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Immunolabeling and Ultrastructural Localization of an *Anaplasma marginale* Soluble Antigen

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ABSTRACT

A soluble Anaplasma marginale antigen was localized on membranes of infected erythrocytes and on parasite bodies by using immunolabeling techniques for light and electron microscopy with a bovine anti-soluble antigen serum. Reaction with normal red cell membrane components was not observed by these methods, but was evident by Western blotting. One of the components of the soluble antigen(s), a 16 kd. protein was indentified on membranes of erythrocytes from normal, infected and convalescent animals. as well as on *Anaplasma* bodies; while the other (36-38 kd.) was only located on infected red cell membranes. An immune reaction with high molecular weight polypeptides was observed on erytrocytic membranes. Since these polypeptides had not been identified previously as components of the soluble antigen(s), we propose three mechanisms by which this antigen is capable of inducing the production of antibodies against itself or other epitopes on red blood cell membranes: a) cross-reactivity of the antibodies developed against the soluble antigen with surface erythrocyte proteins. b) deposition or affinity of the antigen alone or complexed, to the red cell membrane, and c) metabolic processing of the soluble antigen from erythrocytic components.

key Words:
Soluble antigen, immunolabel, *Anaplasma marginale*, ultrastructural localization.

INTRODUCTION

Bovine anaplasmosis is an infectious hemotropic disease, caused by the obligate intracellular parasite *Anaplasma marginale* [1,2]. Its' host cells are erythrocytes from bovines or other ruminants.

The initial contact with the erythrocyte may be mediated by specific receptors and stimulated by hemodynamic forces [3]. Next, the red cell invaginates to internalize the parasite body which mantains itself and reproduces in a parasitophore vacuole [4].

During this intracellular phase, the host cell suffers a series of structural., biochemical and functional alterations that include the appearence of new polypeptides of 155 and 140 kd, and four glycoproteins of 90, 40, 28 and 21 kd. [5] the increment of superficial carbohydrates and affinity toward lectins [6, 7, 8] , a decrease in the levels of cholesterol and membrane phospholipids and an increase in the activity of enzymes from the glycolitic pathway and nucleotidic metabolism [9, 10, 11, 12]. Additionally, the membranes modify their ultrastructural electron density and during the exode of the *Anaplasma*, a fribilar and granulous material surrounds the parasite body [13].

The acute phase of this infection is characterized by an increase of parasitaemia, fever and a dramatic decrease of the hematocrit. In anaplasmosis, as in most hemotropic diseases, the number of parasitized erythrocytes does not correlate with the anemia [14]. For this reason, the existence of an

immunologic mechanism capable of sensitizing normal and infected red blood cells for the phagocytosis by macrophages and nuclear polymorphs has been proposed [14, 15, 16]. Autohemmaglutinins, opsonins and autoantibodies have been identified in serum and on erythrocytic membranes [14, 16, 17, 18, 19, 20].

Many of the molecular events associated with the pathology of anaplasmosis have not been dilucidated and it is necesary to identify antigens related to the anemia and other pathogenic consequences of the disease.

Several researchers identified different soluble antigens or exoantigens of ***Anaplasma marginale*** from northamerican isolates [21,22,23]. Aso (1985) [24] isolated a soluble antigen from infected erythrocytes by Sephadex G-200 and DEAE-celulose. This antigen was identified as a protein with similar characteristics to another antigen isolated from supernatants of short term cultures, which suggests it may be the same antigen that the parasite secretes to the plasma during its infectious cycle. De la Rosa (1990) [25] isolated and purified a soluble antigen from Venezuelan isolates (Yaracuy) by molecular exclusion and ionic exchange chromatography. By sandwich ELISA, this antigen was found present in bovine immune sera, closely to the peak of parasitaemia. SDS-Page and Western blotting of this soluble antigen showed two polypeptides, a 16 kd. protein found on fractions isolated from infected erythrocytes and one of 38 kd. present in fractions isolated from normal and infected red cells.

Soluble antigens have been identified as responsable for pathologic efects in other parasitic diseases such as malaria [26] and african bovine trypanosomiasis [27]. In the present work we used an anti-soluble antigen sera from a hyperimmunized animal to study the structural localization of this ***Anaplasma marginale*** antigen on erythrocytes from infected animals. In this way and with the compliment of other immunolabeling techniques, we also intented to establish its possible relationship to pathologic events of the disease.

MATERIALS AND METHODS

Sera: Sera were obtained from a splenectomized calf, hyperimmunized with a soluble *Anaplasma* antigen isolated from the supernatant of infected erythrocytes centrifuged at 100.000 g, precipitated and partially purified by exclusion crhomatography. The antigenicity of the fractions was monitored by immunodiffussion and ELISA. Immunizations were performed in three subcutaneous boosts of 4 mg. antigen with incomplete Freund's adjuvant and later infected with a venezuelan isolate of ***Anaplasma marginale*** (Yaracuy), [25]. The negative controls were normal sera (pre-immune), the anti-solute antigen serum was a positive serum to the antigen (post hyperimmunization and prior to infection) and positive controls were immune ***Anaplasma*** sera, post peak of parasitaemia.

Indirect immnuofluorescence asays: Tests were performed according to Montenegro et al. (1985) [28]

Animals: Bovines were inbred Swiss-brown, 6 months old. The references to healthy or normal animals were made toward calves not infected nor immunized; infected animals were those in acute phase of anaplasmosis and convalescent animals were calves that maintained a carrier condition (very low parasitaemia), but did not suffer from the symptoms of the disease.

