

branch *D. limbata* biosynthesizes polygodial (1), which constitutes its chemical defense and is stored in the mantle. Thus the ability of a nudibranch to elaborate its chemical defense has been demonstrated. *Dendrodoris limbata* also synthesizes the mixture of sesquiterpenoid esters (2) found in the digestive glands. We believe that these esters should be regarded as products of further metabolism of polygodial as a result of a detoxication process. Polygodial is a reactive molecule that readily interacts with NH₂ groups (16) and therefore could be toxic for *D. limbata* itself if stored for a long time. When the animal is molested, the compound is secreted through the skin. The esters (2), which are found into the hepatopancreas only, could represent the normal excretory metabolites of polygodial.

GUIDO CIMINO
SALVATORE DE ROSA
SALVATORE DE STEFANO
GUIDO SODANO
GUIDO VILLANI

*Istituto per la Chimica di
Molecole di Interesse Biologico,
Consiglio Nazionale delle Ricerche,
Arco Felice, Napoli, Italy*

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14. Radioactive counts were determined in a Tri-Carb liquid scintillation counter (Packard PRIAS, model PLD) equipped with an absolute radioactivity analyzer; the quenching was corrected by external standardization. The samples (0.5 to 2 mg) were dissolved in Insta-Fluor II (Packard) scintillation counting fluid.
15. No attempts were made for the localization of the label at the single carbon atoms of the polygodial skeleton.
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need an effective, nontoxic drug that will kill the parasite and some form of therapy to suppress the major autoimmune mechanisms that produce the lesions of Chagas' disease.

HUMBERTO R. A. CABRAL
*Instituto de Biología Celular,
Facultad de Ciencias Médicas,
Universidad Nacional de Córdoba, C.
Postal 362, 5000 Córdoba, Argentina*

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Rheumatoid Factors and Chagas' Disease

Clarkson and Mellow (1) report that they found antibodies with rheumatoid factor properties in the serum of lactating rats. They point out that such immunoglobulin "accounts for the unusual resistance of previously uninfected lactating rats and their suckling pups to infection with *T. lewisi*," and that "a similar rheumatoid factor . . . which is induced late in the usual course of infection with *T. lewisi* in nonlactating rats, amplifies an earlier IgG response and terminates the infection." They then draw a parallel between *Trypanosoma lewisi* and *T. cruzi* infections and suggest that "it might be possible to treat Chagas' disease by temporarily inducing the production of rheumatoid factor." Although the results of their experiments are interesting, I do not agree with certain aspects of their conclusions and I offer the following comments:

In early work with my colleagues (2) it was shown that humans infected with *T. cruzi* (Chagas' disease) produce rheumatoid factor-like antibodies. Such factors are highly reactive with heterologous γ -globulin (3) and are demonstrable with the Waaler-Rose reaction (4); they are composed of immunoglobulin M (IgM) and are less responsive to human γ -

globulin (3) prepared for the Singer and Plotz test (5). High titers of these rheumatoid factors were found in the serum of 95 percent of patients (infants, children, and adults) from the time of clinical onset (acute phase) of the disease to about 1 year (3). However, these factors were also found, intermittently, in 25 percent of patients with chronic Chagas' disease (3). Such factors were found in patients with or without evident clinical lesions. These findings suggest that there is no correlation between the presence of rheumatoid factor and the course of infection in Chagas' disease, and that IgM therefore plays a marginal role in modulating *T. cruzi* infections.

Although it is possible that the rheumatoid factors produced during Chagas' disease are a natural protective response of the host to *T. cruzi*, it is also possible that these factors, because of their reactivity with γ -globulin, are autoantibodies. There is evidence that several tissue lesions in Chagas' disease result from humoral and cellular autoimmune reactions (3, 6).

It seems unlikely, therefore, that the rheumatoid factors will be useful in the treatment of Chagas' disease. For adequate treatment of this disease we still

Cabral (1) raises doubt about the possible extension of our findings (2) with *Trypanosoma lewisi* in rats to *T. cruzi* in humans. Although we considered this possibility only as a hypothesis to guide future research, there is other evidence that implicates a positive role for rheumatoid factors (RF's) in *Trypanosoma* infections as well as in autoimmune disease.

Cabral notes that RF appears in the serum of most patients with Chagas' disease, generally before the development of a complement fixation titer (CFT), and states that RF is not correlated with protection. However, a negative CFT does not imply absence of parasite-specific immunoglobulin G (IgG). Also rheumatoid factors have a broad range of potential epitope specificities; an immunoglobulin M (IgM) that is specific for one epitope on a particular IgG subclass may be protective, whereas another RF, although generally reactive with bound or aggregated IgG, may not be protective. An analogy can be made to the overall immunoglobulin response to *T. cruzi* and many other infectious agents. With rare exceptions, all infected animals show an increase in serum immunoglobulins whether they resist or succumb to the disease. If one did not know that the specificity of these immu-

noglobulins varied enormously and that passively transferred serum from some infected animals but not others provides protection, one might conclude that immunoglobulins as a whole were ineffective. We induced RF in rats by using Freund's complete adjuvant (FCA) and by using lipopolysaccharide (LPS). The serum of such rats agglutinated IgG-coated *T. lewisi* at reciprocal titers even higher than those observed with serum from noninfected lactating rats [32 to 64 (3) and 4 to 8 (4), respectively]. However, when challenged with *T. lewisi*, such FCA- and LPS-treated rats did not show the enhanced resistance to *T. lewisi* shown by lactating rats. Therefore, the FCA- and LPS-induced RF's are not protective although the lactation- and infection-induced RF are protective (2).

