

THE SIGNIFICANCE OF INAPPARENT INFECTIONS IN CHAGAS' DISEASE AND OTHER FORMS OF TRYPANOSOMIASIS

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Introduction: a short tribute to Carlos Chagas and the Brazilian School of Parasitologists

Our understanding of Chagas' Disease has been dominated these last 70 years by the events which took place in 1909 in Lassance, Minas Gerais. The original scepticism which had greeted Chagas' ideas on the pathogenicity to man of *Trypanosoma cruzi* was then entirely replaced by the enthusiasm which followed the baptism of the illness in its various forms under the name of "Chagas' Disease". The ceremony was attended by Oswaldo Cruz and by a number of other eminent Brazilian scientists. The event bears a striking resemblance to the demonstration at Val de Grace of the malaria parasite by Laveran to a circle which included Pasteur and Roux who were entirely convinced thereby.

As an introduction to my paper, I should like to pay a short tribute to Carlos Chagas and to the School of Parasitologists for which he was responsible.

He must have been a very nice man, untouched by ambition except for a desire to ameliorate the lot of his fellow men: the mission of the doctor. His genius

was admired but not envied by his colleagues, and the charisma surrounding him, spread far beyond his country: it was like a beacon shining across the Atlantic to Europe and attracting some of the most famous figures in parasitology to Brazil. He still lived in the age when inspiration was sought in that continent and French was spoken in all intellectual circles here. One of his pleasantest, and most to be copied, characteristics was his insistence that his name as Director should not be added to all the papers emanating from his Institute. In this, he followed the example of his predecessor and spiritual Father, Oswaldo Cruz.

The contributions which Chagas made to *malaria research* have not received their due recognition. He was probably the first (in 1906) to emphasize the paramount importance of the adult female mosquito and its domiciliary habits. This led directly to (1) the use of bed nets and (2) the attack on the domestic anopheline by pyrethrum and later by DDT; the success of these measures is the result of his observations regarding the behaviour of the insect.

Carlos Chagas was the greatest of a famous School of Brazilian parasitologists. It is invidious to mention names, and the following incomplete list only mentions those who have departed this life; first, Chagas' elder son Evandro, then Oswaldo Cruz his master and colleague and his other companions at the Institute — da Rocha Lima (entomologist), de Beaurepaire Aragão (renowned Director of the Institute, who first demonstrated exoerythrocytic schizo-

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gony in 1908 of the common malaria parasite of pigeons — *Haemoproteus columbae*), Gaspar Vianna (the excellent pathologist who revealed in 1911 the true nature of the pseudocysts in heart muscle in Chagas' Disease and who introduced the antimony cure of American leishmaniasis), Travassos ("Grandfather of Brazilian helminthology"), Emmanuel Dias (malariologist and sanitarian, whose son, João Pinto Dias, gave the most remarkable exposition at the Centenary Congress); the names of Pessôa, da Costa Lima, Lutz, Wucherer and Pinotti are familiar to all. I would like to refer also to Flavio da Fonseca who unravelled the life history of so many interesting parasites of animals, Muniz the discover of toucan malaria, Pedreira de Freitas and José Pellegrino (whose tragic end we all so much deplored). Fortunately Brazil still possesses men of the greatest international fame; there are only two men out of many living examples whom I must mention — Torres, still alive and well at the age of 90 years, and of course, the brilliant scientist, Carlos Chagas Filho, who organized this centenary meeting and who is a worthy successor to his immortal Father.

Inapparent infections of *Trypanosoma cruzi*

The subject of my paper, which I introduce with some trepidation, is "Inapparent Infections". These represent the exact opposite of Chagas' original contention of the excessive pathogenicity of *T. cruzi*. There remains today no doubt about the truth of this view, but alongside it "half-hidden like a violet in the hedgerow" — are the people who are symptomless.