Erythrocyte and Anaplasma bodies: Bovine red blood cells from normal, infected and convalescent animals were isolated from complete blood by standard procedures and ***Anaplasma marginale*** bodies were obtained following Schillinger & Helwig (1985). [29]

Immunocytochemical reactions: The procedure followed was modified from Bretaña et al. (1986, 1992) [30, 31]. The cells were washed three times in 0.01M sodium phosphate buffer saline (PBS), pH 7.2 and then prefixed with glutaraldehyde 2.5% in PBS for 30 min. at room temperature. Subsequently they were incubated with the corresponding. Serum (normal, immune or anti-soluble antigen) diluted 1:20 in PBS, washed and incubated with the IgG-peroxidase conjugate (1:10). The red blood cells reactions were revealed through the

incubation of 100 ul. of a solution of 1 mg. 3-3 diaminobenzidine-HCl (Sigma Chemical Co., USA) in 2 ml. TRIS-HCl 0.01 M. pH 7,2 buffer with 0,85% sodium chloride and 5 ul. 30% Hydrogen peroxide, during 45 minutes at room temperature. Controls without peroxidase-IgG conjugates were incubated simultaneously. Cells were washed and processed for electron microscopy. All labeling procedures were performed by triplicate.

Processing for electron microscopy: All immunolabeled cell sediments and their respective controls were postfixed in 1% osmium tetroxide in distilled water during one hour at room temperature. Samples were then centrifuged at 1500 g and the supernatant removed with a micropipette without disturbing the sediment. The cell pellets were washed three times in distilled water and the sediment carefully detached from the bottom of the centrifuge tube with fine wood aplicators and placed in small vials for their dehydration in increasing concentrations of ethanol. The samples were embedded in Maraglas resin (Ladd Research Industries Inc.. Burlington, Vermont, USA). Blocks were sectioned at 40-90 nm. and some were counterstained with uranyl acetate and lead citrate. Other sections were examined without counterstaining. Electron micrographs were taken with a Hitachi H-500 transmission electron microscope at 75 KV.

Isolation of Erythrocyte and Parasite Membranes: The isolation was done following Dodge et al. (1963) [32]. The infected red blood cells were taken at the peak of parasitaemia (22% by direct determination with Wright-Giemsa stain) for the isolation of membranes and parasites. All extractions were performed in the presence of proteolysis inhibitors and total protein was measured by Lowry before gel electrophoresis.

SDS Gel Electrophoresis: Gel electrophoresis (SDS-PAGE) was performed at 12%, discontinuous system under dissociating conditions [33].

Western Blotting: The blotting procedures used were described by Towbin et al. (1979) [34], Adams et al. (1986) [35] and Bittner et al. (1989) [36].

RESULTS

Light microscopy: The reaction of an *Anaplasma* positive immune serum with infected red blood cells is shown in **Fig.** 1a. Note the fluorescence of the parasite related to that of the erythrocytic cells. The same smear incubated with the anti-soluble antigen serum, shows a weaker reaction. The fluorescence seems to distribute more evenly between parasite and host cell (Fig. 1b.). On contrary, a normal bovine serum did not react with the parasite. Fig. 1c shows the basic red blood cell fluorescence seen with this negative serum.

The rections of these sera with smears of normal red cells are shown on Figs. 1d, 1e and 1f. The positive (1d) and anti-soluble antigen serum (1e) seem to have a slightly higher fluorescence than the negative control (1f), although it is not clear enough as to consider the sera reactive to normal erythrocytes.

Electron microscopy: Normal red cells did not show any immunoreactivity when incubated with preimmune, immune or anti-soluble antigen sera (not shown). The membranes of these cells appeared free of any precipitate and reactions were only evident when the plasma membrane was distortioned or fractured, exposing granular material from its cytoplasm. Fig. 2 shows the immunolabeling observed on the membrane of apparently non-parasitized erythrocytes from infected blood. The phenomenon described is showed on Figs 2a and 2b, were homogeneously distributed labeling on the red blood cell membranes is also evident. Bovine infected erythrocytes reacted positively with anti-soluble antigen serum. as shown on Fig. 3. The immunostaining is seen on the contact zone between the parasite and host cell and on the whole erythrocytic membrane. A diffuse precipitate attached to the cell membrane is also evident, although not present in all red blood cells (Fig.s 3a and 3b). Positive controls showed a similar reaction to the one observed with the antiserum to soluble antigen. Normal sera did not show any reaction (not shown).

The anti-soluble antigen serum immunostained ***Anaplasma marginale*** bodies. It recognizes epitopes on all the parasitic membrane, as shown in Fig. 4. The intensity of

