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Immunogenic Activities of Subcellular Components of *Trypanosoma gambiense* in Mice

MASATO FURUYA

Department of Parasitology, School of Medicine, the University of Tokushima, Tokushima

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Introduction

Many workers have examined the physical, chemical, biological, and serological properties of fractionated antigens of trypanosomes, a member of subgenus Trypanozoon, which have been shown to possess a fair number of phenotypes. On the basis of those informations, Seed (1974) classified these antigens into three major groups, namely, a) common antigens, b) host-like antigens, and c) variant specific antigens. Among the three, common antigens presumably include numerous enzymes and structural components of the cells and are apparently not involved in protection against infections of the parasites (Risby *et al.*, 1969). Host-like antigens are seemingly connected with serum components of infected animals participating in disguising the parasites as a host constituent (Damian, 1964; Ketteridge, 1972). However, the real biological role they play during infection is not elucidated yet. Variant specific antigens are likely related with surface coat which envelopes the limiting membrane of parasite cells and are also likely to be indispensable to the variation of antigenic type, agglutination reaction, and protection against infections of the parasites (Weitz, 1960; Brown and Williamson, 1964; Williamson and Brown, 1964; Gray, 1965; Vickerman and Luckins, 1969).

Of particular interest is the recent report that a ribosomal fraction obtained from pathogenic bacterial cells which possess a number of type-specific antigens on their cell surface as trypanosomes, is highly immunogenic in animal experiments (Youmans and Youmans, 1965; Venneman *et al.*, 1970). In a series of studies in our laboratory on experimental trichomoniasis, mice immunized with ribosomal antigen in the presence of Freund's complete adjuvant demonstrated a significant resistance to fatal infections of *Trichomonas foetus* (Oka *et al.*, 1967, 1970). In experimental trypanosomiasis, however, little attention has been paid to the immunogenicity of cell components although many an examination of the above properties of fractionated antigens has been made. This present report is on immunogenic (protective) activity of antigens prepared from both nontreated and trypsin-pretreated living parasites, and also on immune responses in mice immunized with subcellular components of the parasites.

Materials and Methods

1. Parasites

Wellcome strain *Trypanosoma gambiense* was obtained from the Department of Parasitology, Research Institute for Microbial Diseases, Osaka University and maintained in our laboratory in mice by serial passages at an interval of 3 or 4 days. Parasites originated in a single cell fishing were prepared by a slight modification of Inoki's method (1960) as follows. Parasites isolated from an infected mouse were diluted to a desirable concentration and a small quantity of the dilution was transferred to a microcapillary tube of approximately 0.2 mm in diameter. After being confirmed microscopically that only a single parasite was contained in the dilution, it was drawn into

a syringe and was inoculated into the peritoneal cavity of mice without delay. Seven or eight days after inoculation, the propagated organisms were used for the experiment.

2. Animals

Female ddY strain mice of 40 to 45 days old (Tokushima Experimental Animal Laboratory, Tokushima, Japan) were used for the experiment.

3. Isolation of parasites

Mice inoculated with trypanosomes were exsanguinated by decapitation at their peak parasitemia into ice-cold phosphate buffered saline-glucose solution, pH 8.0 (PBSG) (0.2 *M* Na_2HPO_4 270 ml, 0.2 *M* NaH_2PO_4 30 ml, saline 300 ml, and 2.5% glucose 400 ml) containing heparin (10 IU/ml PBSG) as an anticoagulant. The pooled blood was diluted 1 : 3 with cold PBSG and the large clots were cleared by filtration through cotton wool. Trypanosomes were then separated from blood cells by passage through a DEAE-Sephadex A-25 column (Lanham, 1968; Lanham and Godfrey, 1970) and were washed twice in cold PBSG by centrifuging for 10 minutes at 1,000×g at 4 C.

4. Preparation of antigens

The following antigens were prepared from the harvested trypanosomes and the preparation procedure is summarized in Fig. 1.

Antigen of cell homogenate (CH): The parasites suspended in a small quantity of 0.01 *M* tris-HCl buffer, pH 8.0 containing 5 m*M* $MgCl_2$ and 15 m*M* KCl were disrupted by freeze-thawing three times with acetone dry-ice and tepid water and were triturated with a teflon homogenizer before the homogenate was adjusted with buffer to a final concentration of 10 w/v %.

Antigens of differential centrifugal fractions: Ten per cent cell homogenate was centrifuged at 1,000×g for 10 minutes and then at 8,000×g for 20 minutes. The supernatant was further centrifuged at 144,000×g for 90 minutes to fractionate into soluble protein fraction (SPF) and microsomal fraction (MF). Sodium deoxycholate was added to MF giving a final concentration of 0.5% and the mixture was left stand at 4 C for 10 minutes. After centrifuging at 8,000×g for 20 minutes, the supernatant was recentrifuged at 144,000×g for 90 minutes to sediment ribosomal particles (RF).

Antigens of cell homogenate and differential centrifugal fractions of the trypsin-treated parasites: The suspension of parasites in cold PBSG was mixed with an equal volume of 0.5% trypsin solution (Difco Laboratories) and the mixture was left stand at 4 C for 15 minutes before the parasites were washed twice by centrifuging with a large quantity of cold PBSG. Antigens of cell homogenate (TCH), soluble protein fraction (TSPF), and microsomal fraction (TMF) were prepared from the above trypsin-treated living organisms according to the preparation procedure of CH, SPF, and MF antigens.

Antigens of heat-treated cell homogenate: These antigens were acquired by heating 10% CH for 30 minutes at 60, 50, and 40 C.

Antigens fixed with formaldehyde (FF) *and glutaraldehyde* (GF): Parasites in cold PBSG were fixd with an equal volume of formaldehyde or glutaraldehyde solution giving final concentrations of 2, 1, 0.5, and 0.2% and the mixture was left stand for 12 hours for formaldehyde and 30 minutes for glutaraldehyde. The parasites were then rinsed 7 times by centrifuging in distilled water to remove the fixative.

