The cells in exponential growth period were labeled with ^{3}H-thymidine (5 μCi/ml) for 10 min, washed twice with Hanks' balanced salt solution, and reincubated in growth medium containing adriamycin (0.01-0.1 μg/ml). Cell harvest for cytological preparations was made at 2, 4 and 6 h after the introduction of the drug, each with a 1-h Colcemid arrest. Autoradiographs showed that in the 2-h samples the metaphases were either unlabelled (G_2) or labelled with the typical late replicating pattern (heavy label over the long arm of the *X*, the *Y*, and the small metacentric elements)[3]. In the 4-h and 6-h samples, metaphases with late-replicating pattern were proportionally more than those found in the untreated control samples; but the majority of metaphases showed label over most chromosomes throughout their lengths.

Figure 1 shows that adriamycin induced both the chromatid- and the isochromatid-types of aberrations, and that their frequencies increased as the durations of treatment increased. The slight increase in the frequencies of chromatid gaps and breaks in the control samples harvested at 4 and 6 h was probably due to the radiation effects of ^{3}H-thymidine. However, aberrations of the isochromatid type, nonexistent in the controls, were also recorded with a relatively high frequency in the drug-treated samples. Most of these isochromatid aberrations were fragments, but dicentrics were occasionally observed. Figure 2A depicts a metaphase (0.1 μg/ml, 4 h) in which one of the No. 2 chromosomes had an isochromatid break near the centromere. The acentric fragment (arrow) is not connected with the centric fragment. In Figure 2B (also from 0.1 μg/ml, 4 h), a similar break occurred in the heterochromatic long arm of the *X* chromosome.

It should be pointed out that isochromatid gaps and isochromatid breaks are cytological terms. Breaks at the 'identical' cytological locations of the two sister chromatids may be thousands of nucleotides apart when DNA sequences are considered. On the other hand, the lesions for a chromosome break must be identical in the two sister chromatids because the break occurs prior to chromosome duplication. In our materials, the 'isochromatid' aberrations induced in the G_2 and S phases did not necessarily represent identical loci between the sister chromatids. Since daunomycin may have specific affinity for dAT base pairs[3] and since adriamycin is a derivative of daunomycin, adriamycin might also damage chromosome segments rich in dAT bases. If a break is induced in one chromatid in an AT-rich chromosome segment and another break is induced in the same segment of the sister chromatid, the locations of these two breaks probably are not identical, but the morphological characteristics of the isochromatid breaks would be indistinguishable from a true chromosome break. It is entirely possible that other chemical clastogens with base specificities may behave in a similar way but the employment of proper test materials and proper concentrations are necessary to detect such actions.

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Actinomycin-Resistant Antiviral Activity Associated with Preparations Containing Chicken Interferon[1]

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Summary. Crude and purified preparations containing chicken interferon show a 2fold antiviral activity. One is inhibited by actinomycin and leads to total inhibition if interferon is added to cells before actinomycin, the other is insensitive to actinomycin and leads to partial inhibition if interferon is added simultaneously with or after actinomycin.

Interferon is supposed to need an intact synthesis of ribonucleic acids (RNA) and protein in order to develop within hours a full state of inhibition of viral growth[4–6]. Therefore interferon is considered to be the inducer of a hypothetical antiviral substance[7]. The cellular gene activated by interferon can be discriminated from that specifying the interferon on basis of genetical evidence. Theoretically then, all crude preparations containing interferon (itself induced by a viral challenge) should also contain the antiviral principle. In the course of experiments on kinetics of interferon action, we found crude preparations to inhibit the synthesis of Semliki Forest Virus (SFV), despite the fact that host RNA biosynthesis had been blocked *before* the interferon application by actinomycin D. (The replication of SFV, an RNA-virus, is not harmed by actinomycin.)

Materials and methods. Cell cultures, media and infection with SFV (Kumba or Zurich strain) were as described[8]. Interferon preparations were from allantoic fluid and chicken embryos (II: 120 units/mg protein; V: 240 units/mg; 10/1: 20,000 units/mg) or from allantoic fluid (447/196: 33,420 units/mg; 489/24: 280,000 units/mg) according to methods established[9]. Antiviral activities were assayed either by measuring the incorporation of [^{3}H]-uridine[10] into acid-precipitable material of SFV-infected chick embryo fibroblast (CEF) monolayers treated with actinomycin D (RNA-test)[8] or with the plaque test[11]. The actinomycin-resistant (non-inductive)

[1] Work supported by the Swiss National Foundation, grant 3.399.70.

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[3] The authors wish to thank Miss E. Browse, Miss U. Kohler and Mr U. Rohrer for excellent technical assistance.

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[10] Abbreviations and reagents: Actinomycin D (Calbiochem); medium Eagle MEM is minimum essential medium – Hanks (BBL, USA); Medium 199 – Hanks (Difco, Detroit); Fetal calf serum (Flow, Scotland); [^{3}H]-uridine, uniformly labeled, 3.5–6.3 Ci/m*M* (Amersham, England).

