Onderstepoort J. vet Res., 54, 233–246 (1987)

PURIFICATION OF *COWDRIA RUMINANTIUM* BY LECTIN CELLULAR AFFINITY CHROMATOGRAPHY

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ABSTRACT

VERMEULEN, N. M. J., NEITZ, A. W. H. & VILJOEN, G. J., 1987. Purification of *Cowdria ruminantium* by lectin cellular affinity chromatography. *Onderstepoort Journal of Veterinary Research*, 54, 233–246 (1987)

This review covers the isolation of *Cowdria ruminantium* by lectin cellular affinity chromatography from different *Amblyomma hebraeum* sources. Cellular affinity chromatography has been reviewed with special attention being given to the application of this technique in the isolation of rickettsiae.

INTRODUCTION

The purification of *Cowdria ruminantium* is important for several reasons (Viljoen, Vermeulen, Oberem, Prozesky, Verschoor, Bezuidenhout, Putterill, Visser & Neitz, 1985). Of prime importance is the need for a preparation free from extraneous antigens to provide a suitable vaccine (Wilson, 1967). Furthermore, investigations into the biochemical, antigenic and immunogenic properties of the organism require the availability of pure preparations. Through such studies, methods for sensitive, specific serodiagnosis could possibly be developed, the nature of the immunity to heartwater disease elucidated, the taxonomic position of the organism more accurately described and morphological studies extended (Pienaar, 1970; Du Plessis, 1970; Du Plessis, 1985; Uilenberg, 1981). In addition, information concerning the vector and host specificity of the organism could be gained. The study of the developmental cycles and distribution in the vertebrate and invertebrate hosts would also be facilitated. Pure preparations are also essential for the study of the presumed toxin produced by these pathogens (Neitz, 1968).

The isolation of sufficient amounts of viable pure *C. ruminantium* has been hampered for many years by their extremely labile nature and difficulties encountered in the cultivation of the organism in chicken yolk sacs and tissue culture (Uilenberg, 1983). The propagation of this pathogen in the laboratory has also met with problems (Du Plessis, 1982). Recently, Du Plessis (1982) and Mackenzie & McHardy (1984) have succeeded in the propagation of certain strains of *C. ruminantium* in mice and Bezuidenhout, Paterson & Barnard (1985) in cell culture, thus providing alternative sources of the organism.

Nine methods have been used to purify rickettsiae organisms (Viljoen *et al.*, 1985). Many of these methods are time-consuming and have detrimental effects on the organisms (Weiss, Coolbaugh & Williams, 1975). Apparently no attempts at utilizing these or alternative techniques for the purification of *C. ruminantium* have been described in the literature.

Cellular affinity chromatography (Sharma & Mahendroo, 1980) has developed recently as an extension of the traditional molecular affinity chromatography (Lowe, 1979; Turkova, 1978; Wilchek, 1984). Cellular affinity chromatography has been used to isolate cells, cell organelles, cell membranes, phages and viruses (Sharma & Mahendroo, 1980). The isolation of eukaryotic cells by lectin affinity chromatography is summarised in Table 1. Immunoadsorbent affinity chromatography of cells was not included in this table since it will be discussed elsewhere (Neitz & Vermeulen, 1987). Viruses, phages and bacteria have been isolated with lectins and antibodies (Table 2). The utilization of cellular affinity chromatography to separate bacterial populations (Table 2) has been largely overlooked. This was also observed by Ferenci (1984). Affinity chromatography with specific lectins is a quick, mild procedure for the isolation of a variety of cells (Sharma & Mahendroo, 1980). Since *Cowdria ruminantium* organisms show staining characteristics similar to those of gram-negative bacteria (Cowdry, 1925), an attempt was made to purify viable *C. ruminantium* by means of wheat germ lectin cellular affinity chromatography (Viljoen *et al.*, 1985). This lectin shows specificity towards N-acetyl-glucosamine (Nagata & Burger, 1974; Goldstein, Hammerström & Sundblad, 1975), which is a characteristic constituent of the cell wall of gram-negative micro-organisms (Salton, 1964). Since very little is known about the isolation of rickettsial or bacterial populations by lectin affinity chromatography, the purpose of this paper was:

(a) To review lectin affinity chromatography of eukaryotic cells with special emphasis on the practical application of the process and how this knowledge can possibly be used to separate procaryotic cells.

(b) To describe the isolation of *Cowdria ruminantium* from different sources with lectin-Sepharose-6MB columns.

Cellular affinity chromatography

Cell membranes are known to have dynamic and complex structures with many characteristic functional roles. The technique of affinity chromatography exploits the specificity of the different binding sites located on the surfaces of cells. Usually cells are separated by a process based on the recognition of specific surface markers, by matrix immobilized antibodies (Lowe, 1979), lectins (Hellström, Hammerström, Hammerström & Perlmann, 1984) and receptor binding ligands, such as α-bungarotoxin (Dvorak, Gipps & Kidson, 1978). The principle of cellular affinity chromatography is shown in Fig. 1.

The process allows the binding of cells recognized by matrix-bound ligand specific for a particular cell surface component. The recovery of the cells is usually possible by elution with a competitive agent in the buffer.

Choice of matrix material

Sharma & Mahendroo (1980) summarized the following requirements necessary for a matrix material in cellular affinity chromatography namely that it should: (a) be in a bead form; (b) be chemically and mechanically stable; (c) have good flow characteristics; (d) does not physically entrap cells; (e) permit convenient covalent coupling of biologically active molecules in an unaltered form; (f) not absorb cells specifically; (g) affect minimally, if at all, the viability of the chromatographed cells. Non-specific adhesion of cells to solid supports, like glass, charge and hydrophobic materials (Wigzell & Andersson, 1969) disqualified the use of a number of supports. Beaded agarose materials, like Sepharose, seem to be the closest to the ideal material for cellular affinity chromatography. Little or no non-specific interaction of cells occurs with CNBr activated Sepharose

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Received 24 March 1987—Editor

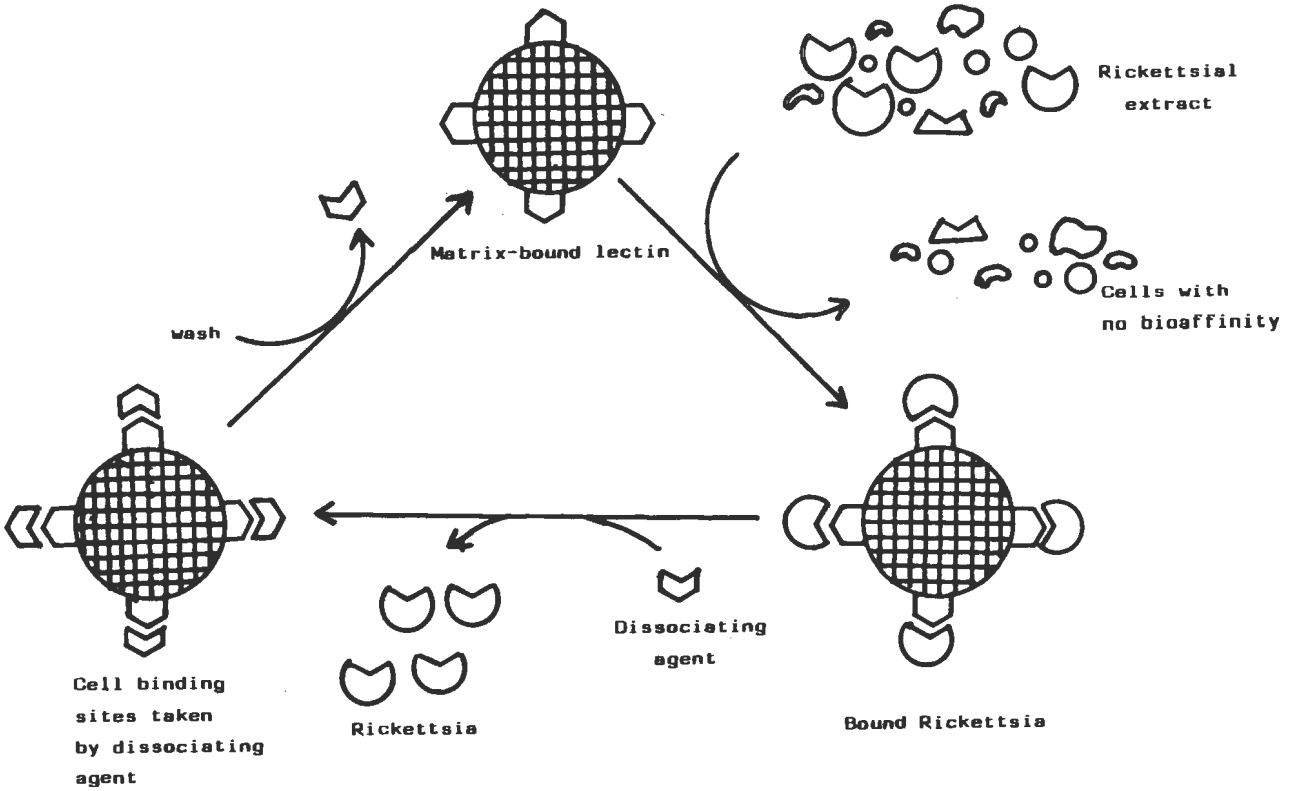

FIG. 1 Diagrammatic representation of cellular lectin affinity chromatography

6MB (diameter 200–300 μM), a specially designed product from Pharmacia with properties which make it a matrix of choice for cellular affinity chromatography (Au & Varon, 1979; Kinzel, Richards & Kubler, 1979; Nicola, Burgess, Metcalf & Battye, 1978).