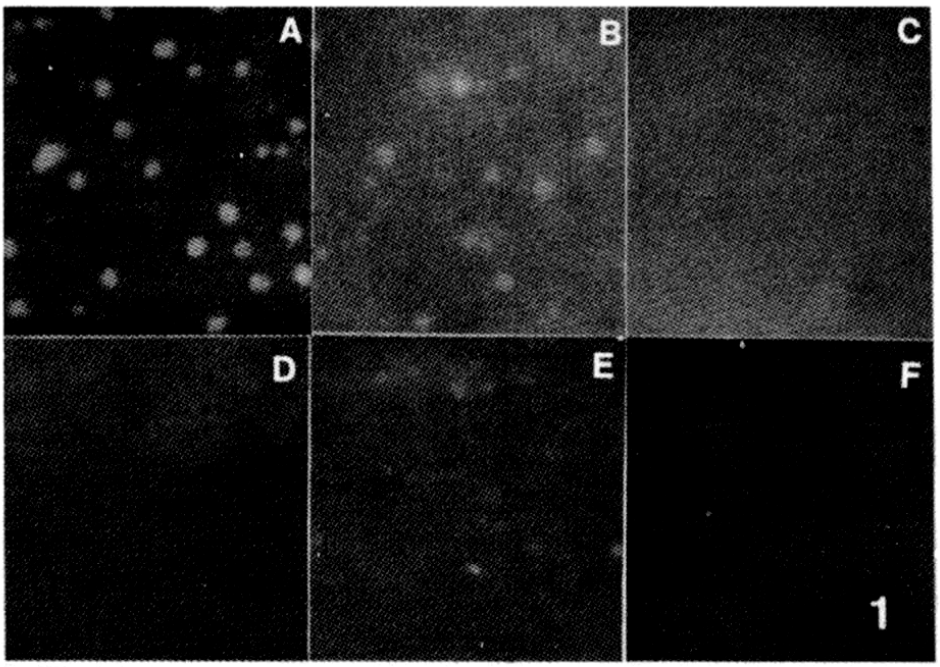

Figure 1.- Results of the immunofluorescence assays. Reactions with infected erythrocytes: A) *Anaplasma* immune serum (positive control) ; B) Anti-soluble antigen serum and C) Normal serum. Reactions with normal bovine erythrocytes: D) Parasite immune serum; E) Anti-soluble antigen serum and F) Normal serum. Sera diluted 1:80 and FITC-conjugate diluted 1:100.

the reaction was evidently higher than with the erythocytes, as can be seen by the electron-dense precipitate on the parasite bodies (Figs. 4a and 4b).

Electrophoresis in SDS-PAGE: Membrane proteins of normal, infected and convalescencet erythrocytes were analyzed by a gel electrophoresis in SDS-PAGE. Parasite bodies and two fractions of the soluble antigen were also included. The pattern observed is shown in Figure 5 , and the relative molecular mass (kd.) of the polypeptides, as well as a measure of their intensity (absorbance) are seen in Table I. There are numerous common proteins between the cell fractions, as those of 178, 98, 85, 71, 62, 43, 38, 36, 16 and 7 kd. The last four are also shared with the soluble antigen (s) (lane 7). Aditionally there are polypeptides that differ between the cells, in presence as in relative concentration, e. g. 105 and 5 kd., proteins only represented on parasite bodies (lane 5), and that of 19 kd. only seen on infected erythrocytes (lane 4). Densitometric differences are also seen. Polypeptides shared by the different fractions as the 16 kd. protein, is observed in various concentractions, ranging from 0.05 to 0.23 O.D.

Western Blotting: Fig. 6 represents the patterns of recognition with negative (6a), positive (6b) and anti-soluble antigen serum (6c). In lane 1 are the molecular weight standards, from lanes 2 to 4 are erythrocytic membranes from normal, convalescent and infected animals, lane 5 shows parasite bodies and lane 6, the soluble antigen (s). The blot with normal serum showed a reaction with two polypeptides, of 43 and 62 kd., repectively (Fig. 6a).

When the fractions were incubated with an ***Anaplasma*** immune serum (positive serum), there was higher number of reactive bands (Fig. 6b). Convalescent and infected erythocytes share an antigenic protein of 85 kd. (lanes 3 and 4), and with the parasite bodies one of 98 kd (lane 5). This positive control showed a recognition of the 43 and 62 kd. bands in all fractions, as seen with the normal sera. There is a reaction to 36 and 16 kd. proteins that was not observed with the pre-immune sera. The soluble antigen (s) (lane 6) shares an antigenic polypeptide of 31 kd. with infected cells and ***Anaplasma marginale*** bodies.

