week-old CBA female mice the LD_{50}[9] of abrin was 5×10^{-13} moles per 25 g b.wt.) Thus although at the lower levels of immunosuppression abrin was around 10^5 times more effective than AMLG on a weight for weight basis, the antibody by virtue of its relative lack of whole body toxicity could be used to produce much higher net reductions in the immune response.

Next, tests were carried out to determine the effectiveness of conjugates of abrin with antibody or non-antibody containing immunoglobulin. Each conjugate was made twice, giving AMLG-abrin preparations 1 and 2 and NIgG-abrin preparations 1 and 2. Using the test described earlier it was found that the LD_{50} of the AMLG-abrin was around 43×10^{-13} moles per 25 g b.wt while that of the NIgG-abrin was not significantly different at around 55×10^{-13} moles; conjugation it seemed had reduced the whole body toxicity of abrin about 10-fold. As can be seen in table 2, preparations 1 and 2 were tested once and twice respectively for effects on PFC production. With doses of conjugate in the $7.5-30\times10^{-13}$ moles per 25 g b.wt range, dose related reductions in PFC were obtained and in every instance the decrease was statistically significantly different from the control (dose 0) group. It was a consistent finding that AMLG-abrin was a more potent immunosuppressive agent than was NIgG-abrin, the differential being around 2-fold.

Anti-lymphocyte globulin has been shown to produce effects on phenomena controlled by lymphocytes while leaving other blood elements relatively unaffected[10]. In as much as this may demonstrate its tissue specificity we have chosen anti-lymphocyte globulin as a model for our studies. The initial aim was to increase its effectiveness by attachment of a potent cytotoxic agent. In a sense this has been achieved, the improvement of AMLG-abrin over AMLG alone being around 10,000-fold. However, it appears from the results that the dominant molecular species in the conjugates is abrin, whose ability to immunosuppress is probably not surprising in view of its known cytotoxicity[11] and tendency to localize in spleen[12]. The whole body toxicity of abrin prevented the use of doses sufficient to give very large effects on the response to sheep erythrocytes and precluded the use of mixtures of antibody and toxin in these experiments. It was a major consequence of conjugation to immunoglobulin that there was a reduction of some 10-fold in LD_{50}.

The AMLG-abrin appears to be about twice as potent as NIgG-abrin and we are encouraged to think that this is due to the ability of the antibody to localize on to target cells better than does normal immunoglobulin. This, together with the great reduction in toxicity of abrin relative to its effectiveness and the extremely small dose levels employed are seen as promising for this method of developing more specific therapeutic agents.

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Effect of inhibitors of protein synthesis from plants on tobacco mosaic virus infection

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Summary. Ricin, abrin, modeccin, gelonin and *Momordica charantia* inhibitor, as well as crude extracts of *Bryonia dioica* seeds and of *Dianthus caryophyllus* leaves, each inhibit protein synthesis in a rabbit reticulocyte lysate and reduce local lesion production by tobacco mosaic virus, thus resembling the effects of the pokeweed antiviral peptide.

Extracts from several plants[2] or seeds[3,4] inhibit replication of plant viruses, and there is evidence that at least some of the active principles may be proteins[2,4,5]. The best characterized is the pokeweed antiviral peptide (PAP) purified from the leaves of *Phytolacca americana*[6,7]. The antiviral activity of this protein is attributed to its inhibitory effect on protein synthesis[8], which in turn is due to enzymic inactivation of the 60S ribosomal subunit[6].

Many seed extracts inhibit protein synthesis[9,10]: from 2 of these, inhibitory proteins have been purified which act in an apparently identical manner to PAP; these are *Momordica charantia* inhibitor[11] (MCI) and gelonin[12], from *Gelonium multiflorum*. These inhibitors, as well as PAP, resemble the A subunit of 3 toxins ricin, abrin and modeccin from the seeds of *Ricinus communis*[13], from seeds of *Abrus precatorius*[13] and from the roots of *Adenia digitata*[14,15] respectively. Each of these toxins consists of 2 polypeptide chains joined by disulphide bonds. The A chain enters cells and inhibits protein synthesis whereas the B chain binds to receptor sites on cell surfaces and behaves like a lectin.