Choice of ligand

Cells can be separated based on 2 major surface marker types: (a) surface antigens detectable by antibodies, and (b) surface saccharides detectable by lectins (Sharon, 1983). Table 3 lists some of the lectins commercially available. Lectins are now known as sugar binding proteins or glycoproteins that agglutinate cells or precipitate glycoconjugates (Goldstein, Hughes, Monsigny, Osawa & Sharon, 1980).

All lectins, a hundred already purified, are oligomeric proteins with several sugar binding sites per molecule, that is, they are multivalent. They combine non-covalently with mono- and oligosaccharides, both simple and complex, in the same way as antibodies bind antigens (Sharon, 1983). It was pointed out that while it is possible to obtain antibody specificity against virtually any organic compound, lectins are only specific against carbohydrates. However, antibodies bind so strongly to cells that they cannot be readily removed from cells (Table 2). Lectins, on the other hand, can easily be removed under mild conditions by competing sugars (Table 1) and with nearly full recovery of cells (Sharon, 1983). In cases where antibody ligands have been used (Table 2), elution was usually only possible with high or low pH conditions. Lectins appear therefore as a natural ligand of choice in the separation of cells.

Isolation of lectins

Lectins can be purified quite easily from readily available sources by affinity chromatography on columns containing sugars with specificty for a particular lectin (Wilcheck 1984; Dillner-Centerlind, Axelsson, Hammerström, Hellström & Perlmann, 1980; Goldstein & Hayes, 1978).

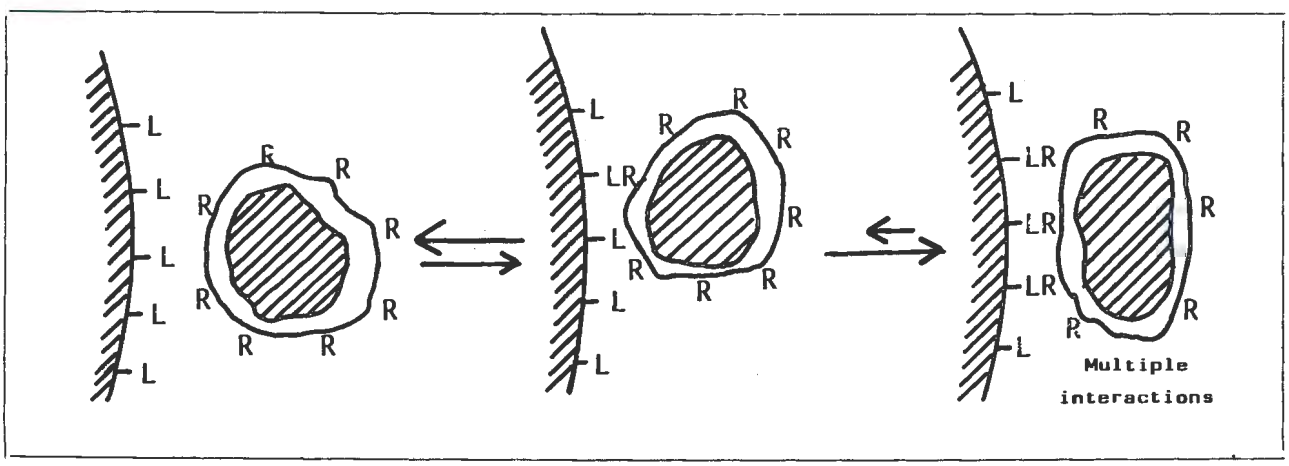

FIG. 2 The effect of prolonged incubation of cells with lectin affinity adsorbents

Coupling of lectins to the matrix

CNBr-activated Sepharose-6MB is most commonly used to couple lectins to the beads (Pharmacia Fine Chemicals, 1976) through cyclic and acyclic imidocarbonate bonds (Axen & Ernback, 1971). The activation of Sepharose-6MB with CNBr, the coupling of the ligand (lectin, antibody or receptor binding compounds), the stability of CNBr-coupled ligands and the problem of leakage of ligands have been described in detail by Lowe (1979). About a 100 purified lectins (Sharon, 1983) with different specificities for carbohydrate binding sites are known. Although ligands are mainly coupled to the matrix through CNBr activation, other coupling methods are also available (Lowe, 1979). It should be kept in mind that lectins are oligomeric proteins (Sharon, 1983) and therefore conditions where dissociation could take place should be avoided (Nagata & Burger, 1974; Ticha, Entlichter, Kostir & Kocourek, 1970; Kalb & Lustig, 1968).

Binding and releasing of cells from affinity columns

Cell affinity chromatography introduces a number of variables not encountered with molecular affinity chromatography. The main differences between these 2 techniques are that cells are much larger and contain many receptors in contrast to smaller molecular compounds, like enzymes with usually only one active site per protein molecule. *Escherichia coli* has approximately 30 000 sites for maltodextrins when fully induced (Ferenci, 1980). Multipoint attachment between lectins and cells (Bonnafous, Dornand, Favero, Sizes, Boschetti & Manni, 1983; Sharma & Mahendroo, 1980) is possible (Fig. 2) and this type of binding increases with time.

(a) *Column and batchwise procedures*

The separation of cells on lectin-coated agarose beads requires a system which avoids non-specific trapping and loss of cells. This has not satisfactorily been achieved with some column procedures (Sharma & Mahendroo, 1980). Batchwise techniques are preferable to the column methods when used under conditions in which the beads permit access of cells to the entire surface, thus utilizing maximum lectin binding capacity. However, with the development of Sepharose-6MB specifically for the separation of cells (Pharmacia, 1984) where a narrow range of bead sizes eliminates physical entrapment of cells, the advantage of the batchwise procedure over the column procedure becomes less obvious (Hammerström, Hellström, Dillner, Perlmann, Perlmann, Axelsson & Robertson, 1978; Hellström, Hammerström, Dillner, Perlmann & Perlmann, 1976). The isolation of cells with the column procedure with no apparent loss of viability has previously been described (Hellström *et al.*, 1984; Au & Varon, 1979). Prevention of non-specific adherence of cells like lymphocytes to glass columns and glass wool can be prevented by siliconization (Hubbard, Schluter & Marchalonis, 1984). The column procedure has another advantage over the batchwise procedure, in that flow rate can be easily manipulated to aid elution of cells, by increasing shear forces (Ghetie, Mota, Sjoqust, 1978; Nicola *et al.*, 1978; Evans, Mage & Peterson, 1969).

(b) *Degree of lectin substitution*

The rate of cell binding and sugar-specific release depends to a large extent on the amount of lectin bound per bead volume (Sharma & Mahendroo, 1980). Pharmacia (1984) suggested that it is desirable to use affinity absorbents with a lower degree of substitution (1–5 mg/mℓ) for cell separations, than would be usual for molecular affinity chromatography. Since multiple interactions (Fig. 2) are possible between the many receptors on cells (Haller, Gidlund, Hellström, Hammerström & Wigzell, 1978; Kinzel *et al.*, 1977) with the lectin molecules on the beads, the degree of lectin substitution should be controlled for ease of removal of the cells from the column (Bonnafous *et al.*, 1983). Higher substitution of lectin on the beads may be beneficial for the depletion of a selected type of cell. However, the higher substitution may lead to an increase in non-specific adsorption, this leading to lower cell recovery.

(c) *Temperature*

The rate of binding and the affinity of cells for ligand-bound Sepharose is temperature dependent (Nicola *et al.*, 1978; Galvez, 1966). With intact lymphocytes the same amount is able to bind to the surface of Con A at either 4 °C or 37 °C; however, binding affinity is 3 times higher at 37 °C. Specific membrane attachment of vesicles to Con A-Sepharose decreases at low temperatures (Brunner, Ferber & Resch, 1977). Insolubilized Con A appears to require at least short-range lateral movement of the Con A receptors. Lateral diffusion of receptors as measured by the capping of receptor molecules is restricted at low temperatures (Yahara & Edelman, 1972). Binding of cells to lectin-substituted Sepharose also involves rearrangement and clustering of lectin receptors. Slower binding and lower affinities of cells for lectins observed at lower temperatures (Nicola *et al.*, 1978; Brunner *et al.*, 1977; Evans *et al.*, 1969) seems to be in agreement with the above observations. Binding of cells (Banga, Hutchings, Tatham, Lang, Gunn, Cooke & Roitt, 1983; Jakobovitz, Eshdat & Sharon, 1981) and a virus (Galvez, 1966) to lectin and antibody-bound beads respectively, is fast and completed within 5–10 min at room temperature. When adsorption of the cells to the ligand-bound beads require lower temperatures, longer incubation times have been used (Viljoen *et al.*, 1985; Galvez, 1966). Higher temperatures are beneficial for the elution of cells from lectin substituted Sepharose columns (Triebel, Gluckman, Chapuis, Charron & Debre, 1985; Nicola *et al.*, 1978).

