

Pan American Health Organization

PAHO/ACMR 14/12
Original: English

FOURTEENTH MEETING OF THE
ADVISORY COMMITTEE ON MEDICAL RESEARCH

Washington, D.C.
7-10 July 1975

INTERNATIONAL SYMPOSIUM ON NEW APPROACHES IN
AMERICAN TRYPANOSOMIASIS RESEARCH

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, or quoted without the consent of the Pan American Health Organization. The authors alone are responsible for statements expressed in signed papers.

CONTENTS

	Page
I. Introduction	1
II. What is <u>Trypanosoma cruzi</u> ?	1
III. How does <u>T. cruzi</u> circulate in nature?	2
IV. How is <u>T. cruzi</u> transmitted to man?	3
V. How can <u>T. cruzi</u> infection be detected?	5
VI. How is man affected by <u>T. cruzi</u> infection?	7
VII. How is <u>T. cruzi</u> evading the host-immune response?	9
VIII. Can <u>T. cruzi</u> infection be cured?	10
IX. What is known about <u>T. cruzi</u> metabolism?	12
X. How can Chagas' disease be controlled?	14
XI. Papers presented at the Symposium on New Approaches in American Trypanosomiasis Research which are cited in this report	16

INTERNATIONAL SYMPOSIUM ON NEW APPROACHES IN
AMERICAN TRYPANOSOMIASIS RESEARCH*

I. INTRODUCTION

The International Symposium on New Approaches in American Trypanosomiasis Research was held at Belo Horizonte, Brazil, on 18-21 March 1975, under the auspices of the Pan American Health Organization, the Government of Brazil, the Wellcome Trust, and the MEDTRONIC Learning Center. Seventy-five participants and invited guests gathered at the Reitoria of the Federal University of Minas Gerais at Pampulha to review current knowledge in all aspects of Chagas' disease and to identify priorities and neglected areas for future research. Of the 75 participants, 48 (64 percent) were from Brazil (Minas Gerais, São Paulo, Bahia, Rio de Janeiro, Federal District, Pará, and Goiás). Several participants, who were not solely committed to the study of Chagas' disease, were invited with the expectation that they would bring a new and original angle to the discussions.

Below are a few pertinent questions and answers extracted from the 63 oral and background papers presented at the meeting, which represent basic issues in the investigation of the disease.

II. WHAT IS TRYPANOSOMA CRUZI?

There is increasing evidence that T. cruzi is actually a biological complex of parasite species that circulate in nature among humans, domestic animals, wild reservoirs, and sylvatic or domiciliary vectors. Fragmentary information has been derived from laboratory study of parasite populations isolated from known sources. These strains had different morphologic patterns, curves of parasitemia, mortality, tissue tropism, capacity of neuronal destruction, susceptibility to chemotherapeutic agents, and antigenic types. Since some of these characteristics are elusive traits, they

*Prepared by Dr. Zigman Brener, Departamento de Parasitologia, ICB, Universidade Federal de Minas de Gerais, Centro de Pesquisas "René Rachou", INERu, Belo Horizonte, MG, Brazil.

cannot, however, be used as reliable markers for strain identification. Because intraspecific variations may be associated with differences in the molecular structure of enzymes, displaying common biochemical functions, identification of soluble enzymes by electrophoresis (which may detect differences in electronic charges as well as in molecular size) is probably a very promising method for strain identification. Preliminary work by Godfrey (9), using three enzyme systems (glucose-6-phosphate-dehydrogenase; malic enzyme; and phosphoglucose isomerase) provided interesting results with 43 cultured isolates of T. cruzi.

A refinement of this procedure consists in the study of enzyme antigenicity. With double-diffusion of the parasite suspensions and antisera, some precipitin lines retain substrate-specific enzyme activity and lead to strain identification after staining.

Another biochemical method, the DNA buoyant density determination, is being used as a taxonomic criterion for Leishmania species isolates. It was suggested that this technique, together with DNA/RNA hybridization, be used in T. cruzi studies.

These sophisticated methods will be of limited value for T. cruzi strain characterization, unless (1) studies are conducted with unmixed parasite populations from defined and known origin, kept in laboratory under standardized and exacting conditions; and (2) a correlation is tentatively established between the subspeciation process that seems to be occurring in T. cruzi and data on the epidemiology and pathogenicity of Chagas' disease.