We report now that gelonin, MCI and the toxins mentioned above, like PAP, reduce local lesion production by tobacco mosaic virus (TMV). A crude extract from *Bryonia dioica* seeds, which inhibits protein synthesis, reduces lesion production by TMV, whereas an extract from *Dianthus caryophyllus* leaves, which has antiviral properties, also inhibits protein synthesis.

Materials and methods. Ricin[16,17], abrin[19] (abrin C), modeccin[15], MCI[11] and gelonin[12] were prepared as described in the respective references. PAP was a generous gift from Dr

J.D. Irvin. Seeds of *Bryonia dioica,* from fields, and leaves from *Dianthus caryophyllus,* from a private garden, were homogenized in a blendor with 10 or 5 volumes of 0.14 M NaCl containing 5 mM Na-phosphate buffer, pH 7.2. After overnight stirring at 4 °C the homogenates were centrifuged at 40,000 × g for 30 min. The supernatants (crude extracts) were stored at −30 °C until used. The native inhibitors were reduced with 2-mercaptoethanol as described previously[15].

Tobacco mosaic virus (TMV) was purified by the method of Gooding and Herbert[18]. Virus was mixed 1:1 with the test substances or with water as controls. The final concentration of protein synthesis inhibitors was 50 µg/ml. Inoculum containing carborundum as an abrasive was rubbed onto leaves of the local lesion host *Nicotiana glutinosa.* Each treatment was replicated 10 times and randomized on whole leaves of the test plants. Protein synthesis was determined with a lysate of rabbit reticulocytes as described by Gasperi-Campani et al.[9].

Results and discussion. All the purified inhibitors tested reduced the number of local lesions induced by TMV (table 1). The inhibitory activity of the substances varied considerably, and in no case reached that of PAP, which was included as a positive control and for comparison. The activity of ricin and gelonin was decreased and that of abrin, modeccin and MCI abolished after treatment with 2-mercaptoethanol which reduces the disulphide bond between the A and B chains of these compounds. As anticipated PAP, a single chain protein similar to the A chain of ricin, was unaffected by 2-mercaptoethanol reduction. Since MCI and gelonin are also single A chain like proteins, the effect of 2-mercaptoethanol on these compounds is not due to dissociation of any subunits but rather to the instability of the proteins per se[12]. An extract from *Bryonia dioica* seeds, which inhibits protein synthesis[9], also gave complete inhibition of viral lesions. Conversely, an extract from the leaves of *Dianthus caryophyllus,* which has a potent antiviral activity[20,21] also had a strong inhibitory effect on protein synthesis (table 2). Possible similarities between these 2 extracts and the other inhibitors exist since materials inhibiting protein synthesis from both of these extracts bind to carboxymethyl cellulose (CM52) as do MCI[11] and gelonin[12] (results not shown).

These results show that toxins and other plant proteins which inhibit protein synthesis, act like PAP and also have inhibitory effects on TMV local lesion production in much the same way as the antiviral action of PAP. Inhibitors of virus infection are present in several parts of various plants[27]. Similarly, seeds and plant tissues from several types of plant contain inhibitors of protein synthesis, and it is postulated that they may be even more frequent, although in concentrations too low to be easily detected[10,12]

Table 1. Effect of inhibitors of protein synthesis and of crude extracts on local lesions production by tobacco mosaic virus

Experiment No.	Additions	Mean number of lesions			
		Native inhibitor	% inhibition	Reduced inhibitor	% inhibition
Purified inhibitors					
1	None	36	-	30	-
	Pokeweed antiviral peptide (PAP)	0	100	0	100
	Abrin	10.8	69	33	0
2	None	57.6	-	52.3	-
	Ricin	8.3	85	43.5	17
	Modeccin	24.4	58	70.6	0
	Gelonin	28.1	51	44.6	15
3	None	48	-	20	-
	Momordica charantia inhibitor (MCI)	27.6	42	24.8	0
Crude extracts					
4	None	38.4	-	-	-
	Bryonia dioica seeds	0	100	-	-
5	None	110	-	-	-
	Dianthus caryophyllus leaves	0	100	-	-

Table 2. Effect of extracts of *Bryonia dioica* seeds and of *Dianthus caryophyllus* leaves on protein synthesis by a rabbit reticulocyte lysate