It appears that higher temperatures supply energy equivalent to that obtained by mechanical aggitation at lower temperatures (Nicola *et al.*, 1978). The large increase in the proportion of bone marrow cells specifically eluted from WGA-Sepharose with GlcNAc at 37 °C, was probably mediated through faster kinetics of both the forward and backward cell-receptor-WGA interactions. Such temperature effects would be expected to be pronounced when multipoint attachment sites are involved, because the increase in the release rate for a given temperature change would increase exponentially with the number of binding sites involved.

(d) *Sample application*

Cell extracts are applied to the drained bed of a column in a small volume of buffer (not exceeding more than 30 % of the volume of the sorbent). For efficient adsorption of the target cells, incubation should be performed for a time on the adsorbent before non-bound cells are washed off. Binding of cells to lectin-substituted Sepharose is rapid, since the binding of erythrocytes to PNA-Sepharose (Jacobovitz *et al.*, 1981), bone marrow cells to WGA-Sepharose (Nicola *et al.*, 1978) and cloned Hela cells to LCL-Sepharose (Kinzel *et al.*, 1977) was completed within 5–10, 10 and 20–30 min respectively. Incubation times of 20 min were found satisfactory for the efficient binding of cells to a number of lectin columns (Triebel *et al.*, 1985; Banga *et al.*, 1983; Schrempf-Decker, Baron & Wernet, 1980; Haller *et al.*, 1978). Prolonged contact (over 30 min) between the lectins and the cells, should be avoided, since this may result in non-specific binding or uptake of the lectins, thus making it impossible to remove the cells with specific sugars (Reisner & Sharon, 1984). These authors also cautioned that mitogenic lectins, with prolonged

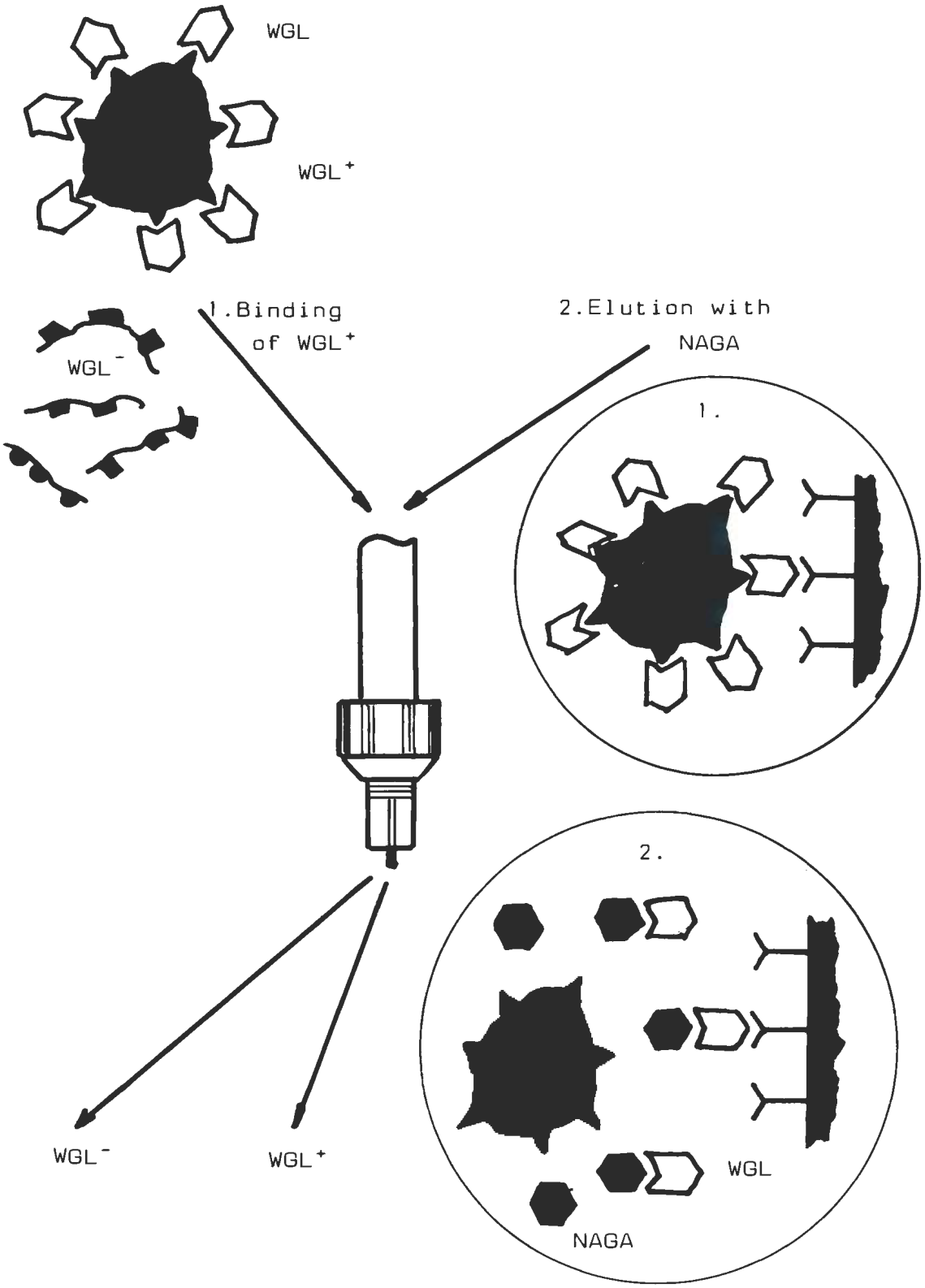

FIG. 3 Fraction of WGA lectin-binding (WGA^+) cells from non-binding (WGA^-) cells using immobilized antibodies against WGA lectin to retain lectin-coated cells

contact, may give rise to lymphocyte stimulation. Kinzel *et al.* (1977) reported that Hela cells bound to Sepharose-2B began to develop a resistance to chemical induced release, and that after 24 h it was impossible to remove these cells by any means. Mechanical agitation and high flow rates prevent binding of cells to lectin ligands (Bonnafous *et al.*, 1983; Nicola *et al.*, 1978; Brunner *et al.*, 1977). Many lectins require metal ions (Table 3) for their biological acitivity (Dillner-Centerlind *et al.*, 1980; Paulova, Ticha, Entlicher, Kostir & Kocourek, 1971), therefore they should be included in the incubation buffer (Viljoen, 1985; Kristiansen, 1975).

(e) *Cell elution*

Following the on-column incubation of sample cells, non-binding cells are recovered by extensive washing with the starting buffer. Numerous methods for the elution of cells from lectin columns have been described

TABLE 1 Examples of cellular affinity chromatography of eukaryotic cells

Substance isolated	Affinity ligand	Solid support	Elution conditions
Eukariotic cells			
Thymocytes, erythrocytes	Concanavalin A lectin	Nylon fibres	50 mM methyl-α-D-mannoside[a]
Human blood lymphocytes	*Lens culinaris* lectin	Gelatin	0,1 M D-glucose + melting 37 °C[b]
Lymphocytes	*Vicia villosa* lectin	Sepharose 6MB	1 mg/mℓ N-acetyl-D-galactosamine (10 min at 37 °C)[c]
Splenocytes	*Helix pomatia* lectin	Sepharose 6 MB	1 mg/mℓ N-acetyl-D-galactosamine + gentle stirring[d]
Human T cells	*Helix pomatia* lectin	Sepharose 6 MB	0,1 to 1 mg/mℓ N-acetyl-D-galactosamine[e]
Human lymphocytes	Wheat germ lectin	Sepharose 6MB	0,1 to 10 mg/mℓ N-acetyl-D-glucosamine[e]
Immunogenic tumour cells	Concanavalin A lectin	Nylon fibres	Methyl-α-D-mannoside[f]
Murine cytotoxic T-lymphocytes	*Vicia villosa* lectin	Sepharose 4B	0,1 M N-acetyl-D-galactosamine + 1 % gelatin[g]
B- and T-lymphocytes	*Helix pomatia* lectin	Sepharose 6MB	2 % N-acetyl-D-glucosamine[h]
Mouse T-hymocytes	Concanavalin A lectin	Merasalyl-trisacryl	50 mM DTT + 2 % methyl-α-D-mannoside[i]
Bovine T-lymphocytes	*Helix pomatia* lectin	Sepharose 6MB	0,1 to 1 mg/mℓ N-acetyl-D-galactosamine[j]
Rat lymphocytes	*Helix pomatia* lectin	Sepharose 6MB	0,1 to 1 mg/mℓ N-acetyl-D-galactosamine[k]
Thymocytes	Anti-peanut lectin anti-serum	Sepharose 6MB	0,1 M Galactose[k]
Synaptosomes	Concanavalin A lectin	Sepharose 4MB	0,25 M methyl-α-D-glucoside[m]
Mouse and human lymphocytes	Peanut lectin	Sepharose 6MB	0,2 M Galactose[n]
Hela, SV3T3	*Lens culinaris* lectin	Sepharose 2B	0,2 M methyl-α-D-mannoside[o] 0,2 M methyl-α-D-glucoside[o]
Mouse bone marrow	Wheat germ lectin	Sepharose 6MB	0,1 M N-acetyl-D-glucosamine[p]

