REPORT OF THE ADVISORY GROUP
ON RESEARCH IN CHAGAS DISEASE

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ADVISORY GROUP ON RESEARCH IN CHAGAS DISEASE

4-7 June 1962, Rio de Janeiro, Brazil

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REPORT OF THE ADVISORY GROUP ON RESEARCH IN CHAGAS' DISEASE

1. INTRODUCTION

The Advisory Group on Research in Chagas' Disease met in Rio de Janeiro, Brazil, June 4-7, 1962.

The meeting was opened by Dr. Hector A. Coll, PAHO Zone V Representative, who welcomed the participants on behalf of the Director of the Pan American Sanitary Bureau and thanked the President of the Brazilian Academy of Sciences, Dr. Artur Moses, for his cooperation in making available the facilities of the Academy.

Dr. Raymond B. Allen, Chief, Office of Research Coordination, PAHO, outlined the research program of the Organization and defined the purposes of the meeting as follows:

a) to evaluate the present status of knowledge on Chagas' disease;
b) to define the most important fields to be studied and to attempt to indicate in each case the type of research best suited for the solution of the problem;
c) to indicate the main studies under way in this field in the Americas.

Dr. Felix Pifano was elected Chairman; Dr. T. Von Brand, Vice Chairman; and Dr. José L. Pedreira de Freitas, Rapporteur.

After approval of the agenda, the activities of the Group were discussed.

* Prepared for the first meeting of the PAHO Advisory Committee on Medical Research, 18-22 June, 1962.
2. PRESENT STATUS OF KNOWLEDGE ON CHAGAS' DISEASE

The fundamental aspects of knowledge on the present status of this problem were summarized in the report of the Study Group on Chagas' disease meeting in Washington, D.C., in March 1960 (WHO Technical Report Series No. 202). This report stresses the seriousness of the problem from the health standpoint not only because of the extensive area affected, which contains an estimated minimum of 7 million infected individuals, but also because increasing knowledge of the disease is bringing to light new pathological processes, thus aggravating the seriousness of the problem under several aspects, including the socio-medical.

The lack of knowledge concerning basic aspects and practical measures of control, as for example those relating to specific therapy for a progressive chronic disease, make the situation even more pressing.

Consequently, it is expected that much will be learned from scientific research in various fields of knowledge and from the contributions of the basic sciences.

3. THE PARASITE

3.1 Characteristics

In reference to the etiological agent, a number of problems were raised concerning the characteristics of this parasite. In effect, although Trypanosoma cruzi (which some authors refer to as Schizotrypanum cruzi) can only be defined zoologically by morphological and ontogenetic criteria, discussion centered on the possibility that this species might actually include a complex of various taxonomic bodies that could only be properly defined by means of other elements. For this reason, data obtained in vivo and in vitro are presented with a view to such a characterization.
Research envisaging a precise definition of variations in such biological attributes as virulence, parasitic specificity, etc., are indicated to determine whether these are actually characteristics linked to genetic patrimony or whether they represent simple variations due to experimental condition. The following criteria are suggested for such a description:

1. Behavior, in vertebrate hosts of different species, with a view to parasitic specificity, virulence as demonstrated through parasitism and mortality, as well as variations in the organ-tropism and other characteristics that may be demonstrated.

2. Behavior in invertebrate hosts, particularly with regard to specific variations of sensitivity.

3. Proof of cross-protection in laboratory animals.

4. Studies on antigenic specificity through agglutination tests following cross absorption and through specific immunochemical techniques in the corresponding topic.

5. Biochemical characteristics in relation to differences in synthesis capacity and studies on the various ways in which carbohydrates and other substances are broken down.

A basic condition for the success of studies in this field and many others is the importance of establishing centers responsible for careful study of a specific number of selected samples and for maintenance of these samples under known conditions to supply accredited researchers.

3.2 CULTURE

To date most methods followed for the culture of T. cruzi have not differed substantially from those used at the beginning of this century. The media are based on whole blood or blood components, occasionally combined with extracts of organs. These conditions make the media difficult to prepare
and unsuited to certain types of research where the possibility of contamination of the organisms by culture media in which they are fixed should be avoided. In recent years some progress has been made in obtaining simpler media, which also make it possible to produce a large quantity of trypanosomes in better conditions for study.

Continued research along these lines is recommended in view of the many benefits it may provide. The need for culture methods to secure predominantly specific cyclic forms present in the vertebrate host should also be stressed.