5. Immunogenicity test of antigens

The protein concentration of each antigen applied was standerdized to 2 mg per mouse. Antigens were emulsified with an equal volume of Freund's complete (FCA) or incomplete (FIA) adjuvant and 0.2 ml aliquots of the emulsion were injected into mice intraperitoneally. Mice injected adjuvant only or nothing were used for controls. On study days 3 and 30, i.e. 3 and 30 days after immunization, $(4\sim2)\times10^3$ parasites of homologous antigenic type were injected intravenously into mice immunized with each antigen to see the vaccine effect. Per cent survival and mean survival days of the dead mice during 20 days after injection were employed as criteria for evaluating the

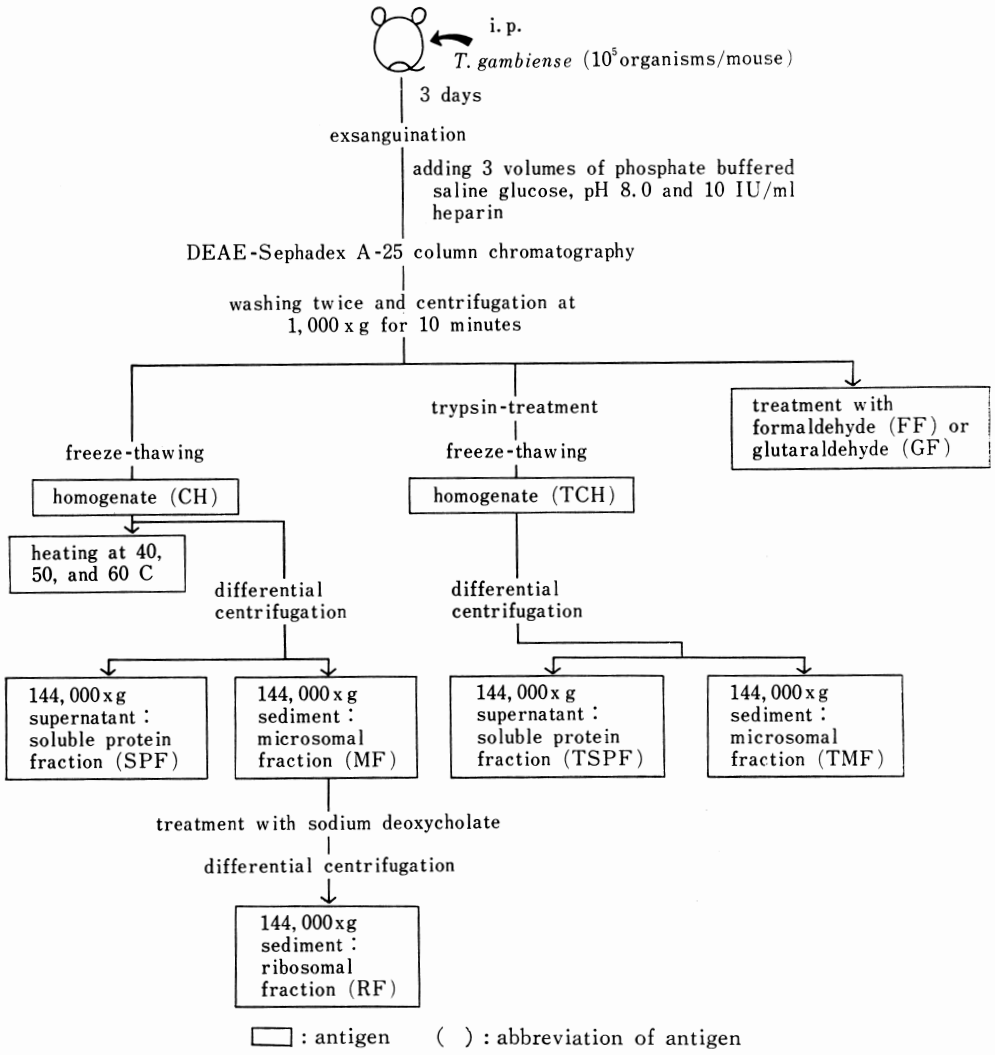

Fig. 1 Preparation procedure of antigens of *Trypanosoma gambiense*.

efficacy of the antigens.

6. Examinations of immune responses in mice

Immune responses in mice immunized with subcellular components in the presence of FCA or FIA were investigated as follows: 1) On study days 2, 4, 6, 8, and 10, body and organ weights of the thymus, spleen and mesenteric lymph node of mice were measured and an index was contrived using a formular {index=organ weight (g)$\times 10^3$/body weight (g)} for estimating the tissue responses to the immune stimulus. 2) For histological examinations, these organs were fixed with 4% formaldehyde and their sections were stained with hematoxyline and eosin. Histological changes in the thymus and increase and decrease of lymphocytes in the thymus dependent area (TDA) and the thymus independent area, i.e. gut associated lymphoid tissue (GALT), of the spleen and mesenteric lymph node were investigated. 3) The antibody titer in sera was measured at intervals of 2 days up to study day 10. An equal volume of parasites (1×10^8 organisms/ml PBSG) was added to the serial

dilutions of mouse serum and the agglutination reaction followed within 10 minutes was observed under the microscope. The highest positive serum dilution was designated as a serum antibody titer.

7. Examinations of the nature of surface coat

The nature of surface coat of the parasite cell was studied electron microscopically in the organisms in part stained with ruthenium red according to Luft's procedure (1964) and in part treated with 0.25% trypsin or amylase for 10 minutes or washed 6 times with 10 m*M* ethylenediaminetetraacetic acid 2Na-salt (2Na-EDTA) before they were fixed in 2% osmium tetroxide and were embedded in Epon. Thin sections of the latter group of specimens were stained with uranylacetate and lead citrate.

Results

1. Immunogenicity of antigens

Results in controls of each experiment are given in Fig. 2. None of nontreated and inoculated mice with $(4\sim2)\times10^3$ parasites survived more than 8 days most of them being died 4 or 5 days after challenge and mean survival days of the dead mice were 4.6±0.1. All of the pretreated mice with FCA or FIA alone died within 6 days after challenge given on study day 3. Mean survival days of the dead mice were similar to those of nontreated mice being 4.3±0.2 and 4.1±0.1 in the groups treated with FCA and FIA, respectively. No significant differences in the mean survival days between groups pretreated with adjuvants and nontreated were observed.

Immunogenic activities of differential centrifugal cell fractions of homogenized antigen prepared from nontreated parasites in mice are shown in Fig. 3. All of the mice received CH antigen with FCA were able to overcome the challenge given on day 3, and a similar extent of protection was also demonstrated in mice challenged on day 30. On the other hand, none and 80 per cent of mice challenged on days 1 and 2 respectively, survived. Per cent survival and mean survival days of the dead mice in the groups given SPF antigen and challenged on days 3 and 30 were 10.7 and 16.7%, and 7.4±0.6 and 6.7±0.9 days, respectively. Mean survival days of these and controls differed significantly ($P<0.01$). The above findings suggest that antigens in SPF were incapable of inducing protection in mice but were causative of the prolongation of survival time. MF antigen also displayed similar protection efficacy to that by CH antigen and per cent survival in mice challenged on days 3 and 30 was 96.3 and 90.0, respectively. Moreover, only one of each mouse in two immunization groups consisting of 27 and 11 mice died 14 days after challenge and the longevity was

