homogenizer. The homogenates were centrifuged at 2000 rpm at 5 °C for 45 min in an MSE refrigerated centrifuge.

Glutamate-oxalacetate transaminase activity was determined in the tissue homogenates and hemolymph by the method of Tonhazy (cf. Bergmeyer[8]). Protein concentration was estimated by the method of Lowry et al.[9]. Isoenzymes of glutamate-oxalacetate transaminase were studied by polyacrylamide disc gel electrophoresis[10] followed by enzyme-specific staining of the gels. About 200 µg of protein from each sample was layered on the gel (5%) and electrophoresis was carried out applying a current of 3 mA/gel at 5 °C. Tris-glycine buffer pH 8.5 was used. The gels were then incubated in 30 ml of 0.05 M Tris-HCl buffer, pH 7.4, containing 50 mM DL-aspartic acid, 20 mM 2-oxoglutarate, 0.05 ml glutamate dehydrogenase (obtained from Sigma Chemical Company, USA.), 20 mg of NAD^+ 12 mg nitro blue tetrazolium (NBT), 2 mg of phenazine methosulfate (PMS).

Results and discussion. The results of the determinations of the glutamate-oxalacetate transaminase activity are given in the table. In fat body and hemolymph of the infected larvae there was a significant increase in the activity of the enzyme whereas the gut tissues did not show any changes. As reported by other investigators[5,11], the viral infection also induces an elevation in the concentration of some of the amino acids, especially glutamic acid, aspartic acid and alanine, which are readily transaminated and used in the virus multiplication process. Increased transaminase activity in the infected fat body indicates that it is the main seat of synthesis of this particular virus. In the case of hemolymph, the increase in the enzyme activity may be due to the release of the enzymes from the disintegrating fat bodies during infection. This fact is also evident from the electrophoretic patterns of the isoenzymes, shown in the figure. The isoenzyme pattern in the intestinal tissues shows no difference, whereas there is a distinct change in the fat body and hemolymph of healthy and infected larvae. The slow migrating bands found in the enzyme from fat body appear to be specific to fat body tissue, since they are not found in either hemolymph or intestine. But these bands appeared in the hemolymph of diseased larvae, indicating that they are liberated from the fat body undergoing destruction caused by the virus. Further, one of the fast migrating bands found in the fat body of healthy larvae is not found in those of infected larvae. This may be due to the destruction of this enzyme along with other tissue proteins. The remaining bands in the fat bodies of infected larvae are intensified, indicating that these isoenzymes are responsible for the increased activity during infection. Thus the changes in the activity and isoenzyme profile of transaminases in the normal and infected tissues can be considered as indicators of the biochemical changes produced by polyhedrosis virus in the insect hosts.

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A lectin in the pollen of marihuana, *Cannabis sativa* L.

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Summary. A lectin was found in the pollen of *Cannabis sativa* L. which reacted with papain treated erythrocytes. The lectin has an affinity for glucose containing receptor sites.

Lectins or agglutinins found in plants have been used to study the structure and topography of cellular membranes[1,2]. Several lectins have been reported which react weakly or not at all with unaltered red cells but react strongly with protease-treated red cells[3]. In addition, lectins have been found throughout some plant species in the seeds and other parts of the growing plant[4].

Cannabis sativa L. is generally regarded as a highly variable species[5] of considerable forensic interest. A lectin has been reported in the seeds of *Cannabis*[6] and in an effort to confirm this result the pollen was examined as a new and unreported source of lectins. The pollen and seeds from locally grown *Cannabis sativa* L. were collected, crushed and extracted into 0.1 m phosphate buffered saline at pH 7.5. The crude extract was tested against untreated human red cells of various blood group specificities without result. When tested against papain treated red cells, the lectin from the pollen showed a low avidity but a titre of $^1/_{128}$ without regard to a recognizable blood group. The reaction with neuraminidase-treated red cells was very weak as was the reaction with ficin treated cells. The activity of the lectin was also apparently directed toward a site different from that of the T, Tn and Tk cryptantigens. The extracts of marihuana seeds behaved similarly although preparations were of a much lower titre.

Inhibition studies with simple sugars showed the lectin to react primarily with glucose and several of its derivatives: glucosamine, N-acetyl D-glucosamine and methyl D-glucopyranoside. The lectin also reacted with galactose to a lesser extent. Concentration by ultrafiltration showed the molecular weight of the lectin to be in excess of 15,000.

The receptor site for the lectin appears to be away from the periphery of the red cell shielded by the protein which is

removed by the protease effect of papain but not by that of ficin. Removal of sialic acid by neuraminidase also had little effect. This site is similar to that described for porcine erythrocytes and the *Nemopanthus mucronatus* lectin[7] and for bovine erythrocytes and the *Amaranthus caudatus* lectin[8].