[a] Edelman & Rutishauser (1975)
[b] Bolt & Lyons (1979)
[c] Banga *et al.* (1983)
[d] Haller *et al.* (1978)
[e] Hellström *et al.* (1984)
[f] Killion & Kollmorgen (1976)
[g] Kimura *et al.* (1979)
[h] Schrempf-Decker *et al.* (1980)
[i] Bonnafous *et al.* (1983)
[j] Morein, Hellström, Axelsson, Johansson & Hammerström (1979)
[k] Swanborg, Hellström, Perlmann, Hammerström & Perlmann (1977)
[l] Irle *et al.* (1978)
[m] Garcia-Segura *et al.* (1978)
[n] Reisner & Sharon (1984)
[o] Kinzel *et al.* (1977)
[p] Nicola *et al.* (1978)

TABLE 2 Examples of cellular affinity chromatography of phages, viruses and bacteria

Substance isolated	Affinity ligand	Solid support	Elution conditions
(a)		*Phages*	
Bacteriophage SKV1	*Shigella sonnei* lipo-saccharide	Sepharose 4B	0,5 M borate pH 7,3 and 0,05 M EDTA[a]
T-4	Poly-D,L-lysine	Sepharose 2B	0,05 M glycine•HCl pH 3,0 and 0,5 M NaCl[b]
(b)		*Viruses*	
Aleution mink disease	Antibody (IgG)	Sepharose 4B	0,5 M NaCl to 0,4 M glycine•HCl/0,75 m NaCl pH 2,0[c]
	Antibody (IgG)	Sepharose 2B	0,2 M Glycine•HCl pH 2,8 in 0,75 M NaCl[d]
Influenza	γ-Globulin	Disulfide linked γ-globulin	pH 11,3; 12,0 and 12,5[e]
Semliki forest	Antibody	Disulfide linked γ-globulin	pH 11,1[f]
Tobacco mosaic	Antibody	ρ-aminobenzyl-cellulose	0,01 M glycine•HCl pH 3,0[g]
Tobacco ringspot	Antibody	Gluteraldehyde crosslinked antibodies	0,1 M glycine•HCl pH 2,8[h]
Virus glycoproteins	*Lens culinaris* lectin	Sepharose 4B	2 % (m/v) methyl-α-D-mannopyranoside in 1 % SDS[i]
Influenza X-31	*Vicia ervilla* lectin	Sepharose 2B	Different sugars[j]
Measels SSPE			
Sendai			
Bovine type 3 paramyo			
Bovine viral diarrhoea	*Crotalaria juncea* lectin	Sepharose 2B	0,2 % lactose[k]
(c)		*Bacteria*	
Escherichia coli	Starch	Sepharose 6B	0,1 M maltose[l,m]
Dictyostelium discoideum	Desilated fetuin	Sepharose 6MB	[n,o]

[a] Romanowska, Lugowski & Mulczyk (1976)
[b] Sundberg & Höglund (1973)
[c] Kenyon, Gander, Lopez & Good (1973)
[d] Yoon, Kenyon & Good (1973)
[e] Sweet, Stephen & Smith (1974)
[f] Wood, Stephen & Smith (1968)
[g] Galvez (1966)
[h] Lapido & De Zoeten (1971)
[i] Hayman, Skehel & Crumpton (1973)
[j] Kristiansen (1975)
[k] Karsnäs, Kristiansen & Moreno-Lopez (1981)
[l] Ferenci & Lee (1983)
[m] Ferenci & Lee (1982)
[n] Enrichment of cells lacking carbohydrate binding protein
[o] Ray, Shinnick & Lerner (1979)

TABLE 3 Specificity and molecular properties of a few commercially available lectins[a]

Abbreviation	Source	Mol. mass $\times 10^3$	Number of subunits	Metals	Carbohydrate specificity	Mitogenic activity
Con A	Jack Bean (*Canavalia ensiformis*)	108	4	Ca^{2+}, Mn^{2+}	α-D-mannose, α-D-glucose	+
WGA	Wheat germ (*Triticum vulgare*)	36	2	n.d.	N-acetyl-D-glucosamine, β-D-GlcNAc-(1→4)-D-GlcNAc	—
HP	Garden snail (*Helix pomatia*)	79	6	—	α-D-GalNAc>α-D-GlcNAc≃ β-D-GalNAc>β-D-GlcNAc	—
PNA	Peanut (*Arachis hypogaea*)	110	4	n.d.	β-D-Gal-(1→3)-D-GalNAc> α-D-Gal	(+)[b]
LCA	Lentil (*Lens culinaris*)	52[c]	4	Ca^{2+}, Mn^{2+}	α-D-Man, D-Glc, α-L-Fuc	+
SBA	Soybean (*Glycine max*)	120	4	Ca^{2+}, Mn^{2+}	α,β-D-GalNAc α,β-D-Gal	(+)[b]
VVL	Hairy vetch (*Vicia villosa*)		4		α-D-GalNAc-(1→3)-D-Gal	—
LTL	Winged bean (*Lotus tetragonolobus*)	120	4		α-L-Fuc	—

[a] Compiled from data cited by Sharon (1983) and Dillner-Centerlind *et al.* (1980).

[b] Sharon (1983)

[c] Stein *et al.* (1971).

(Sharma & Mahendroo, 1980; Nicola *et al.*, 1978; Kinzel *et al.*, 1977). Competitive elution of cells from immobilized lectins, with the specific complementary sugar for the lectin, is the gentlest and most specific method and is often sufficient to release bound cells from the adsorbent (Reisner & Sharon, 1984; Banga *et al.*, 1983; Schrempf & Decker *et al.*, 1980; Haller *et al.*, 1978; Hellström, Perlmann, Robertsson & Hammerström, 1978; Kinzel *et al.*, 1977). On-column incubation with the competing sugar for periods of time has been used to improve recovery of cells from lectin-columns (Banga *et al.*, 1983; Kumura, Wigzell, Holmquist, Ersson & Carlsson, 1979; Edelman, Rutishauser & Milette, 1974).

Elution of cells or membrane vesicles from certain lectin adsorbents are often difficult (Dornand, Bonnafous & Mani, 1978; Haller *et al.*, 1978; Brunner *et al.*, 1977), from Con A which has a high affinity for its receptors (Bonnafous *et al.*, 1983). Mechanical treatment, temperature or flow rate may however be necessary, alone or in combination with sugar, to release tightly bound cells from lectin adsorbents. Thus it has been reported that stirring (Kumara *et al.*, 1979; Brunner *et al.*, 1978), high flow rates (Nicola *et al.*, 1978), and higher temperature (Banga *et al.*, 1983), increases the recovery of cells. *Lens culinaris* lectin binds the same sugar as Con A but with about 50 times lower binding association constant (Stein, Howard & Sage, 1971). Therefore, substitution of *Lens culinaris* for Con A should be considred if cells bind too tightly to this latter adsorbent. This principle has successfully been used in the isolation of a lymphocyte membrane fraction (Dornand *et al.*, 1978).

The relative drastic elution conditions which are sometimes necessary in lectin cell affinity chromatography have led to the development of new milder methods. Cell affinity chromatography with ligands immobilized through cleavable mercury-sulphur bonds has been developed by Bonnafous *et al.*, 1983. In this particular case, Con A was immobilized through the cleavable mercury-sulphur bond to the support. Mouse thymocytes could be recovered from this adsorbent with thiol treatment. A problem with this method is that methyl-α-D-mannopyranoside had to be added in the elution buffer to prevent Con A-induced agglutination of the released cells. Others (Irle, Piquet & Vassali, 1978) have circumvented this problem by coupling rabbit antibodies against peanut lectin to Sepharose 6MB matrix to provide a lectin specific immunoadsorbent. Lectin-binding thymocytes could be adsorbed on to the column and they are easily released by addition of 0,1 M galactose. A similar example with wheat germ lectin is shown in Fig. 3. An advantage of this indirect method of purifying cells is the ease of preparing an adsorbent of adequate binding capacity that still permits desorption of cells under mild conditions.

The elution of cells with complementary sugars from lectin-matrix is an excellent procedure. Elution of mammalian cells from lectin columns requires that sugar concentrations be adjusted to iso-osmolarity, with the aid of non-binding sugars (Kinzel *et al.*, 1977). Osmolarity should play a minor role in the isolation of autonomously growing bacteria, since it is known that they can survive under conditions of variable osmolarity (Davis, Dulbecco, Eisen, Ginsberg & Wood, 1973). The effect of osmolarity on obligate parasites like rickettsiae is quite devastating (Dasch, 1981; Elisberg & Bozeman, 1979; Bovarnick & Schneider, 1960; Bovarnick *et al.*, 1950). In one case, however, it was posible to isolate viable, infective *Cowdria ruminantium* with WGA-Sepharose using a moderately hyperosmolalic sugar solution (Viljoen *et al.*, 1985). It was reported by Weiss (1973) that various factors, such as moderately high osmolarity, a high K^+/Na^+ ratio, retard leakage and stabilize rickettsiae.

(f) *Purity of cell preparations*

The question of cell purity has been addressed by Sharon (1983). In contrast to chemial substances of identical molecules which are judged pure by physicochemical criteria, the molecular complexity of cells is such that a truly homogeneous preparation of cells cannot be obtained. Even cloned lines of cells remain heterogenous with respect to a variety of metabolic, mitogenic, macromolecular and shape-related criteria. Thus, purity may be defined in terms of a single phenotypic trait; typically one that is expressed on the cell surface. *C. ruminantium* organisms isolated by means of Percoll density gradient centrifugation from infected brain tissue and *A. hebraeum* nymphae possessed distinctly different densities (Neitz, Viljoen, Bezuidenhout, Oberem, Putterill, Verschoor, Visser & Vermeulen, 1986).