The reaction with the anti-soluble

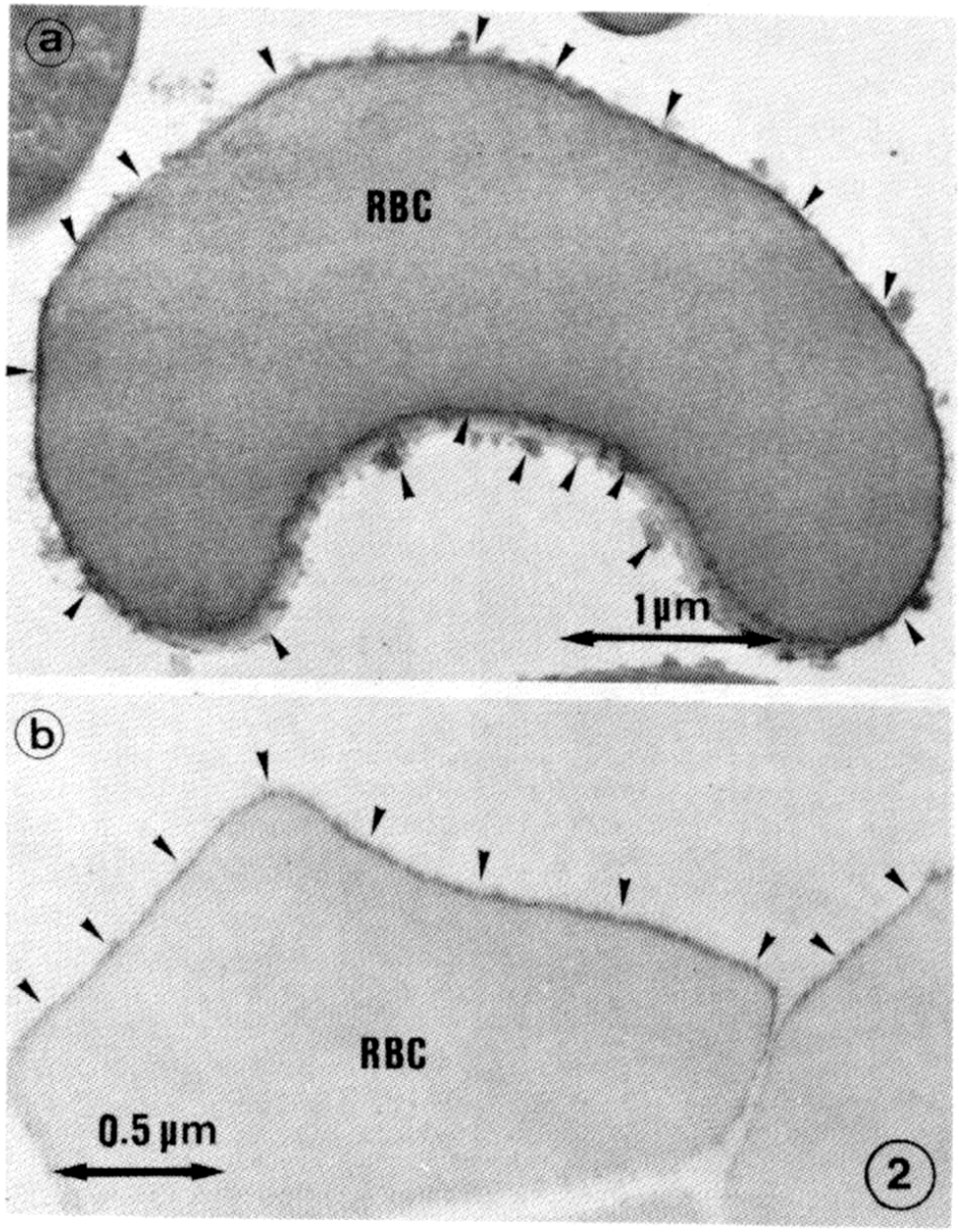

Figure 2.- Electron micrograph showing non-parasitized erythrocytes from an infected blood sample incubated with the anti-soluble antigen serum. The arrows point at the label over the red blood cell (RBC) membranes. There is a granulous material surrounding the cell (2A).

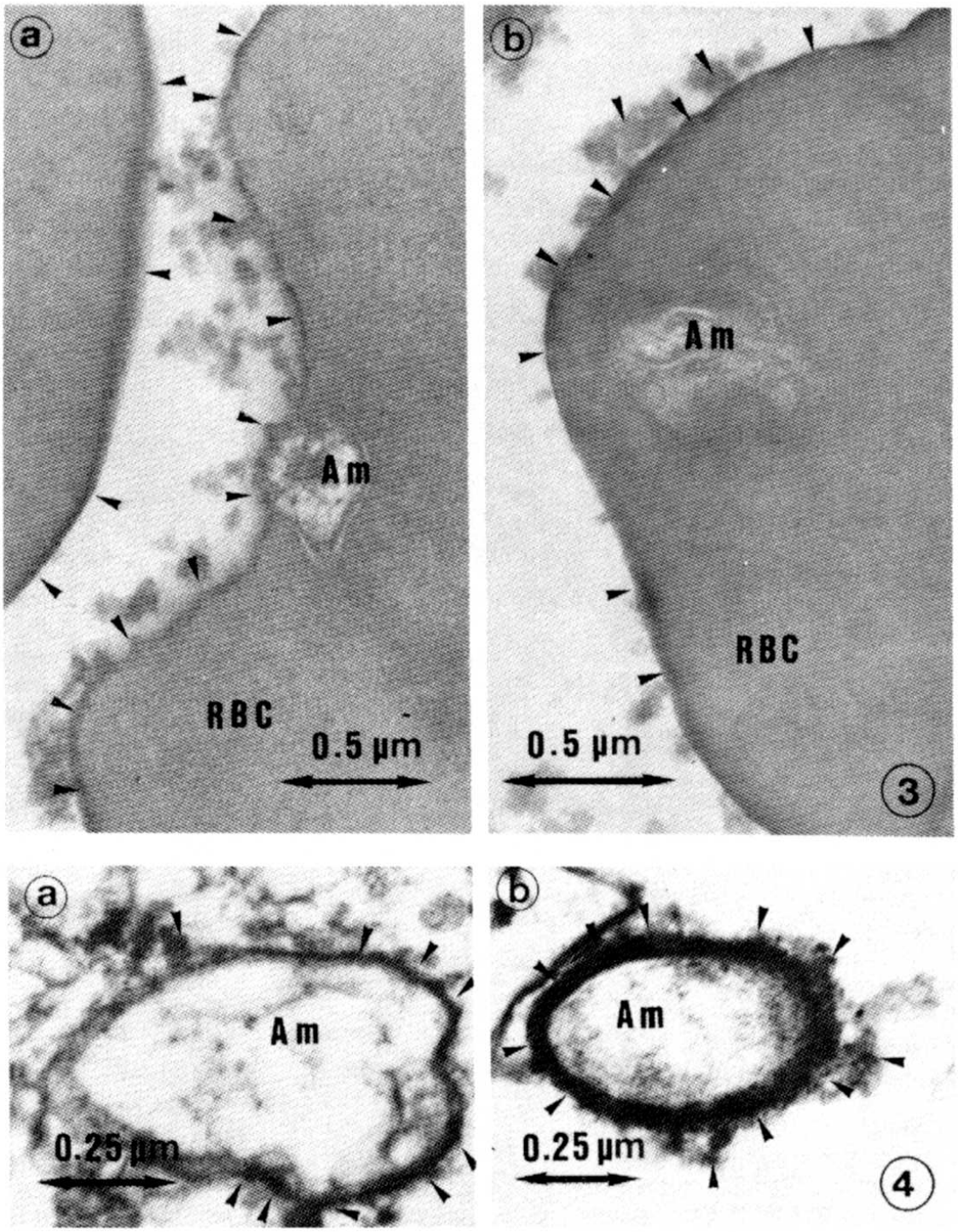

Figure 3.- Electron micrograph of the reactions observed on infected red blood cells incubated with the antiserum to the soluble antigen (problem serum). The arrows show a labelling of the cell membranes and granulous material. There is an evident reaction near the parasite (Am).