3.3 NUTRITIONAL REQUIREMENTS AND METABOLISM

As to the nutritional requirements of T. cruzi, the literature shows very few results. This organism has resisted attempts at culture in chemically specific media, which are a necessary condition for proper treatment of the problem.

Inferior trypanosomes, such as Leptomonas, Crithidia, Encopelti, Fasciculata and, more recently, L. tarentolae, were cultivated in a synthetic medium and their nutritional needs established.

In the particular case of higher trypanosomes such as T. cruzi, little known factors must be involved, since it has been demonstrated that it cannot grow in these media. In view of the fundamental nature of information on nutritional needs, especially for its possible importance in orienting therapeutic research, the Group recommends that continuation of this work be encouraged.

The Group was able to analyze the important progress made in the field of carbohydrate metabolism in trypanosomes. In reference to T. cruzi, fundamental aspects are known, as for example characteristics of its metabolic processes and its characteristics that differentiate from trypanosomes of anterior evolution. These biochemical data have made it possible to
establish a correlation with morphological studies on the establishment of phylogenetic relations. Enzymes operating under the Embden-Meyerhoff scheme, as well as the existence of terminal oxidases were demonstrated. Very recently the mechanism of succinic acid accumulation was discovered, and it was demonstrated, with the use of labelled CO\(^2\), that this is actually a partial reversal of the Krebs cycle. Nevertheless, considerable research is still needed, primarily to investigate certain metabolic by-passes on enzymes acting in the metabolism of phosphate pentoses, as well as to investigate the problem of terminal oxidases that are responsible for specific inhibition by cyanide but which are uninhibited by CO.

In addition, consideration should be given to studies on the biosynthesis of nucleotides suggesting possible therapeutic uses. Very recently important studies were carried out on the mechanism of incorporating substances in the medium, demonstrating that in the model of competing carbohydrates active transportation phenomena are involved. Unfortunately, practically nothing is known about the metabolism of proteins and lipids. High concentrations of lipids were found in trypanosomes, including cholesterol and also phospholipids, which were highly concentrated, although nothing is known of the most elementary facts concerning their biosynthesis and breakdown.

4. IMMUNOLOGY

In considering immunity phenomena, the Group emphasized their importance in understanding the pathogenic processes involved in the evolution of \textit{T. cruzi} infection, as well as the contribution they have made to perfecting methods of serological diagnosis, which are particularly important in the chronic phase of Chagas' disease.

A later chapter analyzes the role of immunological phenomena in the
development of pathogenic processes, particularly as related to Chagas cardiopathy, and outlines the research required.

In view of the experimental results already obtained, as well as the demonstration of Sanarelli-Schwartzmann phenomenon in patients with Chagas' disease, it is recommended that more extensive research be carried out in order to show the role of allergic phenomena in the pathogenesis of Chagas' disease.

Two fundamental aspects of immuno-protection phenomena were considered:
1. Their application as a method of study for the characterization of strains, and in attempting to establish the individuality from the genetic standpoint.
2. Their possibilities from the viewpoint of immunization.

The first aspect has already been considered in the preceding chapter. Although it recognizes the limitations of active vaccination with live or killed organisms, from the standpoint of effectiveness and ease of application, the Group recommends that research on this aspect be continued with a view to exploring its possibilities in the future.

The advisability of producing avirulent strains for vaccine experimentation was noted and the need for rigorous long-term control was emphasized, given the chronic nature of Chagas' infection and the T. cruzi visceral tropism.

With regard to passive immunization, it was considered advisable to undertake research designed to secure concentrated specific antibodies, in view of the results already obtained in other infections such as malaria.

The important role of cellular immunity has been demonstrated, among other protective mechanisms against Chagas' infection. The Group recommends that studies designed to explain the intimate mechanism of these processes
and the active antibodies be continued.

Special mention was made of the importance of immunochemical investigations as a contribution to solution of basic problems. Among the problems mentioned were those concerned with finding new diagnostic methods facilitating a more exact evaluation of the magnitude of the problem in the hemisphere as well as a definition of the degree of specific homogeneity of the parasite and the role of hypersensitivity phenomena in the pathogenesis of the disease.

It was acknowledged that these studies are still in a rudimentary phase owing to the inherent difficulties in the study material which has complex characteristics and is difficult to obtain in the large quantities often required.

The following studies were indicated:

1. Antigenic composition of strains, using methods based on diffusion in gel and immunoelectrophoresis, in order to determine possible differences.

2. Antigenetic fractionation and study of the behavior of such fractions vis-à-vis serum from patients suffering from Chagas' disease.