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Table 1. Characteristics of the interferon preparations used

A Interferon Number	B Interferon Source	C Units/ml used	D Percent inhibition in RNA-test	E
			Total activity	Actinomycin-insensitive activity
II	allantoic fluid	30	98	33
	and embryos	240	98	47
V	allantoic fluid	60	98	40
	and embryos	240	98	65
10/1[9]	allantoic fluid	20,000	98	21
	and embryos	40,000	98	45
447/196[9]	allantoic fluid	20	95	0
489/24[9]	allantoic fluid	15	90	0

Results in columns D and E are from RNA-tests in CEF-cultures with the concentrations indicated in colomn C. *Total activity* of inhibition of incorporation into viral RNA was measured after pretreating cell cultures with interferon for 14 h (without actinomycin before infection) (column D); *actinomycin-insensitive activity* was measured by adding interferon and actinomycin together, 1 h after infection (column E). Harvest was at 9 h after infection.

Table 2. Dependence of residual level of incorporation into viral RNA (RNA-test) on time of addition of interferon, using constant concentrations

		Time of addition of compounds relative to infection			
		3 h before infection	1 h before infection	1 h after infection	2.5 h after infection
A. Actinomycin D alone, 1 μg/ml		114	126	113	100
B. Interferon II	1	14	28	61	–
alone, 10 units/ml	2	15	36	61	–
C. Actinomycin D (1 μg/ml and interferon II (10 units/ml) together	1	62	61	61	–
	2	66	58	67	–

Actinomycin D (A) or 100 μg (= 10 units) crude interferon II/ml (B) or both together (C) were added at the times indicated to CEF cultures in medium 199. After infection with SFV the viral suspension was replaced by Eagle's minimum essential medium containing again the additions mentioned. 2.5 h after infection cultures of experiment B received actinomycin. 3 μ Ci [^{3}H]-uridine per culture were given always 3 h after infection. Experiments were performed at 37 °C to 9 h after infection. Incorporation into acid precipitable material was determined as described[8]. All values are expressed as percentage of the control (experiment A, actinomycin added 2.5 h after infection) and represent means of 3 cultures. B 1 and C 1: interferon preparation used as such. B 2 and C 2: interferon preparation extensively dialyzed before use.

Table 3. Actinomycin-insensitive activity of interferon. Dependence of residual incorporation into viral RNA (RNA-test) on time of addition of constant concentrations of interferon

	Time of addition relative to infection (t = 0) in hours (before –; after +)												
Actinomycin D (1 μg/ml)	–3	–3	–3	–3	–3	–3	+1	+1	+1	+1	+1	+1	+1
Interferon II (10 units/ml)	none	–3	–2	–1	+1	+2.5	none	+1	+2	+3	+4	+5	+6
[^{3}H]-uridine (3 μ Ci/culture)	+2.5	+2.5	+2.5	+2.5	+2.5	+2.5	+6.5	+6.5	+6.5	+6.5	+6.5	+6.5	+6.5
Residual incorporation in percent of control (time of harvest = 9 h after infection)	100	52	55	50	49	60	100	50	60	70	80	90	100

Experiments were similarly performed as stated in the legend to Table 2. Harvest was at 9 h after infection.

Table 4. Actinomycin-insensitive activity of interferon. Dependence of residual incorporation into viral RNA (RNA-test) on time of *removal* of constant concentrations of interferon

	Time of addition of actinomycin D, interferon and [^{3}H]-uridine and time of removal of interferon relative to time of infection (t = 0) in hours (after infection +)							
Actinomycin D (1 μg/ml)[a]	+1	+1	+1	+1	+1	+1	+1	+1
Interferon II (10 units/ml) added	none	+1	+1	+1	+1	+1	+1	+1
Interferon removed[a]	–	+1.5	+2	+2.5	+3	+3.5	+4	+8
[^{3}H]-uridine (3 μ Ci/culture)	+4	+4	+4	+4	+4	+4	+4	+4
Residual incorporation in percent of control (time of harvest = 9 h after infection)	100	90	92	80	84	82	78	50

[a] At removal of interferon cell sheets were washed and fresh medium containing only actinomycin was added. Values are from 3 cultures, and expressed as percent of the control. Harvest was at 9 h after infection.

activity was discriminated from the actinomycin-sensitive (overall) activity by adding actinomycin at *the same time* or *earlier* than interferon. In this case actinomycin does a dual job: 1. it prevents the RNA synthesis of the host and therefore also the development of the antiviral activity induced by interferon; 2. it prevents the incorporation of radioactive precursors into host RNA.