Figure 4.- Electron micrograph showing the strong label of the problem serum to the *Anaplasma marginale* bodies.

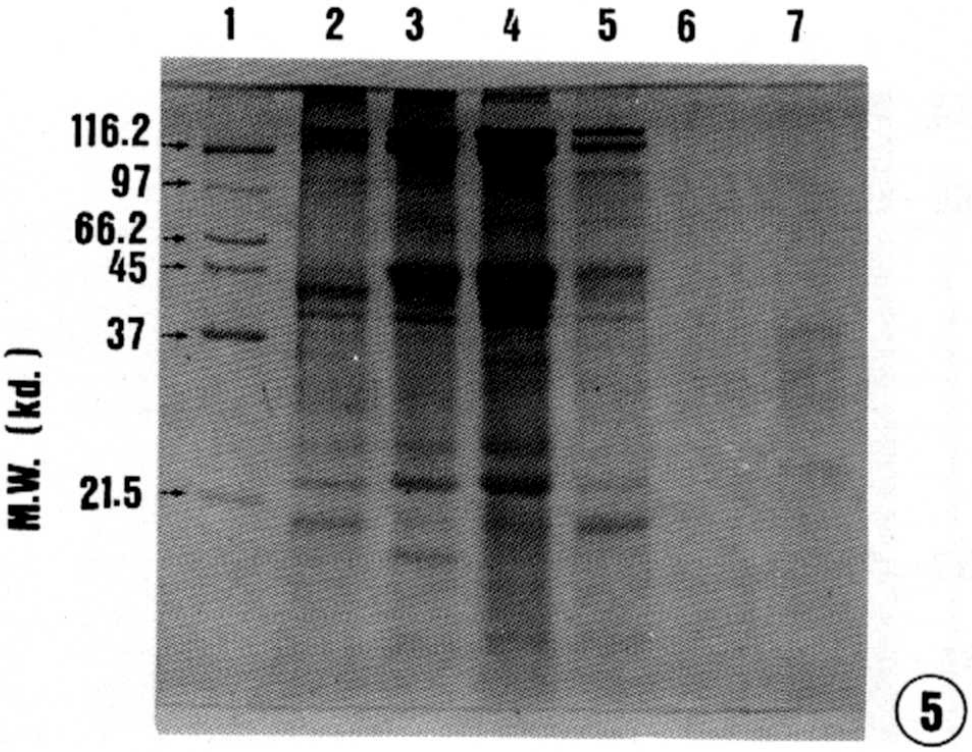

Figure 5.- Gel electrophoresis in SDS-PAGE 12% acrylamide under dissociating conditions. Lane 1: Molecular weight standards. Polypeptidic pattern of: Normal red cell membranes (lane 2) ; Erythrocytic membranes of convalescent (lane 3) and infected animals (lane 4) ; *Anaplasma marginale* bodies (lane 5) ; purified soluble antigen (lane 6) and crude soluble antigen (lane 7). Coomasie blue stain.

antigen serum is shown in Fig. 6C, in which the reaction to high molecular weight proteins (178. 98 and 85 kd.) is seen with normal, convalescent and infected erythrocytes. The two unspecific bands of 62 and 43 kd. are also evident. The serum also recognizes proteins of 36-38 kd. and 16 kd. in most fractions.

Table II shows a summary of all reactions with their relative molecular weight.

DISCUSSION

Soluble antigens may be involved in the parasitic strategies to evade the host response or to assure their survival and fitness, as has been reported for some parasites as *Plasmodium falciparum* [26] or *Trypanosoma brucei* [27]. These antigens may not be of immunologic relevance, but play essential roles in the pathology of the disease and consequently could represent appropriate targets for the development of diagnostic or prophylactic methods.

In the present study, the soluble antigen (s) obtained from venezuelan isolates by De la Rosa (1990) [25] was localized by immunocytochemical techniques, for light and electron microscopy, as well as by a polypeptidic and blot analysis.

Normal red blood cells showed a low or absent labeling with the anti-soluble antigen serum by indirect immunofluorescence (Fig.1) and electron microscopy.

Infected erythrocytes showed a strong immunoreactivity by immunofluorescence and electron microscopic procedures, when incubated with the anti-soluble antigen serum. The reactions were localized on the parasite bodies or distributed along the red cell membrane. This result allows us to suggest that the soluble antigen is present on the membrane of infected erythrocytes, and may be involved in an interaction of the host cell and parasite or as a modification of the red cell surface. The label could be considered as a possible transit of the antigen from the parasite to the host membrane and/or to the serum to its soluble condition. De la Rosa (1990) [25] detects the soluble antigen on

