from the sponges *A. aaptos* and *S. domuncula,* together with the relative retention times (RRT) in gas chromatograph and the percentage of the sterol components. From these results it may be seen that the sponges *A. aaptos* and *S. domuncula,* classified in the order Hadromerida[17], have rather similar sterol composition. They contain stanols widely distributed in the marine environment, cholestanol and 24-ethylcholestanol being the principal sterols present. Minor amounts of cholest-7-en-3β-ol and cholesterol were also present. Nuclear saturated sterols predominate in other species of Hadromerida order[5-7], and their origin is difficult to determine. It was pointed out that they may be arise from dietary Δ^5sterols[3].

1 We thank Prof. L. Boniforti (Istituto Superiore di Sanità, Roma) for CG-MS analysis, Prof. R. Pronzato (Istituto di Zoologia dell'Università di Genova) for identifying the sponges and A. Senatore for G.C. measurements. This research was supported by grants of Ministero P.I. and C.N.R.

2 Goad, L.J., in: Marine Natural Products, vol. 2, p. 75. Ed. P.J. Scheuer. Academic Press, New York 1978.
3 Goad, L.J., Pure appl. Chem. *51* (1981) 837.
4 Djerassi, C., Pure appl. Chem. *53* (1981) 873.
5 Delseth, C., Tolela, L., Scheuer, P.J., Wells, R.J., and Djerassi, C., Helv. chim. Acta *62* (1979) 101.
6 Sica, D., De Simone, F., Senatore, F., and Zollo, F. Gazz. chim. ital. *110* (1980) 147.
7 Sica, D., De Simone, F., and Zollo, F., Gazz. Chim. ital. *108* (1978) 575.
8 Sheikh, Y.M., and Djerassi, C., Tetrahedron *30* (1974) 4095.
9 Kanazawa, A., Teshima, S., and Hyodo, S., Comp. Biochem. Physiol. *62B* (1979) 521.
10 Erdmann, T.R., and Thomson, R.H., Tetrahedron *28* (1972) 5163.
11 Sica, D., De Simone, F., Ramundo, E., and Zollo, F., Biochem. Syst. Ecol. *6* (1978) 77.
12 Khalil, M.W., Djerassi, C., and Sica, D., Steroids *35* (1980) 707.
13 Voogt, P.A., Neth. J. Zool *26* (1976) 84.
14 Dini, A., Minale, L., Pizza, C., Riccio, R., and Zollo, F., Comp. Biochem. Physiol. *71B* (1982) 285.
15 Ballantine, I.A., Lavis, A., and Morris, R.J., Comp. Biochem. Physiol. *63B* (1979) 119.
16 Di Giacomo, G., Dini, A., Falco, B., Marino, A., and Sica, D. Comp. Biochem. Physiol. *74B* (1983) 499.
17 Bergquist, P.R., Sponges. Hutchinson, London 1978.

0014-4754/84/020170-02$1.50 + 0.20/0

Induction of heart alterations by immunization with subcellular fractions from *Crithidia fasciculata*

P.M. Cabeza Meckert, J.J. Cazzulo, E.L. Segura, M. Esteva, A.M. Ruiz, R. Gelpi and R.P. Laguens

Centro de Investigaciones Cardiovasculares, Facultad de Medicina de La Plata, and INDIECH Fatala Chaben, Buenos Aires (Argentina), 14 June 1982

Summary. Immunization of mice with subcellular fractions of *C. fasciculata* led to myocarditis and electrocardiographic alterations similar to those induced by immunization with *T. cruzi,* the etiological agent of Chagas' disease, suggesting the presence of similar cardiotoxic antigens in both trypanosomatid flagellates.

It has been postulated that immunological mechanisms participate in the pathogenesis of tissue damage in Chagas' disease[1]. This opinion is partly supported by the fact that in the mouse[2] and rabbit[3] heart lesions, resembling those appearing in human Chagas' disease, can be induced by immunization with subcellular fractions of *Trypanosoma cruzi,* the etiological agent of South American Trypanosomiasis. In order to determine whether this ability is shared by other trypanosomatid flagellates, experiments were performed in which mice were immunized with subcellular fractions obtained from *Crithidia fasciculata,* an insect parasite not infectious for mammals.