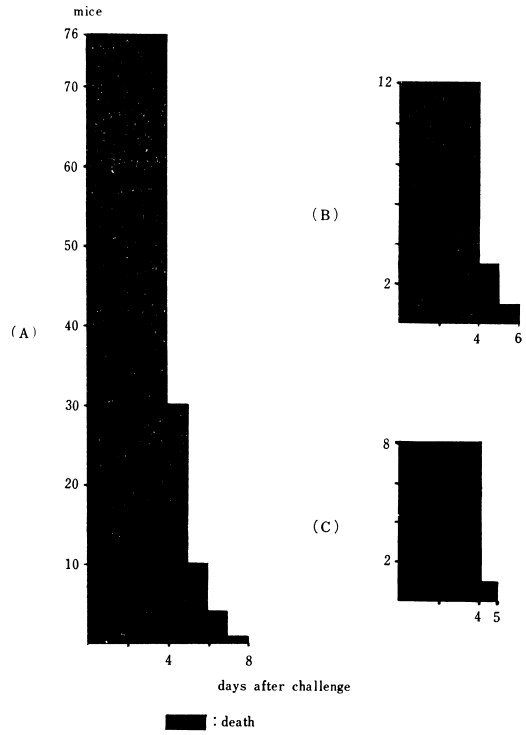

Fig. 2 Pathogenicity of *Trypanosoma gambiense* in mice inoculated with Freund's complete or incomplete adjuvant.
(A) : nontreatment
(B) : treatment with Freund's complete adjuvant
(C) : treatment with Freund's incomplete adjuvant

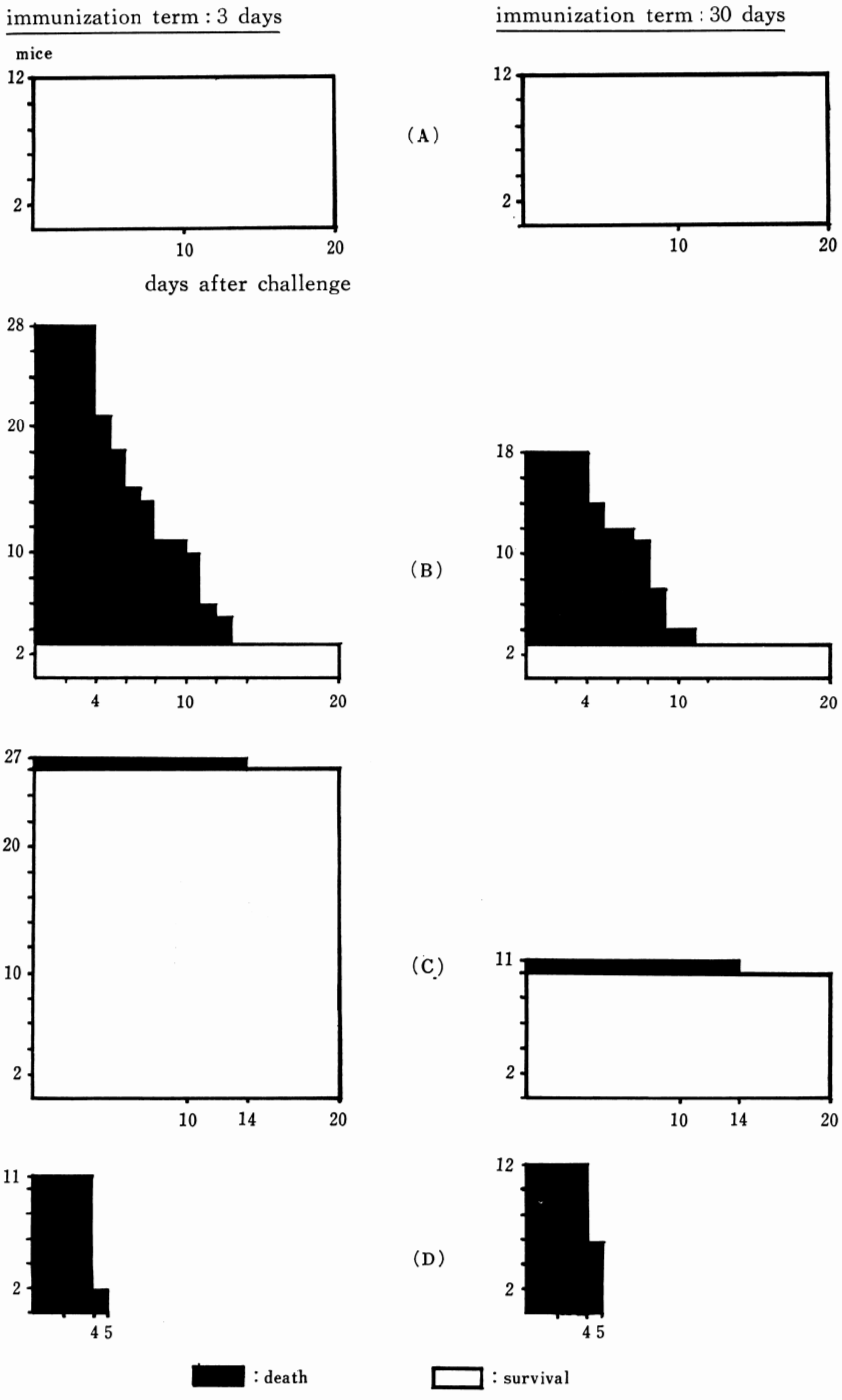

Fig. 3 Immunogenicity of differential centrifugal fractions of homogenized antigen of *Trypanosoma gambiense* in mice.

(A) : cell homogenate (CH) (B) : soluble protein fraction (SPF)
(C) : microsomal fraction (MF) (D) : ribosomal fraction (RF)

greater than in controls. Neither protection nor longevity was induced by immunization with RF antigen obtained by treating MF with sodium deoxycholate.

Mice which conquered the challenge given 3 days after immunization with MF antigen were able to overcome another challenge with organisms of homologous antigenic type even 7 months after the former one. This evidence indicates that specific resistance once acquired to infections of *T. gambiense* is well maintained for a long term. The immunity acquired, however, was found to be completely ineffective against challenges with organisms of heterologous antigenic types.