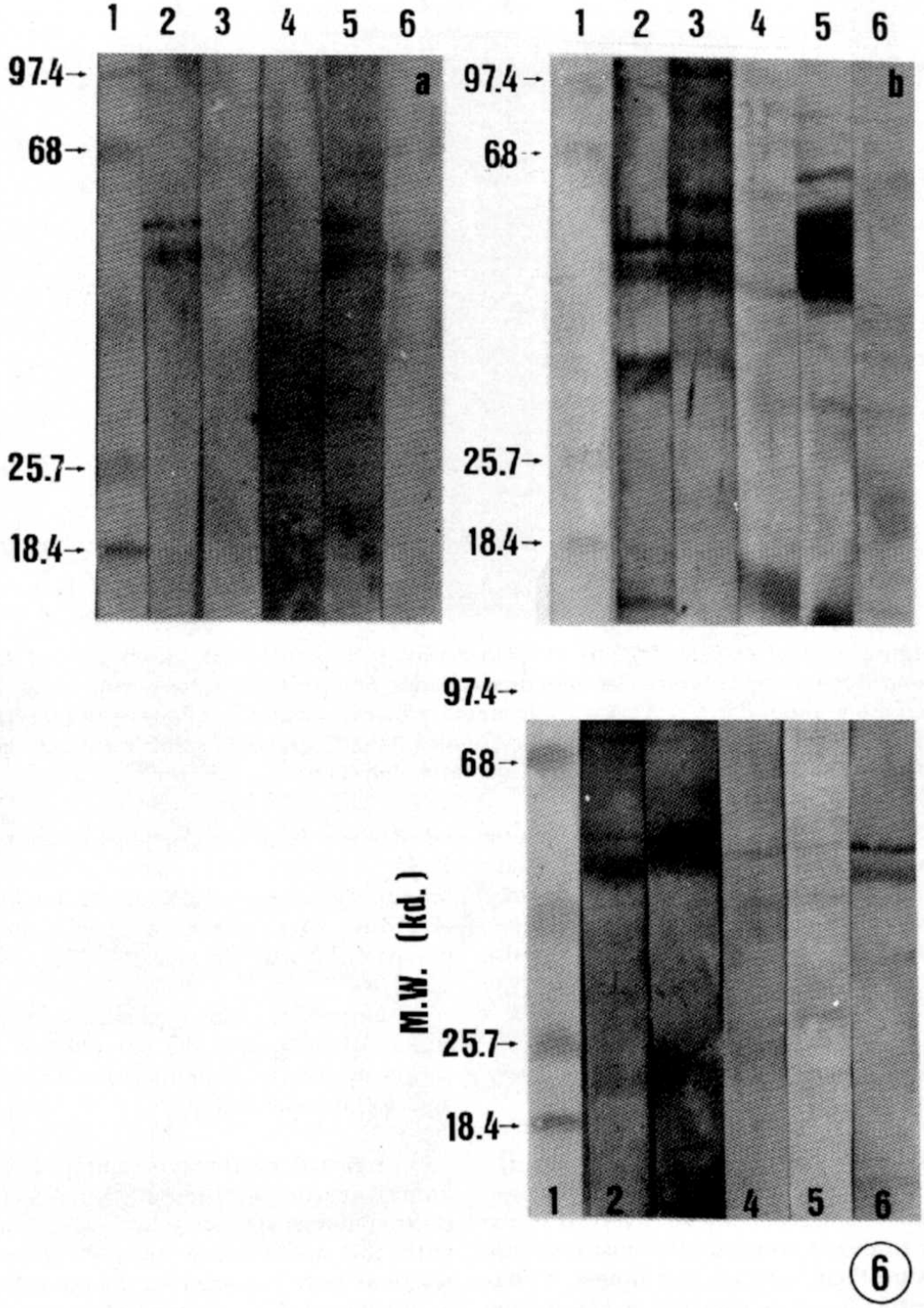

Figure 6.- Western blots showing the reactions of normal bovine serum (A) , *Anaplasma* immune serum (B) and the antiserum to soluble antigen (C) towards surface antigens of normal erythrocytes (lane 2) , convalescent erythrocytes (lane 3) , infected red blood cells (lane 4) , parasite bodies (lane 5) and *Anaplasma* soluble antigen (s) (lane 6). Molecular weight standards stained with amido black are on lane 1.

TABLE I

RELATIVE MOLECULAR MASS (Kd.) OF POLYPEPTIDES
OBSERVED BY ELECTROPHORESIS SDS-PAGE

NRBC	CRBC	IRBC	AB	SAP	SAC
	**181				
*178	**178	**178	*178		
**126	**126		*126		
	*120	**120			
			105		
100		*100			
98		98	98		
85	*85	*85	85		
71	71	*71	71		
62	*62	62	62		
	**60		*60		
*59		**59	59		
*54		**54			
43	*43	**43	43		
38		*38	38	*38	*38
	36	*36	*36		*36
	34	*34	34		
31	31		31		*31
		*19			
16	*16	*16	16	*16	*16
13					
8	8	8	8		
7	7	7	7	*7	*7
		6	6		*6
			5		
			3		

NRBC = Normal Red Blood Cells;
IRBC = Infected Red Blood Cells;
SAP = Soluble Antigen Purified;
* Optic Density = 0.1 - 0.3
** Optic Density = 0.3 - 1.2

CRBC = Carrier Red Blood Cells
AB = Anaplasma Bodies
SAC = Soluble Antigen Crude

TABLE II

SUMMARY OF REACTIVE POLYPEPTIDES
DETECTED BY WESTERN BLOTTING

	NRBC	CRBC	IRBC	AB	SAG
NORMAL SERUM				75	
	62	62	N.D.	62	N.D.
	42	42	N.D.	42	42
IMMUNE SERUM		98	98	98	
		85	85		
	62	62	62	62	
	43	43	43	43	43
	36	36	36	36	36
	31		31	31	31
		16	16	16	16
				7	7
ANTI-SOLUBLE ANTIGEN SERUM	178	178	178		
	98	98			
	85	85			
	71				
	62	62	62	62	62
	43	43		43	43
			38		38
	16	16	16	16	16
					7

N.D. = Not Determined; NRBC = Normal Red Blood Cells
CRBC = Convalescent/Carrier Red Blood Cells
IRBC = Infected Red Blood Cells; AB = *Anaplasma* Bodies
SAG = Soluble Antigen

bovine sera from intectd animals, finding the maximun concentration on days of the peak of parasitaemia and prior to them. The presence of the antigen in serum is described up to 180 days after infection. This suggests that the antigen is secreted to the blood plasma during the infection, but it is not known whether this takes place from the intracellular phase or during the exode of the ***Anaplasma*** to another red cell. Giardina et al. (1983) [13] described the presence of granular electron dense material seen in the electron microscope during the exit of the parasite towards other infective units.

