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## Identification of radioiodinated cuticular proteins and antigens of *Onchocerca gibsoni* microfilariae

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### Summary

The filarial parasite of cattle, *Onchocerca gibsoni*, has been used to establish procedures of antigen identification with a view to applying these techniques to studies on human filarial parasites. Emphasis has been placed on methods suitable for use with small numbers of parasites. Microfilariae (mf) of *O. gibsoni* were extracted from nodular worms, purified and <sup>125</sup>I-labeled using IODO-GEN in solid-phase. Radioactivity was shown to be confined to the cuticle of sectioned mf using the technique of electronmicroscope autoradiography. Radiolabeled mf were analysed by two-dimensional gel electrophoresis. Autoradiographs of <sup>125</sup>I-labeled proteins of *O. gibsoni* mf were relatively complex, there being at least 32 proteins ranging in molecular weights from 20,000 to 120,000 and displaying considerable charge heterogeneity. Evidence was obtained that at least the major serum proteins of the host, albumin or immunoglobulin, were not adsorbed on to the surface of these uterine mf and detectable in the labeled surface protein patterns. Sera from infected cattle immunoprecipitated 5 labeled proteins from a Triton X-100 extract of <sup>125</sup>I-labeled mf. Sera from either of two calves which had been given multiple injections of mf subcutaneously, and which had no detectable skin mf, recognised 6 additional proteins in this extract as well as 3 of the proteins recognised by sera from infected cattle.

*Key words:* *Onchocerca gibsoni*; microfilariae; antigens; <sup>125</sup>I-labeling; gel electrophoresis; immunoprecipitation.

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## Introduction

The surfaces of certain life cycle stages of filarial parasites and other parasitic nematodes have been shown to contain antigenic targets for immunological effector mechanisms in vitro (Subrahmanyam et al., 1976; Chaicumpa and Jenkin, 1978; Mackenzie et al., 1978; Tanner and Weiss, 1978; Haque et al., 1980; Kazura and Grove, 1978). Moreover, antibody-dependent cell-mediated destruction of *Dipetalonema viteae* microfilariae (mf) has been demonstrated in vivo by Weiss and Tanner (1979). The appearance of anticuticular antibodies in the sera of *D. viteae*-infected hamsters (Weiss, 1978), *Litomosoides carinii*-infected albino rats (Bagai and Subrahmanyam, 1970), *Loa loa*-infected drills (Duke, 1960), and *Brugia pahangi* or *Dirofilaria immitis*-injected dogs (Wong, 1964) correlates with suppression of microfilaremia. Similarly, relatively high titers of antibodies to the surface of *Wuchereria bancrofti* or *Brugia malayi* mf are present in the sera of amicrofilaremic individuals compared with the sera of patients with patent microfilaremia (Piessens et al., 1980; McGreevy et al., 1980). Despite this evidence indicating the importance of nematode surface antigens in host-protective immunity, little is known about the molecular and immunochemical nature of relevant surface antigens.

Recent studies of surface antigens by Parkhouse et al. (1981) have defined two major cuticular antigens of *Trichinella spiralis* third stage larvae (L3). However, similar information on human filarial parasites such as *W. bancrofti* and *Onchocerca volvulus* will be considerably more difficult to obtain due to the problems of collecting sufficient parasite material. To develop strategies of antigen identification, isolation and characterisation for small amounts of filarial parasite material, we have used a readily available cattle parasite, *Onchocerca gibsoni* to study the surface antigens of mf. This parasite, like *O. volvulus*, resides as an adult in subcutaneous nodules and mf are found in the skin of infected cattle (Heydon, 1927). Procedures based on conventional radioiodination techniques have been established to surface label as few as  $5 \times 10^3$  to  $10^4$  mf. Cuticular proteins recognised by sera from *O. gibsoni*-infected cattle as well as calves immune to mf, have been analysed by two-dimensional gel electrophoresis.

## Materials and methods

**Parasites.** The brisket regions of *Onchocerca gibsoni*-infected cattle were collected during April to October, 1980, from carcasses held overnight in a 4°C chiller at abattoirs in Townsville. Nodules were removed from this tissue in a sterile hood, transported to Melbourne at 4°C and dissected approximately 15 h after collection. Large numbers of mf were obtained by dissection of nodules and teasing out fragments of adult worms. Mechanical disruption of adult worms by pipetting allowed the release of uterine mf when dissected fragments were suspended in Leibowitz (L-15) medium (Gibco, New York, USA) with 0.15% glucose (w/v) and 200 units of penicillin and 200 µg of streptomycin (Glaxo, Melbourne, Australia) per ml. The mf were maintained overnight in L-15 and then purified as outlined in Forsyth et al. (1981) using a Ficoll-paque method. Uterine mf

purified by the above method contained less than 5% eggs and less than 0.1% contaminating bovine cells.

*Antisera.* Sera were obtained from 2- to 3-year-old *O. gibsoni*-infected cattle shown to have high numbers of skin mf by the technique of Beveridge et al. (1980). Calves given multiple injections of uterine mf subcutaneously and shown to have no detectable skin mf were also bled for antisera. Control bovine serum was taken from a calf maintained in a clean facility and exposed to viral and bacterial stimuli but no known metazoan parasites. Antiserum to purified mf was raised in a rabbit by intradermal and subcutaneous injections of  $5 \times 10^4$  purified mf in Complete Freund's Adjuvant (CFA) (Difco Laboratories, Detroit, Michigan, USA), followed by two booster injections of  $5 \times 10^4$  purified mf in Incomplete Freund's Adjuvant (IFA).