It is essential to view the whole spectrum of the infection. Charles Nicolle first put "infections inapparentes" on the map in his lectures on the "Destin des Maladies Infectieuses" in 1933 at the Collège de France. His theories were based on his experiences in Tunisia where he had the opportunity of watching the course of infections of bacteria, protozoa, rickettsia and viruses in man and animals. He concluded that in many of them, e.g. poliomyelitis, leptospirosis, some forms of relapsing fever and brucellosis, the flagrant disease in man was uncommon, and inapparent infec-

tions were the rule; similar examples were noted in animals e.g. typhus in dogs, rinderpest in rabbits, *Trypanosoma equiperdum* in donkeys and *Leishmania tropica* in the gerbil. Many more examples have been discovered since Nicolle's time — I could mention the asymptomatic cases of African trypanosomiasis, the large number of inapparent infections of kala-azar in the epidemic which occurred in Emilia Romagna 5 years ago, the absence of pathogenicity accompanying many infections on *Entamoeba histolytica* and particularly, the predominantly inapparent infections associated with *Toxoplasma gondii*. This organism was discovered simultaneously in 1908 by Nicolle in Tunis and by Splendore in São Paulo in wild rodents and rabbits respectively. The human disease was not recognized until 1923, since when cases have been reported with increasing frequency and with the introduction of the dye test by Sabin and Feldman, the enormous extent of the infections in the human population has been demonstrated; the annual incidence of the disease runs into thousands, but about a quarter of the population of the whole world has the *inapparent infection*. Right at the extreme limit of inapparent infections in man lies *Trypanosoma rangeli* which is always asymptomatic not only in man, but also in many vertebrate animals.

What is the place occupied by Chagas' Disease in this spectrum?

My own very limited experience of *T. cruzi* in the field was first acquired when I visited Brazil in 1955 — specifically to learn a little about the two famous protozoal diseases of the New World: Espundia and Chagas' Disease. Mauro Pereira Barretto was my instructor in chief and he took me to places where these diseases were highly endemic. Naturally he thought that I wanted to see actual cases and he managed to procure a little girl (Sonya) with a typical chagoma. We fed clean *Triatoma infestans* on her ankle and 18 days later we discovered metacyclic trypomastigotes in the faeces. I took the infected triatoma to England and established the so-called Sonya strain (Garnham 1956) in mice. The heart of these animals became heavily parasitised in 10 days after the appearance of *T. cruzi*

in the blood, and the mice died in an oedematous condition after a further week.

Barreto later showed me various people suffering from mega, including a miserable man with *mal de engasgo*. (Incidentally, he kept my nose very much to the grindstone, day and night, but we did have a day off as my visit happened to coincide with the "jaboticaba" season — October — and we gorged on these delicious fruits in a plantation).

But back to work, in Ribeirão Preto where Fritz Köberle enthralled me with his macabre collection of hearts, oesophagus, colon etc. which he had collected at autopsies.

Naturally I came away from this visit fully convinced of the *pathogenicity* of *T. cruzi*. I was very familiar in East Africa in travelling through the "Country of the Blind" (due to onchocerciasis) but here I found myself in the "Land of Sudden Death"!

But nobody on my first trip in Brazil had whispered a word about the inapparent infections: I had only seen the sensational tip of the iceberg. Below this lies a submerged mass of inapparent infections. How great is this mass and how is it to be ascertained?

Probably the best technique is serological, because the demonstration of the organism itself may be difficult. *T. cruzi* is often occult in the blood stream and may even fail to be revealed by xenodiagnosis, culture or inoculation of blood into laboratory animals. Minter et al (1978) compared the efficacy of the various diagnostic procedures in a series of papers, chiefly relating to recent experiences in Bahia and concluded that haemoculture was no less sensitive than xenodiagnosis for chronic infections, provided that susceptible strains of triatomine bugs are used. The specificity of the simpler serological reactions may be doubtful; Kagan et al (1976) showed that the indirect haemagglutination test was the best. They found no evidence of cross reactions in the latter test between *T. cruzi* and *T. rangeli*.

How often in a community are the first signs of Chagas' Disease diagnosed? — e.g. the Chagoma, Romana's sign or the less specific fever and headache? If the signs are visible at all, then strictly the infection is not inapparent, but only undiagnosed. The subsequent course of Chagas' Disease runs from these barely recognizable lesions or entirely without symptoms, through a latent period lasting often for 20 years and terminating in mega or sudden from heart failure.

What we want to know is *which people* with these inapparent infections will eventually develop the severe disease? This subject has of course been much discussed and Aluizio Prata (1975) in a paper on the Natural history of Chagasic cardiomyopathy states that half the patients in the San Felipe study had the "indeterminate" form of the disease and that the heart changes were frequently asymptomatic, stationary or with a very slow evolution (less than 5 per cent). The expression "*forma indeterminada*" should not be considered synonymous with the "infections inapparentis" of Nicolle in which symptoms are *absent* throughout the course of the infection, however protracted.

