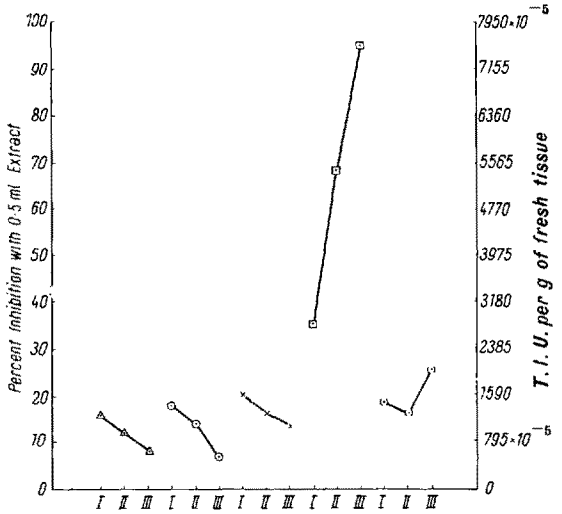
organs of the plants and the accumulation of the proteins explains the increase in the inhibitor activity of the seeds as they grow.

Fig. 3.–Trypsin inhibitor activity of various organs of the field bean plant.



△—△ roots; ○—○ stems; x—x leaves; ⊡—⊡ seeds; (·)—(·) skins

Plant Organs at Three Stages of Development
(I = tender; II = semimature; III = mature)

Whether the inhibitors in the plant tissues function as stabilizers of the associated proteins and act as regulators in the protein synthesis and breakdown or otherwise, is a question that should be left open at this stage. In fact, a study of the interaction of the plant inhibitors with the plant proteases themselves should preceed such an enquiry.

A detailed account of the investigation will be published elsewhere.

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Institute of Science, Department of Biochemistry, Bombay, April 3, 1956.

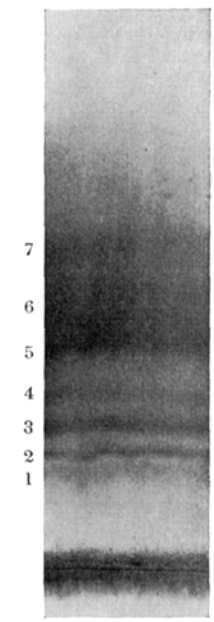
Résumé

L'activité inhibitrice de la trypsine contenue dans les différentes parties de *Faba vulgaris* Mœnch et *Dolichos lablab* L., pendant germination, et des plantules et de leurs parties en voie de croissance a été déterminée à trois différents stades, à savoir aux états: 1° de tendre, 2° de demi-maturité et 3° de maturité. On l'a constatée aux trois stades. Sauf dans les graines, l'activité inhibitrice diminue avec la maturation. Dans les graines c'est l'inverse, l'activité inhibitrice de la trypsine augmente avec la maturité. On suggère le rôle possible de ce principe comme régulateur dans le processus de formation et de rupture de la protéine.

Paper Electrophoresis of the Soluble Proteins of the Central Nervous Tissue

Reports in the literature on the electrophoretic separation of the proteins of the nervous tissue are, as far as we are aware, very scarce. Keil[1] described the separation, by paper electrophoresis, of the proteins extracted from the obturatorious nerve of ox and demonstrated 4 components.

In the same year Nakamura, Hayashi, and Tanaka[2] studied, by boundary electrophoresis, the soluble proteins from ox brain, demonstrating 8 fractions.



Paper electrophoresis of the soluble proteins of the cerebellum after 12 h of electrophoresis. Borate buffer pH 8·6 μ 0·06.

In the present communication the techniques used and the results obtained in the separation, by paper electrophoresis, of the soluble proteins of central nervous tissue of albino rats are outlined. Brain and cerebellum (pooled separately) of albino rats weighing about 200 g were used. Animals were killed by exsanguination under ether anaesthesia and the head was immediately perfused through the ascending aorta with 100 ml of ice-cold 0·25 *M* sucrose solution. Brain and cerebellum were quickly dissected, frozen over solid CO_2 and set aside to await completion of collection.

The pooled material was then dispersed in a Potter-Elvehjem glass homogenizer kept at ice-water temperature, with 0·25 *M* sucrose (5 ml/g of tissue). The dispersion was centrifuged for 1 h at 25000 × *g* in a refrigerated high-speed centrifuge (Pirouette Phywe) and the decanted supernatant, which had a slightly opalescent appearance, was dialyzed for 12 h in a cold room at 2°C, with mechanical agitation, against several changes of distilled water. After the dialysis the liquid was again centrifuged for 1 h at 25000 × *g* and frozen-dried.

Before use, the dry material was dissolved in boric acid–Na_2SO_4–NaOH buffer (Adjutantis: personal communication) pH 8·6, μ 0·06, to give a final protein concentration of 10% (*w/v*).

[1] A. W. Keil, Pflüger's Arch. *259*, 146 (1954).