Bovine IgG was prepared by isolation of IgG from a serum pool from uninfected bovines after sodium sulphate precipitation and subsequent Sephacryl S-300 chromatography (Pharmacia Fine Chemicals, Uppsala, Sweden). Rabbit anti-bovine Ig was prepared by immunisation of rabbits with 0.5 mg of purified bovine IgG in CFA given intradermally, subcutaneously and intramuscularly followed by a boost of 0.5 mg of purified bovine IgG in IFA. Rabbits were then bled routinely 10 d after repetition of the above boosting procedure. Affinity purified rabbit anti-bovine IgG was prepared according to the procedure outlined by Haustein and Warr (1976). Rabbit anti-bovine IgM was obtained commercially from Miles Laboratories, Elkhart, Indiana, USA, and used after establishing specificity by testing its capacity to specifically immunoprecipitate bovine IgM which was  $^{125}\text{I}$ -labeled using the chloramine T radioiodination procedure (Greenwood et al., 1963). An antiserum to bovine serum albumin (BSA) was raised by injecting BALB/c mice with 10  $\mu\text{g}$  of Fraction V BSA (Armour Pharmaceutical, Eastbourne, England) in CFA into the footpads, boosting 1 month later with 100  $\mu\text{g}$  of BSA in saline given intraperitoneally, and bled 1 week after this injection.

*Radioiodination of intact *O. gibsoni microfilariae*.*  $10^5$  purified uterine mf were washed  $3 \times$  in bovine tonic phosphate buffered saline (BTPBS) and added in 100  $\mu\text{l}$  of BTPBS to a glass tube coated with 10  $\mu\text{g}$  of IODO-GEN (Pierce Chemical Company, Rockford, Illinois, USA) as described by Markwell and Fox (1978). 200  $\mu\text{Ci}$  of  $\text{Na}^{125}\text{I}$  (Sodium iodide; Amersham Searle Corp., Arlington Heights, Illinois, USA) in 5  $\mu\text{l}$  was added, the reaction allowed to proceed at room temperature for 10 min and then terminated by addition of ice-cold BTPBS and transfer to a non-coated glass tube. The mf were then washed  $3 \times$  in BTPBS and were found to be 90–100% viable. Approximately  $10^4$  mf were stored at  $-70^\circ\text{C}$  for subsequent gel analysis in BTPBS containing 5% normal rabbit serum (NRS), 5 mM phenylmethylsulphonyl fluoride (Sigma, St. Louis, Missouri, USA) (PMSF) and 5 mM EDTA.  $9 \times 10^4$  mf were incubated for 30 min in 0.05 M Tris-HCl (pH 8), 0.15 M NaCl buffer containing 1.5% Triton X-100 (BDH, Poole, England), 5% NRS, 5 mM PMSF, 5 mM EDTA on ice, sonicated, and thereafter maintained on ice for 30 min. The mixture was centrifuged at 12,000 g for 10 min; the supernatant contained 60–70% of the total radioactivity of which 70–80% was precipitable with 20% trichloroacetic acid (TCA).

*Immunoprecipitation of  $^{125}\text{I}$ -labeled antigens of *O. gibsoni microfilariae*.* Supernatants of solubilized  $^{125}\text{I}$ -labeled mf (200,000 TCA precipitable counts per minute) were precleared with 100  $\mu\text{l}$  of a 10% (v/v) suspension of heat killed and formalin fixed *Staphylococcus aureus* of the Cowan I strain (Commonwealth Serum Laboratories, Parkville, Victoria, Australia). This procedure removes denatured protein and materials that bound to staphylococci in the absence of antisera (Cullen and Schwartz, 1976). The cleared supernatant was then added to 10  $\mu\text{l}$  of the appropriate rabbit, mouse or bovine serum and incubated for 2 h on ice. Three methods of precipitating immune complexes were used. (a) An amount of rabbit anti-bovine IgG (optimised to precipitate all the IgG in 10  $\mu\text{l}$  of *O. gibsoni*-infected cattle serum) was added and the mixture kept for 1 h at room temperature followed by an overnight incubation at  $4^\circ\text{C}$  to allow a precipitate to form. (b) Complexes were isolated by binding to protein A-bearing *S. aureus* as described by Kessler (1975). (c) Affinity-purified rabbit anti-bovine IgG was coupled to cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) by the procedure outlined by the manufacturer. 50  $\mu\text{l}$  of a

50% suspension of rabbit anti-bovine IgG Sepharose was incubated with antigen/antibody complexes for 45 min on ice.

In all three procedures,  $^{125}\text{I}$ -labeled microfilarial lysates were diluted in a 0.05 M Tris-HCl buffer, pH 8 containing 0.15 M NaCl, 0.05 M EDTA, 0.5% Triton X-100 and precipitated immune complexes were washed  $3\times$  in the above buffer containing only 0.05% Triton X-100 as described by Kessler (1975) except for procedure (c) where the washing buffer also contained 10% NRS for all but the last wash. Washed precipitates were stored at  $-70^\circ\text{C}$ .

