Both donors and recipients were female mice with a b.wt of 20–23 g. Before collection of peritoneal cells, donors were flushed i.p. with buffered physiological solution containing 10% BSA and cells were collected and counted according to Katiyar and Sen[11] and Wintrobe[12] 21 days after infection. Within 4 h after collection, approximately 26×10^4 cells were injected i.p. into each recipient mice; group A received singly sensitized, group B repeatedly sensitized and group C non-sensitized (control) peritoneal exudate cells. 7 days after cell transfer, each recipient was challenged with a single dose of 500 infective *A. caninum* larvae and subsequent recoveries were made from different organs and muscles at 4-h intervals by the Baermann method after digestion in artificial gastric juice.

Results and discussion. Results of recoveries made from the 3 groups of recipients are shown in table 2 and they show remarkable variations. Most of the larvae were expelled from recipient group A mice which received cells from singly sensitized donors. Here comparatively more larvae (62.5%) were expelled during the 1st 4 h after challenge as compared to 60.6% expelled from group B recipients. Thereafter the expulsion rate in group A mice proceded rapidly and, at 36 h after challenge, the percentage of larvae expelled in both groups A and B was about the same, 97.8% in group A and 97.6% in group B mice. Control recipients with cells from normal non-infected donors gave baseline readings of larvae expelled as 5.4% at 4 h and 40.8% at 36 h after challenge.

Table 2. Percentage of *A. caninum* larvae recovered from experimental and control groups of recipient mice at 4-h intervals during 36 h after a challenge dose of 500 larvae

Duration of infection at necropsy (h)	A Recipients with singly sensitized cells	B Recipients with repeatedly sensitized cells	C Recipients with nonsensitized cells
4	37.5	39.4	94.6
8	23.8	26.8	86.2
12	20.3	25.6	81.6
16	17.2	21.8	74.2
20	10.9	12.8	66.2
24	8.9	6.0	61.6
36	2.2	2.4	59.2

Readings are based on mean of recoveries made from 3 animals.

It is clear from the results that transferred sensitized (immune) peritoneal exudate cells from singly infected isologous donors produced rapid expulsion as compared to repeatedly immunized and non-immunized cells. However, at 36 h after challenge, expulsion was almost identical in the 2 experimental groups with an almost equal immune response. From 24 to 36 h, group A expelled more larvae (6.7%) in comparison with group B (3.6%), probably due to the presence of increased histamine in the intestine of former on the 1st day after infection (Vardhani and Johri[13]). The entire worm load within the alimentary tract was expelled within 24 h in both groups A and B. Lung migration did not take place and larvae migrated into the muscles within 4 h after challenge in group A mice, whereas this did not occur in groups B and C mice. Comparatively less larvae in the gastrointestinal tract suggests the induction of strong immunity due to rapid homing and dissemination of sensitized cells into the lumen of crypt at a faster rate in group A recipients where more larvae resorted to migration to muscles. Thus, the sensitized bowel became untenable for the larvae resulting in their allergic immobilization or death and eventual expulsion. Coupled with this, local cellular injuries result in the production of histamine which also aids in expulsion. These results confirm those of Murray et al.[14,15] who also found vasoactive amines during expulsion of *N. brasiliensis* from infected rats. Allergic inflammations, fragmentation of the intestinal villi and alterations in the biochemical environment also act as intolerable factors. Though muscles are a favourable and suitable site, the migrating larvae perish due to diffused infiltration of inflammatory cells which also sensitize and partly destroy muscle fibres. Lee et al.[16] also reported migration of *A. caninum* larvae into the muscles within 4 h and the infiltration of inflammatory cells in infected mice. Larsh[17] found that larvae that did not enter the lungs during migration perish readily, and this may account for the rapid expulsion of larvae from recipients of group A.

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The lectin from *Viscum album* L. purification by biospecific affinity chromatography

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Summary. A lectin from *Viscum album* which specifically binds to D-galactose was isolated by affinity chromatography on O-lactosyl-, O-galactosyl-polycarylamide or hydrolized sepharose 4 B. Some serological and physicochemical properties of the agglutinin are reported.

Of the lectins described in the literature showing anti-D-galactose specificity, those from *Ricinus communis*, *Momordia charantia* and *Abrus precatorius* have been isolated by biospecific affinity chromatography on agarose gel beads[1]. But those from *Bauhinia purpurea alba*, *Crotalaria juncea*, *Sophora japonica* and *Wistaria floribunda* do not react with agarose[2,3]. However these lectins react with a galactan gel prepared by acid treatment of agarose gel beads as described by Ersson et al.[2]. The lectin from *Viscum album* shows similar properties. Recently Franz et al. reported on the isolation of a lectin- and toxin-fraction from *Viscum album* by affinity chromatography on insolubilized serum proteins and subsequent fractionation by gel chromatography using sephadex G_{75}[4]. In this paper we describe a method to isolate and purify the lectin by affinity chromatography on

O-lactosyl-polyacrylamide, O-galactosyl-polyacrylamide or hydrolized sepharose 4 B, and some serological and physicochemical properties of the agglutinin.