The development of cryopreservation methods and of "trypanosome banks", in which T. cruzi strains may be isolated, characterized, and stored at low temperature, should be encouraged. To obtain more homogeneous isolates in the laboratory, studies on the behavior of clone populations derived from single bloodstream or culture forms should also be stimulated.

III. HOW DOES T. CRUZI CIRCULATE IN NATURE?

Natural foci of American trypanosomiasis with infected wild reservoirs and sylvatic triatomine vectors have been detected in a large area of the

American Hemisphere, between 42°N in the United States and 43°S in Argentina. Infection with cruzi-like tripanosomes have been detected in over 100 mammalian species from several orders. Barretto (2) discussed the importance of the displacement of T. cruzi from the natural foci to artificial biotopes and, then, to a domiciliary cycle of the disease. Invasion of those artificial biotopes by infected wild vectors and mammals, domiciliation of wild triatomines in new areas penetrated by man, temporary contact of domestic mammals in natural foci, and intrusion of man into natural foci are some of the mechanisms by which T. cruzi seems to be displaced from the sylvatic to a domiciliary cycle. Pires (20) talked of the potential risks of this sequence of events in areas very recently occupied by man, such as the Amazon region. The profound changes in the environment and the construction of human dwellings favoring vector colonization, may contribute to development of microclimates suitable to the introduction of local or foreign vector species in the house. The internal migration, bringing infected humans to these areas may contribute to create new domiciliary foci of the disease.

New and reliable ecological methods for marking, trapping, and sampling vectors in the field are needed to study bug dispersion and trace possible flows of vectors between sylvatic and domiciliary ecotopes. Some kind of characterization of T. cruzi isolates is also essential to determine the degree of overlapping of domestic and sylvatic cycles and the rate of introduction of "wild" strains into the domiciliary cycle. Miles (16) pointed out that more studies on sylvatic triatomines are necessary to determine which species are likely to emerge as important domestic vectors of T. cruzi and establish new domestic foci following vector control programs.

Rodrigues da Silva (23), on the other hand, described the gradual transformation of American trypanosomiasis into an urban disease as a consequence of the internal migration to the big cities. Prospective studies in such groups of patients living in nonendemic areas may help to evaluate the role of reinfection in the pathogenesis of Chagas' disease.

IV. HOW IS T. CRUZI TRANSMITTED TO MAN?

In endemic areas, transmission of Chagas' disease readily occurs with susceptible humans, active domiciliary vectors, and viable parasites. In other

areas, such as in the United States and the Amazon, the presence of these three elements of the cycle does not necessarily entail human Chagas' disease, and the sylvatic cycle is practically the only one existing there. Chagas' disease is, however, gradually escaping from the strict dependence of the vector, and there has been an increase in other transmission mechanisms such as blood transfusion and congenital disease.

Minter (17) and Zeledón (26) reported on some aspects of vector transmission, which, to date, had been only partially investigated, namely the effects of insect behavior on T. cruzi transmission. Zeledón suggested that a gradient of man-insect relationship exists. It would begin with insects well adapted to houses, pass through species occasionally found in or around houses but unable to thrive there, and, finally, end with totally wild insects.

Species in the evolutionary process of adaptation to the domestic or peridomestic environments, and passive transportation of species with high adaptability may contribute to the expansion of endemic areas and reinvasion of areas under prophylactic control. Minter (17) described vector feeding patterns and host preferences, as indicated by bloodmeal identification.

Rabinovich (21) talked of an ecologic mathematic model ("an imperfect copy of the real world") to study the possible channels by which T. cruzi reaches man and the main characteristics of Chagas' disease transmission. This model would be based on such parameters as number of vectors that can be supported by one host, number of insect bites, rate of natural vector increase, and others. The simplified model simulated a house inhabited by defined numbers of infected and noninfected humans and domestic animals in addition to uninfected insects, and included the possibility of wild population (reservoirs and vectors) migration towards the house.