*Polyacrylamide gel electrophoresis.* One-dimensional polyacrylamide gel electrophoresis (PAGE) was carried out in the presence of sodium dodecyl sulfate (SDS) according to the method of Laemmli (1970), using 10% acrylamide slab gels.  $^{125}\text{I}$ -labeled mf were prepared for electrophoresis by addition of a Tris-HCl buffer, pH 6.8, containing 3% w/v SDS, 6 M urea, 50% glycerol, and 5% v/v  $\beta$ -mercaptoethanol. Alternatively, samples were electrophoresed in two dimensions by the method developed by O'Farrell et al. (1977), using 10% acrylamide slab gels for the second dimension. Prior to two-dimensional gel analysis, immune complexes precipitated by procedures (a), (b) and (c) were incubated for 15 min at room temperature in 25  $\mu\text{l}$  of SDS-lysis buffer which was identical to the O'Farrell isoelectric focussing (IEF) buffer (O'Farrell et al., 1977) except that the Triton X-100 was replaced with 2% SDS (w/v). 100  $\mu\text{l}$  of IEF-lysis buffer was then added and the mixture incubated for 15 min at room temperature. Unsolubilized material was removed by centrifugation at 10,000 g using a Beckman microfuge (Fullerton, California, USA). The supernatant which was then ready for isoelectric focussing, contained 90–95% of the total immunoprecipitated counts. Gels were run with molecular weight standards (LMW calibration kit, Pharmacia Fine Chemicals, Uppsala, Sweden): phosphorylase b (Mr 94,000), bovine serum albumin (Mr 67,000), ovalbumin (Mr 43,000), carbonic anhydrase (Mr 30,000), soybean trypsin inhibitor (Mr 20,000), and  $\alpha$ -lactalbumin (Mr 14,400) and stained with 0.01% Coomassie Blue in 50% methanol/10% acetic acid. They were destained in 7% acetic acid and autoradiographed using Kodak X-Omat S film and Dupont Cronex Lightning plus intensifying screens (DuPont Wilmington, Delaware, USA) (Laskey and Mills, 1977). Exposure times varied between 1 and 20 d. Isoelectric focussing gels were run using standards supplied in the broad pI calibration kit (pH 3–10) (Pharmacia Fine Chemicals, Uppsala, Sweden).

*Neuraminidase treatment of  $^{125}\text{I}$ -labeled *O. gibsoni* microfilariae.*  $2\times 10^4$  mf which had been  $^{125}\text{I}$ -labeled as outlined above were incubated at  $37^\circ\text{C}$  for 30 min in 200  $\mu\text{l}$  of Hepes buffer (Sigma, St. Louis, Missouri, USA), 0.5 M NaCl pH 6.5 containing 50 units/ml of neuraminidase from *V. cholerae* (Calbiochem-Behring, La Jolla, California, USA). Similarly,  $2\times 10^4$  mf were either incubated in the above buffer containing no neuraminidase for 30 min at  $37^\circ\text{C}$  or stored immediately after iodination in 5% NRS, PMSF and EDTA at  $-70^\circ\text{C}$ . After incubation, the  $^{125}\text{I}$ -labeled mf were washed  $3\times$  in BTPBS with 5% NRS and stored at  $-70^\circ\text{C}$  in 5% NRS and PMSF + EDTA. Labeled mf were then analysed by two-dimensional gel electrophoresis to determine whether neuraminidase treatment altered the charge of *O. gibsoni* mf proteins.

*Reduction and alkylation of  $^{125}\text{I}$ -labeled proteins of *O. gibsoni* microfilariae.* To assess whether disulphide bond dependent aggregation of mf labeled proteins was occurring,  $2\times 10^4$  mf were subjected to Triton X-100 solubilization as outlined above with the addition of either 10 mM iodoacetamide (BDH, Poole, England) or 2 mM dithiothreitol (BIO-RAD Laboratories, Richmond, California, USA) or both reagents to the standard 1.5% Triton X-100 extract buffer (pH 8) (Glazer, 1976). Extracts were then analysed by two-dimensional gel electrophoresis.

*Autoradiography of sections of labeled microfilariae.* Ultrathin sections of araldite embedded microfilariae were placed on collodion-coated glass slides, which were then carbon-coated: Ilford L4 liquid emulsion was used to coat slides, which were stored in light tight boxes for 2 weeks at  $4^\circ\text{C}$ . Exposed slides were developed using Microdol X (Kodak, Melbourne, Australia) according to the manufacturers specifications. Sections were stripped from the glass slides and mounted on 400 mesh copper grids on 1 mm aperture discs before examination by electronmicroscopy.



*Lentil-lectin binding of radioiodinated mf surface proteins.* Two hundred-thousand counts per minute precipitable with 20% TCA of the Triton X-100 extract of  $^{125}\text{I}$ -labeled mf were incubated with 500  $\mu\text{l}$  of a 50% suspension of lentil-lectin Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) in a 0.5% Triton X-100 buffer described by Kessler (1975) and containing 1 mM  $\text{MnCl}_2$  and 1 mM  $\text{CaCl}_2 \pm 0.5 \text{ M } \alpha$ -methylmannoside for 2 h on ice. Pellets were washed in the above mentioned buffer and bound proteins removed by incubation in the buffer used for preparation of samples for one-dimensional SDS-PAGE. SDS-PAGE analysis was then carried out.