Effects of treating the parasites with 0.25 % trypsin on immunogenic activity were in-

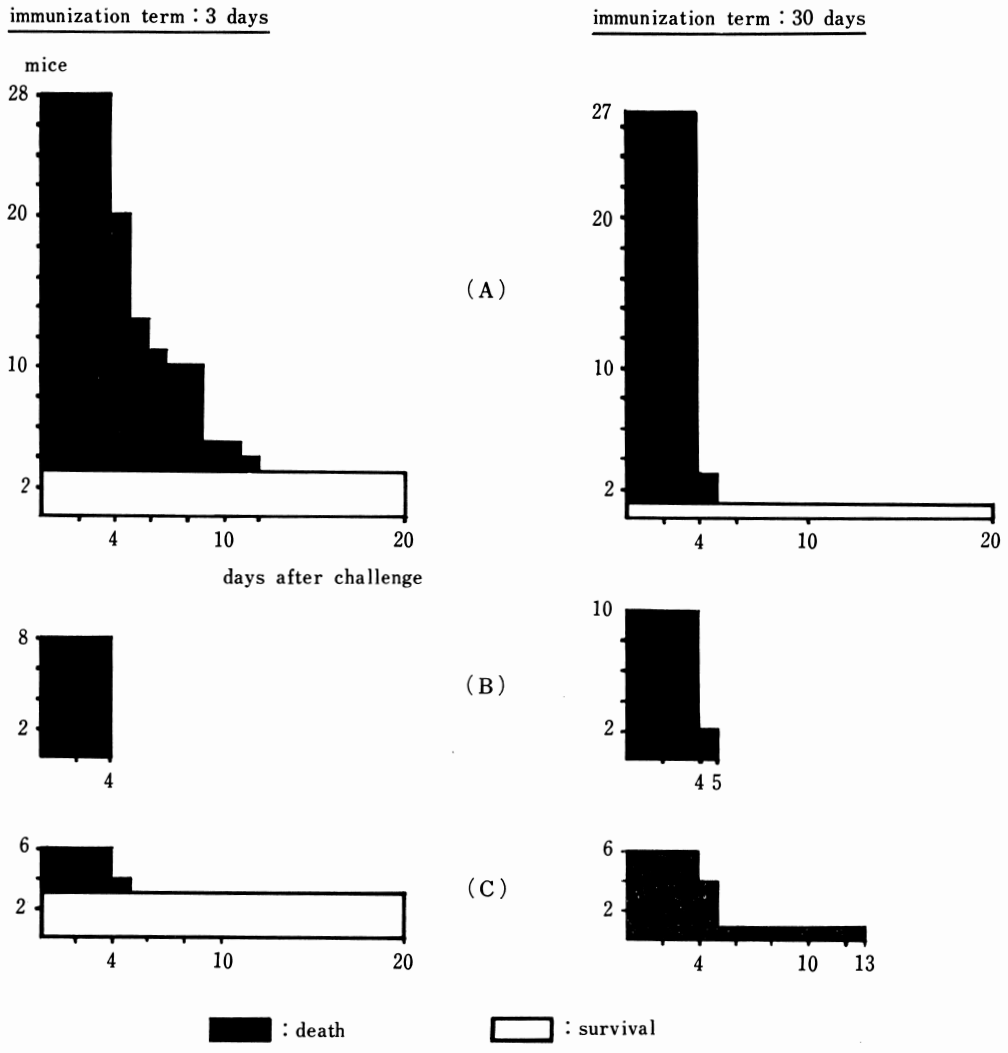

Fig. 4 Immunogenicity of differential centrifugal cell fractions of homogenate antigen of *Trypanosoma gambiense* pretreated with trypsin in mice.

(A) : cell homogenate (TCH)
(B) : soluble protein fraction (TSPF)
(C) : microsomal fraction (TMF)

vestigated. Approximately 40% of the parasites were destructed by treating for 60 minutes but the destruction was less than 10% by treating for not more than 20 minutes and the surface coat was seen removed from the limiting membrane without causing any noticeable damage in the limiting membrane and cytoplasm of the cells under the electron microscope (Fig. 8 B). The immunogenic activity of TCH antigen prepared from the trypsin-treated living parasites was far lower than that of CH antigen and per cent survival in mice given TCH and challenged on day 3 was 10.7 but mean survival days of the dead mice was extended to 6.2±1.4. No protection was induced in mice immunized with TSPF antigen but TMF antigen was proved to be immunogenic and a half of the mice received this antigen survived the challenge made on day 3 (Fig. 4).

From the examination directed to the effect of heat treatment of CH antigen on immunogenic activity in mice, this antigen was found to become ineffective by heating at 60 and 50 C for 30 minutes but a quarter of the mice immunized with heat-treated CH antigen at 40 C for 30 minutes survived and mean survival days of the dead mice were prolonged. Furthermore, one third of the mice challenged on day 30 were able to overcome the infection (Table 1).

Effects of aldehyde fixatives of the parasites on immunogenic activity in mice were studied and the immunogenicity was found to be denatured by 2% FF or GF (Table 1). Approximately one half of the mice immunized with fixed antigens with 1% FF or GF survived and mean survival days of the dead mice were also lengthened. When

Table 1 Effects of treatment of *Trypanosoma gambiense* antigen with heat, formaldehyde, and glutaraldehyde on immunogenic activity in mice

Antigen treatment	No. of mice	Term between immunization and challenge in days	Per cent survival	Mean survival days* of the dead mice
Deating				
40C	12	3	25.0	9.1±1.0
	12	30	66.7	4.3±0.3
50C	13	3	15.0	6.0±0.6
	12	30	0	5.2±0.3
60C	13	3	0	4.6±0.2
	12	30	0	5.3±0.3
Fixation with formaldehyde				
2 %	13	3	0	4.2±0.1
	14	30	0	4.6±0.2
1 %	6	3	66.6	15.0±3.0
0.5%	5	3	100	
	5	30	100	
0.2%	5	3	80	13.0±0
	5	30	100	
Glutaraldehyde				
2 %	9	3	0	6.4±0.9
	9	30	11.1	4.4±0.2
1 %	6	3	50.0	7.7±2.7
0.5%	6	3	66.6	14.5±0
	5	30	100	
0.2%	6	3	100	
	5	30	100	

* : mean survival and standard error

0.5 or 0.2% FF or GF was used as a fixative, 80 to 100 per cent of mice survived showing that immunogenicity of the parasites was not impaired.

2. Immune responses in mice immunized with subcellular components and FCA or FIA

The above results were obtained by immunizing mice with antigens in the presence of FCA. Next, effects of the type of adjuvant on immunogenic activity of subcellular components of the parasites were studied in mice and the results obtained are indicated in Fig. 5. Challenges were given on day 3 and all of the mice immunized with SPF antigen died 4 to 6 days after challenge irrespective of the type of adjuvant added being no better than the results in controls presented in Fig. 2. Immunizations of mice with MF antigen together with FCA or FIA resulted in a mighty resistance to challenges and per cent survival was 75 and 71, respectively. With FIA, 2 of 7 mice died 14 days after challenge and with FCA, 2 of 8 died 13 and 20 days after challenge. These results differed significantly from those of controls and of groups immunized with SPF in the presence of FCA or FIA.