Blood transfusion is the second most important mode of Chagas' disease transmission in endemic and nonendemic areas. This is probably related to the above mentioned gradual transformation of American trypanosomiasis into an urban disease. In some endemic areas the prevalence of Chagas' disease among possible blood donors has been higher than 20 percent; about 10-20 percent of patients who received blood from chagasic donors became infected. This percentage may be as high as 50-60 in series of hemophilic patients

submitted to repeated transfusions in endemic areas. The importance of the problem may be realized by the fact that in São Paulo about 500,000 blood transfusions are performed every year. Two percent positive donors could provide 10,000 blood samples potentially capable of transmitting Chagas' disease. These samples should be selected either by careful screening of presumptive donors or sterilized by active drugs (4). Good results have been obtained with gentian violet added to banked blood, but there is still room for new colorless, soluble, atoxic, inexpensive, prophylactic drugs. These could be found through specific extensive screening programs, designed to find agents active against bloodstream forms.

In the last few years, over 100 cases of congenital Chagas' disease (half of them from Chile), have been reported in the pediatric and parasitologic literature. This figure is probably an underestimate of the number of cases occurring in endemic areas. Infected newborns often have severe disease with parasitemia and, if untreated, high mortality. Prospective, cooperative studies in endemic areas should be conducted through blood examination and immunofluorescence detection of IgM-specific antibodies. An international registry and long-term follow-up should be established to assess the possibility of mental retardation and learning disabilities in congenital cases.

V. HOW CAN T. CRUZI INFECTION BE DETECTED?

Chronic T. cruzi infection may be diagnosed by a number of serologic tests (complement fixation, immunofluorescence, hemagglutination). These tests have been comparatively evaluated by Camargo (4). Lack of standardization will, however, lead to extensive variations in test sensitivity and specificity. Adequate studies were recommended on the composition of antigenic extracts, antigenic characterization of different strains, and on different developmental stages, using sera from qualified reference centers,

Extensive serum epidemiology surveys require a different technology than do clinical case diagnoses, for which a battery of serologic test is recommended to insure maximal sensitivity. For mass surveys which necessitate testing of hundreds of samples, collection of digital blood samples on

filter-paper is probably the best method, but it should be standardized for immunofluorescence and other serologic techniques. More studies are needed on the practical details regarding the logistics of mass surveys in all or most endemic areas, persistence of antibodies in filter paper under different conditions (temperature, humidity), serologic techniques to be employed, and evaluation of results.

Parasitemia is very low in chronic Chagas' disease, reflecting the steady balance between host and parasite. In this case, trypomastigotes are not found by fresh blood examination or related methods. Hemoculture and inoculation into animals provide very poor results. Xenodiagnosis is still widely used. This is a rather crude method by which clean laboratory-bred bugs are allowed to feed on patients and then are dissected after 1 to 2 months, to look for flagellates in the insects' gut. The positivity rates are low unless large numbers of insects are employed and tests repeatedly performed. Serial xenodiagnosis performed in 508 chronic patients over 3 years' observation, using every month 80 insects, gave an overall positivity of 70.5 percent (6). Studies on the susceptibility of different vector species and investigations on intraspecific differences in the susceptibility of laboratory-reared populations have been recommended. There is some evidence that genetic factors may affect bugs' susceptibility to T. cruzi infection; the progeny of insects that failed to acquire experimental infection were significantly less susceptible than the general population. Selection of populations better fitted to be used in xenodiagnosis could be done this way. Besides, more basic studies on T. cruzi life cycle in the vector, and the factors that can affect its development, such as temperature, presence of endosymbionts, rate of blood digestion, host-parasite relationships between insects and parasites from different geographic origins, should be encouraged.

The presumable potentialities of hemoculture methods were reviewed by Minter-Goedbloed (18). There is a need to develop suitable media and to analyze factors that can handicap the growth of bloodstream forms, such as the presence of antibodies or other inhibitory factors, and the different competence of evolution of the parasites.

Alternative methods like infection of very susceptible cell lines in tissue culture with blood of human patients have also been suggested.

VI. HOW IS MAN AFFECTED BY T. CRUZI INFECTION?

Most patients in the chronic phase of Chagas' disease are asymptomatic. A variable number of patients (around 20-30 percent) present clinical or electrocardiographic evidences of chagasic cardiopathy, which affects mostly people under 40 years of age. In these patients, a large range of alterations, from minor electrocardiographic changes to heart failure and sudden death, are found. Disturbances in the motility of the esophagus and distal colon as well as pathologic dilatations of these organs (megaesophagus and megacolon) have been associated with Chagas' disease in some endemic areas. In a series of 966 patients with mega syndromes in Central Brazil, 90 percent of them had positive serologic tests for Chagas' disease, and half of them showed evidence of chagasic cardiopathy (22).