## Results

### *Surface labeling of O. gibsoni microfilariae*

Using a solid phase radioiodination procedure,  $10^5$  uterine mf were labeled routinely. This resulted in 30–40% incorporation of total added  $^{125}\text{I}$  radioactivity. As few as  $5 \times 10^3$  mf could be labeled successfully by this procedure in which 10–20% incorporation of total added  $^{125}\text{I}$  was achieved. Autoradiography of cut sections of radiolabeled mf, and examination by electronmicroscopy, indicated that radioactivity was essentially localised in the cuticle apparently in association with hypodermal cell membranes (Martinez-Palomo, 1978) (Fig. 1).

Use of the procedure of Zimmerman and Chapman (1977) to enrich for glycolipid indicated that less than 5% of the total number of counts incorporated into  $^{125}\text{I}$ -labeled mf was found to be in the glycolipid fraction.

One-dimensional SDS-PAGE analysis (under reducing conditions) of  $^{125}\text{I}$ -labeled mf revealed the presence of 4 major bands and 2 minor bands of apparent Mr 67,000, 50,000, 36,000, 28,000 and 120,000, 20,000, respectively. The Mr 50,000 protein appeared to bind to lentil-lectin Sepharose as did some unresolved material in the high molecular weight region of the gel. It was a consistent finding that resolution of labeled proteins was poor using one-dimensional gel techniques.

High resolution analyses of the microfilarial cuticular proteins that were radioiodinated were carried out by two-dimensional gel electrophoresis. An autoradiograph of separated radioiodinated cuticular proteins showed that there were at least 32 labeled proteins varying in isoelectric points from 3.5–8.15 and ranging in Mr from 20,000 to 120,000 (Fig. 2 A). The distortion of the Mr 67,000 protein in this autoradiograph can be accounted for by the presence of an overload of protein due to carrier NRS in the whole extract of  $^{125}\text{I}$ -mf. The apparent charge heterogeneity of these labeled proteins was unchanged by treatment of intact, viable  $^{125}\text{I}$ -labeled mf with neuraminidase.

### *Solubilization of $^{125}\text{I}$ -labeled microfilariae*

When  $^{125}\text{I}$ -labeled mf were initially solubilized in the isoelectric focussing lysis buffer described by O'Farrell et al. (1977), which contains Triton X-100 as well as urea (9 M) and dithiothreitol (20 mM), 95–100% of the total radioactivity incorporated into radiolabeled mf was solubilized. In contrast, when  $^{125}\text{I}$ -labeled mf were solubilized for immunoprecipitation studies in a 1.5% Triton X-