Immunoreactivity was also observed in the parasites located inside the erythrocytes (Fig. 3) and on the membranes of isolated Anaplasma bodies (Fig. 4.) This points towards a specificity of the antigen to the parasite. Still, there is a possibility that the antigen may be a metabolic product of the parasite from erythrocytic material, since the ***Anaplasma*** bodies are isolated from infected red cells and generally some contamination from cytoplasmic or membranous material remains. Amerault & Roby (1967) [22] isolated an ***Anaplasma*** soluble antigen which was considered a metabolic product from the host cell proteins. Ristic et. al. (1962) [37] described two soluble lipoproteic antigens that could be metabolic products from the erytrhocytic stroma. This could represent one of the factors responsible for the decrease of membrane phospholipids in the acute stage of the disease as has been reported [12,38]. On all immunocytochemical reactions performed for electron microscopy, a label was observed whenever the cell membranes were fractured, chacacterized by a granular material. This precipitate possibly corresponds to enzimatic reactions (redox) performed in the presence of hydrogen peroxide (substrate) and DAB. Red blood cells have catalase and peroxidase activities [39].

The polypeptidic analysis of normal, convalescent and infected red cell membrane constituents, ***Anaplasma*** bodies and soluble antigen (s) by electrophoresis and Western blotting permitted the identification of antigenic proteins recogniced differentially by normal, immune or anti-soluble antigen sera. The negative control serum (normal bovine serum) reacted in all cases with two polypeptides of 43 and 62 kd. (Fig. 6, Taable II). Immunoblots performed as controls by incubating the nitrocellulose papers with the conjugated secondary antibody only (anti-IgG-peroxidase), showed a weak but evident reaction to the 62 kd. band which could represent the heavy chain of bovine IgG.

When comparing the polypeptides present on the cell fractions by SDS-PAGE (Fig. 5) with those recognized by the different sera, it is evident that their concentration is not proportional to their antigenicity. Most proteins that had an optic density higher than 1.0 were not relevant antigens.

The membrane proteins of normal erythrocytes, contrary to what was observed by electron microscopy and immunofluorescence methods, were reactive to normal, immune and problem sera by Western blotting. This technique offers some advantages like its high sensitivity and the lack of drastic procedures for the preservation of the cellular material (fixation, dehydration). These procedures could implicate damage, make antigenic changes or modify the accesibility of the to cell.

Additionally it mantains and concentrates antibodies or otner reactants to the protein separated by their electrophoretic mobility. It is also possible that we are dealing with an immunorecesive antigen whose label is not evident by the methods used in the present study.

It is important to consider that the antigen presentation plays an essential role in its recognition, and we do not discard that its configuration on the surface of normal red blood cells after the treatment for electron microscope studies, is not appropriate for it to react positively.

There are some alternative tecnniques for this suceptible material, like the use of acrilic resins like LRwhite or LRgold recomendended for labile antigens or vegetal tissues [40]. Other possibilities are label-fracture cytochemistry or crioultramicrotomy, succesfully used in the localization of surface antigens and glycoconjugates [41,42,43].

De la Rosa (1990) [25] described two basic constituents of the soluble antigen or two

antigens, of 16 and 36-38 kd. aproximately. The 16 kd. polypeptide appeared as an antigenic protein on the surface of normal, infected and convalescent erythrocytes, as well as on parasite bodies and on the soluble antigen fraction. Palmer et al. (1986) [44] described a group of proteins from different northamerican isolates of ***Anaplasma marginale*** that remain present on all phases of infection, foccusing on two polypeptides, of 86 and 15 kd. that are conserved among the isolates. The latter may represent a similar or identic protein to that of the soluble antigen (16 kd.)

The other polypeptide of 38 kd. is recognized by the anti-soluble antigen serum and immune sera on infected red blood cells. Proteins that range from 36-38 Kd, have been described as important immunogens of venezuelan and american isolates of ***Anaplasma marginale*** [16,45,46,47] . Am36 (a 36 kd. *Anaplasma* protein) was identified as present on vector and bovine stages, and proved to induce protection against homologous and heterologous isolates, even when there exists an heterogeneity in its molecular weight among the different geographical stocks [45,48]. If the components of the soluble antigen (s) effectively are present on erythrocytic membranes of different stages of infection as it has been found in the present study, the proteins of 16 and 38 kd. may be related to those reported by other groups.

Since these two low molecular mass antigens have been the only constituents described for the soluble antigen, the reaction of the problem serum (anti-soluble antigen) with high molecular weight proteins on normal, infected and convalescent erythrocytes calls to attention. 178,98,95 and 70 kd. bands are evidently recognized, as seen on Fig. 6. This phenomenon and other factors described along the discussion led us to propose three models (Figure 7) that may explain characteristics and possible role of the soluble antigen:

a) The antigen may be a parasitic antigen liberated to the plasma where it remains in a soluble form and antibodies developed to neutralize it recognize common epitopes of surface erythrocytic proteins. This could establish an identity reaction. Specially when they are glycoproteins a cross-reaction may sensitizes these red blood cells for being phagocitized (Fig. 7a)

There are many antigenic determinants that can establish an identity reaction. Specially when they are glycoproteins or glycolipids whose epitopes include the sugars. Nordelo & Ysern-Caldentey (1982) [5] described four new glycoproteins that appear during the accute stage of anaplasmosis, stage in which the affinity to many lectins is modified [8]. This may indicate that the host cells alter their surface expossing more linking groups of polysaccharidic origin.

b) The antigen may have affinity for erythrocytic membranes where it can be deposited after liberated to its soluble condition. This could occur with the antigen alone or complexed with other serum proteins (Fig. 7B).