To see immune responses in mice immunized with SPF and MF antigens in the presence of FCA or FIA, body and organ weights of each mouse were measured at intervals of 2 days up to study day 10. All of the immunized mice weighed less than

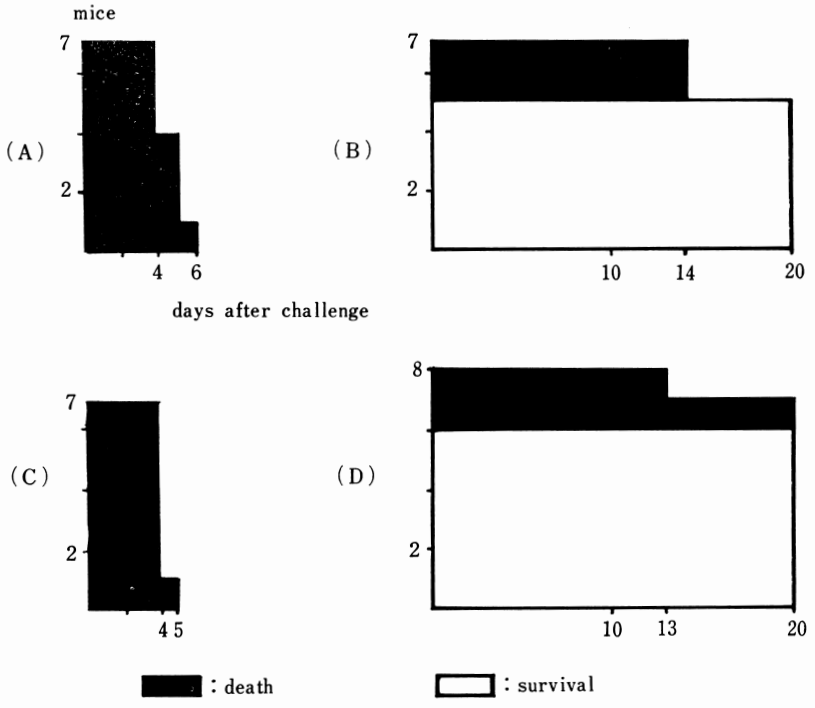

Fig. 5 Effects of Freund's complete or incomplete adjuvant on manifestation of immunogenicity of *Trypanosoma gambiense* subcellular components in mice.
(A) : soluble protein fraction (SPF)+FIA
(B) : microsomal fraction (MF)+FIA
(C) : SPF+FCA
(D) : MF+FCA

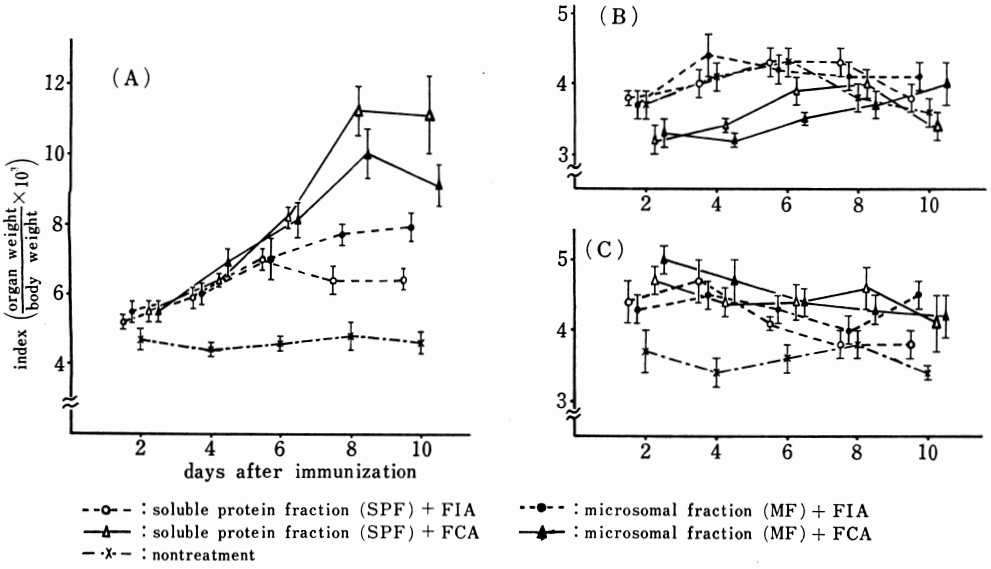

Fig. 6 Fluctuation in spleen, thymus and mesenteric lymph node indices after immunization with subcellular components of *Trypanosoma gambiense* in the presence of Freund's complete or incomplete adjuvant.
(A) : spleen (B) : thymus (C) : mesenteric lymph node

controls on day 2, and thereafter they gained the weight daily reaching approximately the level of controls in 4 more days. The thymus indices of immunized groups with FCA were far smaller than those of groups given FIA and of controls up to day 6 (Fig. 6 B). The mesenteric lymph node indices of immunized groups were similar to each other and were larger than those of controls. As a whole, there were remarkable differences in the index values between immune and control groups ($P<0.01$) except for those of on day 8 in the FCA groups and of on days 2 and 8 in the FIA groups (Fig. 6 C). The values for spleen index were noticeably larger than those for other organs. Conspicuous rises in the index value were seen in all of the experimental groups especially in those immunized with MF on and after day 2. The rise continued up to day 6 and the values became stable showing approximately index 7 in the FIA groups, and the rise in the FCA groups lasted as long as 8 days until the values reached indices 10 to 11. Prominent differences in the spleen index value were noted on days 6 to 10 between FIA and FCA groups but not between SPF and MF groups.

Histopathologic studies on the thymus, TDA and GALT of the spleen and mesenteric lymph node were performed in mice immunized with subcellular components and adjuvants. In the thymus, a slight narrowing of the width of cortex and decreases of cortical lymphocytes were observed up to day 8 in the groups added FCA (Fig. 7 A). In the mesenteric lymph node, a striking increase of lymphocytes was observed in the GALT but no recognizable changes were observable in the TDA (Fig. 7 B). Increases of lymphocytes in the groups added FIA and in the SPF group with FCA lasted for 4 and 4 to 10 days, respectively, and those in the MF group with FCA for 10 days. Moreover, plasma cells were seen increased in the pulp cord of the mesenteric lymph node for 6 days after immunization with MF antigen irrespective of the type of adjuvant employed. Quantitative changes of lymphocytes in the TDA and GALT of the spleen was not intensive for the conspicuous rise of their index values. However, the number

of erythroblasts and immature medium-sized lymphocytes were increased in the red pulp cord of the spleen on days 6 to 10.

Agglutination antibody titers in the serum of mice were measured at intervals of 2 days up to study day 10. In the MF groups with FCA or FIA, positive reaction was noticed in undiluted sera on day 2 and the titer climbed day by day reaching a peak as high as 1 : 8. On the other hand, in the SPF groups with FCA or FIA, positive reaction was observed only in undiluted sera on days 8 to 10.

3. The characteristics of surface coat

Treatment with 0.25% amylase also deprived the limiting membrane of the surface coat (Fig. 8 C) whereas washing the parasites 6 times with PBSG containing 2Na-EDTA failed to remove the coat (Fig. 8 D). The surface coat was found to contain a polysaccharide electron microscopically in the ruthenium red-stained preparation. A stratiform structure was observable occasionally in the surface coat but it was uncertain whether it was an artifact (Fig. 8 E).

