Riassunto

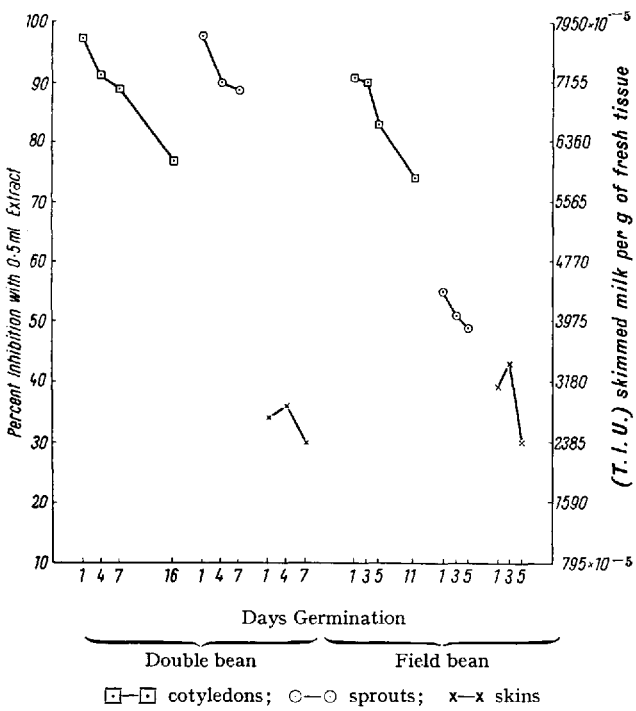
La Piritiamina, somministrata per bocca in una singola dose di 0,5 mg a topolini di 12–14 g che ricevono 2 μg di tiamina *pro die*, non produce la sindrome neuromuscolare caratteristica dell'avitaminosi B_1 e non modifica nè la piruvicemia nè il livello di vitamina nel fegato, mentre lo fa abbassare nel muscolo e nel cervello. Se i topolini non ricevono tiamina, la stessa dose di Piritiamina dà perdita di peso, sindrome neuromuscolare in tutti gli animali trattati e cospicuo abbassamento del livello di vitamina B_1 nel cervello. Nelle stesse condizioni sperimentali, l'Ossitiamina, somministrata *per os* una sola volta in dosi di 0,5 e 2 mg, non dà alcuna sintomatologia neuromuscolare e non modifica nè il peso corporeo, nè il piruvato ematico, nè il contenuto in vitamina B_1 del muscolo e del cervello, solo abbassa il livello vitaminico del fegato.

Questi risultati non sono in favore dell'ipotesi di una funzione della tiamina distinta da quella della cocarbossilasi.

Trypsin Inhibitor in Plant Metabolism

SOHONIE and AMBE[1] have recently reported the crystallization of trypsin inhibitors from the Indian double bean (*Faba vulgaris*) and field bean (*Dolichos lablab*). The same pulses have been further investigated for the inhibitor activity throughout their plant life-cycle. For comparison, the inhibitor activity residing in equal amounts of cotyledons, sprouts and skins of the germinating grains and also of roots, stems, leaves, legumes, etc., of the growing plants has been determined at three stages of development.

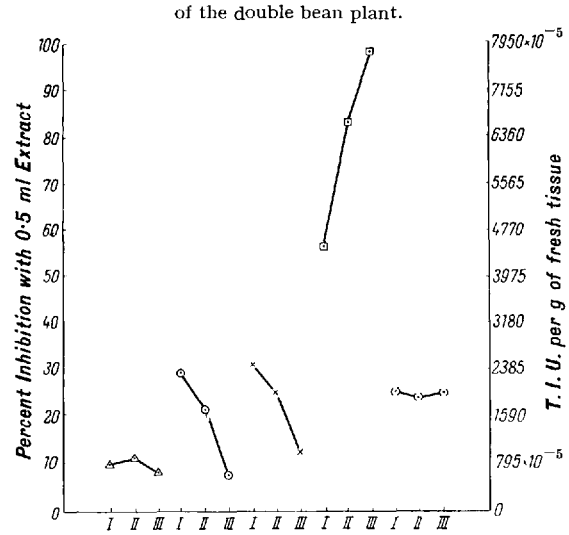
Fig. 1.–Trypsin inhibitor activity of various parts of the germinating double bean and field bean.



