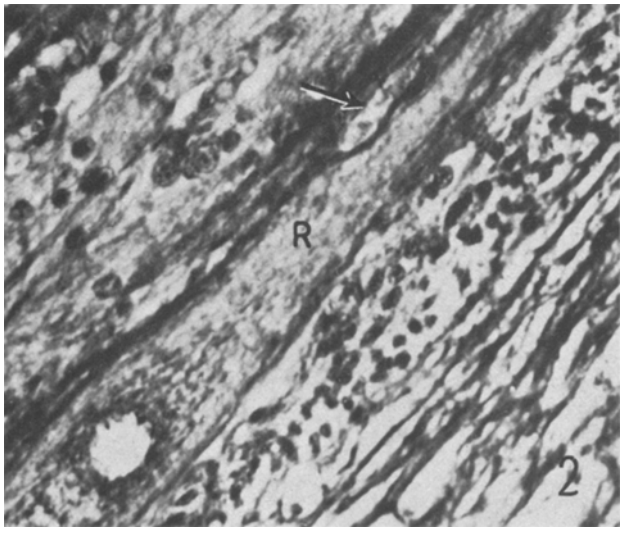

Fig. 2. Same as figure 1 showing an intimate contact between a blood capillary and the rostral process (arrow). × 600.

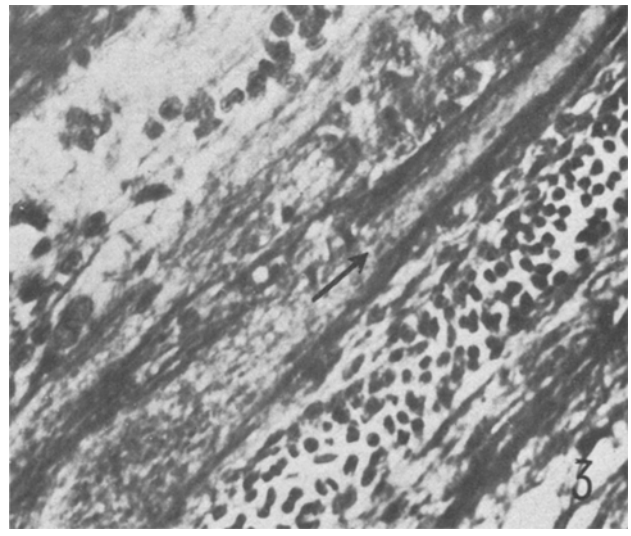

Fig. 3. Same as figure 2, showing neurosecretory grains in the rostral process (arrow). × 600.

common carp and roach, in the catfishes, the rostral processes are also associated with the transmission of the neurosecretory substance.

It may be recalled that in these catfishes, as compared to ordinary teleosts, the posterior part of the spinal cord, in which the neurosecretory cells are situated is more heavily vascularized. It seems likely that unlike the caudal process, the rostral process might be doing the job independent of the agency of the neurohaemal organ, the urophysis. This mediation of rostral processes in the transmission of the neurosecretory substance may be looked upon as a device to supplement the activity of the caudal processes in the event of faster rate of synthesis of the neurosecretory material in the bipolar neurosecretory cells, which are about 4 times larger than the ordinary neurosecretory cells. It is also possible that the rostral process reflects a more primitive mode of transmission of neurosecretory material comparable to that obtaining in primitive bony fish group, the Chondrostei. In Acipenseridae[4] a urophysis is lacking and the neurosecretory cells, unlike those of teleosts, discharge their neurosecretory material into the blood vessels of the meninx in the spinal cord.

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The effects of legume seed extracts on plant virus infection

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Summary. Extracts from the seeds of 15 species of legume inhibited the infection of plants by viruses. Extracts could be divided into those with marked inhibitory activity reducible on heating and those with less marked inhibitory activity which increased on heating. Evidence is given to suggest that seed extracts contain both virus inhibitors and augmenters and that the inhibitors are high molecular weight proteins possibly related to lectins.

Although numerous plants extracts have proved inhibitory to the replication of plant viruses, relatively few reports of the effects of seed extracts have been made[1].

Following the observations that some seed extracts may enhance virus local lesion production whilst others cause inhibition, a theory was proposed to account for the variability in the seed transmission of viruses based on the concept of seeds containing a mixture of virus-enhancing and virus-inhibiting compounds[2]. In order to examine this theory further a range of legume seeds was studied.

Materials and methods. Whole seeds from 15 species of legume were each ground to a powder and 1.0 g samples mixed with 10 ml water. After 10 min the slurry was centrifuged at 3000 × g for 15 min to remove cell debris.

The clear supernatant was mixed with an equal volume of tobacco necrosis virus (TNV) prepared by the method of Kassanis[3] in 0.06 M phosphate buffer pH 7.0. Inoculations were made onto the leaves of 13-day-old french bean plants (*Phaseolus vulgaris* L. cv. the Prince) using 600 grit carborundum as an abrasive. Similar inoculations were made

with extracts heated to 100 °C for 10 min, cooled and then mixed with virus. Control plants were inoculated with virus mixed with buffers instead of seed extracts. Each treatment was replicated on 10–15 leaves. The results were analyzed by the t-test using converted numbers of lesions (z) derived from the counted lesion numbers (x) using the Kleczkowski[4] transformation i.e. $z = \log_{10}(x + c)$ where c was 10.