Discussion

It has been reported that a complete or at least a partial protection could be obtained against trypanosomes by immunization with homogenate antigen of the parasites (Seed, 1969; Seed and Gam, 1966; Herbert and Lumsden, 1968). In this present experiment, mice immunized with CH antigen (2 mg protein/mouse) and FCA were able to acquire a resistance in a very short time to overcome challenges with the parasites of homologous antigenic type and the resistance was maintained for a long term after immunization. It was also demonstrated that the antigens were highly immunogenic and extremely heat labile. The protectivity induced in mice by homogenate antigen (TCH) prepared from the trypsin-treated parasites was far trifling for that by CH antigen from the nontreated ones. These findings, with the fact that treatment with trypsin deprived the limiting membrane of the surface coat, suggest that one of constituents closely related to the antigen which is protective in *T. gambiense* is contained in the components of surface coat.

Equally sufficient vaccine effects with those by CH antigen were exhibited in mice by MF antigen and 50% of mice immunized with TMF antigen prepared from TCH were able to survive the challenge given 3 days after immunization. Consequently, protective antigen contained in MF antigen is to be originated in the components of the coat. Other reasons why MF antigen was highly immunogenic in mice might be adduced as follows. 1) The recognition of immunogens by lymphoreticular system of the host was easily accomplished since MF antigen was remained insoluble. 2) The membrane structure (lipoprotein) in MF antigen has possibly acted as an adjuvant. From the fact that only mice immunized with CH and MF antigens containing the cell membrane have shown a mighty protection against challenges, however, it is assumed that not only the components of surface coat but also those of cell membrane are to be related to the induction of specific resistance to *T. gambiense* infections in mice.

Only a slightest resistance to the challenge given on days 3 and 30 was demonstrated in mice immunized with SPF antigen and no resistance was noted in mice immunized with TSPF antigen obtained from TCH. Seed (1972), Lanham and Taylor (1972), Taylor and Lanham (1972), and Cross (1975) reported that the components of surface coat could be purified partially from the soluble protein fraction which corresponds to SPF in this experiment and that animals received this antigen were able to overcome the challenge with the parasites of homologous antigenic type when they were given several boosters and long term immunizations. These results differ apparently from the findings shown in Fig. 3 but the above investigators have performed only a single immunization with the antigen without success.

Evidence have recently been presented that the information as "protective antigen" is located in ribosomal particles of microbial cells such as *Mycobacterium tuberculosis* (Youmans and Youmans, 1965), *Salmonella typhimurium* (Johnson, 1972; Venneman *et al.*, 1970), *Vibrio cholerae* (Jensen *et al.*, 1972), and *Diprococcus pneumoniae* (Thompson and Snyder, 1971) and protozoan cells such as *Trichomonas foetus* (Oka *et al.*, 1967, 1970) and *Leishmania enriettii* (Preston and Dumonde, 1971). The mechanisms of protection against infections of these microbial and protozoan cells have been studied from the stand point of cell-mediated immunity. It is of great interest that these organisms have a great deal in common with *T. gambiense*, for instance, they possess a number of antigenic phenotypes and *Leishmania enriettii* is belonged to the same family of trypanosomatidae. In this experiment, however, not a bit of specific resistance to fatal infections with *T. gambiense* was induceable by immunizing mice with RF antigen. Furthermore, no essential differences in per cent survival were observed in mice immunized with a combination of MF antigen and FCA or FIA. Thus, it is to be hypothesized that protection against *T. gambiense* infections in mice is based on humoral immunity. In prospect of possibility that the dissolution of "protective antigen" in MF by treating with sodium deoxycholate is adduced as one of reasons for the ineffectiveness of RF antigens, an immunization test with soluble fraction obtained by treating MF with sodium deoxycholate was performed in mice but all of the mice died before challenge with the parasites and the problems are remained unsolved.

It has been reported that at least an incomplete protection could be obtained against trypanosome infections by immunization with irradiated avirulent parasites (Duxbury and Sadun, 1969; James *et al.*, 1973) and attenuated or killed parasites treated with β-propiolacton or formaldehyde (Soltys, 1967). Lanham and Taylor (1972) reported that the parasites treated with formaldehyde did not give a rise in agglutination antibody in animals. In an experiment where the parasites were treated with 2~0.2% formaldehyde or glutaraldehyde, the higher the concentration of the fixative used, the more the protective activity of the antigen became diminished. Aldehyde of high concentrations reacts with amino groups of protein and the structure of agglutinogens in the surface coat may be subject to alteration. Accordingly, a lower concentration of aldehyde fixatives than 0.5 % must be used for the preparation of killed vaccine of trypanosomes.

Results that the components of surface coat were well stained with ruthenium red and that the coat was removed from the limiting membrane by treating with trypsin suggest that the components may be composed of glycoprotein complex containing acid polysaccharides, and they support previous chemical data on the components of surface coat (Humphryes, 1970; Allsopp *et al.*, 1971; Allsopp and Njogu, 1974) from morphological features. Taylor and Godfrey (1970) reported that even after washing 12 times in phosphate saline-glucose buffer, trypanosomes appeared to be normal and the surface coat was intact. In contrast, Vickerman (1969) reported that extensive washing in saline resulted in removal of the coat. In this present experiment, it was shown electron microscopically that even washing 6 times with buffered solution containing 2Na-EDTA failed to remove the coat from the cell membrane. Furthermore, only a slightest protection was induced by immunization with SPF antigen fractionated from CH. From the above, there is little possibility that the components of surface coat are slovenly combined with cell membrane through the aid of metallic salt.

It is of deep interest that lymphocytes in the GALT of the mesenteric lymph node increased markedly in mice in early immunization with subcellular components for those in the GALT of the spleen. This is to be interpreted that antibody production in the mesenteric lymph node may have

started earlier than in the spleen. In fact, a number of plasma cells were observed in the pulp cord of the mesenteric lymph node of mice immunized with MF antigen and the antibody produced was thought to be IgM. Cornille (1969) reported in his immunocytoadherence test, i.e. rosette formation test in trypanosomes that antibody synthesizing cells increased rapidly in the spleen 5 days after intraperitoneal immunization in rabbits. In the present experiment, however, lymphocytes in the TDA and GALT of the spleen did not increase distinctly in proportion to the rise of index values. A number of immature medium-sized lymphocytes were observed in the red pulp cord of the spleen on and after day 6 but immunological role they may play was remained unidentified.