It is important to estimate to what extent inapparent infections constitute a reservoir — for as Nicolle emphasized, such infections may be very important, especially in young children — he affirmed that they sometimes acted as the genesis of epidemics. There is little information in the literature, but let us examine a few examples:

Cerisola a few years ago quoted a figure of 1% of the total infections in a population in the Argentine which had enough symptoms to attract the attention of their physicians. 99% were inapparent. On the other hand, a WHO Report in 1960 estimated that there were 7 million people with *T. cruzi* and that 20% had electrocardiographic abnormalities compatible with Chagasian lesions. The Report of PAHO in 1974 was probably more realistic when it stated that in the total at that time of 10 million cases the number with the clinical disease was still ill-defined.

Maria Teixeira (1977) carried out a neat little survey in Salvador, in which she examined 982 people living in Triatoma-infested huts; half of these had a negative serology and this group was followed up in 8 examinations over 16 months: 14 became positive in the course of this period — 9 asymptomatic and 5 had the acute disease. Three of the 9 inapparents had a normal ECG — the other 6 all showed abnormalities of the cardiac rhythm in spite of absence of symptoms.

Coura (1975) and his colleagues here kept 260 people (from clinics) under observation in this City for 14 years. They had practically all come from different parts of Brazil and notably from Bahia and Minas Gerais and were all serologically positive. They were classified according to symptoms: 60% had cardiomyopathy and/or mega, while 40% were inapparent; 36% of the symptomatic group gave a positive xenodiagnosis but only 22% of the asymptomatic. These 260 people had all lived outside the endemic zone for many years, and unfortunately no information regarding the early clinical history was available. Thus a certain number of the so-called "inapparent cases" may have exhibited symptoms in the acute phase in childhood. Marsden rightly points out that patients with chronic syndromes often give no history of the acute phase which may have been entirely asymptomatic or have occurred in childhood and been forgotten.

Aristoteles Brasil (1964) went one step further and kept under observation 1000 patients for up to 10 years. He concluded that only 10 per cent of sub-clinical cases of chronic Chagas' Disease, and aged on an average 40 years, developed clinical forms of cardiomyopathy *in spite of abnormal electrocardiograms*. He assumes that the cases evolve much more benignly than is usually thought. However, he qualifies this optimistic view, with the prevision that the severity of the infection varies from place to place; the prognosis is of course worse in Chagas' Disease as compared with "Chagas infection" — Brasil gives a death rate of 43% for the disease and 13% for the inapparent infection.

Anselmi and Moleiro (1974) hesitate about criteria for prognosis, and doubt

the value of the size of the heart, or of ECG changes — sudden death for instance may bear no relation to the degree of cardiomyopathy.

Köberle (1974) on the basis of his histopathological research is convinced that the lesions in the heart or other organs begin very early — during the acute disease, and that the destiny of the patients is determined at that time. Presumably he would extend this idea both to apparent and inapparent infections of *T. cruzi*.

If we assume that inapparent infections are at least as numerous as — and probably much in excess of the clinical disease, the question of prognosis must be of immense interest to the patient — whether he is a laboratory worker who has cut himself doing an autopsy or whether he is an inhabitant of one of the endemic areas of the country.

I do not suppose that this consideration entered into the mind of Berenice, the two-years old patient of Chagas in Lassance in 1909, who had the flagrant disease at that time, who still had trypanosomes in the blood 52 years later but whose electrocardiogram was normal and who had no other apparent Chagasian lesions, except for fantastic delusions about "barbeiros"! Two years later still in 1963, Berenice was exhibited to many of us at the 7th International Congress of Tropical Medicine and Malaria in Rio. João Amilcar Salgado saw her in 1961 and has continued to study her case up to the beginning of 1979, when she was still comparatively well in this anniversary year! He continues to cryopreserve the Berenice strain — the "type"!?

The case of Berenice must be a record, but as she has continued to live in an endemic zone, she may have been re-infected. Maekelt records an example of a healthy man who continued to show *T. cruzi* in the blood after being more than 23 years outside the endemic area.