Nemazee and Sato (5) subsequently but independently developed a similar yet broader concept of a positive role for RF. They studied the interactions of monoclonal antibodies and their antigens and observed that some monoclonal RF's interacted only with idiotype-anti-idiotype matrices and that others interacted with idiotype-antigen matrices. They found specificity for a subclass of IgG in their monoclonal RF's which has also been observed in (a polyclonal) RF-containing antiserum (6). Nemazee and Sato referred to these RF's as "enhancing antibodies." Since this term already has a very different meaning (7), we prefer the term "amplifying antibody."

Davis and colleagues (8-11) have found evidence that RF provides protection from the immune complex-induced tissue damage associated with the autoimmune disease systemic lupus erythematosus (SLE). For example, (i) RF can inhibit complement fixation by immune complexes (8); (ii) when RF is injected intraperitoneally into rats together with immune complexes or aggregated IgG, RF enhances their clearance and decreases the observed serum complement depletion (9); (iii) there is a positive correlation between the age of a patient at the onset of SLE and the appearance of RF, and a negative correlation between these and kidney disease associated with immune complex deposition (10); and (iv) RF directly inhibits complement-mediated immune complex deposition on human kidney glomeruli in vitro (11). Indeed, RF has recently been found to protect against immune complex-induced kidney damage in schistosomiasis (12).

More direct experimentation will be required to establish the relation between RF and *T. cruzi* infection. However, we have confirmed and extended the

finding by Krampitz and Disco (13) that lactating mice are more resistant than nonlactating mice to *T. cruzi* (3). Whereas 67 percent of (20 of 30) lactating S/W mice survived challenge with *T. cruzi*, only 18 percent (6 of 34) of nonlactating mice survived. The differences in the patterns of parasitemia in our *T. cruzi*-infected lactating and nonlactating mice (3) were similar to those in our lactating and nonlactating *T. lewisi*-infected rats (4); initially the parasitemias increased at the same rate, but the parasitemias in the lactating mice had a lower peak and a shorter patent period. In *T. lewisi* infections this was due to the lactation-induced RF (2, 4). The time at which the parasitemias in the two groups of *T. cruzi*-infected mice deviate is correlated with the time at which IgG first appears on circulating parasites (14). We transferred protection from lactating to nonlactating mice by injecting 0.3 ml of serum from the former into the latter: nine out of ten mice survived the challenge that was fatal to the ten control mice that received 0.3 ml of serum from nonlactating donors. We suspect, but have not proved, that the transferred protective factor is an RF.

Although not pointed out by Cabral, we did omit a procedural detail in our report (2) which allowed a reasonable alternative explanation of our results. Immunoconglutinins are similar to RF's except that they are directed against bound, activated complement components (ACC) (15). Since we inactivated both of the sera containing the sensitizing IgG and the RF by heating, thus eliminating ACC participation, an immunoconglutinin could not have been responsible for our results.

Although we certainly agree with Ca-

bral that better drugs and a better means of combating the pathology are major goals for research on Chagas' disease, we also believe that new approaches to controlling the disease may be derived from studies not directly associated with *T. cruzi*.

ALLEN B. CLARKSON, JR.

GEORGE H. MELLOW*

Division of Parasitology,
Department of Microbiology, New York
University School of Medicine,
New York 10016

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* Present address: Department of Pathology, University of Medicine and Dentistry of New Jersey, Piscataway 08854.

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Mass Extinctions in the Fossil Record

Raup and Sepkoski (1) statistically examine the marine fossil record to test the proposition that apparent "mass extinctions" might simply represent stochastic variation in an underlying extinction process. They perform a linear regression on the disappearance rates of marine families over the Phanerozoic and conclude that (i) overall extinction rates of families appear to have declined over time and (ii) four events (late Ordovician, Permian, Triassic, and Cretaceous, and possibly a fifth in the late Devonian) are distinct outliers, not adequately explained by the regression model. They postulate that these disappearances are likely to represent phenomena qualitatively

different from the typical extinction processes.

Within the limitations of poor preservation and taxonomic uncertainty, Raup and Sepkoski's conclusion that familial extinction rates have declined over evolutionary history is convincing (Fig. 1A). The second conclusion, that four "mass extinctions" are statistically discernible from the regression appears to depend on the mechanisms assumed for the "background" extinction process. Raup and Sepkoski do not state a mechanistic model of extinction. Their regression technique is based on the assumption that the number of extinctions in each time period is independent of both the