The presence of the lectin in the pollen of the plant as well as in the seeds of *Cannabis sativa* L. shows the synthesis of the protein to occur at a very early stage in the development of the plant. The lectin was also present in higher quantities in the pollen than in the seeds.

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Isolation and partial characterization of cuticular collagen from the parasitic nematode *Gaigeria pachyscelis*

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Summary. The cuticle from adult *Gaigeria pachyscelis* was isolated by solubilizing the internal tissues with sodium dodecyl sulphate (SDS) at 37 °C. Cuticular protein was extracted with guanidine-HCl and β-mercaptoethanol and purified by ammonium sulphate fractionation and DEAE-cellulose chromatography. SDS-polyacrylamide gel electrophoresis of purified protein revealed 2 polypeptides with apparent mol. wts of 58,000 and 74,000. As judged from their hydroxyproline content both of them are collagenous in nature. Results of gel filtration indicate that cuticular collagen exists in two forms, a non-associated form at low concentration and an associated form at high concentration.

Nematodes are surrounded by a tough cuticle, consisting of 3 layers[2,3], which, in parasitic forms, is important in their intricate relationship with the host[4]. At present little is known about the biochemical nature of the cuticle of most nematodes. *Ascaris* cuticle is composed of 3 genetically distinct collagenous polypeptides[5] and recently the presence of 2 genetically distinct collagen chains has been reported in *Caenorhabditis*[6].

The present study reports the isolation and partial characterization of cuticular collagen from the parasitic nematode, *Gaigeria pachyscelis* (Ancylostomatid), a hookworm of sheep and goat.

Materials and methods. Adult parasites, after extensive washing with distilled water, were incubated in 1% sodium dodecyl sulphate (SDS) at 37 °C overnight[7]. This treatment selectively solubilizes the internal tissues of the worm, as could be confirmed by light microscopy.

Isolated cuticles were extracted with 8 M guanidine-HCl and 2% β-mercaptoethanol at 4 °C overnight, and centrifuged at 10,000 × g for 30 min. The clear supernatant was dialyzed against 0.2 M NaCl-0.05 M Tris (pH 7.5)-0.1% β-mercaptoethanol and made 50% with respect to ammonium sulphate. Further purification of precipitates obtained by ammonium sulphate fractionation was carried out on a DEAE-cellulose column[8]. Gel filtration studies of purified collagen, at concentrations of 500 µg/ml and 5 mg/ml, were carried out on Sephadex G-200.

Relative amount of various polypeptides and their apparent molecular weights

Polypeptide	Hydroxyproline	Percentage	Molecular weight
1	Present	42	58,000
2	Present	57	74,000
3	Absent	1	79,000

Relative amount (expressed as percentage) of polypeptides was calculated from the areas of the bands after scanning the polyacrylamide gels.

Electrophoresis was performed on 5% polyacrylamide gels[9]. After staining, gels were scanned at 580 nm and the positions of the bands were compared with those of standard proteins for molecular weight determination. Gel portions containing the bands were cut, washed with distilled water and extracted with 0.2 M NaCl-0.05% SDS at 37 °C[10]. After centrifugation, the supernatant was dialyzed against distilled water and lyophilized. Hydroxyproline was estimated after hydrolysis of the protein with 6 N HCl at 110 °C for 24 h[11]. Hexosamine was determined as described by Ashwell[12].

Results and discussion. Because of the small size of the parasite, it is difficult to isolate the cuticle by physical means. The isolation of the cuticle by solubilizing the internal tissues with SDS is simple and highly satisfactory. Guanidine-HCl (8 M) and β-mercaptoethanol (2%) solubilizes 80–90% of the cuticle. The need for a reducing agent during solubilization indicates that disulphide bonds are probably involved in the organiza-

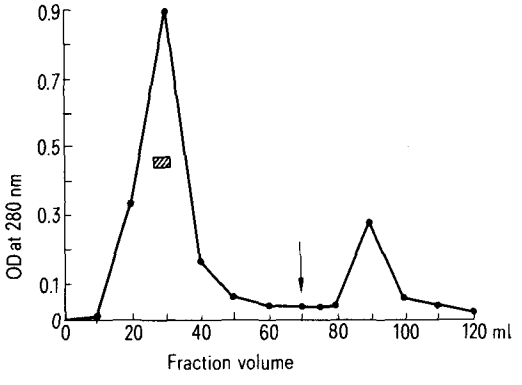

Figure 1. Ion exchange chromatography on DEAE-cullulose. Arrow indicates the elution of bound components (peak 2) with 1 M NaCl.