Experiment No.	Additions	Concentration (µg extract protein/ml)	Protein synthesis (dpm incorporated/5 min)	Inhibition %
1	None	-	3084	-
	Bryonia dioica seed extract	10.0	35	99
		1.0	878	72
		0.1	2566	17
2	None	-	2748	-
	Dianthus caryophyllus leaves extract	10.0	21	99
		1.0	751	73
		0.1	1773	35

Reaction mixtures contained, in a final volume of 125 µl: 10 mM Tris/HCl buffer, pH 7.4, 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM phosphocreatine, 6 µg of creatine kinase, 0.05 mM L-amino acids (minus leucine), 0.19 µCi of L-(^{14}C) leucine, the appropriate amount of extract and 50 µl of a rabbit reticulocyte lysate. Incubation was at 27°C for 5 min and the radioactivity incorporated into protein was measured on 25-µl samples.

PAP prevents the replication of some animal viruses[22,23], and therefore it is possible that the inhibitors described here have similar action.

These inhibitors are probably one type of compound amongst many substances controlling virus replication. Virus inhibitors separated from plants have been variously identified as proteins, glycoproteins and polysaccharides[2]. Extracts with lectin-like properties have also been implicated in antiviral activity[4] and, interestingly, some lectins also inhibit protein synthesis[11]. Furthermore, evidence is accumulating that some antiviral compounds are induced in plants in response to virus infection[24,25]; such compounds, like the antiviral compound from *Dianthus* sp.[26], have been compared to interferon[28].

The antiviral activity of plant extracts is exerted largely against viruses in plants different from the ones from which the extracts are prepared and this has led to the conclusion that they act on the host plant rather than on the virus[29]. If these antiviral proteins act enzymically on ribosomes[9], they should act on ribosomes different to their own; this has been shown with PAP which inactivates ribosomes from wheat germ and from cowpea but not those pokeweed[8]. It may be concluded that many, and possibly all, plants contain enzymic proteins either free (like PAP), or similar to the A chain protein of ricin and related toxins, being bound to a 2nd B chain protein. Such enzymic proteins, capable of recognizing and inactivating foreign ribosomes, may be a selective RNAse of the type recently demonstrated in eukaryotic cells (Hela cells)[30].

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Observations on calcareous corpuscles using a scanning electron microscope

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Summary. Calcareous corpuscles were observed in a scanning electron microscope, and the presence of Ca was examined by means of elemental X-ray analysis.

Calcareous bodies, round or oval in shape, often described as 'calcareous corpuscles' in the literature, are known to occur in the parenchyma of cestodes and to store large amounts of calcium and magnesium carbonates in addition to phosphates and phospholipids[1,2]. It is reported that the binding of the Ca^{2+} to phospholipids is stronger than that of Mg^{2+}[3]. Several workers have attempted to study: a) the formation of these structures in different species of cestodes by the use of the light and electron microscope[4–10], and b) their inorganic composition using X-ray diffraction or emission spectrographic methods[11–14]. In this study the shape of calcareous corpuscles was examined using the scanning electron microscope (SEM), and the presence of Ca was investigated by means of elemental X-ray analysis.

Materials and methods. Plerocercoid of *Diphyllobothrium erinacei* obtained from the snake, *Rhabdophis tigrinus*, was employed as the material. Plerocercoid was fixed with formalin solution and cut with a razor to small pieces. After washing with distilled water to remove formalin, the specimens were freeze-dried and coated with carbon. The specimens thus prepared were examined in an ISI-30 scanning electron microscope, and elemental X-ray analysis was performed[15].

Result and discussion. There are several studies regarding the structure of the calcareous corpuscles using the light microscope, and the transmission electron microscope (TEM). There are, however, few reports on investigations using scanning electron microscopy. A scanning electron micrograph of the parenchyma containing the corpuscles is shown in figure 1. At high magnification of the area outlined in figure 1, calcareous corpuscles can be clearly observed as spherical or ellipsoidal bodies in the parenchyma (figure 2). Their surfaces appear to be smooth. It is reported that their diameter may be up to 30 μm[6,16]. Using the SEM they were also seen to vary in size, the range was between 10 and 20 μm. The chemical composition of calcareous corpuscles has been examined with histochemical methods[7,17], X-ray diffraction and emission spectrogra-