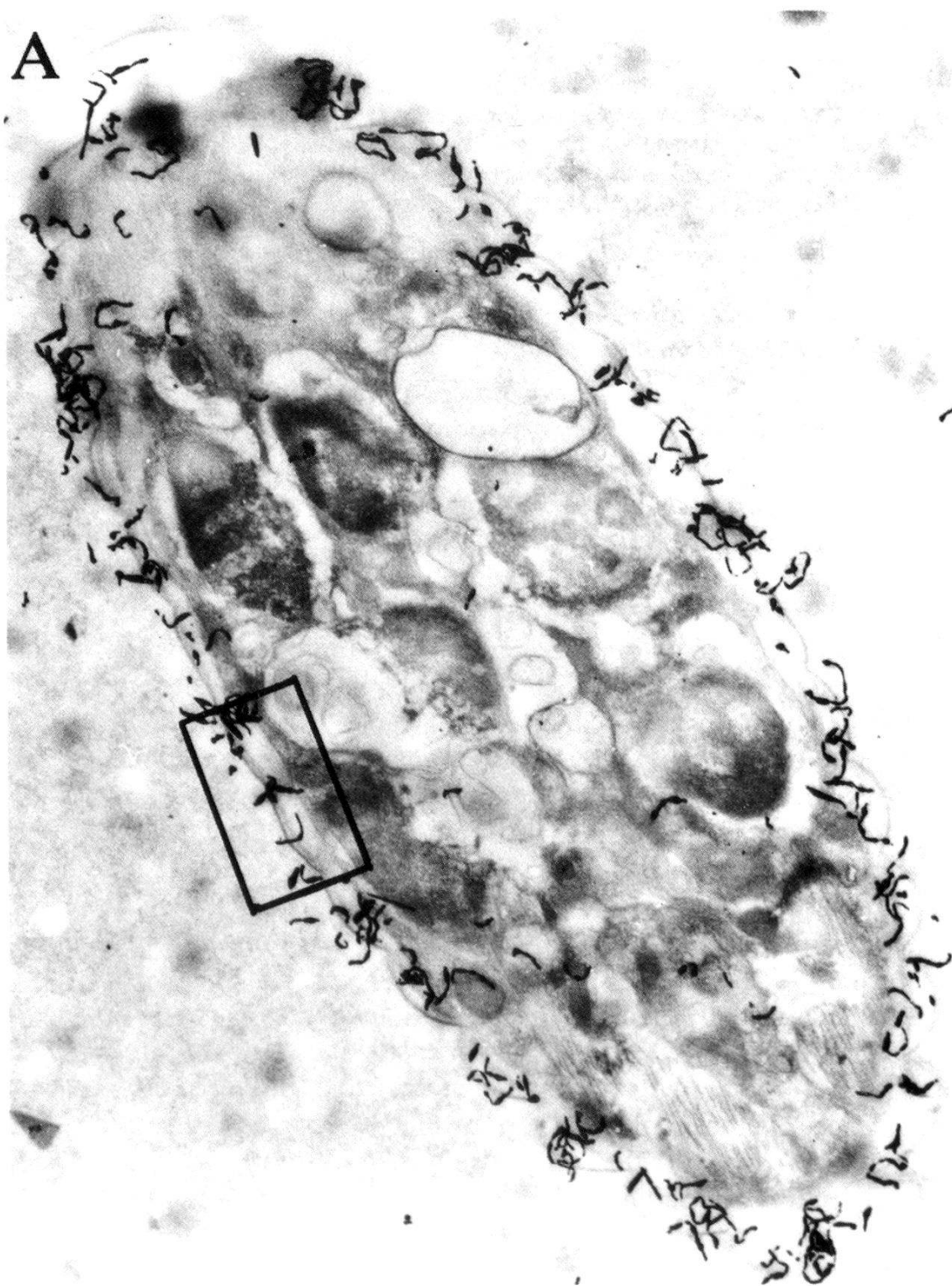


Fig. 1. Tangential section of *Onchocerca gibsoni* microfilaria showing peripheral distribution of  $^{125}\text{I}$ -labeled protein in an unstained electron micrograph ( $\times 14,700$ ) (A). Rectangular ruled area in (A) is enlarged to a higher magnification ( $\times 74,000$ ) in showing association of grains with the cuticle (B).