For comparative purposes, *C. fasciculata* was grown in a biphasic culture medium developed for *T. cruzi*[4]. When cultures reached the late logarithmic phase, the cells were harvested by centrifugation at 5000 × g for 15 min at 4 °C, and washed twice in 5 mM KCl 0.25 M sucrose (SKS). The parasites were disrupted by compression-decompression in a Sorvall Ribi Cell Fractionator and the homogenate was fractionated by differential centrifugation, following the experimental protocol developed for *T. cruzi* epimastigotes[5]. The pellets obtained at 5000 × g and 105,000 × g, resus-

Histopathological and electrocardiographic results obtained from mice immunized 3 months before with subcellular fractions of *Crithidia fasciculata*

Immunogen	Number of pathologic electrocardiographs/ total number of mice	Number of mice with myocarditis/total number of mice studied
5,000 × g pellet	1/12	5/11
105,000 × g pellet	7/15	6/15
Cytosol	4/11	3/11
SKS	0/20	0/20
Serum albumin	2/10	0/10
Culture medium	2/13	0/13

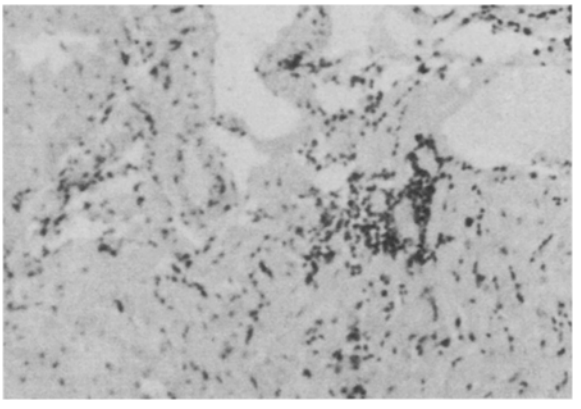
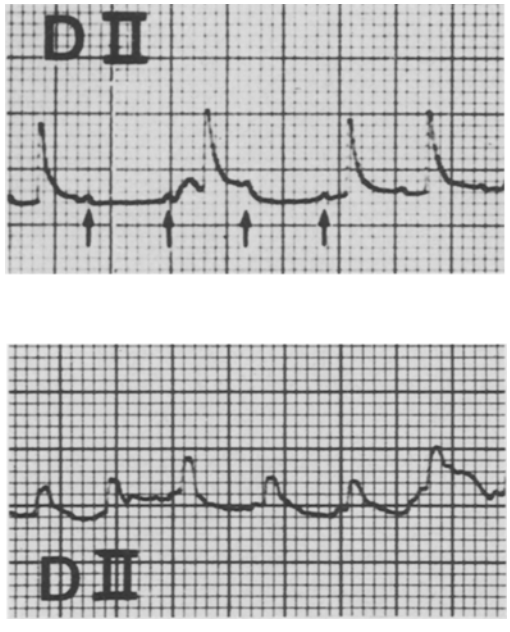


Figure 1. Low power view of atria showing a nodular mononuclear infiltrate in the interstitium. H and E, × 125.

pended in SKS, as well as the 105,000 × g supernatant (cytosol) were inoculated i.p. in 30-day-old Swiss mice, in 3 doses at 15-day intervals. Each animal in the group inoculated with the 5000 × g pellet received in total 0.8 mg of protein[6]; those inoculated with the 105,000 × g pellet and the cytosol received 1.5 and 3.2 mg of protein, respectively. Control mice were injected with culture medium (1.4 mg of protein per mouse), SKS or bovine serum albumin (0.75 mg per animal). Three months after the last immunizing injection electrocardiograms were obtained under light ether anesthesia, and the animals were bled until death. Samples of heart, skeletal muscle and large bowel were obtained for histologic study; the presence of antibodies against *T. cruzi* was studied by direct agglutination[7] and by immunofluorescence, and that of anti-heart and skeletal muscle antibodies was investigated by immunofluorescence techniques. In some cases sera containing anti-striated muscle antibodies were absorbed overnight at 4 °C with *C. fasciculata* 1/1, v/v. Details of these techniques have been published elsewhere[8].



a)

b)

Figure 2. *a* ECG showing an A-V block of 2nd degree in a mouse immunized with cytosol fraction. The arrows points to p waves. *b* Increase in the length of the QRS complex in the ECG of an animal immunized with the 105,000 × g fraction.

Figure 3. Immunofluorescence. Normal murine skeletal muscle incubated with serum from a mouse immunized with 105,000 × g fraction. A brilliant fluorescence can be seen on the sarcolemmal area. × 250.