The pathogenesis of the clinical disorders in Chagas' disease is still controversial. It is now well established, on the basis of clinical and experimental evidences, that T. cruzi causes a marked decrease in the number of ganglionic cells in the CNS but chiefly in the autonomic system of the heart and of the myenteric plexus. The mechanism involved in the neuronal destruction seems to be triggered by substances released by degenerated host cells and/or intracellular parasites that elicit an inflammatory process and produce regressive lesions of the ganglia and nerve fibers (24). Disturbances in the peristalsis of affected esophagus and colon has been explained by hypersecretory activity of the remaining healthy neurons, which apparently increase their synthesis and liberate active biogenous amines (24).

Teixeira (25) suggested, however, that heart lesions could be produced, instead, by a delayed hypersensitivity mechanism. A cytotoxic interaction of T. cruzi-sensitized rabbit lymphocytes with parasitized and nonparasitized allogeneic heart cells was observed in tissue culture experiments. Further studies showed that subcellular fractions of heart cells and of T. cruzi homogenates contain a cross-reacting antigen that induces a strong cell-

mediated immune response and is responsible for the cytotoxic interaction. An autoimmune mechanism would then explain the ongoing tissue injury that takes place during the chronic phase of the disease.

Andrade (1) has studied the pathology of the right bundle branch block associated with left anterior hemiblock, which, in 30-60 percent published reports, was found to be the most common electrocardiographic disorder in Chagas' disease cardiomyopathy. By examination of 2,000 to 6,000 serial sections on transparent plastic tapes of several studied hearts, Andrade confirmed the selectivity of the lesions in the right half of His's bundle, an "enigmatic curiosity" waiting for a plausible explanation. Another unexplained and puzzling condition discussed by Andrade was the relatively frequent occurrence of aneurismal dilatations of the left ventricular apex and thromboembolic phenomena seen with and without congestive heart failure.

Longitudinal and cross-sectional studies to describe the "natural history" of Chagas' disease cardiomyopathy in endemic areas are now underway in some areas of Brazil (19). A 5-year longitudinal study in São Felipe, Bahia, demonstrated that about 5 percent of the cardiomyopathic patients showed an evolution of their cardiac disturbances during each study year and that in all sudden death cases electrocardiographic changes (half of them with complete A-V block) had occurred. Mortality in the T. cruzi infected population was 10.3 per 1,000. About 57 percent of the deaths were directly caused by Chagas' disease and of these, 37.5 percent were from sudden death.

Surveys in Venezuela and Central America showed that mega syndromes are seldom or not at all found in endemic areas, whereas they are extremely frequent in Central Brazil. There is no explanation for these geographical differences. Rezende (22), reported on 1,679 patients with mega syndromes whom he had personally examined in Central Brazil.

Dvorak (7) said that some of the crucial points related to the harmful effects of T. cruzi infection and pathology of Chagas' disease will only be satisfactorily answered when we have a better knowledge of some basic biological facts, such as cell tropism of the parasite, effects of host and/or parasite genetics, and physiology of the disease. An in-vitro system of tissue culture

using a perfusable culture chamber has been developed by Dvorak to study T. cruzi/vertebrate cells interaction. Dynamics of cell invasion and differentiation of intracellular stages are being studied with this system. The probable genetically controlled feedback regulation of intracellular reproduction in various host cells and influence of physical parameters in cell penetration are being studied.

VII. HOW IS T. CRUZI EVADING THE HOST-IMMUNE RESPONSE?

Chronic Chagas' disease is characterized by a balance between host and parasite. No spontaneous cure nor new acute phase occur under normal conditions both in experimental and human disease during the chronic phase. The infected host usually builds up a strong immunity, so that a challenge infection will not induce new outbreaks of high parasitemia. The nature of the immune response is not quite clear. Cell-mediated immunity (CMI) has been detected in vitro, as inhibition of macrophage migration and blast transformation of lymphocytes. In-vitro evidence of CMI participation in the host resistance has been provided by enhancement of infection by neonatal thymectomy and administration of specific antithymocyte sera as well as by transference by sensitized lymphocytes. Activated macrophages were resistant to infections of T. cruzi culture forms (13). More basic investigations are, however, recommended to determine the role of CMI, such as data on specific and unspecific macrophage activation, on adoptive immunity transference of T and B lymphocytes, and selective action of cellular immunity against tissue and bloodstream stages.