Many workers have attempted to make clear the mechanisms of protection against trypanosome infections from the stand point of humoral immunity (Seed, 1963; Seed and Gam, 1966; Takayanagi and Enriquez, 1973) and certain indications in their favor were given in this present study. It is problematic, however, whether the invading protozoa were removed only by antibody in vivo because the agglutination antibody titer in sera in early immunization was not sufficiently high, the agglutinating protozoa were being lively for hours and no phenomena such as cytolysis of protozoa were being presented. Patton (1972) and Takayanagi *et al.* (1974) have reported that phagocytosis of trypanosomes by macrophages was enhanced immensely in the presence of specific antibody. Greenblatt and Tyroler (1971) demonstrated that macrophages in the spleen of immune mice to *Trypanosoma lewisi* were highly activated. Takayanagi and Nakatake (1975) pointed out that adherent cell subpopulations in the spleen cell suspension in early immunization were effective in preventing experimental trypanosome infections in nontreated mice. Thus it is hypothesized that the mechanism of protection against experimental trypanosomiasis in mice is based rather on the clearance of the invading parasites in the spleen and some other organs being enhanced by opsonin than on the cytolytic reaction.

The present experiment made it sure that specific antibody against surface coat plays a key role in the protection aginst infections with *T. gambiense* of homologous antigenic type but the problem whether the mechanism of protection, if any, capable of overcoming the challenges with the parasites of all of the antigenic types is based on humoral immunity or cell-mediated immunity is remained unsolved. This is a controversial point to be worked out in the future in relation to the development of vaccination against malaria and other protozoan diseases including African trypanosomiasis.

Summary

Immunogenicity of antigens obtained from *Trypanosoma gambiense* and immune responses in mice immunized with subcellular components of the parasites were examined.

Mice were inoculated with antigens (2 mg protein/mouse) intraperitoneally with an equal volume of Freund's complete or incomplete adjuvant, and $(4\sim2)\times10^3$ parasites of homologous antigenic type were given intravenously 3 and 30 days after immunization to see the protective effects of the antigens.

1. Mice given 10% cell homogenate disrupted first by freeze-thawing with acetone dry-ice and tepid water, and next with a teflon homogenizer were able to overcome the challenges. By heating at 50 C for 30 minutes, the homogenate became ineffective.

2. A comparable extent of protective efficacy was demonstrated by microsomal fraction (144,000×g sediment) of the homogenate prepared by centrifugal differentiation and the efficacy was maintained when Freund's incomplete adjuvant was added to the fraction. On the contrary, mice given soluble protein fraction (144,000×g supernatant) or ribosomal fraction of the homogenate were not able to overcome the challenges.

3. Cell homogenate obtained from the trypsin-treated living parasites also exhibited protectivity although the activity was not as high as that of the homogenate obtained from nontreated parasites. The parasites treated with trypsin were devoid of surface coat. Thus the components of surface coat are probably closely related to the "protective antigen".

4. By fixing with 0.5 and 0.2% formaldehyde or glutaraldehyde, the immunogenicity of the parasites was fully maintained but when treated with 2 per cent of either one of them, the activity was vanished.

5. In the mesenteric lymph node of mice immunized with subcellular components, increases of lymphocytes in the thymus independent area and of plasma cells in the pulp cord were observed in early immunization. No quantitative changes of lymphocytes in the thymus dependent and independent areas of the spleen was noted but spleen index {organ weight (g) $\times 10^3$/body weight (g)} was higher than that of other organs.

6. The surface coat was seen removed from the limiting membrane electron microscopically by treating with 0.25% trypsin or amylase whereas washing even 6 times with buffered solution containing 10 m*M* ethylenediaminetetraacetic acid 2Na-salt failed to remove it from the membrane. The surface coat was found to be a glycoprotein complex containing acid polysaccharide in the ruthenium red-stained preparations.

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Trypanosoma gambiense 遠心画分の免疫原性と宿主の免疫応答

古　谷　正　人
(徳島大学医学部寄生虫学教室)

Trypanosoma gambiense から得た各種抗原の免疫原性と遠心分画物抗原で免疫されたマウスの免疫応答について検討を行つた．抗原は 2 mg タンパク/マウス を等量の Freund の完全アジュバントと共に マウスの 腹腔内に接種し，3 日目及び 30 日目に (4～2) × 10^8 個の同一抗原型原虫を静脈内に接種して免疫効果を判定した．

1. 凍結融解とテフロンホモジナイザー によつて 破砕した原虫の細胞ホモジネート免疫は 3 日 及び 30 日 目の原虫攻撃に対してマウスを耐過生残させる．この能力は 50 C 30 分の熱処理で消失した．

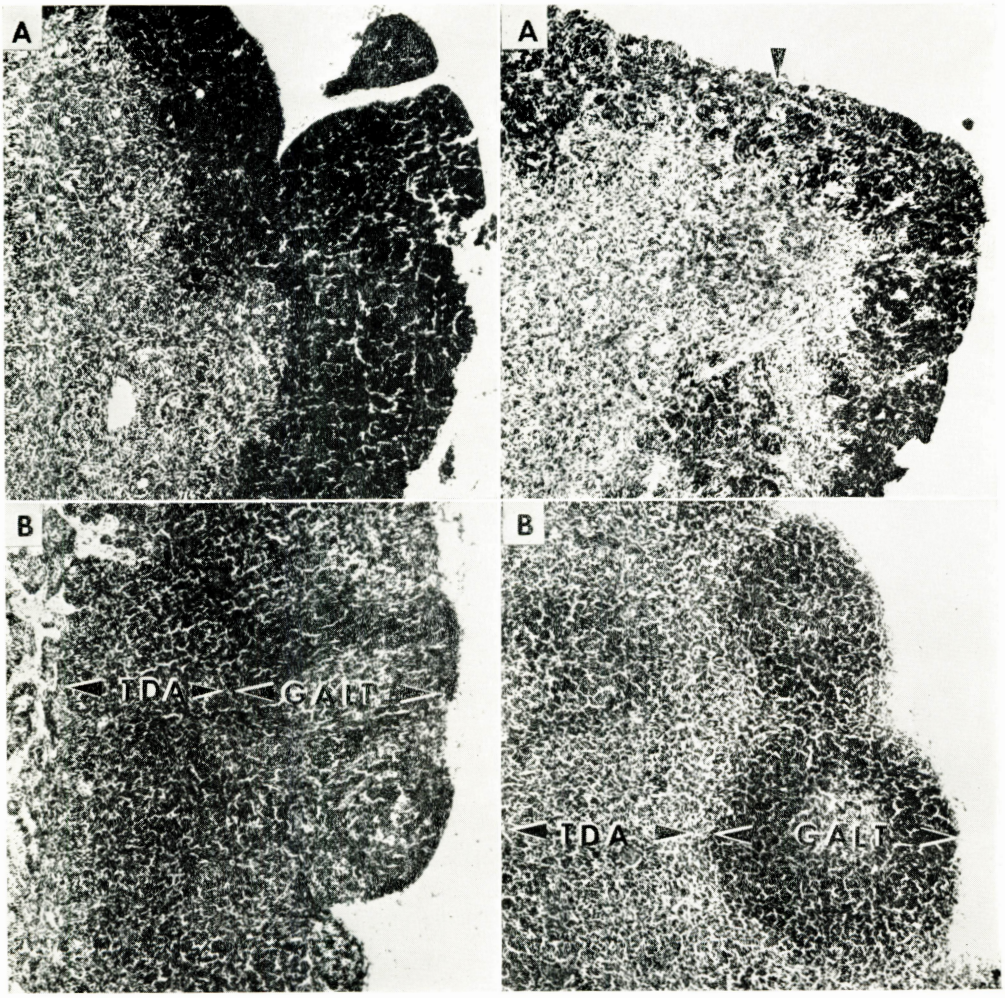
2. 遠心分画抗原で は マイクロソーム 画 分 (144,000 ×g 沈渣) に細胞ホモジネートと 同じ 効果が 認め られた．この効果は Freund の不完全アジュバント を 添加して免疫した場合にも認められた．可溶性タンパク質画分 (144,000×g 上清) 又はリボソーム 画分の免疫では マウスに防御能を付与できなかつた．