(g) *Effect of lectins on cells*

Certain lectins are mitogenic (Dillner-Centerlind *et al.*, 1980) and they can stimulate a number of cells such as lymphocytes to grow and divide (Sharon, 1983). Thus prolonged contact between lectins and cells may lead to lymphocyte stimulation (Reisner & Sharon, 1984). Whether similar problems occur in bacteria, viruses and

rickettsiae is not clear. Many lectins, however, agglutinate bacteria, precipitate bacterial polysaccharides specifically (Lis & Sharon, 1973; Sharon & Lis, 1972) and inhibit growth of certain fungi (Mirelman, Galun, Sharon & Lotan, 1975). The above facts must be borne in mind when well-defined populations of cells from different sources are isolated by lectin affinity chromatography (Sharon, 1983).

(h) *Nutritional requirements of isolated rickettsiae*

The purification of *C. ruminantium* is more difficult than the purification of other rickettsiae, owing to their extremely labile nature (Uilenberg, 1983) Rickettsiae like *C. ruminantium* are obligatory intracellular parasites. The *Rickettsia* can only grow in the cytoplasma of eukaryotic cells (Winkler, 1976; Weiss, 1973). In contrast, *C. ruminantium* grows only in the membrane bound vacuoles (Prozesky & Du Plessis, 1985; Pienaar, 1970) and therefore they resemble the *Ehrlichia* and *Chlamydia* (Moulder, 1985; Avakyan & Popov, 1984). Once isolated from their host, rickettsiae are deprived of the supply of cytoplasmic substrates. Although no universal cell-free growth or sustaining medium is known for rickettsiae in general, and certainly not for *C. ruminantium,* some knowledge on the requirements for growth and sustenance of metabolic acitivity is known (Weiss, 1973). The SPG medium formulated by Bovarnick, Miller & Snyder (1950) favours the survival of a number of strains of rickettsiae. Recently, Birnie, Endris & Logan (1986) compared a number of diluents for the preservation of the viability of the Kwanyanga isolate of *C. ruminantium*. They found that the Snyder 1 diluent was significantly better than other media at maintaining *C. ruminantium* at room temperature.

Materials and Methods

The methods and procedures used for the isolation of *C. ruminantium* with WGA-lectin chromatography have been reported in detail (Viljoen *et al.*, 1985). Since this is the first time that a rickettsiae has been isolated by cellular lectin affinity chromatography, the preparation of isolates and the chromatography will be described in detail.

Preparation of crude brain and nymph extracts

Owing to the extremely labile nature of *C. ruminantium* all procedures were performed at 4 °C. The crude brain extracts were prepared, using 200–300 g of frozen, infected or control non-infected brain. These were quickly thawed and homogenized at 4 °C at low speed for 5 min in a Waring blender in 100–150 mℓ of a 0,05 M HEPES-0,154 M NaCl buffer, pH 7,4 (subsequently referred to as HEPES buffer).

Infected and non-infected *A. hebraeum* nymphae were homogenized for 10 min in the same buffer and blended at a dilution of 10 nymphae per 5 mℓ of buffer.

The brain and tick homogenates were centrifuged for 30 min at 1 000 × g in a Rotor 19 in a Beckman L5-65 ultracentrifuge. This centrifuge was also used in all subsequent centrifugations with half maximum acceleration and braking. The supernatants were then centrifuged for 30 min at 10 000 × g with a Rotor 30. The resultant supernatants were centrifuged at 30 000 × g for 30 min in a Rotor 30. The sediment was resuspended in 12 mℓ of HEPES buffer.

Preparation of crude endothelial extract

The infected and non-infected crude cell cultures (10 mℓ) were sonified for 5 s to release *C. ruminantium* from the endothelial cells. A Sonifier Cell Disruptor B-30 (Branson Sonic Power Company, Danbury, U.S.A.) and microtip at 40 % continuous duty cycles and a setting of 5 on the output control were used. Centrifugation was

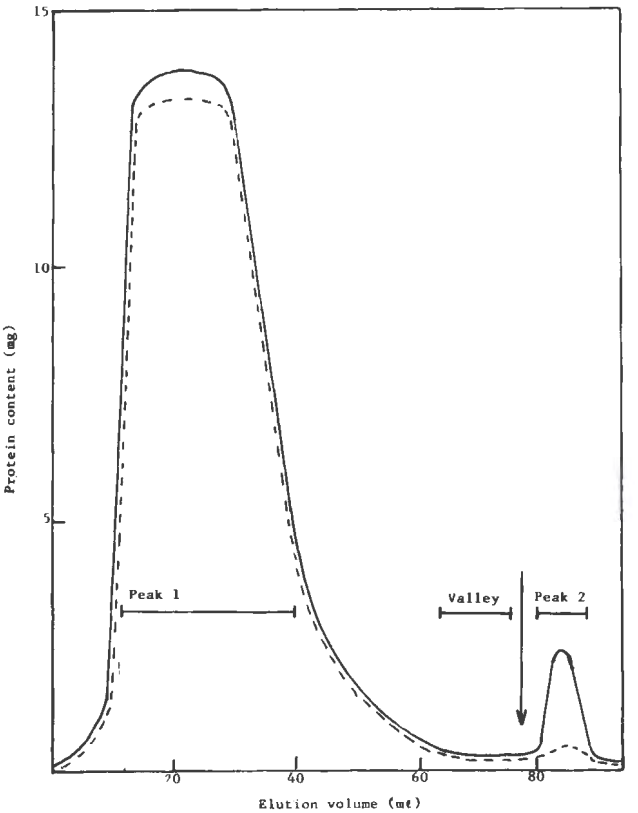

FIG. 4 Representative chromatogram of nymph crude extracts on a wheat germ lectin Sepharose 6MB column (void volume: 10 mℓ). The column was equilibrated at 4 °C with a 0,05 M HEPES; 0,154 M NaCl, pH 7,4 buffer. Crude extracts (10 mℓ) were applied to the column and left for 2 h to bind. The column was then washed with equilibrating buffer. The arrow indicates the application of elution buffer (c. 20 mℓ) containing N-acetyl-D-glucosamine (1 g/10 mℓ). Infected nymph crude extracts (—), non-infected nymph crude extracts (- - -). Flow rate was 30 mℓ/h.

carried out for 15 min at 300 × g in a Piccolo bench top centrifuge. The supernatant was then centrifuged for 30 min at 10 000 × g with a Rotor 30. The supernatant was further centrifuged for 30 min at 30 000 × g in a Rotor 40 in a Beckman L5-65 ultracentrifuge, maximal acceleration and half maximal braking. The sediment was resuspended in 10 mℓ of a 0,05 M HEPES, 0,154 M NaCl buffer, pH 7,4 for lectin affinity chromatography.

Wheat germ lectin chromatography

Method A

Wheat germ lectin Sepharose 6MB (10 mℓ) was packed into a Pharmacia column C10/20 (1,0 × 13,5 cm). The binding capacity of the column was 1 mg ovomucoid per mℓ bed volume. The void volume of the column was *ca.* 10 mℓ.

The gel was regenerated with 100 mℓ of 0,1 M Tris-HCl, 0,5 M NaCl, 0,02 % (w/v) NaN_3, pH 8,5 followed by 100 mℓ of 0,1 M sodium acetate, 0,5 M NaCl, 0,02 % (w/v) NaN_3, pH 4,5, and equilibrated with 100 mℓ of HEPES buffer containing 0,02 % (w/v) NaN_3. The column was also stored in the latter buffer. Before use, the column was washed with 500 mℓ of HEPES buffer to remove the azide. The re-suspended sediments of the crude extracts (10 mℓ) were applied to the column and incubated for 2 h. The non-adsorbed material was eluted from the column with HEPES buffer before a pulse of N-acetyl-D-glucosamine was applied (20 mℓ of HEPES buffer containing 2 g of carbohydrate). The column fractions were analysed for their protein content, infectivity and antigenicity. Fractions were also investigated electron microscopically.