A humoral response in Chagas' disease is demonstrated by the early increase of IgM levels and production of agglutinating and precipitating antibodies. Complement fixation, immunofluorescent, and hemagglutination tests are usually positive in the chronic phase. The protective action of serum antibodies, however, remains controversial in spite of a few reports on successful passive transfer of immunity. In-vitro incubation of bloodstream forms with immune sera agglutinates parasites and decreases the infectivity of the treated parasites inoculated into normal hosts (15).

The possibility of parasites being sensitized by specific immunoglobulins and then destroyed by macrophages or complement-dependent lysis should be investigated. The specific role played by both humoral and cellular immunity is so far difficult to define: challenged thymectomized rats present exacerbated high parasitemia despite normal amounts of antibody produced, which suggests that CMI is responsible for the resistance; on the other hand, however, antithymocyte serum apparently does not affect acquired immunity in the chronic phase, which would indicate that humoral immunity plays a fundamental role.

Although the mechanisms of immunity are not yet completely known, it is nevertheless clear that the parasite is equipped to evade the host's immune response and produce life-long infections. Parasite-antigenic variations described in African trypanosomes and Plasmodium infections, have not been so far demonstrated with certainty in T. cruzi infections. Variant antigens, which in the case of T. brucei are glycoproteins found on the surface layer of the bloodstream trypomastigote, have not yet been investigated in T. cruzi. The possibility that parasites bind host-antigens to their surface and thus avoid attachment of specific antibodies by a disguised phenomenon similar to that in schistosomiasis infection should also be investigated.

Prospects for Chagas' disease vaccination have been reviewed by Gonzalez Cappa (10). Attempts to induce acquired immunity by vaccination with parasites killed by physical or chemical agents usually cause only partial or even negligible resistance. Live or attenuated vaccines represent a potential risk of inducing subpatent or cryptic infections that may prove harmful in long-lasting diseases such as American trypanosomiasis.

VIII. CAN T. CRUZI INFECTION BE CURED?

Compounds suitable for use against T. cruzi are still being investigated on an empirical basis (11). Nevertheless, a number of drugs showing suppressive action have been described and some of them have reached the stage of clinical trials. Results are, however, rather disappointing, and no drug is at present available to cure completely Chagas' disease and still be safe in clinical and mass treatment.

In experimental chemotherapy of T. cruzi infections, suppressive drugs, especially nitrofurans, have been shown to eradicate T. cruzi infection when given to animals on long-term schedules. These experimental findings strongly stimulated clinical trials in many endemic areas of Latin America where hundreds of patients had been treated for long periods of time (2-3 months' treatment). Clinical data are not, however, reliable criteria for drug evaluation since irreversible organic damage exists, chiefly in patients in the chronic phase, which is not likely to be affected by treatment. Accordingly, criteria for drug assessment in the chronic phase have been established, as described by Cançado (5). Patients should be divided into two comparable subgroups, one receiving placebo, and the other, medication, on a double-blind basis. Since parasitemia is very low in the chronic phase and some patients may have repeated negative xenodiagnosis (Cançado studied a chagasic patient who had 29 consecutive negative tests), only patients showing a tendency to give repeated positive xenodiagnostic results before treatment should be selected; at least three serologic tests (complement fixation, immunofluorescence, and hemagglutination) should be done in the pretreatment period; after drug administration, xenodiagnosis (with 40-48 insects) and serologic tests should be performed twice a month for at least 1 year, in both groups.

Using nearly the same methods, investigators from Argentina and Brazil employed a nitrofurfurylidene compound in series of chronic patients and reached the following conclusions: in both countries, patients repeatedly had positive serologic tests after treatment; the rate of negative xenodiagnosis after 15-36 months of control was 96.5 percent in Argentina (6), whereas in Brazil it was only 30.8 percent after a control period of 4 years (5). The persistence of positive serologic tests in all treated patients and the discrepancy of results in parasitologic "cures" remain unexplained, which illustrates the difficulties in the assessment of clinical trials in Chagas' disease.