The situation that I have been briefly described (and there must be many others), can best be classified into 4 groups all serologically positive, as follows:

1. No primary signs or symptoms, but serology becomes positive and *T. cruzi* may be demonstrable; no late manifestations: *the true inapparent infection*.

2. Like 1, but inconspicuous chagoma, Romana's sign, fever or headache; *undiagnosed (mild) Chagas' Disease*.

3. Acute phase was present, but no late manifestations of disease: *Chagas' Disease*. (Berenice case is an example).

4. Acute phase followed by late manifestations usually after a latent or symptomless period: *chronic Chagas' Disease*.

Large surveys of the population in Latin America have been done in the past and are being actively pursued at present in Brazil, Venezuela, Mexico and elsewhere, but the results are rarely classified in the above way, i.e. to indicate inapparent infections as such. The surveys may indicate people who fall into the first 2 categories but unless they are followed up by longitudinal studies information about the last two groups will inevitably be missing.

Factors concerned in susceptibility

There are many factors influencing the insusceptibility of an individual to the *disease* and leading to the state of inapparent infection. The factors responsible for heightened susceptibility may give a clue to our problem, for if these are absent inapparent infections may be more numerous. Zeledon (1974) has discussed these factors but chiefly in relation to disease. I have tried to divide the "negative" factors into 3 groups:

Extrinsic — The number of any pathogenic organisms which are introduced into a host often influences the subsequent course of the infection — a low number followed by slow multiplication enables the immunity mechanism of the host to destroy quickly the invader and the person may either show no symptoms (inapparent infection) or the disease is mild and quickly overcome. There is little direct evidence on whether the introduction of small numbers of metacyclic *T. cruzi* affects the

nature of the infection in man, but immunity starts early and may abort the development. Neva has discussed this subject in detail.

The portal of entry of the parasite may be significant, and this varies from place to place — Romana states that over 90% of infections in Argentine are acquired through the conjunctiva, and I wonder how frequent inapparent infections are in that country. Elsewhere, transmission through the scratched skin or through the mouth may be equally important. These are the natural routes and there may be a smaller number of trypanosomes than in the unnatural routes where the blood is directly invaded e.g. after blood transfusion, laboratory infections or congenital passage. How many transfusions of infected blood are followed by inapparent infection — we only hear of the clinical cases?

Many people including Chagas himself and some of the older workers like Muniz (1962) and Neghme (1962) emphasize the role of repeated infections in the aetiology of mega and cardiomyopathy — but it should be remembered that there is usually a long latent or inapparent period before these syndromes disclose themselves — the insidious destruction of nerve ganglia is often asymptomatic.

Intrinsic factors — Much has been written about the low susceptibility of *T. cruzi* in certain circumstances, without any special reference to the prevalence of inapparent infections. A variety of factors exist and I will discuss a few:

Strain of T. cruzi. In speaking of mega and cardiac syndromes, people always point to this country as, par excellence, the home of the severe forms of Chagas' Disease — just as they do in reference to espondia. Different strains of the parasite have been postulated for the severe and the milder forms and recent work on serology by Araujo and Nunes (1978) and on biochemistry by Miles et al (1979) has at last confirmed the existence of distinctive sero — and zymodemes respectively. Such data may throw light on the identity of inapparent infections. Artificial strains of low virulence have been produced by Menezes (1972) in Ribeirão Preto.

The low virulence of strains of several pathogens including *T. cruzi* seems to be associated with transmission of the parasite directly from a wild animal. If this is true, there should be an excess of inapparent infections in these circumstances.

It is difficult to know if the mildness of *T. cruzi* in Central America, Mexico and the United States is due to special strains, poor vectorial capacity of the triatomid (e.g. Petana 1971) or minimal contact between man and bug. Perez-Reyes is carrying out a large survey of Chagas' Disease in Mexico and relates the low incidence of infection in Oaxaca in certain species of bugs to the mildness of the disease in the population. But as a general rule, the species of triatomid, unlike the species of *Anopheles* in malaria has little relevance to its susceptibility to the parasite, and of course the habits of particular species of bug may not bring them into contact with man.

Other intrinsic factors which may contribute to the failure of *T. cruzi* to elicit a clinical response, includes age, genetic characters in the host, diet and nutrition (see Zeledon 1974), but the evidence is contradictory and little research has been done especially on the genetic aspects until the recent work of Neva and his associates. Prata believes that the severity of clinical forms may be related to *stress*, but says that the importance of nutrition, physical exercise, immunity status etc are factors yet to be determined.

