

Table 4 is compatible with this idea: actinomycin and interferon in constant amounts were given simultaneously to the cell cultures, but interferon was removed after variable time intervals. The results show that the inhibition is the higher, the longer 'interferon' acts in presence of actinomycin.

On one hand, a rapid uptake should lead to an extensive inhibition despite a short presence of the material on the cell cultures; on the other hand, a high intracellular turnover rate of the material taken up should allow a full viral replication. A slow uptake is also indicated in Figure 1, where it can be seen that a full inhibition and saturating concentrations are never reached, despite high quantities of the preparations. There are arguments that the RNA-test is prone to artifacts. However, quite a different testing approach (titration of extracellular infectious units, rather than intracellular viral RNA), the plaque test, presents the same results. Figure 2 shows the slightly enhancing effect on virus formation of actinomycin alone (curve 1); the heavy inhibition of formation of infectious particles under fully inducing conditions of interferon (curve 4); and the intermediary level established by the actinomycin-insensitive activity (curve 3).

We excluded the following possibilities of artifacts: isotope dilution by nucleotides introduced with the crude preparation; interference between SFV and New Castle

disease virus used to provoke the interferon, toxicity and RNase activity of the preparations, escape synthesis of antiviral messenger RNA at low concentrations of actinomycin, mock preparations. As the onset of the inducing action of interferon is surprisingly fast<sup>13</sup>, we conclude that, in certain preparations containing interferon, there are additional antiviral factors. These may come from an intracellular pool of virus-infected chicken embryos and may be lost during purification of interferon (Table 1). The effect described need not be a physiological one, because eventually this antiviral principle is only forced into cells at high extracellular concentrations, a specific uptake mechanism lacking in the cell membrane. On the other hand, interferon itself might be the antiviral principle<sup>14-18</sup>.

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## Effects of Plant Hormones on Leucine Aminotransferase in Pea (*Pisum sativum*)

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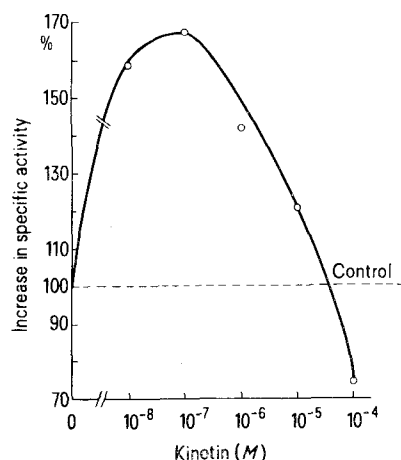
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**Summary.** The work reported here shows a specific effect of kinetin in enhancing LAT activity in pea buds and indicating its possible mode of action at the translational level. Other hormones tested did not show any appreciable effect on the enzyme activity.

Although extensive work has been done on the hormonal regulation of aminotransferases in animals, very few reports exist on the effect of plant hormones on the regulation of the synthesis or activity of aminotransferases in higher plants<sup>2-6</sup>. The present study demonstrates that certain plant hormones increase the level of

leucine aminotransferase in pea shoots, and evidence is presented that the effect of the hormone is at the translational level.

Pea seeds (*Pisum sativum* var. coll 191) were germinated in petri dishes under light. Enzyme was isolated from 5-day-old pea buds by homogenizing them in presence of 0.05 M Tris-HCl buffer, pH 8.4, 10<sup>-4</sup> M mercaptoethanol and 10<sup>-4</sup> M EDTA. The homogenate was centrifuged at 16,000 g in Janetzki K 24 Centrifuge and the supernatant was used for enzyme assay. Leucine aminotransferase (LAT) activity was assayed by the method of AKI and ICHIHARA<sup>7</sup>, and protein by the method of LOWRY et al.<sup>8</sup>. One unit of enzyme activity has been defined as the formation of 10 µg of α-ketoisocaproate per 20 min per mg protein.



Effect of different concentrations of kinetin on leucine aminotransferase activity in vegetative buds of 5-day-old *Pisum sativum* seedlings grown in light. The treatments were given for 24 h. The observations are expressed as percentage increase over control.