B

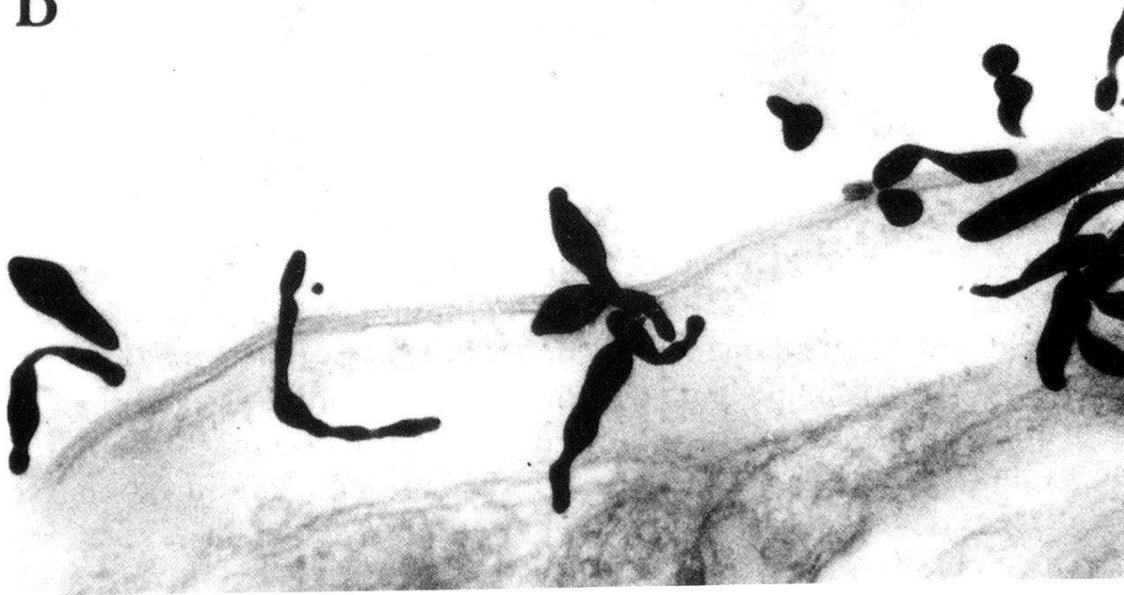


Fig. 1 B

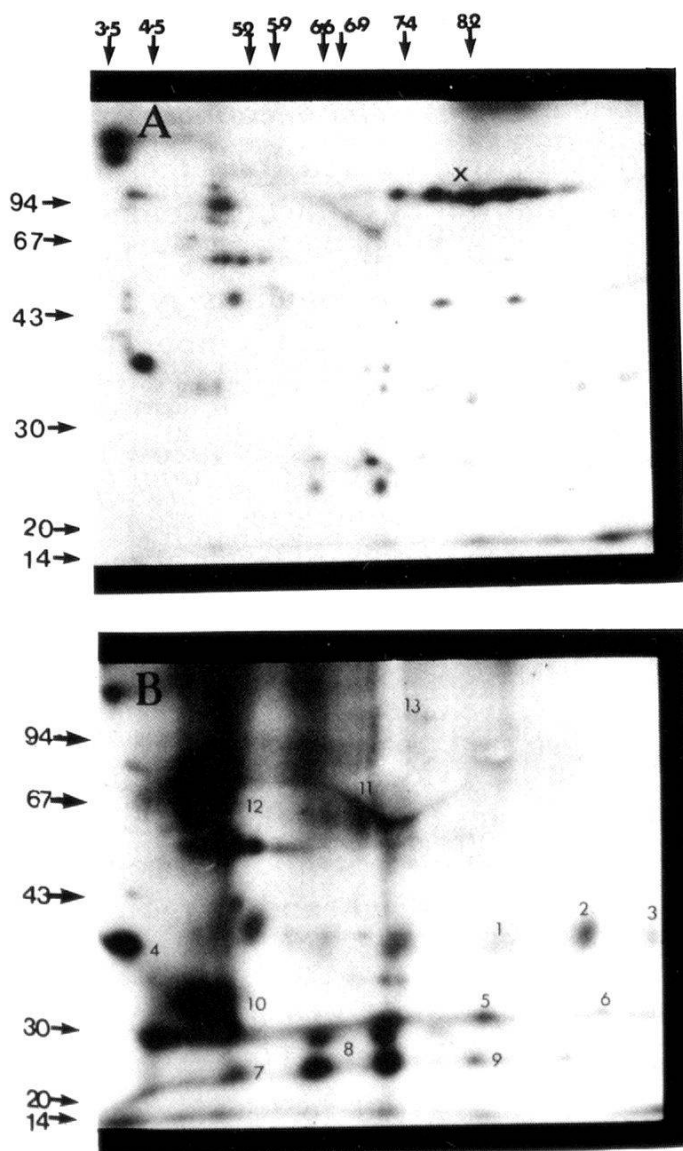


Fig. 2. Autoradiographs of two dimensional gels (under reducing conditions) of  $^{125}\text{I}$ -labeled mf (A) and the Triton X-100 extract of  $^{125}\text{I}$ -labeled mf (B). Numbers to the left of Figs. 2 A, B represent Mr standards and the numbers above Fig. 2 A represent isoelectric point standards.



100 buffer, which contained no urea or reducing agents, only 60–70% of the total counts incorporated were solubilized. Two-dimensional gel analyses of  $^{125}\text{I}$ -labeled mf solubilized by both the above mentioned procedures are shown in Figs. 2 A, B. The major difference between the 2 extracts is that a protein complex of considerable charge heterogeneity and apparent Mr 90,000 (designated x in Fig. 2 A) was present only in the extract prepared in urea and under reducing conditions. Secondly, a protein complex of apparent Mr 60,000 (designated 11 in Fig. 2 B) and present in the Triton X-100 extract, did not appear to be in the extract prepared under reducing conditions. However, this protein could be seen as a minor component after longer exposure of the gel in Fig. 2 A.

The possibility of disulfide bond dependent aggregation was examined by addition of 10 mM iodoacetamide  $\pm$  2 mM dithiothreitol or 2 mM dithiothreitol alone to the 1.5% Triton X-100 extract buffer prior to solubilization. No apparent affect of reduction and/or alkylation was observed on the mf proteins by a comparison of two-dimensional gels analyses of  $^{125}\text{I}$ -labeled mf Triton X-100 extracts prepared in the presence or absence of iodoacetamide and/or dithiothreitol.

#### *Examination for the presence of bovine serum proteins on the microfilarial surface*

Although mf used in these experiments were derived from the uteri of female adult worms, there was a possibility that, during extraction, contact with nodule fluid may have resulted in 'contamination' of the mf surface. This fluid contains antibody to mf as detected by solid-phase radioimmunoassay (Forsyth K. P., unpublished observations) as well as other host serum components. To assess this, the Triton X-100 extract of  $^{125}\text{I}$ -labeled mf was incubated with 10  $\mu\text{l}$  of each of the following sera; (a) rabbit anti-bovine IgG, (b) rabbit anti-bovine IgM, (c) mouse anti-bovine serum albumin, (d) rabbit anti-*O. gibsoni* mf, (e) NRS and (f) normal mouse serum (NMS). Immune complexes were isolated with protein A bearing *S. aureus* and counted. No significant levels of radioactivity above background were immunoprecipitated by sera (a), (b), (c), (e) or (f), whereas the positive control (d), immunoprecipitated 15-fold the number of background counts. Furthermore, the radioiodinated mf proteins recognised by the rabbit serum to *O. gibsoni* mf could be detected by two-dimensional gel analysis and autoradiography (Fig. 3 C). Further evidence that the mf did not have significant amounts of bovine serum proteins bound to their surface was the failure to find proteins in the autoradiograph of the two dimensional gel pattern of  $^{125}\text{I}$ -labeled mf with the known molecular weight and charge characteristics of bovine IgG, IgM and serum albumin.

#### *Detection of microfilarial cuticular antigens*

Three immunoprecipitation systems were assessed for their ability to detect radioiodinated mf antigens recognised by bovine antibodies. From Table

Table 1. Immunoprecipitated radioactivity (in cpm) using three different immunoprecipitation systems with bovine sera and <sup>125</sup>I-labeled *O. gibsoni* mf extracts

Serum*	Experiment 1		Experiment 2	
	Protein A	R anti B IgG**	Protein A	R anti B IgG: Sepharese
<i>O. gibsoni</i> .....	4900	2880	5620	5590
<i>O. gibsoni</i> .....	4850	2940	5730	5620
Control .....	1780	780	1760	1180
Control .....	1650	690	1790	1220

\* Sera were obtained from two cows in Queensland known to be infected with *O. gibsoni* and a calf maintained in a clean facility with no exposure to metazoan parasites. The control was an amount of purified IgG from this calf equal to the amount of IgG in sera from *O. gibsoni*-infected cattle.