The histological study showed in about half the animals injected with any of the subcellular fractions from *C. fasciculata* the presence of inflammatory infiltrates in the atrial myocardium. The lesions were nodular and appeared to consist of mononuclears located in the interstitium or beneath the epicardium (fig. 1). No difference in the intensity or cellularity of the infiltrates could be demonstrated in mice injected with the different fractions. The skeletal muscle and intestine did not show alterations. The electrocardiographic study showed definite abnormalities (QRS > 0.04 sec and atrio-ventricular (AV) block, fig. 2) in the groups immunized with the 105,000 × g pellet and cytosol). The incidence of ECG abnormalities in the animals immunized with the 5000 × g pellet, and in the control groups, was significantly lower. The results of the morphological and ECG studies are summarized in the Table. The search for anti-*T. cruzi* antibodies rendered constantly negative results when direct agglutination tests were employed. However, by IF techniques all animals showed cross-reacting antibodies. The highest titers (> 1/132) were found in mice immunized with the 5000 × g pellet. In the remaining groups titers ranged between 1/32-1/64. Anti-skeletal and anti-heart muscle antibodies with a sercolemmal pattern were detected in all mice immunized with the 5000 × g or 105,000 × g pellets (fig. 3).

No anti-muscle antibodies were detected in the animals injected with cytosol, or in the control. Absorption of positive sera with *C. fasciculata* rendered fluorescence negative.

Our results show that immunization with subcellular fractions of *C. fasciculata* leads to heart alterations, both from the morphologic and the electrocardiographic points of view. These observations suggest that this non-pathogenic flagellate shares with *T. cruzi* antigens able to induce heart damage. The presence of antigens common to both species is also supported by the fact that some degree of protection against infection with *T. cruzi* can be obtained by previous immunization with *C. fasciculata*[9]. The fact that some antigens apparently cross-react with striated muscle, as is also the case for *T. cruzi*[10], supports the antigenic similarity between both parasites. However, the fact no anti-*T. cruzi* agglutinating antibodies were found in the sera of the mice immunized with subcellular fractions of *C. fasciculata* indicates that this antigenic similarity is only partial and probably restricted only to intracellular and not to surface antigens. No correlation could be established between the presence of myocarditis and electrocardiographic alterations. In addition, although some fractions (105,000 × g and cytosol) induced both myocarditis and ECG alterations, another fraction (5000 × g) induced only myocarditis. A similar situation exists when mice are immunized with subcellular fractions of *T. cruzi*[2], suggesting that different pathogenetic mechanisms operate for the induction of electric or inflammatory lesions in the heart, and that they are related to different parasite antigens. Although it is possible to induce myocarditis in different species by immunization with pathogenic strains of bacteria, or allo or xenogeneic heart antigens[11], our experiments are the first in which heart damage has been induced by immunization with antigens of a protozoan non pathogenic for mammals.

The taxonomic proximity of *C. fasciculata* and *T. cruzi* gives interest to these studies, not only for the investigation of pathogenetic mechanisms of tissue damage, but also because of the mandatory requirement of investigating the potential harmfulness of immunogens from lower trypanosomatids, if they are to be tested as potential immunogens for the preventation of Chagas' disease.

1 Autoimmunity in Chagas' disease. Editorial, Brit. med. J. *5* (1977) 1243.
2 Segura, E.L., Cabeza Meckert, P., Esteva, M., Gelpi, R., Campanini, A.R., Subias, E., and Laguens, R., Medicina, B. Aires *40* (1980) 807.
3 Teixeira, A.R.L., Teixeira, M.L., and Santos Buch, C.A., Am. J. Path. *80* (1975) 163.
4 Gerez de Burgos, N.M., Burgos, C., Blanco, A., Paulone, I., and Segura, E.L., Acta physiol. latinoam. *26* (1970) 10.
5 Segura, E.L., Vazquez, C., Bronzina, A., Campos, J.M., Cerisola, J.A., and Gonzales Cappa, S.M., J. Protozool. *24* (1977) 540.
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
7 Vattuone, N.H., and Yanovsky, J.V., Exp. Parasitol. *30* (1971) 349.
8 Laguens, R.P., Cabeza Meckert, P., and Gelpi, R., Medicina, B.Aires *41* (1981) 35.
9 Pereira, N.M., Souza, W., Machado, R.D., and Castro, F.T., J. Protozool. *24* (1977) 511.
10 Cossio, P.M., Diez, C., Szarfman, A., Kreutzer, E., Candiolo, B., and Arana, R.M., Circulation *49* (1974) 13.
11 Laufer, A., and Davies, A.M., Methods and Achievments in experimental Pathology, vol.3, p.183. Eds E. Bajusz and C. Jasmin. Karger, Basel/New York 1967.