3. 0.25% トリプシン処理生原虫からのホモジネートは，完全な原虫から得たものに比べてその免疫原活性が低下していた．更にトリプシン処理原虫は surface coat を欠いていた．これらの結果は surface coat 構成成分が感染防御と密接な関係にあることを示唆した．

4. 2%ホルムアルデヒド又はグルタールアルデヒド処理によつて 原虫の免疫原性は 変性したが，0.5 及び 0.2 %処理ではその能力を保持していた．

5. 遠心分画物抗原で免疫されたマウスの 腸間膜 リンパ節では免疫後早い時期に胸腺非依存領域でのリンパ球の増加と髄索でのプラスマ細胞増加が認められた．脾臓指数 (臓器重量×10^3/体重) は顕著に増加したが，胸腺依存及び非依存領域でのリンパ球はさほど増加しなかつた．

6. surface coat の特性については電子顕微鏡観察も行つた．surface coat は 0.25 % トリプシン あ る い は 0.25 %アミラーゼ処理によつて 原虫細胞膜 から 除去されたが，10 m*M* 2 Na-EDTA を含む溶液での 6 回遠心洗浄でも除去されなかつた．surface coat が酸性多糖類を含む糖タンパク複合体で構成されることがルテニウム赤染色で確認された．



nontreated mice　　immune mice

Fig. 7 Sections of the thymus and mesenteric lymph node of mice immunized with MF antigen of *Trypanosoma gambiense*.

GALT : thymus independent area
TDA : thymus dependent area
A : thymus ; A slight narrowing of the width of cortex and a decrease of cortical lymphocyte are observed in immune mice.
B : mesenteric lymph node ; A striking increase of lymphocytes is observed in GALT in immune mice.

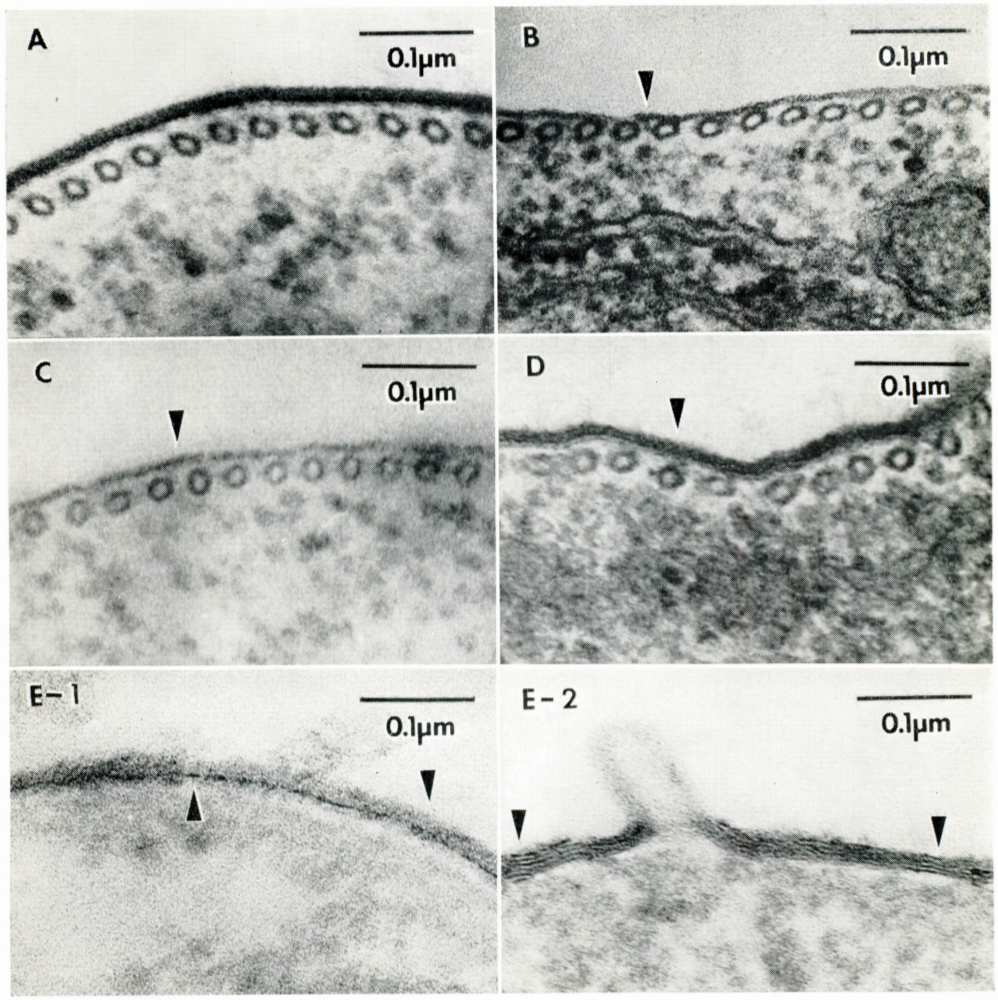


Fig. 8 Electron microphotographs of the surface coat of *Trypanosoma gambiense* treated with 0.25% trypsin, 0.25% amylase, and 10 m*M* 2Na-EDTA, and stained with ruthenium red.

A : nontreatment B : treatment with 0.25 % trypsin for 10 minutes ; The surface coat is completely removed from the limiting membrane (arrow) while no damage is noticeable in the limiting membrane and cytoplasm of the cell. C : treatment with 0.25 % amylase for 10 minutes ; The surface coat is removed from the limiting membrane of the cell (arrow). D : washing 6 times with 10 m*M* 2Na-EDTA ; The surface coat is remained (arrow). E-1 and E-2 : staining with ruthenium red ; The surface coat and limiting membrane of the cell are well stained (arrow) (E-1) and stratiform surface coat (arrow) is observed (E-2).