\*\* R = rabbit; B = bovine.

It is apparent that all 3 systems gave similar precipitable counts, but SDS-PAGE analysis and subsequent autoradiography identified the *S. aureus* immunoprecipitation system as giving the clearest resolution of the higher molecular weight antigens. Although protein A of *S. aureus* is known to bind only one subclass of bovine IgG significantly (Goudswaard et al., 1978) it appeared to isolate a greater amount of each antigen/antibody complex than did the other systems. This suggests that there may be little subclass restriction of antigen recognition and that, perhaps, like goat and sheep IgG subclasses which do not normally bind to protein A, bovine IgG<sub>1</sub> binds better in the form of immune complexes (Langone, 1980; Kessler, 1975).

Two-dimensional gel analysis of the radioiodinated proteins in the Triton X-100 extract precipitated by the sera from any one of 10 *O. gibsoni*-infected cattle revealed the presence of 5 proteins specifically immunoprecipitated, i.e. proteins designated 1 to 5 in Fig. 3 A. Sera from either of 2 calves given multiple injections of uterine mf, but having no detectable skin mf, immunoprecipitated 9 proteins (Fig. 3 B). Three of the 9 radioiodinated proteins immunoprecipitated were also recognised by sera from infected cattle, i.e. proteins designated 1, 4, 5 in Fig. 3 A, B. However, proteins 7, 9 to 13 were only immunoprecipitated by the sera from the 2 above mentioned calves exposed only to mf and apparently immune to mf establishment. It should be noted that the protein complex designated 8 in Fig. 2 B was also immunoprecipitated weakly by sera from the mf injected calves, but there was insufficient radioactivity to give 4 intense spots on the gels. The sera of 2 rabbits immunised with purified mf recognised 8 proteins designated 4, 5, 7, 9 to 13 in Fig. 3 C.

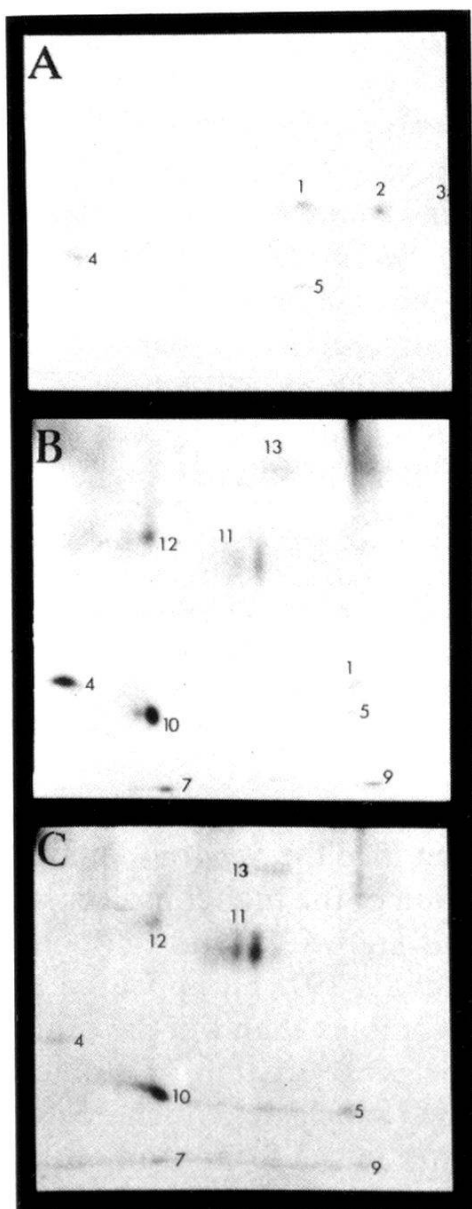


Fig. 3. Autoradiographs of two dimensional gels (under reducing conditions) of immunoprecipitates of the Triton X-100 extract of  $^{125}\text{I}$ -labeled mf. A representative immunoprecipitation pattern with a serum from each of the following groups of animals is shown: 10 *O. gibsoni*-infected cattle (A), 2 calves injected with mf (B), 2 rabbits immunised with mf in FCA (C).

## Discussion

A procedure for the radioiodination of *O. gibsoni* mf has been described and shown to label only the cuticle. The pattern of 6 major bands observed by one-dimensional SDS-PAGE was shown to be more complex when solubilized radioiodinated mf were analysed by two-dimensional gel electrophoresis. Using the reducing conditions of 9 M urea and 20 mM dithiothreitol, two-dimensional gel analysis of  $^{125}\text{I}$ -labeled mf revealed that the radioiodinated cuticular proteins of *O. gibsoni* mf displayed considerable charge heterogeneity. This heterogeneity was not reduced by treatment of viable  $^{125}\text{I}$ -labeled mf with neuramini-

dase prior to solubilization. Therefore, the range of charges of the radioiodinated proteins cannot be attributed to the presence of at least neuraminidase-sensitive sialic acid residues on cuticular glycoproteins.

