figure 1) in the early stage of development (7th day after flower formation) consisted of only 3 bands. As the development of seeds proceeded, new bands progressively appeared.

In contrast to the cotyledons during development, the pattern of carboxylesterases of seeds in the early stages of germination (5 h dermination) consisted of 11 bands (Figure 1, sample 5). As the germination proceeded, new bands were observed, while others gradually disappeared, resulting in a quite different pattern (compare sample 5 with 10).

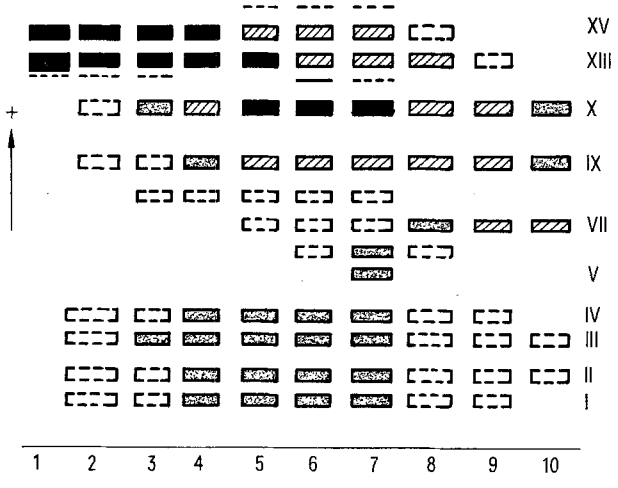

Fig. 1. Electrophoretic patterns of carboxylesterases from cotyledons of *M. scutellata*. Nos. 1 to 4, extract from ripening cotyledons 7, 18, 22 and 40 days of age respectively, Nos. 5 to 10, extract from germinating cotyledons of 5 h, 3, 4, 10, 14 and 33 days of age respectively. Explanation of the symbols: Black rectangles, high activity; rectangles with cross hatching medium; with stippling low, and with dotted outlines, very low activity.

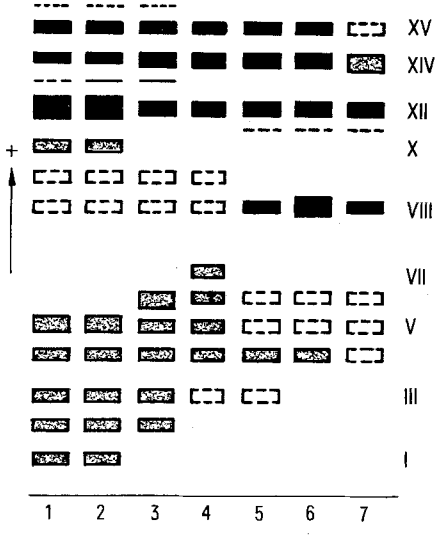

Fig. 2. Electrophoretic patterns of carboxylesterases from roots of *M. scutellata*. Sample Nos. 1 to 7 extracts from roots of 5 h, 2, 3, 4, 7, 10 and 33 days of age respectively.

Roots. After 5 h germination the pattern consisted of 13 bands (Figure 2). As germination proceeded new bands gradually appeared, while others progressively disappeared. The appearance of the band VII (sample 4) is very characteristic, because it coincided with the emergence of the root hairs. At a later stage, the branching of the main root coincided with the disappearance of the band (VII) and the simultaneous increase in the activity of another band (VIII) (sample 5). Roots from the advanced stages of seed germination (33 days old) had a very different pattern (compare sample 1 with 7).

Root nodules. The pattern of root nodules is characterized by the great number of bands, the high activity and the numerous changes during development (Figure 3). Although the degree of morphological differentiation in the root nodules did not seem to be very significant, nevertheless the carboxylesterases differentiation was significant.

Hypocotyls. The pattern of young hypocotyls was similar to that of cotyledons (Figure 1, sample 5). During development, the activity of 1 band increased considerably (very characteristic) while the activity of others rather decreased.

Stem. The pattern of stem was similar to that of young cotyledens. No significant changes were observed during development.

Leaves. The pattern of single leaves (the first emerging leaves) consisted of 5 bands (IV, VII, X, XIII, XIV, Figure 1, sample 7), while in the early stage of the first emerging triple leaves, 8 new bands (I, II, III, V, VI, VIII, IX, XI, Figure 1, sample 7) appeared. During development, the activity of most forms decreased, so that only 2 forms of high activity and high mobility remained.

Pericarp. The pattern of pericarp at an early stage of fruit development resembled the one of young triple leaves. As fruit development continued, the activity of most of them decreased so that in the dry, grey, hard and coiled pericarp only 3 forms of high activity and high mobility remained.

Seed-coat. The pattern from the seed-coat during the early stage of seed germination consisted of only 2 forms of carboxylesterases of high mobility and high activity, and it did not change during the later stages.

Flower. The pattern of green calyx was very similar to that of young triple leaves. In the pattern of coloured corolla, many bands disappeared and the activity of the remains decreased. Many more changes occurred in the pattern of pollen grains.