3. Detection and identification of presumed substances with toxic action suggested as the agents responsible for lesions in the vertebrate host.

4. Mechanism of lysis of crithidial forms of T. cruzi cultures by sera from certain animal species.

5. As a basic condition for a number of immunological investigations, it was suggested that research be carried out on methods to obtain sera with an antibody titer higher than that obtained to date.
5. DIAGNOSTIC METHODS

Diagnosis of Chagas' disease can be made by demonstration of the parasite or through search for specific antibodies.

5.1 Parasitological Diagnosis

The main problems open to investigation in this field are:

a. In relation to menodiagnosis, processes that make it possible to increase its sensitivity and to simplify the technique of examination.

b. In relation to in vitro culture, development of media permitting greater efficiency and faster results.

c. In relation to inoculation of laboratory animals, search for more sensitive animals and evaluation of the results of sub-inoculations.

5.2 Immunological Diagnosis

Available information have shown two serological reactions to be the best immunological methods for the diagnosis of Chagas' disease: the precipitin test and the complement fixation test.

The first, which has been used with a fraction extracted from culture forms of T. cruzi by formamide, although it is simple to perform, is limited in application to the acute phase and consequently detects only about 20% of chronic cases. On the other hand, the complement fixation test, for which several antigens and techniques have been employed, despite its reliability for chronic forms, presents difficulties that are inherent in the method, which constitutes an obstacle to its general use, particularly in areas where personnel and material are scarce.

Since the diagnosis of chronic forms has been based on serological methods in view of the difficulty of demonstrating a parasite in its phase,
research should be carried out with a view to obtaining simpler working procedures.

This will require:

1. The preparation of antigens capable of providing more uniform and stable results. Purer antigenetic fractions could help to solve this problem.

2. Based on results obtained in other infections, particularly viral diseases, it is recommended that plate techniques of complement fixation be perfected for the diagnosis of Chagas' disease.

Other tests have been described for the immunological diagnosis of this parasite, such as agglutination, conditioned hemolysis, research on incomplete antibodies, hemagglutination tests or the use of fluorescent antibodies.

Although to date none of these tests has been shown to be superior to those previously considered, they should be perfected or new techniques should be developed.

The difficulty of evaluating therapeutic results in chronic forms, which will be examined below, requires the development of immunological techniques that permit their application for this purpose.

The need for obtaining comparative results in epidemiological surveys in various endemic areas requires the establishment of regional centers that will be responsible for preparing uniform antigens for distribution as standard reference antigens to accredited laboratories. Furthermore, these centers would be responsible for testing antigens prepared in different laboratories.

The PAHO is requested to furnish technical and financial cooperation for the establishment and operation of these centers.
6. PHYSIOPATHOLOGY

In studying the pathology of Chagas' disease the acute form should be dealt with separately from the chronic form. Between these two forms, an intermediate phase, generally designated as latent but better identified as an indeterminate phase, can be considered.

The acute form is characterized basically by a hematic parasitism as a reflex of an extensive and accentuated parasitism of the tissues. Its pathology and pathogeny can, consequently, be explained primarily on a parasitic basis.

The chronic phase is characterized by scant parasitemia, and in many cases it becomes almost impossible to detect the parasite by the usual research procedures. Seeing that there is no known cure for this infection and spontaneous regression is unheard of, and seeing that it is evolutional and often terminates with serious clinical manifestations which are frequently fatal, this phase makes the disease highly important from the public health standpoint.

The indeterminate phase, which varies in duration and is not well known, is characterized basically by a progressive diminishing of parasitemia while specific antibodies can be detected in the blood. Although during this period clinical manifestations may not appear, investigation with more precise diagnostic methods (electrocardiogram, X-rays, etc.) can now reveal the involvement of various organs or systems.

The problem open to research is the pathogenic mechanism by which the chronic form is established, particularly with respect to the possible role of outbursts of parasitemia derived from the infection itself or from superinfections of exogenic origin. This accentuates the need for studying the influence of environmental, nutritional, constitutional, and other factors.
in this evolution.

A solution to this important problem calls for widespread epidemiological longitudinal studies among groups with a high rate of T. cruzi infection.

The best known manifestation in the chronic phase of Chagas' disease is chronic cardiopathy, whose characteristics and seriousness are well defined in numerous publications. However, there are a number of problems open to investigation in this connection:

1. Determination of the percentage of chronic cardiopathies originating from Chagas' disease in Latin America and the importance of other factors in its genesis, together with a determination of other etiological factors, unrelated to T. cruzi, that may produce cardiopathy with similar characteristics.