Finally, the climate may be of direct significance – in the words of Zeledon (1974) climate may affect not only the distribution of the vector but also the transmission of the disease. But the subject remains controversial – there are some reports that a low ambient temperature may attenuate a strain of *T. cruzi*; if this could be confirmed, the door might be opened to the production of a vaccine, which by inducing inapparent infections, would have a prophylactic value (see Menezes 1972). But there are more reports – largely based on experiments on laboratory animals – that a high temperature may abolish entirely cardiac involvement. Such were the experiments done by Marinkelle and Rodriguez

(1968), who suggested also that “healthy carriers” of *T. cruzi* were more frequent in warm climates.

T. cruzi as a zoonosis has been much discussed and Prof. Barretto has considered the subject in masterly detail in the Rio Congress. It is desirable to consider this problem from two standpoints (1) the true or primary zoonosis associated with opossums, armadillos and various wild rodents and (2) the secondary zoonosis involving domestic animals such as dogs, cats and domestic rodents. The infection in all or most of the wild animals seems to be inapparent – in contrast, for instance, to the wild canids – e.g. *Lycalopex* etc – which suffer very severely from *Leishmania chagasi*: the zoonotic host of human kala-azar in Brazil. I should like to make a plea, here, that instead of spending so much time in studying infections of *T. cruzi* in laboratory mice, workers should concentrate attention on the behaviour of the parasite in the true host – to opossum etc. etc.

Inapparent infections of T. rangeli

Here in this continent, there is an excellent example of another human trypanosome which is always inapparent and offers a good model for the study of the phenomenon. *T. rangeli* is occult in the human host. This trypanosome is such a curious contrast to *T. cruzi*: *T. cruzi* kills the vertebrate host but is harmless to the invertebrate; *T. rangeli* is inapparent in the vertebrate, but kills the bug! The scientific history of this trypanosome began eleven years after that of *T. cruzi*, when Tejera in Venezuela in 1920 described it from the dejecta of *Rhodnius prolixus*, but human infections were not recognized until 1936 when Romeo de León found four children infected with the trypanosome in Guatemala. The infection is found in most South and Central American countries but the true prevalence is unknown, as *T. rangeli* is the supreme example of an inapparent infection and it is only easily demonstrable by culture or xenodiagnosis. Then, by the use of such methods, the incidence is seen to be high: e.g. Pifano found over 50% of people positive in Venezuela.

Here, may I refer to a method for demonstrating very scanty parasites in the

blood? This is the technique of anion-exchange centrifugation recently devised by Lumsden et al (1979). They have adapted it for field use and it will detect trypanosomes in as low a density as 18 per cu millilitre. They used it in inapparent infection of African trypanosomiases and it seems that it could well be useful in *T. rangeli*.

The question arises: why does *T. rangeli* remain occult in the vertebrate host, including man? The usual explanation is that the parasite does not multiply in the vertebrate — either in the blood or tissues. But even this statement may not be correct, for a wave of parasitaemia has been demonstrated (by Afiez in my laboratory) after a mouse has been bitten by heavily infected *Rhodnius*. This wave rises from the first day up to a density in which as many as two trypanosomes may be visi-

ble per field, under X40 objective, on about the 9th day when the numbers start to decline. How, can this be explained, if not by multiplication — unless there is a cryptic development at the site of the bite? Moreover, Afiez has shown that splenectomy of a baby mouse will result in a doubling of the parasitaemia. These phenomena are only seen after infection *by bite* and not after inoculation of metacyclic trypanosomes either from culture of salivary glands.

Some of these experiments may seem to be “academic” — but I feel that more attention should be paid to “les infections inapparentes de Charles Nicolle”. One objective in malaria control used to be the attainment of a state of premunition, a comparable objection in Chagas’ Disease would be to make all the infections inapparent.