Some experimental evidence show that different strains may display differences in their susceptibility to nitrofurans and nitroimidazole derivatives, a fact that may have implications in human therapy (3).

Gutteridge (11) critically reviewed the current knowledge about experimental chemotherapy of Chagas' disease and confirmed that results were discouraging.

He suggested the following leads for investigation: studies on the biochemistry of intracellular stages of the parasite so that selection of targets for attack and compounds to be tested could be done in a more rational basis; suitable direct screening systems against intracellular stages in cell-free systems; and more data on active drugs' mode of action.

In view of the increasing importance of transmission by blood transfusion, more emphasis should be given to the development of a screening test by which soluble, colorless, stable, nontoxic compounds active against T. cruzi bloodstream forms could be safely added to banked blood.

IX. WHAT IS KNOWN ABOUT T. CRUZI METABOLISM?

Data on the biochemistry of T. cruzi usually applies to culture forms obtained in cell-free media: bloodstream forms cannot as yet be readily separated from blood cells even with anion-exchange columns or other methods, and pure tissue forms obtained from infected vertebrate hosts have only recently been obtained (12).

T. cruzi does not store polysaccharides; its endogenous respiration is supported by utilization of lipids and proteins. A large proportion of glucose is metabolized through glycolysis using a tricarboxylic acid cycle. Two key enzymes of the pentose-phosphate pathway have also been detected. A cytochrome C-containing respiratory chain has been described and a low cytochrome-oxidase activity has been shown in culture, blood, and tissue forms. However, an L- α -glycerophosphate oxidase or equivalent system has not been detected. These findings present a general interest since in salivarian African trypanosomes an alteration of a cytochrome to a noncytochrome metabolic pathway seems to be occurring in different developmental stages of the parasite, the cytochrome system being replaced in certain forms by a cyanide insensitive L- α -glycerophosphate - oxidase system. Such differences in energy metabolism between different stages remain to be demonstrated in T. cruzi cycle. Organelles similar to extramitochondrial peroxisome-like bodies, which usually contains L- α -glycerophosphate oxidase have been described in T. cruzi bloodstream forms but the enzyme activity has not been detected.

Little is known about synthesis or catabolism of proteins. Uptake by active transport of a few aminoacids in culture media have been reported, but nothing is known about their utilization in protein synthesis or about the functioning of ribosomes. The pathways used for the utilization of proteins as a source of energy have not been investigated either but trans-aminase activity was described.

Although lipids account for about 20 percent of T. cruzi dry weight, little is known about their function and metabolism. Composition of the growth media influences the lipid composition of the parasite: there is no cholesterol in flagellates cultivated in cholesterol-free media, but ergosterols are present. Cholesterol seems to be, therefore, of exogenous origin and is not further metabolised. No information is available on fatty acid synthesis nor on fat catabolism pathways for energy production.

T. cruzi is becoming very popular among molecular biologists interested in structure and function of extranuclear or mitochondrial DNA. Like other flagellates, about 20 percent of its total DNA is located in a still obscure organelle, the kinetoplast. Because it is one of the few pathogenic Trypanosomatidae cultivated in cell-free media, T. cruzi has been used as a tool for the investigation of kinetoplast DNA base composition, molecular structure, ability of coding proteins of polypeptides, and function on the biogenesis of mitochondria.

Ultracentrifugation in isopyknic Cs, Cl gradients of culture forms demonstrated the presence of a major and satellite band, with different base composition, which corresponds to nuclear and kinetoplast DNA. Recent work on the two DNA components from culture, bloodstream, and intracellular forms, showed that there is more KDNA in blood forms than in the other two stages. The buoyant densities of both DNA had been extensively studied. DNA-dependent RNA polymerase have not, however, been isolated and characterized and nothing is known about how and when the genetic information contained in KDNA is transcribed and translated.

Studies of the metabolism of nucleic acids in T. cruzi culture forms showed that those stages apparently cannot synthesize purines and pyrimidines de novo and that they depend instead of exogenous preformed purines and

pyrimidines (salvage pathway). Studies with intracellular stages from tissue cultures and isolated amastigotes indicate that a de novo pathway is actually used for purine and pyrimidine synthesis. As the dependence of flagellate forms on preformed pyrimidines is unusual among Trypanosomatidae cultivated in defined media (which obtain pyrimidines by de novo synthesis), this problem should be experimentally reviewed.