[2] S. Nakamura, Y. Hayashi, and K. Tanaka, J. Biochem. *41*, 13 (1954).

If homogenization was carried out with a minimum of isotonic sucrose, protein concentrations adequate for electrophoresis could be obtained, omitting dialysis and freezing-drying; in this case the results were similar to those observed with the usual procedure.

Electrophoresis was carried out with a constant potential of about 6–8 V/cm for periods variable from 4 to 12 h. After drying in an oven at 120°C, the strips were stained with amido-schwarz 10 B according to GRASSMAN, HANNIG, and KNEDEL[3]. The relative amounts of the components were determined cutting the corresponding portions of the stained paper, eluting the dye with 5 ml of 5% solution of phenol in water and reading the optical density at 640 mμ.

The Figure shows the patterns of the soluble cerebellum proteins after 12 h of migration in borate buffer. 7 components are indicated with the numbers from 1 to 7 in the order of their increasing mobility. Some material is detectable ahead of fraction No. 7 indicating the presence of proteins with higher mobility, which represent 16·2% ($\pm$ 1·1) of the total amount.

After runs lasting only 6–8 h, this material appeared distributed in 2 fairly definite bands. The band visible at the point of application of the protein solution on the paper is considered to represent particulate matter not sedimented by the centrifugal force employed.

The Table shows the relative amounts of the separated fractions obtained by dye elution.

Relative amounts of electrophoretic components of cerebellum proteins, obtained by dye elution, after 12 h of electrophoresis (means $\pm$ S.E.M.)*.

Fraction No	% Content	S.E.M.
1	6·61	0·71
2	8·33	0·81
3	11·27	0·59
4	7·33	1·45
5	16·45	0·37
6	17·97	1·04
7	15·13	1·43
Fast moving material	16·20	1·10

* The immobile material located at the point of application of the protein solution on the paper corresponds to 5% of the total amount of the soluble proteins.

Similar results were obtained with proteins extracted from the brain.

As resulting from the researches of NAKAMURA *et al.*[2] and from the present results, the patterns of the soluble proteins of the central nervous tissue appear differently from those of the nerve[1] and from patterns observed in other organs (liver[4]; muscle[5]).

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Istituto di Patologia Generale and Istituto di Semeiotica Medica, Università di Pisa, November 10, 1955.

[3] W. GRASSMAN, K. HANNIG, and M. KNEDEL, Dtsch. med. Wschr. *76*, 333 (1951).

[4] G. ADJUTANTIS, Nature *173*, 539 (1954); *174*, 1504 (1954).

[5] G. TOSCHI and A. MARIANI, R. C. Accad. Lincei, Cl. Sci. fis. mat. e nat. [8] *16*, 365 (1954).

Riassunto

Le proteine idrosolubili estratte dal sistema nervoso di ratti sono state separate mediante elettroforesi su carta.

Il quadro elettroforetico appare costantemente riproducibile nelle condizioni sperimentali impiegate.

The Transformation of a Tail into Limb After Xenoplastic Transplantation

(1) One unexpected result obtained from the graft of a tail bud of a Urodele (*Triton cristatus; Axolotl*) on to an Anuran embryo at the neurula stage (*Discoglossus pictus*) is that the tail which differentiates from the transplant, may become transformed into a limb[1]. This, however, only occurs if the graft is made into a limb field of the host, and starts at, or close to, the time at which the metamorphosis of the host is initiated. This transformation occurred in 15 of 180 *Triton* grafts, and in 2 of 120 *Axolotl* transplants.

1 *2* *3*

Figs. 1, 2 and 3.–Axolotl tail bud grafted on to Discoglossus, respectively 13, 28 and 38 days after transplantation.

The stages of the transformation can be set out as follows:

(a) The graft develops into a normal tail, complete with fin, nerve cord, notochord and symmetrically arranged myotomes. It is slender and lanceolate in form (Fig. 1). Chromatophores, arranged in the pattern typical of the donor, are to be observed. The tail moves both spontaneously and upon stimulation.

(b) When the initial process of morphological and histological differentiation is complete, the tail undergoes regression, a process which particularly affects the fin. The tail ceases to be lanceolate, and instead assumes a cylindrical shape (Fig. 2). Concurrently it becomes fleshy and turgid and develops an intensely black pigmentation. The peduncle bends at right angles and at the distal extremity appears a variable number of digits (Fig. 3). It is, however, neither sensitive nor motile.

The limb persists for a considerable time. Meanwhile at its base a normal limb grows from the limb field of the host. In some cases an additional limb develops, which may fuse with the transplant, thus producing a chimera. A study of the histology of the transformed tail shows the nerve cord to be degenerating; in most specimens, remains of the notochord are present, but the musculature is almost completely lacking, a few sporadically

[1] N. FARINELLA-FERRUZZA, Boll. Zool. *17*, 113 (1950); Riv. Biol. *45*, 523 (1953).