Material and methods. 100 g ground plant material from *Viscum album* L. grown on *Robinia pseudoacacia* was stirred with 750 ml 0.5 M NaCl solution for 3 h at room temperature, then filtered, and solid $(NH_4)_2SO_4$ (60 g/100 ml) was added to the clarified extract. The precipitate was allowed to settle overnight, collected by centrifugation and resuspended in a minimum volume of 0.5 M NaCl solution. The solution was applied directly to a column (60 × 3 cm) of sepharose 4B, hydrolized for 3 h with 0.2 M HCl as described by Errson[2] or to a column of 300 ml O-lactosyl- or O-galactosyl-polyacrylamide copolymerized by the method of Hôrejši and Kocourek[5]. It is also possible to apply the clarified crude extract after dialysis against 0.5 M NaCl solution for 16 h to the columns. The columns were washed protein-free and the lectin was eluted with 0.5 M NaCl containing 0.15 M D-galactose or lactose. The fractions containing the active protein were pooled, dialyzed against distilled water and freeze-dried.

Haemagglutinating activity was determined with 1% suspension of washed human erythrocytes using the microtitrator of Takatsy. The haemagglutination inhibition test was carried out as follow: to 0.05 ml of a 2fold serial dilution of carbohydrates 0.025 ml lectin solution with an agglutinating activity of 2 units was added. After incubation at 37 °C for 1 h 0.025 of a 2% erythrocyte suspension was added. After 1 h at 37°C the degree of agglutination was assessed.

Carbohydrate content of the lectin, after chromatography on sephadex G_{25}, was determined by the phenol-H_2SO_4 method with reference to glucose[6]. For mol. wt determination a column of sephadex G_{200} (90 × 1.5 cm) equilibrated with 0.9% NaCl was calibrated by applying proteins of known molecular weight as ovalbumin 45,000, bovine serum albumin 67,000 and its dimer 134,000 and human IgG 150,000. TLC for determination of the mol. wt was performed with sephadex G_{200} superfine. For polyacrylamide-gel disc electrophoresis the method of Maurer[7] was used, which involved electrophoresis in columns of 7.5% polyacrylamide-gel in buffer of pH 4.3 and 8.9 respectively. The mol. wt of lectin subunits was obtained in 10% polyacrylamide-gel in the presence of sodium dodecylsulphate, by comparison with marker proteins.

Results and discussion. The *Viscum album* lectin cannot react with the internal galactosyl residues of long linear galactan molecules. One way to increase the number of end groups is to split the galactan chains without completely degrading the network. Sepharose 4B, treated with 0.2 M HCl as described, was chosen for the isolation of *Viscum album* lectin by affinity chromatography. Another simple method of isolation of the lectin involved the use of O-galactosyl- or O-lactosyl-polyacrylamide. The amount of lectin obtained from 100 g dry plant material was about 40 mg. The value of sugar content obtained by the phenol-H_2SO_4 method was 10.1% with glucose as a standard. The sugars were not identified further. Mol. wt estimated by gel chromatography and TLC was about 115,000. The lectin exhibited a single band by polyacrylamide disc electrophoresis at pH 4.3 and 8.9 respectively. A mol. wt of 29,000 and 34,000 was obtained for the subunits in 10% polyacrylamide gel in the presence of sodium dodecylsulphate. In contrast to the findings of Luther et al.[8] with mistletoe crude extract, we found for the purified lectin no specificity to human B erythrocytes, rather the agglutinin possesses non-specificity to human red cells (table 1). The haemagglutination was inhibited by lactose, D-galactose, raffinose and galactan from the larch in that order of potency (table 2). The results obtained corroborate earlier reports for crude extract[9]. The following sugars showed no interaction with the lectin: D-glucose, D-glucosamine, N-acetyl-D-glucosamine, D-mannose, D-mannosamine, N-acetyl-D-mannosamine, L-fructose, L-rhamnose, D-galactosamine, N-acetyl-D-galactosamine and D-galacturonic acid. The activity of the lectin is unchanged by warming to 50 °C for a short time, but heating to about 60 °C destroyed the agglutinating activity (table 3).

Recently we found a report about isolation of a lectin from *Viscum album* using granulated Agar[10] with a mol. wt of 160,000 and a sugar content of 9.3%. Further studies about structure and toxicity of the lectin are underway in our institute.

Table 1. Hemagglutinating activity of *Viscum album* lectin

Origin	Human A_1	A_2	A_1B	A_2B	B	0	Rabbit	Mouse	Sheep
Titer	1:16	1:16	1:16	1:16	1:32	1:16	1:8	1:128	0

A 1% erythrocyte suspension and a 0.02% lectin solution were used.

Table 2. Hemagglutinating inhibition test

Sugar	Minimum sugar concentration for inhibition
D-galactose	50 μM/ml
Lactose	25 μM/ml
Raffinose	75 μM/ml
Galacatan from larch	20 mg/ml

0.05 μl of a 2fold serial dilution of sugars were mixed with 0.025 μl of *Viscum album* lectin (25 μg/ml) and 0.025 μl of a 2% human erythrocyte suspension.

Table 3. Thermal stability of *Viscum album* lectin

Temperature (°C)		10′	20′	30′
20	Titer	1:32	1:32	1:32
30		1:32	1:32	1:32
40		1:32	1:32	1:32
50		1:32	1:32	1:16
60		1:8	1:8	1:4
70		0	0	0

A 0.02% lectin solution was heated on a water bath for different intervals.

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