Discussion. From these results, it is concluded that each organ of *Medicago* presents a characteristic pattern of carboxylesterases. These patterns undergo several changes during the development of the organs. The degree of these changes is seen to be correlated with the degree of organ differentiation, as is concluded especially from the developement of cotyledons, roots, flower and seed-coat. Such correlations have also been observed[10–16] in other plants. These changes of carboxylesterases may be explained as representing the activation or inactivation of the genes which are responsible for their synthesis during ontogenesis. The switching on and off of these

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genes may be explained according to the hypothesis of JACOB and MONOD[17] and BRITTEN and DAVIDSON[18,19]. The different distribution of hormonic content during plant differentiation[20,21], the changes in light and diet (because the root, for instance, is growing in the absence of light and receives food from the cotyledons in the early stages of its development, whereas later it receives food from the soil) may all be important factors contributing to the changes which have been observed. The dramatic changes of carboxylesterasic pattern of root nodule (Figure 3) may be due to the polyploidy of the nodule cells[22]. On the other hand, the *Bacteria* (Rhizobiaceae), which always live symbiotically in the root nodules may release some esterasic bands into them. In the root nodules of *Lotus pedunculatus*, however, only quantitative changes in esterase pattern were observed[23].

Riassunto. Il modello elettroforetico delle carbossilesterasi solubili nel corso dell'ontogenesi del *Medicago scutellata* è stata studiata con elettroforesi orizzontale su gel-amido. Il modello di ogni organo esaminato include una somma di molteplici forme molecolari di carbossilesterasi, la maggior parte delle quali è sottoposta a considerevoli alterazioni nel corso dell'ontogenesi della pianta.

T. PAPACHRISTOS and C. CHRISTODOULOU

University of Patras, Laboratory of Biology, Patras (Greece), 3 July 1974.

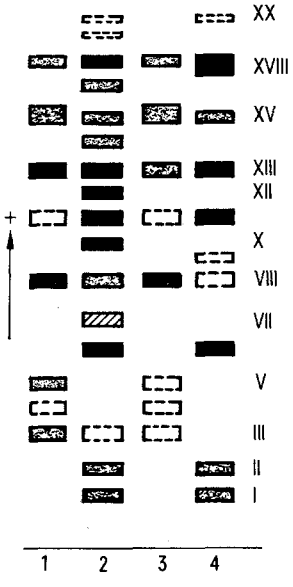

Fig. 3. Electrophoretic patterns of carboxylesterases from root nodule of *M. scutellata*. Nos. 2 and 4, extracts from root nodules of 30 and 90 days of age respectively. Nos. 1 and 3, extracts from roots of 30 and 90 days of age respectively.

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The Anatomy and Innervation of the Ecdysial Glands of the Mature Larva of Castor Silk Moth, *Philosamia ricini* Hutt.

Ecdysial glands have been described in a large number of insects and one of the issues on which workers disagree is whether or not the glands are innervated. Those who describe innervation also differ greatly among themselves. ARVY and GABE[1], WILLIAMS[2], SRIVASTAVA and SINGH[3] in *Tenebrio molitor*, *Hyalophora cecropia* and *Papilio demoleus* respectively have reported innervation whereas SRIVASTAVA[4] in *Tenebrio* and HERMAN and GILBERT[5] in *Hyalophora cecropia* find no trace of nerves in the glands. Apart from Lepidoptera, detailed structure of the glands has not been described, except by HERMAN and GILBERT[5] in *H. cecropia*.

This paper describes the structure and innervation of the glands in a well-known experimental Lepidopteran *Philosamia ricini* Hutt. 3–4-day-old 5th instar larvae were used for the study. The glands were dissected out, fixed and stained with haematoxylin-eosin, paraldehyde fuchsin, lacto-aceto-orcein or methylene blue.

Each gland consists of a cluster of cells lying immediately dorsal to the prothoracic spiracle inwards of the lateral longitudinal tracheal trunk (Figure 1). Anterior, posterior, median and lateral branches radiate irregularly from the cluster and the glands are thus spread in the prothoracic segment and reach the anterior part of the mesothoracic segment. The cells of the anterior branch reach close to the prothoracic ganglion and run along with the prothoracic anterior nerve (pan, Figure 5), branches of which innervate it. The posterior branch is comparatively smaller but reaches the anterior region of the mesothoracic segment. The cells of the ventral strands lie in close association with the transverse nerve of the prothoracic ganglion. The shape and size of the gland cells of the central mass, as well as those of the branches, vary greatly. They are block-like, conical or elongated (Figure 2). The distal cells of the branches are mostly triangular, with the ends drawn out. The smallest cells occur in the distal part of the gland. The gland cells are enclosed in a thin, acellular, closely applied membrane (Figure 3). From the ends of the strands it is drawn out as long filaments attached to the neighbouring tissues or adjacent

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