REFERENCES

- AÑEZ, N. (1979) – Personal Communication.
- ANSEMI, A. & MOLEIRO, F. (1974) – Pathogenic Mechanism in Chagas' Cardiomyopathy. In "Trypanosomiasis and Leishmaniasis with special reference to Chagas' disease". Amsterdam, Excerpta Medica. (Ciba Symposium).
- ARAGÃO, H. de B. (1908) – *Arch. Protist.* 12 :154-167.
- ARAUJO, F.G. & NUNES, R.M.B. (1978) – Immunology of Chagas' Disease. III. *Rev. Inst. Med. trop. São Paulo.* 20 :315-317.
- BRASIL, A. (1964) – Proc. XX Confr. Bras. Cardiologíe e Brasilia p. 365-380. Prophylaxia do Impaludismo. *Brasil Med.* 20
- COURA, J.R. (1975) – Evolutive pattern in Chagas' Disease and the life-span of *Trypanosoma cruzi* in human infections. In "International Symposium on New Approaches in American Trypanosomiasis Research" Belo Horizonte PAHO – Washington – p. 378-383.
- GARNHAM, P.C.C. (1956) – *Trans. Roy. Soc. Trop. Med. Hyg.* 50 :613. Isolation of a new strain of *Trypanosoma cruzi*.
- KAGAN, I.G., GOLDSMITH, R.S., ZARATE-CASTAÑEDA, R. & ALLAIN, D.G. (1976) – *Bull. Panam. Hlth. Org.* 12 :341-348.
- KÖBERLE, F. (1974) – Pathogenesis of Chagas' Disease. In "Trypanosomiasis and Leishmaniasis with special reference to Chagas' disease".
- LUMSDEN, W.H.R., KIMBER, C.D., EVANS, D.A. & DOIG, S.J. (1979) – *Trypanosoma brucei*. Miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Trans. Roy. Soc. Trop. Med. Hyg.* 73 :312-317.
- MARINKELLE, C.J. & RODRIGUEZ, E. (1968) – The influence of environmental temperature on pathogenicity of *T. cruzi* in mice. *Exp. Parasit.* 23 :260-263.
- MENEZES, H. (1972) – Imunização ativa contra a tripanossomose subamericana. *Rev. bras. Med.* 10 :519-524.
- MILES, M.A., BARRETT, T.V., HOFF, R.H. & MOTT, K.E. (1979) – Epidemiological studies of Chagas's Disease in Brazil. *Trans. Roy. Soc. Trop. Med. Hyg.* 73 :135-136.
- MINTER, D.M., MINTER-GOEDBLOED, E. & MARSHALL, T.F.C. (1978) – *Trans. Roy. Soc. Trop. Med. Hyg.* 72 :84-91.
- MINTER-GOEDBLOED E., MINTER, D.M & MARSHALL, T.F.C. (1978) – *Trans. Roy. Soc. Trop. Med. Hyg.* 72 :217-225.
- MUNIZ, J. (1962) – Imunidade da Doença de Chagas, An. Congr. Intern. Doença de Chagas, 1003-1063.
- NEGHME, A.R. & SCHENONE, F. (1962) – Enfermedad de Chagas en Chile; Veinte años de investigación. Anais do Congresso Internacional sobre a Doença de Chagas. Vol. III. Rio de Janeiro.
- NICOLLE, C. (1933) – Les infections inapparentes. *Scientia* 181-271.
- PETANA, W.B. (1971) – American trypanosomiasis in British Honduras X. *Ann. Trop. Med. Parasit.* 65 :169-178.
- PRATA, A. (1975) – In "International Symposium on New Approaches in American trypanosomiasis research". Belo Horizonte (Washington PAHO, 191-193).
- SALGADO, J.A. (1962) – Revisão clínica atual do primeiro caso humano descrito da doença de Chagas. *Rev. Inst. Med. trop. São Paulo.* 4 :330-337. (1979, Personal Communication).
- TEIXEIRA, M.G.L.C. (1977) – Thesis "Doença de Chagas: Estudo da forma aguda inaparente". Rio de Janeiro.
- VIANNA, J. (1912) – Tratamento da leishmaniose pelo tártaro emético. *Ann. 7 Congr. Bras. Med. Chirurg.* 4 :426-428.
- VIANNA, J. (1911) – Contribuição da Anatomia patológica da moléstia de Carlos Chagas. *Mem. Inst. Oswaldo Cruz.* 3 :276-294.
- ZELEDON, R. (1974) – Epidemiology of Chagas' Disease. In "Trypanosomiasis and Leishmaniasis with special reference to Chagas' disease". Amsterdam, Excerpta Medica. (Ciba Symposium).