Ysern-Caldentey & Romero (1988) [12], with the purpose of identifying and characterizing the proteins that act as receptors in ***Anaplasma marginale*** described five polypeptides of 105, 91, 61, 38 and 29 kd. with high affinity for erythrocytes. Some of these may represent the soluble antigen (38 kd.) or related antigenic complexes. Nevertheless, these polypeptides could not necessarily have any relation to the soluble antigen and represent receptors in the parasite that bind easily to the host cell membrane.

Aso (1985) [24] described that isolated and purified fractions of soluble antigens were often contaminated by serum albumin, to which it could be complexed. Other authors isolated a soluble antigen complexed with albumin from culture supernatants [23].

c) The antigen could represent a metabolic product of the microorganism processed from erythrocytic components, for which there could be a cross-reactivity with the host cell (Fig. 7c).

Any of the mentioned possibilities could explain the reaction with high molecular weight proteins on normal, infected and convalescent erythrocytes, as well as pointing possible roles of the antigen in the pathology of the disease and more specifically, with the

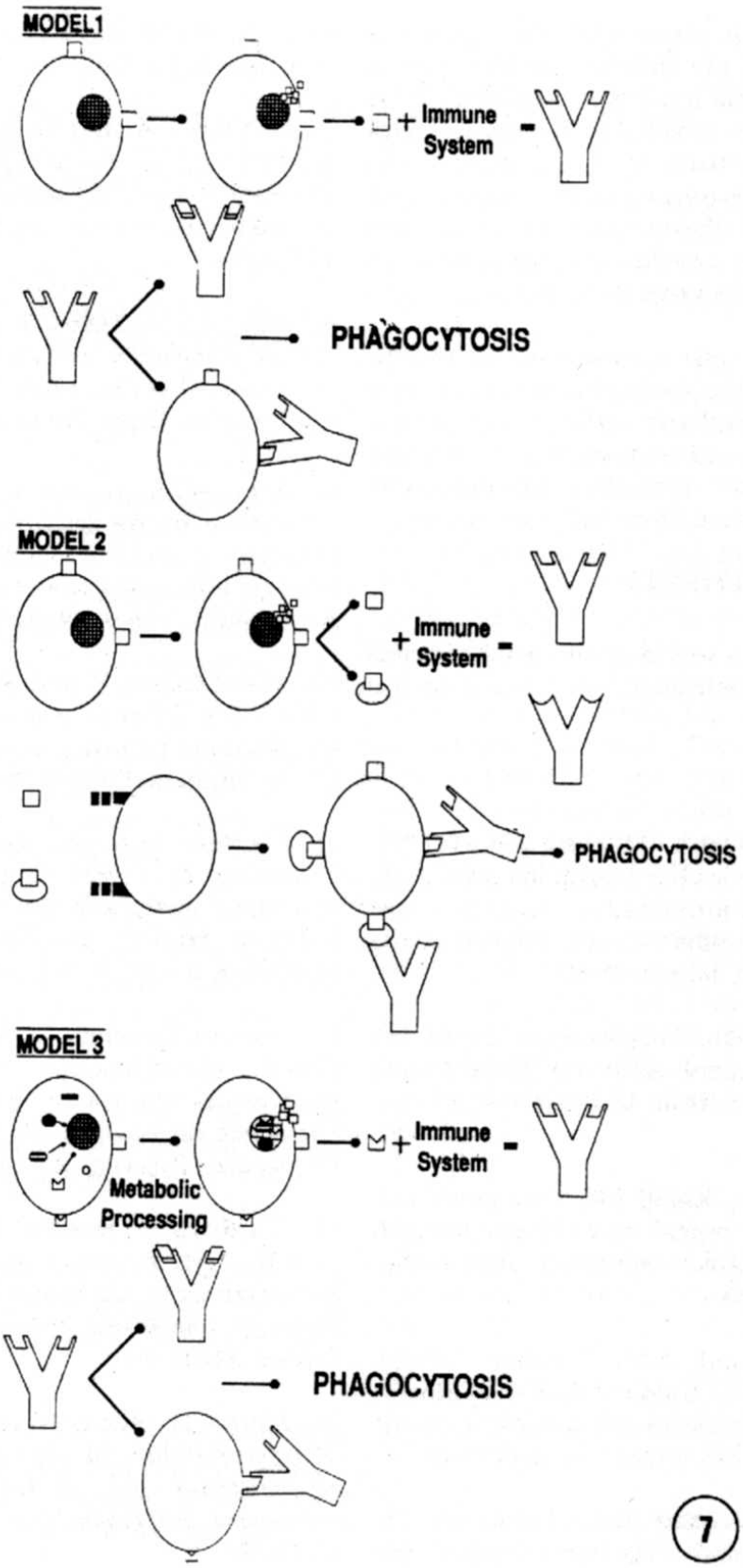

Figure 7.- Three possible alternatives for the role of the soluble antigen (s) in the pathology of bovine anaplasmosis.

severe anemia. It seems that the parasite is able to distract the immune system with a soluble antigen that it needs to neutralize, but is at the same time sensitizing the erythrocytes for being phagocitized, by one or more of the mentioned menchanisms. In this way, it may represent one of the factors that causes the anemia and it would be implicated in autoimmune mechanisms in the disease.

There are many questions that remain unsolved regarding the molecular events that cause the pathologic effects of bovine anaplasmosis, but it is important to understand the mechanisms that the parasite has developed to achieve its evolutionary success.

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