X. HOW CAN CHAGAS' DISEASE BE CONTROLLED?

Control of Chagas' disease transmission is usually achieved with residual insecticides such as benzene-hexachloride (BHC) and organophosphorous derivatives. BHC is active for 3 to 6 months and has been widely used in endemic areas of Brazil. Insecticides greatly reduce the vector population and therefore the transmission risks and are still the best approach to Chagas' disease control. Nevertheless, some actual disadvantages can be identified and future risks foreseen as a consequence of the massive and repeated campaigns needed for successful control. Unless dramatic changes are made in rural housing, insecticide spraying will have to be performed repeatedly and areas apparently free of vectors submitted to a long surveillance period, which increases costs. Animal and human populations are likely to accumulate long-lived chlorinated hydrocarbons. Finally, insecticide resistance may emerge in the future. There is already evidence that species of vectors nearly eradicated by insecticides may be replaced by other species after interruption of campaigns.

The possible use of juvenile hormone mimics in vector control has been suggested as a prospective alternative in Chagas' disease prophylaxis (8). Very low doses of such hormones or their analogues block the development to fertile adults; the substances interfere with the metamorphosis of arthropods and related phyla but do not apparently affect mammals and, therefore, there is no great risks of environmental contamination. Some laboratory and field trials with the hormone incorporated into a dust have been performed with encouraging results. More data, however, are necessary to evaluate the percentage of juvenilized bugs and their life span in treated houses.

Cost evaluation, now under investigation, depends on how long the compound will last in field conditions. Prophylaxis of transmission by blood transfusion is still based on the rejection of donors presenting positive serologic tests for Chagas' disease and addition of gentian violet in banked blood. Improvement of serologic tests and selection of drugs to be added to blood have already been discussed. Study of housing improvement methods is very scarce.

XI. PAPERS PRESENTED AT THE SYMPOSIUM ON NEW APPROACHES IN AMERICAN
TRYPANOSOMIASIS RESEARCH WHICH ARE CITED IN THIS REPORT

1. ANDRADE, Z. Pathology of heart lesions in Chagas' disease.
2. BARRETTO, M. P. Possible role of wild mammals and triatomines in the transmission of American trypanosomiasis.
3. BRENER, Z. Aspects of experimental chemotherapy.
4. CAMARGO, M. E. Serological diagnosis of Chagas' disease.
5. CANÇADO, J. R. Clinical trials in Chagas' disease.
6. CERISOLA, J. A. Xenodiagnosis.
7. DVORAK, J. A. Dynamics of cell invasion, biology, and differentiation of intracellular stages.
8. GILBERT, B. Possible use of juvenile hormone mimics in vector control.
9. GODFREY, D. G. Biochemical strain characterization of trypanosomes.
10. GONZALES-CAPPA, S. Antigenic variation, antigenic typing, exoantigens, and prospects for vaccines.
11. GUTTERIDGE, W. E. The biochemistry of Trypanosoma cruzi.
12. ----- . Experimental chemotherapy of Chagas' disease.
13. HOFF, R. Recent advances in cell-mediated immunity to T. cruzi.
14. HOWARD, J. E. Clinical aspects of congenital Chagas' disease.
15. KRETTLI, A. U. and Z. BRENER. Humoral immunity in Chagas' disease (short communication).
16. MILES, M. A. Sylvatic bugs and sylvatic cycles.
17. MINTER, D. M. Feeding patterns of some vector species.
18. MINTER-GOEDBLOED, E. Haemoculture compared with xenodiagnosis for the detection of T. cruzi infection in man and in animals.
19. PRATA, A. Natural history of chagasic cardiopathy.

20. PIRES, F. A. Ecologic methods in animal reservoir studies.
21. RABINOVICH, J. E. Mathematic models and ecology of Chagas' disease.
22. REZENDE, J. M. Regional differences in mega syndromes.
23. RODRIGUES DA SILVA, G. Chagas' disease as an urban problem.
24. TAFURI, W. L. Fine structure of Chagas' disease lesions.
25. TEIXEIRA, A. R. L. Autoimmune mechanisms in Chagas' disease.
26. ZELEDÓN, R. Effects of triatomine behavior on trypanosome transmission.