Parkhouse et al. (1981) have recently shown that the major radioiodinated surface proteins of <sup>125</sup>I-labeled *T. spiralis* third stage larvae (L3) are a glycoprotein of Mr 47,000 and another protein of Mr 55,000. This lack of complexity of radioiodinated surface proteins reported by Parkhouse et al. (1981) contrasts with that obtained in this study using an entirely different life cycle stage of another parasitic nematode, *O. gibsoni*. The complexity of the *O. gibsoni* radioiodinated cuticular protein pattern may be attributed in part to possible binding to the surface of the following: (1) excretory/secretory (ES) products of mf akin to the situation in *Schistosoma mansoni* (Riley, 1979), (2) host proteins at the time of extraction of mf from nodular worms although two major bovine serum proteins, albumin and immunoglobulin, were not found on the surface of *uterine* mf (but see below), and (3) various parasite proteins of adult or egg origin which may adsorb to mf in utero. Alternatively, there are indeed numerous innate protein components of the *O. gibsoni* mf cuticle detectable by radioiodination.

Solubilization of <sup>125</sup>I-labeled mf in 1.5% Triton X-100 was somewhat selective, in that a Mr 90,000 protein complex designated x (Fig. 2 A) was not significantly solubilized. Maximum solubilization of the radioiodinated cuticular molecules was only achieved with a minimum concentration of 6 M urea which denatures proteins making immunoprecipitation impractical. Disulphide bond dependent aggregation after solubilization did not appear to occur since the presence of reducing and/or alkylating agents during Triton X-100 solubilization had no effect on the molecular weights of solubilized proteins.

Immunoprecipitation studies with sera from either *O. gibsoni* infected cattle or calves given multiple injections of mf but having no detectable skin mf, revealed important differences in the response of the host to mf. The additional polypeptides recognised by sera from mf-injected calves may be a consequence of depletion of antibody specificities from the sera of naturally infected cattle due to the high and persistent parasite antigen load. Alternatively, and more interestingly, the 6 labeled polypeptides recognised only by sera from calves exposed to mf may be weak antigens in naturally infected cattle. Reduced apparent immunogenicity of these molecules may reflect the operation of phenomena such as antigenic competition or selective immunosuppression in infected animals. In addition, the mf which escape from the nodule may be a very selected subpopulation of parasites which are weakly immunogenic in contrast to the bulk of *uterine* mf which may be relatively sequestered. Relevant to this are the studies of Wegerhof and Wenk (1979) who observed that *uterine* mf of *L. carinii* were more potent at inducing anti-mf responses compared with blood mf in rats. The 6 polypeptides recognised by sera of the immunised calves rather than infected animals may be candidate immunogens with respect to



stimulation of host protective anti-mf responses. An experiment to test the reason for apparent weak immunogenicity in infected cattle of the above mentioned 6 polypeptides is to inject uterine mf into infected cattle; if antibodies to the 6 appear, the sequestration of these mf antigens in the nodules of infected animals is a possible explanation; if antibodies are not produced, then immunosuppression may be responsible. The 2 polypeptides immunoprecipitated by sera of infected cows (albeit weakly), but not mf-injected calves, may be extremely weak immunogens requiring years of exposure for induction of antibody specificities.

When interpreting the immunoprecipitation results with bovine sera it must be remembered that the animals from which sera were taken were cattle from the field which would most certainly be infected with at least 1 if not 2 other *Onchocerca* species and most likely exposed to or infected with other metazoan and protozoan parasites. Hence the presence or absence of antibody specificities in sera of *O. gibsoni*-infected cattle which recognise *O. gibsoni* mf cuticular antigens may be influenced by other parasites. Sera from *O. gibsoni* monoinfected cattle are not yet available.

Since as few as  $5 \times 10^3$  mf can be successfully radioiodinated by the procedure outlined above, it will be possible to label skin snip mf which can only be obtained in limited numbers from a heavily infected bovine. The apparent high immunogenicity of *L. carinii* uterine mf (Wegerhof and Wenk, 1979) suggests that a comparison of the cuticular proteins and/or antigens of uterine mf versus skin mf may highlight differences. Histologically, host reactions to viable mf in skin are not obvious. Conceivably, the presence of adsorbed host proteins identified by  $^{125}\text{I}$  surface labeling of skin snip mf, may lead to reduced availability of parasite antigen and assist the mf in evading immune attack. In human filariases, circulating mf may display the same blood group antigens as the host (Ridley and Hedge, 1977). Preliminary indications are that labeled albumin is present in autoradiographs of two-dimensional gels of  $^{125}\text{I}$ -labeled *O. gibsoni* skin mf (Forsyth K. P. and Copeman D. B., unpublished observations).

The radioiodination procedure outlined in this paper offers the possibility of identifying the major cuticular proteins and/or antigens of mf of human filarial parasites, i.e. parasites which are available in limited numbers. For instance, the antigenic variability which has been observed in extracts of *O. volvulus* (Bryceson, 1976) and the differences in pathology in the cornea of eyes of rabbits produced by *O. volvulus* microfilariae (Garner et al., 1973) taken from either savannah or forest dwellers, could be characterised in terms of cuticular antigens of microfilariae. Furthermore, this procedure can be applied to identification of stage specific and species specific cuticular antigens of filarial parasites as has been done for *T. spiralis* (Philipp et al., 1980). Hopefully, such studies will lead to identification and characterisation of candidate molecules for immunodiagnosis of filarial infections or immunisation designed to prevent immunopathology or to reduce transmission.



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