[1] K. SOHONIE and K. S. AMBE, Nature *175*, 508 (1955).

The various plant organs were extracted under identical conditions with dilute hydrochloric acid and the inhibition caused due to equal portions of extracts were evaluated. A method similar to that described by ANSON[2] was employed using digestion mixtures of skimmed milk and commercial trypsin (Merck's) at pH 7·6, at 37°C. The inhibitor activities of the various parts of the germinating pulses and their plants have been presented graphically (Fig. 1, 2. 3).

Fig. 2.–Trypsin inhibitor activity of various organs of the double bean plant.



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

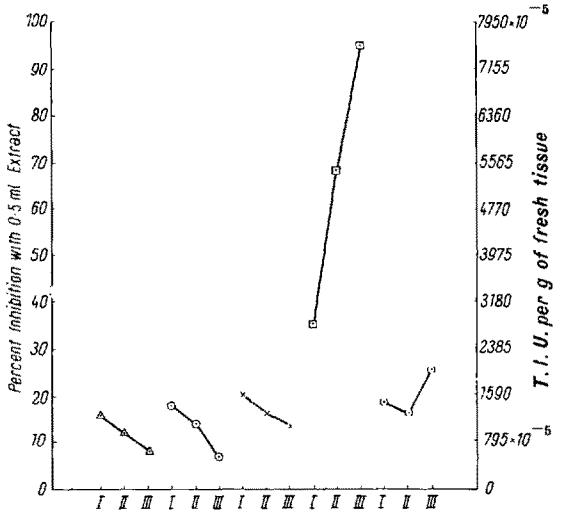
It is interesting to note that the trypsin inhibitor is present in all the parts of the germinating pulses and their plants at all the stages of growth. A study of the curves depicted here reveals that the inhibitor activity in the different parts of either plant is found to be maximum in the seeds and least in the roots; the leaves, stems, etc., fall in between. The cotyledons and the sprouts show decrease in the inhibitor content with germination, while the skins do not exhibit any significant variations. On the plants, in the same organ, however, with the exception of the legume seeds, the power of inhibition diminishes with maturation. The seeds, on the other hand, show an increase in the inhibiting capacity with development. A parallel investigation on the plants of potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), green grams (*Phaseolus aureus*) and Asiatic Yam (*Dioscorea alata*) at different stages of development has also confirmed the same trend of results. The activity of the extracts of leaf-stem portions is found to decrease with maturation whereas the tubers and the seeds exhibit a reverse phenomenon (unpublished).

These results suggest a parallelism between the changes occurring in the inhibitor activity and the protein synthesis in the plant tissues. BURSTRÖM[3] has shown that the latter declines with age; and the same seems to be true, with the exception of the seeds, in the case of the trypsin inhibitor concentrations in different parts of the plants analyzed. The seeds form the storage

[2] M. L. ANSON, J. gen. Physiol. *22*, 79 (1938).
[3] H. BURSTRÖM, Bot. Arch. [B] *30*, 1 (1943).

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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K. S. Ambe and Kamala Sohonie

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

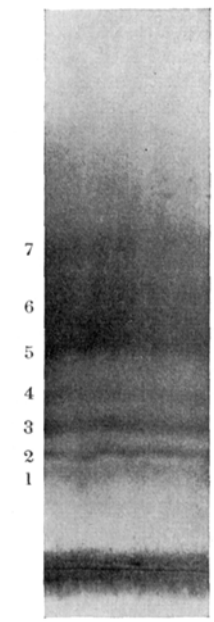
Résumé

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).