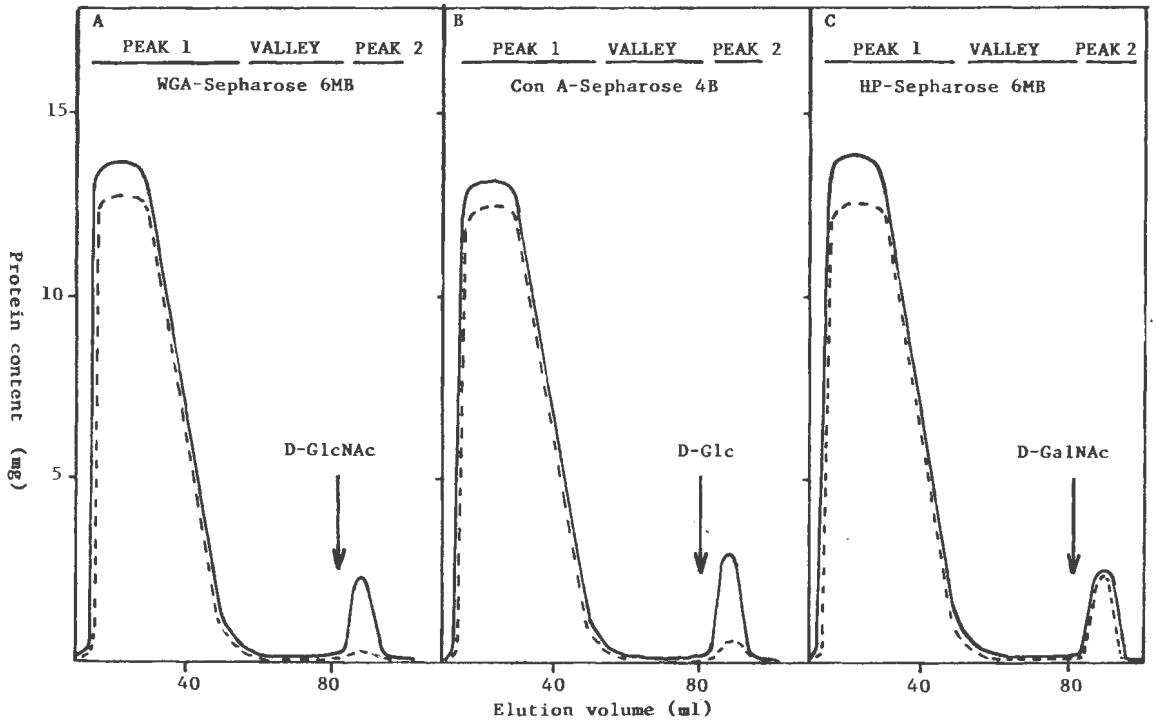

FIG. 5 Representative chromatogams of infected (—) and non-infected (- - -) nymph crude extracts on (A) wheat germ lectin-Sepharose 6MB, (B) Concanavalin A lectin-Sepharose 4B and (C) *Helix pomatia* lectin-Sepharose 6MB. The arrows indicate the application of elution buffer containing the applicable carbohydrate at 1 g per 10 mℓ HEPES buffer. The flow rate was 30 mℓ/h.

TABLE 4 Properties of heartwater infected and non-infected crude extracts and column fractions

Source amount			Protein content (mg)			
			Crude extract	Peak 1	Valley	Peak 2
Wheat germ lectin:						
(Ball 3 strain)						
Nymph:	2 400 NN	+	3 600[1]	3 582[1]	0	13,5[1]
	400 NN	+	1 919[3]	1 860[3]	0	12,6[3]
	200 NN	+	843[1]	828[1]	0	11,7[1]
	200 NN	−	758[3]	750[3]	0	2,7[3]
	200 NN	−	712[3]	678[3]	0	2,1[3]
Brain:	224 g S	+	1 728[2]	1 716[2]	0	11,0[5]
	207 g S	+	1 476[2]	1 452[2]	0	12,3[5]
	122 g S	+	864[4]	846[3]	0	12,7[3]
	134 g S	+[7]	912[3]	892[3]	—	15,6[3]
	187 g G	+	996[4]	982[2]	0	11,3[2]
	89 g G	+	708[2]	694[2]	0	12,9[2]
	214 g S	−	1 452[3]	1 446[3]	0	2,6[3]
	197 g S	−	1 164[3]	1 158[3]	0	2,7[3]
(Kümm strain)						
Liver/spleen:	12 g M	+	29[6]	24[6]	0	4,5[6]
	9 g M	−	27[3]	25[3]	0	1,4[3]
	15 g M	+	32[3]	25[3]	0	6,2[3]
Concanavalin-A lectin:						
(Ball 3 strain)						
Nymph:	100 NN	+	387[1]	362[2]	0	21,3[1]
	25 NN	+	95[1]	85[2]	0	7,2[1]
	100 NN	−	329[3]	325[3]	0	4,1[3]
Helix pomatia lectin:						
(Ball 3 strain)						
Nymph:	100 NN	+	362[1]	346[1]	0	9,5[2]
	25 NN	+	89[1]	81[3]	0	3,1[2]
	100 NN	−	297[3]	281[3]	0	8,9[3]
Total volume/fraction:			10 mℓ	60 mℓ	30 mℓ	9 mℓ

M — Mouse liver and spleen material
NN — Engorged *A. hebraeum* (Spes Bona strain) nymphae
S — Sheep brain material
G — Goat brain material
\+ — Heartwater infected material
− — Heartwater non-infected material
[1] — Heartwater infective as determined in sheep
[2] — Heartwater non-infective as determined in sheep
[3] — Biologically not tested
[4] — Died within 24 h after injection
[5] — Sheep showed for one day high rectal temperature, but had no heartwater protection when challenged
[6] — Heartwater infective as determined in mice
[7] — Batch method (peak 1 represent the non-adsorbed fraction and peak 2 the adsorbed fraction)
(Viljoen, 1985)

TABLE 5 Determination of *Cowdria ruminatium* in lectin affinity chromatography by ELISA[1]

Antigen source	Antibody source	Crude extract	Peak 1	Valley	Peak 2
Wheat germ:					
Ball 3 strain: brain	Isolated IgG	1,83 ± 0,4	1,89 ± 0,4	1,00 ± 0,1	2,80 ± 0,5
	Infected serum[2]	1,74 ± 0,5	1,97 ± 0,4	1,00 ± 0,1	2,55 ± 0,4
	Non-infected serum[3]	1,00 ± 0,1	1,00 ± 0,1	1,00 ± 0,1	1,00 ± 0,1
Ball 3 strain: nymph	Infected serum[2]	1,46 ± 0,3	1,36 ± 0,3	1,02 ± 0,1	2,28 ± 0,3
	Non-infected serum[3]	1,00 ± 0,1	1,00 ± 0,1	1,00 ± 0,1	1,01 ± 0,1
Ball 3 strain: nymph[7]	Infected serum[4]	1,43 ± 0,2	1,32 ± 0,2	1,00 ± 0,1	2,49 ± 0,1
Kümm strain: liver/spleen[5]	Infected serum[4]	1,30 ± 0,2	1,33 ± 0,3	1,05 ± 0,1	2,78 ± 0,2
	Infected serum[6]	1,23 ± 0,1	1,28 ± 0,1	1,01 ± 0,1	2,32 ± 0,3
Concanavalin-A:					
Ball 3 strain: nymph	Infected serum[2]	1,31 ± 0,1	1,12 ± 0,2	1,05 ± 0,1	3,15 ± 0,3
	Non-infected serum[3]	1,00 ± 0,1	1,01 ± 0,1	1,00 ± 0,1	1,03 ± 0,1
Helix pomatia:					
Ball 3 strain: nymph	Infected serum[2]	1,42 ± 0,1	1,52 ± 0,2	1,00 ± 0,1	1,12 ± 0,2
	Non-infected serum[3]	1,02 ± 0,1	1,01 ± 0,1	1,00 ± 0,1	1,00 ± 0,1

[1] — n=6

[2] — Serum from Onderstepoort Ball 3 vaccine, inoculated sheep

[3] — Serum from non-infected Ball 3 nymphae homogenate, inoculated sheep

[4] — Serum from infected Kümm strain mouse organ homogenates, inoculated sheep

[5] — Kümm strain infected liver and spleen homogenates as signal and non-infected liver and spleen homogenates as background

[6] — Serum from infected Kümm strain mouse organ homogenates, inoculated mice

[7] — Ball 3 strain infected nymphae as signal and non-infected nymphae as background (Viljoen, 1985).