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To study the effects of hormones, vegetative buds from seedlings grown for 120 h were excized and dipped in the hormone solution. The control set was dipped in hormone solution at 0°C. After soaking for the required time, buds were washed thoroughly with distilled water and employed for enzyme assay. Of all the different

hormones tested, kinetin was found to be effective in enhancing the enzyme activity. As shown in the figure, the optimal concentration was  $10^{-7}$  M, where about 70–80% increase was noticed, higher concentrations were found to be inhibitory. The kinetics of the hormone treatment showed that the enzyme activity started increasing after 4 h and reached a maximum at 24 h. Other hormones did not have much effect. IAA at  $10^{-6}$  M could enhance by only 11–16% and  $GA_3$  at  $10^{-6}$  M enhanced enzyme activity by 22–29% (table). Another cytokinin, benzyladenine was totally ineffective. Cyclic AMP, tested at  $10^{-6}$  M,  $10^{-5}$  M and  $10^{-4}$  M, also did not show any effect. To find out the mechanism of action of kinetin, effect of different inhibitors was tested. RNA synthesis inhibitors, such as cordycepin (20 µg/ml) and  $\alpha$ -amanitin (5 µg/ml) had no effect. Actinomycin-D sometimes increased the enzyme level by 10–15%. However, cycloheximide, a potent inhibitor of protein synthesis, stopped the increase of LAT activity of kinetin by almost 100%. We have confirmed that all these inhibitors do act at the concentrations tested, in pea, by radioactive precursor incorporation study (Sihag, Sopory and Guha-Mukherjee, unpublished) and also they do not have any significant effect on total protein content of the tissue.

Effect of different hormones on Leucine aminotransferase activity. Here kinetin is tested at  $10^{-6}$  M for the sake of comparison

Hours of treatment	Percentage increase in specific activity of enzyme		
	$GA_3$ ( $10^{-6}$ M)	IAA ( $10^{-6}$ M)	Kinetin ( $10^{-6}$ M)
4	0	0	20
8	2.8	11.9	26
12	11.2	16.5	30
16	16.5	7.8	32
24	29.9	6.08	54

Effect of Various Factors on the Activity of Trehalase from the Larvae of *Sesamia inferens* Walker (Insecta)

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**Summary.** Trehalase from the salivary glands and the midgut of *Sesamia inferens* showed optimum activity at pH 5.8, and at temperatures of 50 and 60°C respectively. The increase in the incubation period, enzyme concentration, and substrate concentration respectively increased the end-product, the hydrolysis, and the rate of hydrolysis of the substrate. Dialysis did not affect, tryptophan accelerated, and other amino acids and end-product inhibited the enzyme activity.

FRAENKEL<sup>2</sup> first detected trehalase ( $\alpha$ - $\alpha$ -glucoside-1-glucohydrolase, E.C. 3.2.1.28) in insects and then FRÈRE-JACQUE<sup>3</sup> found its optimum activity at pH 5.8. GILMOUR<sup>4</sup> did not include it among the digestive enzymes, although it was detected in the gut or/and salivary glands of *Chilo simplex*<sup>5</sup>, aphids<sup>6</sup>, *Lucilia serricata*<sup>7</sup> and *Leucophaea maderae*<sup>8</sup>. The trehalase activity seems generally to be

greatest in the salivary glands and gut<sup>9</sup>, although it is distributed in most of the insect tissues. An excellent review on trehalase has been published by WYATT<sup>9</sup>. Properties of trehalase from the salivary glands and midgut of *S. inferens* have been studied separately. **Materials and methods.** The reaction mixture containing 0.2 ml of 1.0% trehalose solution, 0.2 ml of suitable buffer and 0.2 ml of enzyme extract<sup>10</sup> was incubated at 37 °C (except in case of temperature experiments) for the appropriate time periods. The reaction was stopped and the amount of reducing sugar formed was estimated<sup>10</sup>. The pH for the optimum activity was found by experiments at different pH values ranging from 3.0 to 10.0 using

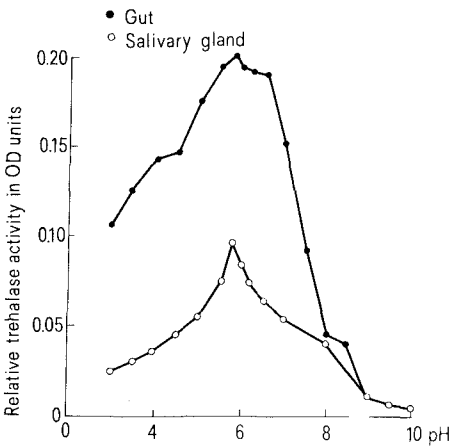


Fig. 1. The pH-trehalase activity curves of the larvae of *S. inferens*.

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