The Group underlines the advisability of carrying out geographical pathology studies relating to Chagas' disease. Since a broad international study is already underway on the pathology of atherosclerosis, in which many pathologists from various Latin American countries are participating, it is recommended that the PAHO request the persons responsible for this project to extend the study to cardiopathy due to Chagas' disease. To this end, it is suggested that the collection of necroscopic material include the heart and other organs whose comparative anato-pathological examination may help clarify the problem under discussion.

2. Explanation of the similarity, from the anato-pathological standpoint, of cardiopathy due to Chagas' disease and non-specific chronic myocarditis and endomyocardiac fibrosis as described in certain regions of Africa.
For the pathogeny of cardiopathy experimental clinical studies are required to determine the role of:

a. the parasite as direct inflammatory mechanical agent.
b. substances derived from disintegration of the parasite.
c. sensitization phenomena either caused by the parasite or resulting from lesions in the cardiac fiber.
d. other mechanisms that might be responsible, either wholly or in part, for this cardiopathy.

The study of cardiopathy caused by Chagas' disease should cover not only those individuals who have open manifestations of the disease, but also and primarily those who have apparently asymptomatic Chagas' disease. This accentuates the need for having such studies directed by researchers familiar with modern histological and physiopathological techniques as well as with methods of exploring cardio-respiratory hemodynamics.

Aside from the cardiac form, important evidence indicates that in the areas where Chagas' disease is endemic the digestive forms, particularly mega-oesophagus and megacolon, may constitute manifestations of Chagas' disease in its chronic form.

Consideration was given to the importance of studying:

1. The prevalence of digestive manifestations, either mega-oesophagus or megacolon or manifestations of digestive dysrhythmia, among the population of endemic and non-endemic areas of the disease.

The need for establishing uniform criteria for the histopathological and physiopathological characteristics of these manifestations was stressed, with a view to obtaining comparative data. To this end, more objective and more easily performed techniques,
such as the use of substances tagged with radioisotopes, should be applied.

2. The possible role of other causes such as neuroviruses or nutritional factors, as the exclusive or a contributing cause, in the genesis of these digestive manifestations.

3. Existing data on the experimental production of digestive organomegaly are scant, and consequently it is recommended that these studies be extended, particularly with reference to the role played by *T. cruzi* in these processes.

In view of the need to extend these studies and adopt comparative methods, it is recommended that PAHO provide facilities to enable researchers to exchange information through personal contacts, fellowships or travel grants, especially for pathologists, and through specialized training of personnel for this type of investigation.

Since the time of Carlos Chagas the existence of nervous and psychic manifestations has been noted in the chronic phase of Chagas' disease. More extensive studies are still lacking to show the frequency and significance of these processes in human pathology.

Experimental work has shown lesion of the nervous system in *T. cruzi* infection.

It is recommended that clinical and experimental studies on this aspect of the problem be intensified.

Recent studies have revealed important metabolic and functional changes in the chronic phase of Chagas' disease. Basically these changes are characterized by exaggerated response to certain stimuli, including pharmacological stimuli, especially of a cholinergic nature. The results obtained to date indicate that the studies being carried out on metabolic
disturbances should be expanded, especially considering that this important field is still almost unexplored.

7. CHEMOTHERAPY

The problem of effective etiological therapy assumes particular importance in connection with Chagas' disease, considering the chronic nature of this parasitosis and the fact that spontaneous cure has not been verified. Taking into account the fact that in addition to the normal mechanisms of transmission by the vector in nature, inter-human transmission by blood transfusion or from mother to child has already been verified, chemotherapy is of even greater health importance.

Several drugs have been shown to have suppressive action, generally in relation to the forms circulating in the blood in the acute phase. However, to date none of these has been demonstrated as guaranteeing a parasitological cure for the infection.

Most of these drugs can be included in the following chemical groups:

1. Bisquinaldines
2. Trivalent arsenicals
3. Phenantidine:
   a. 6-Methoxy 6-aminoquinolines
4. Nitrofurazones
5. Purine analogues, especially the ribofuranosilpurines

It was emphasized that the suppressive action of these drugs although it is occasionally intense has only been observed in the acute phase of infections both in laboratory animals and in humans. This points up the need for research designed to discover substances which have a more radical action in this phase and which can also act on chronic infection.

The need for establishing rigorous and well-defined criteria for evaluating the therapeutic action of drugs in experimental and human Chagas' disease was underlined.

In the study of new drugs in experimental animals, it is suggested that
the following criteria be taken into consideration:

1. Initial evaluation of therapeutic activity through determination of the levels of parasitemia or the mortality rates for animals inoculated with highly virulent strains.