Results and discussion. According to Table 1, preparations containing interferon exhibit two activities: one, that leads to a total inhibition of incorporation of [^{3}H]-uridine into viral RNA in the concentrations used, if interferon is added long enough before actinomycin (column D); and one, that leads to a partial inhibition of incorporation, if interferon is added after or together with actinomycin (column E). This is true for more or less crude preparations made of allantoic fluid and chicken embryos; the actinomycin-insensitive activity lacks in highly purified preparations of allantoic origin. Table 2 depicts the behaviour of incorporation in function of the beginning of the treatments relative to the time of infection. Actinomycin alone, if given up to three hours before infection, enhances the incorporation[12] (line A); interferon alone has an increasing antiviral effect, the longer it acts without actinomycin before infection[4–6] (line B); simultaneous addition of interferon and actinomycin results in an intermediary antiviral state (line C) between full replication and full inhibition. Table 3 shows a finer analysis of the actinomycin-insensitive activity: if actinomycin and interferon are added between 3 h before infection and 2 h after infection, there is always an equal state of inhibition with the concentration used; later addition results in increasingly low inhibition. These results point to the possibility that, in crude preparations of interferon, there is an additional antiviral factor, which is *slowly* taken up into the cells and acts on early phases of the replication cycle. The experiment of

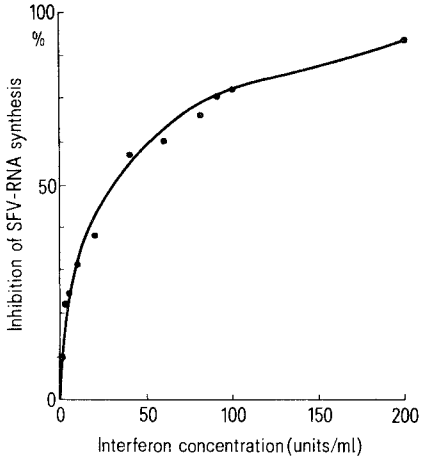

Fig. 1. Actinomycin-insensitive activity of interferon. Dependence upon concentration in the RNA-test.
Actinomycin D (1 μg/ml) and interferon II in the concentrations indicated were added together 1 h after infection of cultures with SFV. Harvest 9 h after infection. All points represent means of 3 experiments and are expressed as percent of the control (no interferon, actinomycin 1 h after infection).

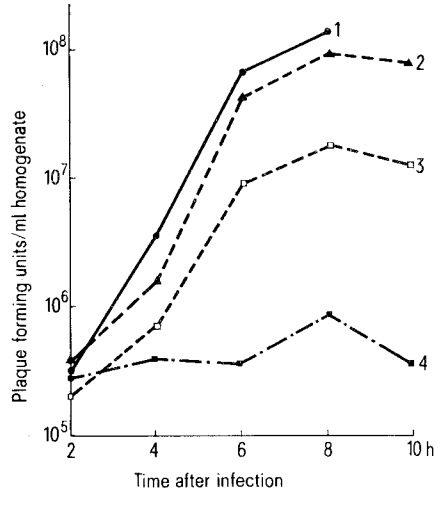

Fig. 2. Actinomycin-insensitive activity of interferon. Time course in the plaque test.
A first set of CEF-cultures (test-set) was infected and received at the same time (3 cultures per point). *1* Actinomycin D (1 μg/ml); *2* no additions; *3* actinomycin D (1 μg/ml) and 4000 units/ml of dialyzed interferon 10/1 together, to measure the actinomycin-insensitive activity; *4* 4000 units/ml of dialyzed interferon as above, to measure the overall activity.
2, 4, 6, 8 and 10 h thereafter cell sheets and culture fluids of the test set were homogenized together, appropriately diluted and given into a second set (indicator-set) of CEF-cultures to measure in the plaque test[11] the infectious units developed in the test set. The hours on the abscissa indicate the time of harvest in the test set; the numbers on the ordinate indicate plaque forming units calculated for 1 ml homogenate of the test set.
Obviously, interferon and actinomycin of the test set are introduced into the indicator set. However, they are diluted equally up to 10^6fold, which results in undetectable levels no longer interfering with the virus titration.

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[13], 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[14–18].

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

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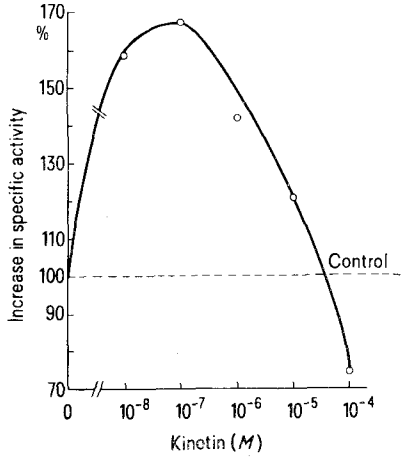
Summary. The work reported here shows a specific effect of kinetin in enhancing LAT activity in pea buds and indicating its possible mode of action at the translational level. Other hormones tested did not show any appreciable effect on the enzyme activity.

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[2–6]. 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 translation level.



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.

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^{-4} *M* mercaptoethanol and 10^{-4} *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[7], and protein by the method of Lowry et al.[8]. One unit of enzyme activity has been defined as the formation of 10 µg of α-ketoisocaproate per 20 min per mg protein.

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