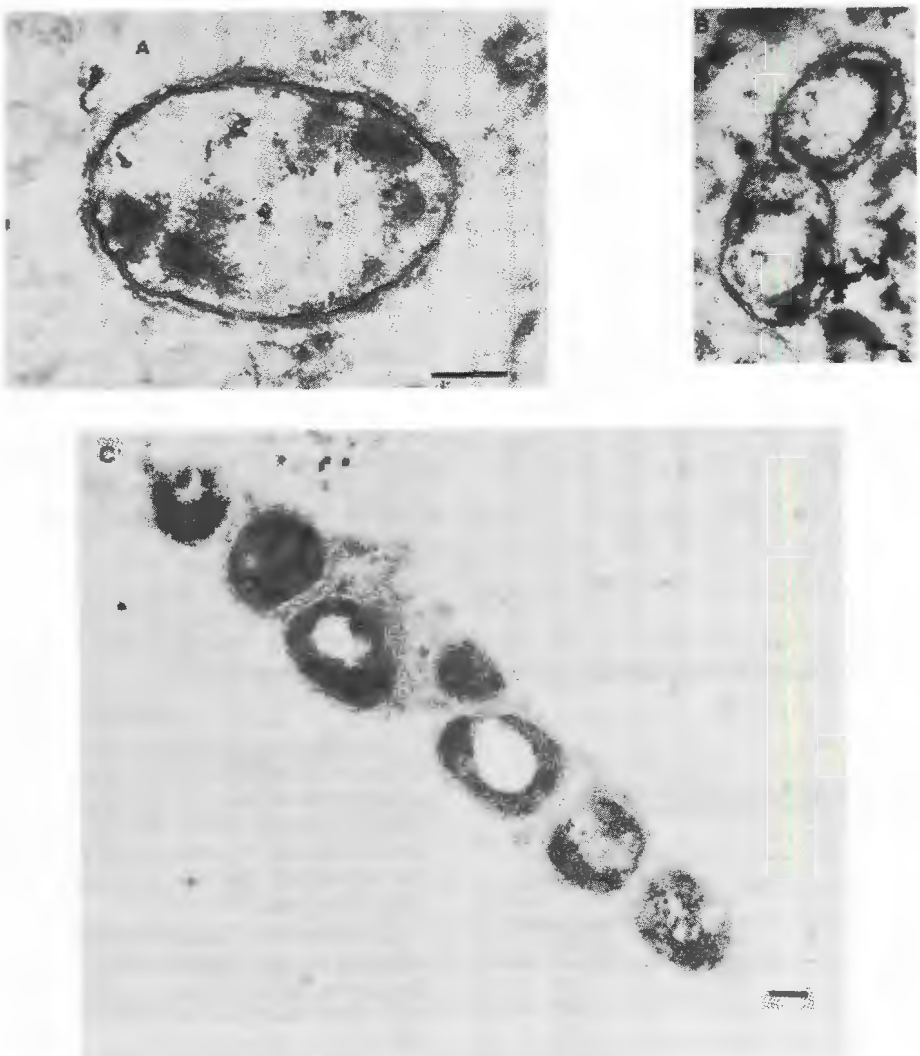

FIG. 6 Electron micrographs of typical *Cowdria ruminantium* organisms isolated on a wheat germ lectin Sepharose 6MB column
(A) Infected *Amblyomma hebraeum* nymphae as starting material
(B) Infected *A. hebraeum* nymphae as starting material
(C) Infected sheep brain as starting material
Bar scale = 0,1 μm

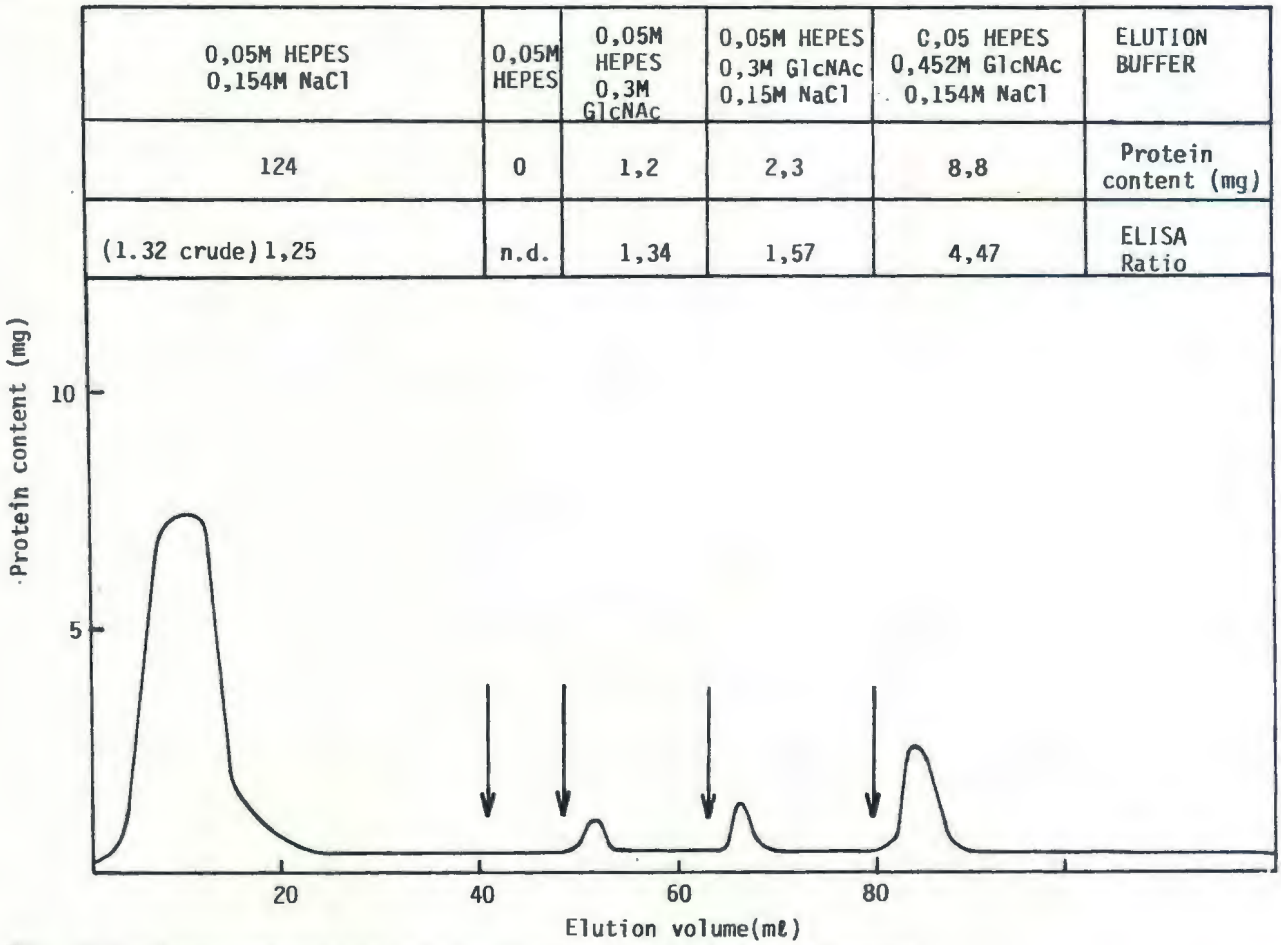

FIG. 7 WGA-affinity chromatography of *C. ruminantium* infected endothelial cells: Effect of osmolarity

TABLE 6 Capacity of WGA-Sepharose for *C. ruminantium*[a]

Nymphae suspension[b]		Peak 1		Peak 2	
Number	Protein[c] content mg	Protein[d] content mg	ELISA[d]	Protein[c] content mg	ELISA[e]
200	843	821	+	12	+
100	422	400	+	12	+
50	211	199	+	12	+
25	106	94	+	12	+
10	42	30	+	12	+
5	21	16,1	−	4,9	+
2,5	11	9,0	−	2	+

[a] Unpublished results
[b] Ball 3
[c] Determined by the method of Dorsey, McDonald & Roets (1977)
[d] Calculated by subtraction
[e] + ratio of about 2
− Ratio of about 1

TABLE 7 ELISA signal to background ratios of adsorbance values for fractions obtained from *C. ruminantium* infected calf endothelial cells[a]

Fraction[b]	Origin of the antigen material[c]	
	Ball 3 strain	Welgevonden strain
Crude suspension	1,32	2,19
peak I	1,25	1,20
peak IIa	1,35	1,12
peak IIb	1,57	1,62
peak IIc	4,47	4,17

[a] Unpublished results
[b] Obtained by WGA-Sepharose 6MB chromatography according to method B. See also Fig. 7
[c] Represents the average values of 3 experiments each

Method B

Method B was the same as method A except that the re-suspended sediments of crude extracts (10 mℓ) obtained from endothelial cells (Bezuidenhout *et al.*, 1985) were applied to the column and incubated for 2 h. The non-adsorbed material was eluted from the column 0,05 M HEPES buffer pH 7,4 containing 0,154 M NaCl (40 mℓ) followed by 0,05 M HEPES buffer pH 7,4 (10 mℓ). The column was then eluted successively with 0,05 M HEPES buffer pH 7,4 containing 0,3 M N-acetyl-D-glucosamine (35 mℓ), 0,05 M HEPES buffer pH 7,4 containing 0,3 M N-acetyl-glucosamine and 0,154 M NaCl (40 mℓ) and finally with 0,05 M HEPES pH 7,4 containing 0,452 M N-acetyl-D-glucosamine and 0,154 M NaCl (50 mℓ). Fractions were analysed as in method A (unpublished procedure).

RESULTS

No difference could be observed between the chromatograms (except for protein quantity) of sheep or nymph crude extracts after chromatography on a wheat germ lectin Sepharose 6MB column. A significant difference in the amount of protein bound to the column (peak 2) was observed, however, between extracts from infected and normal material (Fig. 4).

Representative chromatograms of infected and non-infected nymph crude extracts on wheat germ lectin-Sepharose 6MB, Concanavalin A lectin-Sepharose 4B and *Helix pomatia* lectin-Sepharose 6MB is shown in Fig. 5.

The protein content and ELISA ratios of heartwater infective and non-infective material is shown in Tables 4 & 5, respectively.

Typical electron micrographs of *C. ruminantium* organisms isolated on a wheat germ lectin column are shown

in Fig. 6. It was observed that peak 1 (Fig. 4, Table 5) in the different isolation procedures still contain *C. ruminantium* organisms, as measured by the infectivity of the fractions. The wheat germ lectin-Sepharose 6MB column (10 mℓ) was therefore titrated with different positive nymphae suspensions to determine its binding capacity. It has been shown that only with a suspension of less than 10 nymphae in 10 mℓ (42 mg protein) was all the *C. ruminantium* retained on the column (Table 6) as measured by the ELISA technique.