Results and discussion. Table 1 shows that of the 15 legume seeds tested all except french bean *(P. vulgaris)* proved inhibitory to local lesion production by TNV.

Heating the extracts showed that they could be divided in 2 groups. In 1 group heating reduced inhibitory activity. In the 2nd group, extracts showed relatively weak inhibitory activity which became stronger on heating the extracts for 10 min at 100 °C. Using soya bean (SB) extracts from the 1st group and french bean (FB) extracts from the 2nd, experiments were performed to show whether other viruses in different local lesion hosts could be inhibited. Table 2 shows that SB extracts were inhibitory to tobacco mosaic virus (TMV) multiplication in *Nicotiana tabacum* var. *Xanthi* - nc. and also to potato virus X (PVX) lesion production in *Gomphrena globosa.* Extracts from FB seeds were much less effective as inhibitors to local lesion production by TMV and PVX than were SB extracts. Loss of inhibitory activity on dilution of the seed extracts showed that they act as inhibitors of virus infection rather than as virus inactivators[5]. Progressive dilution of the FB extracts resulted in a change from extracts with no significant effect to extracts at 10^{-5} dilution showing significant inhibitory properties (table 3). This suggests that FB and 2nd-group seeds contain both inhibitors and virus-enhancing or augmenter compounds such that extracts from these have little effect on virus infection. Dilution of the extracts results in inhibitors becoming evident because the enhancing compounds have been diluted beyond their effective concentration. In the 1st group of seeds including SB the inhibitory activity is sufficiently high initially to give inhibitory extracts. To examine this hypothesis further, 2 ml samples of SB and FB seed extracts were each passed through a 1.5×40 cm Sephadex G100 column, using 0.03 M phosphate buffer, pH 7.0, as eluent.

Similarities between the 2 seed extracts became apparent when elution profiles were determined by UV light absorption at 280 nm (figure). SB seed extracts showed 3 peaks with elution volumes of 17 ml (peak I), 39 ml (peak II) and

Table 1. Effects of legume seed extracts on local lesion production by tobacco necrosis virus

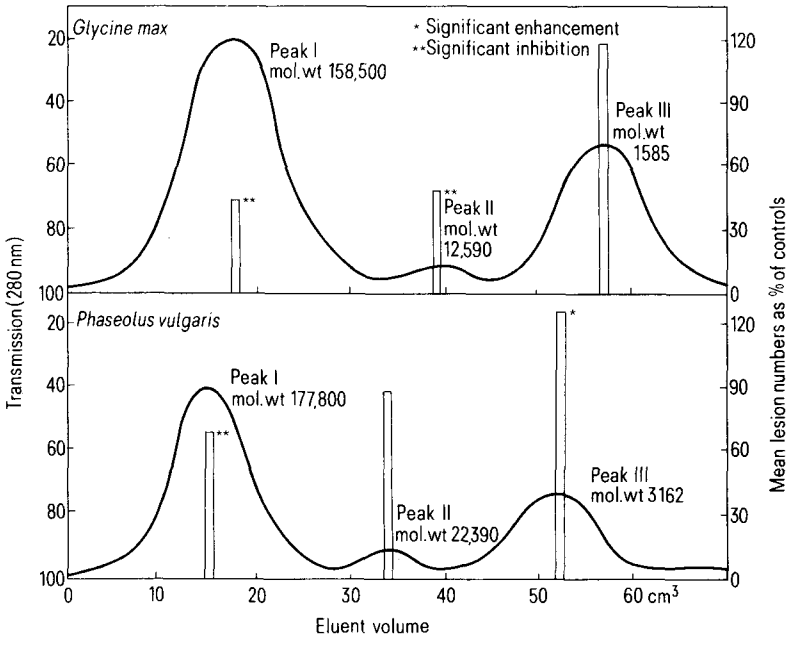
Seed extract	Lesion numbers as percentage of controls	
	Unheated extract	Heated extract
Canavalia ensiformis DC.	17	89
Cicer arietinum L.	52	79
Glycine max Merr.	8	22
Lens esculenta Moench	9	56
Lotus tetragonolobus L.	22	94
Phaseolus aureus Roxb.	25	52
P. coccineus L.	64	34
P. limensis Macf.	68	47
P. mungo L.	13	36
P. vulgaris L. (haricot bean)	68	32
P. vulgaris L. (french bean)	102	54
Pisum sativum L.	11	84
Robinia hispida L.	9	40
Securigera securidaca DC.	46	65
Vigna sinensis Savi.	16	44

Table 2. Effects of *Phaseolus vulgaris* and *Glycine max* seed extracts on local lesion production by tobacco mosaic virus and potato virus X

Seed extract	Virus	Lesion numbers as percentage of control
Phaseolus vulgaris (french bean)	TMV	85
	PVX	104
Glycine max (soya bean)	TMV	36
	PVX	16