0014-4754/84/020171-03$1.50 + 0.20/0

Erythrocyte membrane lipid peroxidation in iron deficiency anemia

M. Ramachandran and G.Y.N. Iyer

Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar (Nigeria), 6 January 1983

Summary. Erythrocytes from normal subjects and from cases of iron deficiency anemia were exposed to hydrogen peroxide and the extent of membrane lipid peroxidation studied. Significantly less peroxidation was observed in intact anemic erythrocytes compared to normal. However, when isolated membrane lipids were subjected to peroxidation, there was no significant difference between the two groups. It is unlikely that lipid peroxidation per se plays a major role in the reported decrease in red cell life-span in iron deficiency.

Considerable evidence is available in the literature which suggests that the life-span of erythrocytes tends to decrease in iron deficiency anemia (IDA)[1-8]. Red cell fragmentation is commonly observed in experimental iron deficiency[9]. It has also been reported that hypochromic red cells survive normally in splenectomized but not in normal individuals[10,11]. However, the exact mechanisms underlying the decrease in the life-span of red cells are not known.

Peroxidation of membrane lipids leading to increased membrane rigidity has long been recognized as an important factor underlying the aging and destruction of red cells[12]. We have, therefore, made a comparative study of the susceptibility to peroxidation of membrane lipids of red cells from normal subjects and IDA patients, in an attempt to evaluate the possible role that oxidant injury might play in the reported shortening of the life-span of red cells in IDA.

Methods. The criteria employed in the selection of cases of IDA are detailed in a previous publication[13].

Red cells were routinely washed in normal saline and suspended in phosphate-saline medium (1 vol. of potassium phosphate buffer, 0.1 M, pH 7.3 mixed with 9 vol. of 0.15 M NaCl) for peroxidation studies. Intact red cells were exposed to H_2O_2 which was either added direct to the suspension medium or introduced by vapor diffusion from the center-well of a Warburg flask[14]. The resultant peroxidation of erythrocyte lipids was measured by estimating the malonyldialdehyde (MDA) formed, using the thiobarbituric acid (TBA) reaction described by Stocks and Dormandy[15].

Erythrocyte lipids were extracted and quantitated as follows: Saline-washed, packed red cells were lysed in 4 times their volume of distilled water. An aliquot of the lysate was used to determine the Hb content. The lipids in the remainder of the lysate were extracted as described by De Gier and Van Deenen[16]. An aliquot of the extract was used for estimation of lipid phosphorus according to Connerty et al.[17] and the relation between lipid phosphorus and Hb in the original lysate was established. Using this relation, the lipid phosphorus content of intact red cells in suspension was calculated from their Hb content.

Peroxidation of isolated lipids from intact red cells or from their ghosts was studied as described by Kurien and Iyer[14]. Red cell ghosts were prepared according to Dodge et al.[18]. Reduced glutathione (GSH) was estimated according to the DTNB colorimetric procedure of Beutler et al.[19].

Treatment of erythrocytes with N-ethylmaleimide (NEM) was carried out according to Morrel et al.[20].

Results and comment. The results of a comparative study of

Lipid peroxidation in normal and anemic erythrocytes (nmoles of MDA formed/mg lipid P)

Lipid source	Mode of addition of H_2O_2 Direct	p	Diffusion	p
A) Intact red cells				
1. Normal (10) *	1878 ± 234		1154 ± 165	
2. Anemia (10)	1988 ± 243	N S	838 ± 272	<0.01
3. Normal (NEM-treated) (4)			1726 ± 191	
4. Anemia (NEM-treated) (4)			1625 ± 266	N S
B) Isolated lipids				
From intact red cells				
5. Normal (5)	456 ± 63			
6. Anemia (5)	497 ± 79	N S		
From red cell ghosts				
7. Normal (5)	478 ± 61			
8. Anemia (5)	515 ± 59	N S		

Group A. 4 ml of a 2.5 % suspension of erythrocytes in phosphate buffered saline was mixed directly with H_2O_2 (10 mM final) and incubated at 37 °C for 1 h. In diffusion experiments, 4 ml of the suspension in the main chamber of a Warburg flask was exposed to H_2O_2 vapors distilling from 0.2 ml of 30 % H_2O_2 in the center-well for 6 h (oscillation 90/min). Other details of experiment were the same as described by Kurien and Iyer[14].
Group B. Emulsions of extracted lipids (0.03–0.04 mg lipid P) treated with H_2O_2, added direct (10 mM final) and incubated at 37 °C for 1 h in presence of Fe^{++} (100 μM final).
* Number of experiments in each group given in parenthesis;
N S, difference between the normal and anemia groups not significant.