The effect of osmolarity on the separation of a suspension of calf endothelial cells infected with *C. ruminantium* (Bezuidenhout *et al.*, 1985), on wheat germ lectin-Sepharose 6MB chromatography is shown in Fig. 7. The fractions obtained were assessed with the ELISA technique, and signal to background ratios are given in Table 7.

Discussion

The results of Viljoen *et al.*, (1985) demonstrate for the first time that a rickettsia, namely, *C. ruminantium*, could be isolated by lectin cellular affinity chromatography in a partially purified state. Three lectins immobilized on Sepharose were studied for their ability to retain material when incubated with *C. ruminantium* infected and non-infected extracts from different sources (Fig. 4 & 5). When the retained material was eluted from the column with their respective sugars, large quantitative differences in the protein content of the bound peak of the positive material in comparison to negative controls were observed only with WGA-Sepharose 6MB and Con A-Sepharose 4B. In contrast no quantitative difference was observed with HP-Sepharose 6MB. The different fractions were analysed for protein content (Table 4), for infectivity in sheep and mice (Table 4), for the presence of *C. ruminantium* in the fractions by ELISA (Table 5) and electronmicroscopy (Fig. 6). The results obtained suggest that both wheat germ lectin and Concanavalin A lectin affinity chromatography are suitable for the purification of *C. ruminantium* from a number of sources.

When the different fractions obtained after lectin affinity chromatography were used as an antigen in ELISA studies, larger signal to background ratios of absorbance were obtained consistently with peak 2 fractions (Tables 4 & 5). The morphology and size of the organisms in peaks 1 and 2 obtained from infected sheep brain and nymphae closely resembled (Fig. 6) those of *C. ruminantium* described in sheep and mice (Prozesky & Du Plessis, 1985; Pienaar, 1970). The presence of *C. ruminantium* in both peaks 1 and 2 was shown to be due to overloading of the column (Table 6). It was shown with the ELISA that a WGA-Sepharose 4MB retains all the *C. ruminantium* only when an extract of less than 10 nymphae (42 mg protein) was applied to the column (Table 6). Some non-specific retention does occur on WGA-Sepharose 4MB and Con A-Sepharose 2B, when extracts of negative material are passed through these columns. Infectivity studies (Table 4) indicate that no *C. ruminantium* was retained on HP-Sepharose 6MB. The sugar specificity for the 3 lectins (Table 3) is also described by Dillner-Centerlind *et al.* (1980). Our data suggest that *C. ruminantium* contains no assessible α or β-D-GalNAc or α-D-Gal on its surface. The presence of β-D-GlcNAc as a monomer, dimer or trimer, α-D-Man and/or α-D-Gluc on the surfaces of this rickettsiae is therefore a strong possibility. It has been known for some time that the fine structure and chemical composition of rickettsiae are entirely similar to those of other gram-negative bacteria (Weiss, 1973).

The outer surface of the outer membrane of gram-negative bacteria is covered by a lipopolysaccharide of marvellous complexity (Metzler, 1977). This outermost layer of the lipopolysaccharide consists of long projecting polysaccharide chains, with specific repeating units that have antigenic properties and are called 0-antigens. The structures of these polysaccharides are so varied that 1000 different "serotypes" of Salmonella are known. A slime layer of polysaccharide has been reported for *Coxiella burnetii, R. rickettsii, R. rhipicephali, R. sibirica, R. typhi, R. tsutsugamushi* and WB-8-2, as summarized by Hayes & Burgdorfer (1982). They reported that all pathogenic rickettsiae studied by them possessed the slime layer. Although no slime layer as such has been described for *C. ruminantium,* it appears that sugars may play an important role on the surface of *C. ruminantium* and rickettsiae. Therefore lectin affinity chromatography is a promising technique for the isolation of these organisms.

Too long an incubation time leads to irreversible adsorption of cells to the lectin column (Kinzel *et al.*, 1977). Together with the incubation time, affinity of the lectin may also play an important role. Con A is known for its strong interaction with its receptors; for instance, 18 % of the influenza virus A_2HK could not be recovered in viable form from Con A-Sepharose. However, 100 % viability could be recovered from *Vicia ervilla* lectin-Sepharose 2B under milder conditions than those used on the Con A column (Kristiansen, 1975). *Lens culinaris, V. ervilla* lectins with Con A specificity (Dornand *et al.*, 1978; Kristiansen, 1975), but with much weaker binding constants may be attractive alternatives for this latter lectin in cell affinity chromatography. The large number of lectins with different specificities and binding constants could be a powerful tool in the separation of different cells or organisms (Sharon, 1983).

The separation of *C. ruminantium* on WGA and Con A columns has not been optimized in terms of incubation times and osmolarity of the elution buffer. *C. ruminantium* from calf endothelial extracts was eluted from a WGA-Sepharose column by step-wise-increases of the osmolarity of the Buffer (Fig. 7). It was possible to obtain 3 fractions by increasing the osmolarity from 0 to 0,76. The antigenic fractions were analysed by ELISA (Table 7). Fractions IIa and IIb had signal to background absorbance ratios similar to that of the crude suspension. However, ratios greater than 4 were obtained with fractions IIc which makes this fraction a good source of antigen for ELISA determinations. The infectivity potential of these fractions are being studied. Preliminary results indicate that *C. ruminantium* fractions of higher purity could be obtained by combination of the wheat germ lectin affinity chromatography and Percoll density gradient centrifugation (unpublished results).

Conclusion and Perspectives

The extremely labile nature of rickettsiae (Weiss, 1973) and the difficulty in maintaining the viability of isolated oganisms (Birnie, Endris, Logan, 1986; Bovarnick, Miller & Snyder, 1950) is well-known. These characteristics should be taken into consideration in the different steps of the isolation procedure and should be optimized for maximum yield and viability.

Rickettsiae contain sugars on their surface (Hayes & Burgdorfer, 1982), like bacteria (Metzler, 1977) and viruses (Kristiansen, 1975). The mild nature of lectin cell affinity chromatography should therefore find general application in the isolation of rickettsiae. The proper control of the following factors may result in isolation of highly enriched rickettsial fractions: (a) lectin load on Sepharose 6MB, (b) the affinity and specificity of the lectin, (c) incubation time on the column, (d) elution conditions, (e) maintenance of viability of isolated organisms.

As described in the introduction multi-point interaction between lectins and cells are possible (Bonnafous *et al.*, 1983; Sharma & Mahendroo, 1980). These interactions increase with lectin substitution on the matrix (Haller *et al.*, 1978; Kinzel *et al.*, 1977), incubation time (Reisner & Sharon, 1984; Kinzel *et al.*, 1977) and high affinity constant of the lectin for its receptor (Dornand *et al.*, 1978; Kristiansen, 1975), which could make elution of cells difficult or even impossible. These factors could be controlled by choosing the correct lectin substitution concentration on the matrix, by substituting a high affinity lectin with a lectin of the same specificity but of lower affinity and, most importantly, to determine the minimum time necessary for binding on to the lectin-matrix by a technique such as phase contrast microscopy (Kinzel *et al.*, 1977). Optimization of the minimum conditions necessary for binding to lectin column will facilitate elution conditions, and in our opinion rickettsiae could be eluted then only with the complementary sugar, without resort to extreme elution conditions which include high flow rates, and high temperature, for instance. Another possible improvement in the elution conditions, could be to incorporate some of the compounds necessary for maintaining viable organisms (Birnie *et al.*, 1986; Bovarnick *et al.*, 1950) in the elution buffer.

The large number of lectins, with different specificities and affinities for a large number of carbohydrates (Sharon, 1983), makes it a powerful technique for the isolation of cells containing glycopolymers on their surfaces. A limitation of lectins is however the fact that they are not as specific as antibodies in recognizing different cell populations (Sharon, 1983). Nevertheless, it has been shown that the minimum concentration that gives agglutination of erythrocytes varies for 5 different lectins from 37 to 325 $\mu g/m\ell$, while one was negative even at 5 000 $\mu g/m\ell$ (Goff, Johnson & Kuttler, 1986). It should therefore be possible to obtain some degree of specificity with lectins. However, the smaller affinity of lectins for the receptors than that of antibodies results in milder elution conditions for the former (Tables 1 & 2). A serious limitation of lectins is the fact that they do interact with glycoproteins (Garcia-Segura, Martinez-Rodriques, Martinez-Murillo, Bogonez & Toledano, 1978; Gombos, Zanetta, Reeber, Morgan & Vincendon, 1974). Also, WGA binds to at least 10 glycopeptides on the surface of human T cells (Torrisi & Da Silva 1982). This limitation can be minimized by the introduction of a low (10 000 *g*) and a high (30 000 *g*) centrifugation step before rickettsial extracts are introduced to the lectin column. The latter centrifugation step sediments the reckettsiae, like *C. ruminantium* and should separate it from soluble glycoproteins. Improved separation should also be obtainable by successive affinity chromatography on different lectins with different specificities and affinities. The optimization of conditions for cell lectin affinity chromatography of *C. ruminantium* has been limited, to some extent, by a number of factors, such as cost of experimental animals and the preparation of infective materials. The availability of rickettsial strains toxic to mice (Du Plessis, 1982) and the availability of *C. ruminantium* infective endothelial cells (Bezuidenhout *et al.*, 1985), should facilitate the optimization of lectin cell affinity chromatography in the near future.

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Printed by the Government Printer, Private Bag X85, Pretoria, 0001