Table 3. Effects of various dilutions of seed extracts on local lesion production by tobacco necrosis virus

Dilution	Lesion numbers as percentage of controls	
	Phaseolus vulgaris seed extract	*Glycine max* seed extract
10^{-1}	107	46
10^{-2}	93	63
10^{-3}	96	62
10^{-4}	95	68
10^{-5}	70	102
10^{-6}	105	99



56 ml (peak III). FB extracts showed 3 similar peaks with elution volumes of 16, 35 and 52 ml respectively. Comparison of the peaks with the elution volumes of known proteins by the method of Andrews[6] showed the SB extract to contain proteins with mol.wt of 158,500 (peak I); 12,590 (peak II) and also material of mol.wt 1585 for peak III. The FB extract contained proteins of mol.wt 177,800, 22,390 and 3162 for peaks I, II and III respectively. Peaks I and II for each extract were not only inhibitory (figure) to local lesion production but could also be separated from peak III by precipitation on adding ethanol to 80% concentration or by using ammonium sulphate. The material in peak III for each extract enhanced TNV local lesion production and appears to be, at least in part, proteinaceous and clearly different from the oxalate augmenter desribed by Benda[7] and Matsashita[8]. The inhibitor fractions of peak I are of much larger molecular weight than previously described plant virus inhibitors[9,10] and resemble more closely lectins which have been extracted from both SB and FB seeds. SB lectin (mol.wt 110,000) and phytohaemagglutinin (PHA), the lectin from FB (mol.wt 128,000), exhibit a wide variety of biological activities including effects on cell surfaces by binding specifically to carbohydrate-containing receptors on membranes[12]. SB lectin and PHA have affinities for N-acetyl-D-galactosamine although SB additionally binds to D-galactose. Initial stages of plant virus infection involve the entry of virus through the plasmalemma with subsequent multiplication of virus particles. Lectins, by attachment to specific carbohydrate-containing regions of the membrane, may block or modify infectible sites so preventing virus entry. Inhibitors of animal virus multiplication by plant lectins comes about, at least partly, by effects on cell surfaces[11]. The seed extracts examined and described in this paper may contain lectin or lectin-like proteins and their virus inhibitor properties may be related to their effects on cell membranes.

Although the rôle of lectins in plants is not understood it has been suggested that they protect plants against diseases induced by bacteria and fungi[13]. Further studies of SB and FB seed extracts and their authentic lectins should help to verify the possible rôle of these proteins in plant defence against disease.

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Base pairing in messenger RNA's for small peptides

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Summary. Longest runs of Watson-Crick pairing in hypothetical m-RNA's for a number of natural peptides were no greater than those in the hypothetical m-RNA's for a large number of randomized amino acid sequences from these peptides. This shows that even if base-pairing in m-RNA were a biological requirement, it would little constrain the amino acid sequence.

An interesting question in the fundamental biology of proteins is what, if any, are the a priori restrictions on the possible amino acid sequences. One such restriction could be the relative instability of some messenger RNA's. The stability of these very long chain molecules would be enormously increased by folding stabilized by Watson-Crick pairing of the bases. This would require many regions of contiguous base pairs. The published base sequences for m-RNA's show the possibilities of stems containing short regions of contiguous pairing, but the information is too limited to be an adequate test. The amino acid sequences of many proteins and peptides are known and from these the possible base sequences of their respective m-RNA's may be conjectured, the true sequence being only one amongst many because of the redundancy in the code.

We had preliminary evidence favouring such pairing from the hypothetical sequences for angiotensinogen, when Polya[1] indicated for somatostatin a sequence of bases that can be bent to allow extensive base-pairing. However, there were 2 deficiencies in these examples: a) only one bending point along the RNA chain was examined; and b) probabilities would be very difficult, if not impossible, to calculate.

We have remedied these deficiencies a) by using a computer programme that generates all possible base sequences for a peptide and examines the runs of contiguous pairs for all possible positions of bending; and b) by basing probabilities on the comparison of the data obtained for the peptide with those obtained for random sequences of the amino acids of the same peptide. We have assumed that all codons for each amino acid are equally likely. To keep the computing effort reasonably small, we have limited our attention to small peptides (table 1), the amino acid sequences for which have been published[2-4]. Furthermore, we have avoided analysis of the possible simultaneous presence of several bending positions or presence of stems with stretches of noncontiguous pairing and we have confined our attention to extensive runs of contiguous pairs. Our findings have made it unlikely that this is a serious limitation. For each peptide the longest run of contiguous pairs was compared with runs obtained from random sequences of those same amino acids.

Results. Except for ranatensin, no more than a hundred peptides generated by random arrangement of the constituent amino acids was needed to yield at least one run of paired bases as long as or longer than the longest obtained for the natural peptide (table 1, column 5). Furthermore, the fraction of random peptide sequences which gave rise to a run equal to or longer than the maximum obtained for the natural peptide is often substantial, and from them one can obtain the 95% confidence intervals for binomial distribution (table 1, footnote). Thus, for example, for ranatensin there is a 95% chance that the 'true' percentage lies between 0–4%.