2. A negative result to an examination of peripheral blood, even when repeated over a long period, does not constitute an indication of parasitological cure.

3. More reliable methods should be used in these apparently cured animals, such as xenodiagnosis, inoculation of young animals and reinoculation of treated animals with the same virulent strain in order to detect immuno-protective phenomena. In determining the activity of drugs proved effective investigation of the cure of treated animals should be supplemented by histological examination of the viscera.

4. The possibility that immunological tests, particularly the complement fixation test, may constitute a valuable additional criteria for the evaluation of therapeutic effect was noted. This is an important problem to be investigated.

5. It was suggested that drugs revealing suppressive action in animals should be used on a prolonged schedule, including the chronic phase. This schedule should be extended to man when the toxicity of the drugs permits, special emphasis being placed on the need for rigorous parasitological and serological control of the results.

6. An additional method used for chemotherapy investigation is the utilization of drugs against *T. cruzi* in tissue culture. More extensive research is necessary in order to evaluate the pos-
sibilities of this method.

Data obtained for *T. cruzi* infections generally reveal a low antibody titer, which has made it difficult to evaluate therapeutic results by serological tests, including the complement fixation test. For this reason, repeated negative results from this test might prove a valuable criterion.

In view of results already obtained from the association of drugs with trypanocidal action with corticoids, it is suggested that new studies be conducted in order to learn more about the nature of the related phenomena and their therapeutic possibilities.

In addition to empirical investigation, it is suggested that studies be undertaken in an attempt to correlate trypanocidal action with anti-metabolic activity.

8. EPIDEMIOLOGY

A better understanding of the various factors involved in the epidemiological structure of Chagas' disease constitutes a vital element in a more objective evaluation of the magnitude of the problem and assigns to it the priority it deserves in the panorama of Latin American health problems, while at the same time it will serve as a basis for a more concentrated attack on this disease.

While recognizing that the great medical and health importance of this protozoosis stems from the high indices of its prevalence and from its very serious effects on man, the fact cannot be ignored, in considering its epidemiology, that since it is a zoonosis it has inherent peculiarities that must also be considered.

An evaluation of the elements involved in the epidemiology of this disease requires:
1. A more precise evaluation of the areas infested by domestic triatomes, with a determination of their indices of infection by T. cruzi.

2. A determination of the prevalence of human infection in these areas by the complement fixation test on samples based on statistical criteria. The selection of these areas should also be based on criteria that will permit a better appreciation of the data obtained.

It is suggested that studies be undertaken with a view to a better evaluation of the results of the complement fixation test on blood collected on filter paper for its use in epidemiology.

3. Where human infection has been verified, evaluation of the seriousness of the disease in samples covering various age groups as shown by a brief clinical examination, an electrocardiogram, particularly for individuals over 20 years of age, and, when possible, a radiological examination, especially of the heart and the alimentary canal.

In order to obtain comparative data that will serve to clarify existing doubts regarding regional differences in the pathology of Chagas' disease, it is highly advisable that these clinical data be secured in a uniform manner.

4. As supplementary information for the evaluation of the magnitude of the problem, notification of acute and chronic cases of this disease should be encouraged.

5. For the same purpose, an attempt will be made to have the antecedent primary cause of death specified in the death certificates of individuals who have died from various manifestations of Chagas' disease.
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5. For the same purpose, an attempt will be made to have the antecedent primary cause of death specified in the death certificates of individuals who have died from various manifestations of Chagas' disease.
Under the chapter on physiopathology, reference has also been made to the advisability of undertaking systematic anatomo-pathological studies in the interests of a better understanding of the role of this disease in the mortality rate for different areas. Studies of this type are already being conducted in a limited area, and it is recommended that this survey be extended to other regions.

Available data on the frequency of Chagas' infection among blood donors and verified cases of accidental transmission by transfusion call for an evaluation of the importance of this problem. This could be done by carrying out seriological surveys among donor candidates and through a careful follow-up of patients receiving blood from individuals who are subsequently found to be chronically infected.

The importance of such research not only in the endemic areas but also in the large centers to which the inhabitants of those areas may move is emphasized.

The fact that cases of transmission of infection from mother to child are being observed on an increasing scale calls for better evaluation of the importance of this mode of transmission through the placenta and through the mother's milk.

The zoonotic nature of Chagas' disease assumes various aspects, with special characteristics that deserve investigation:

1. In the endemic areas with domestic vectors, dogs, cats, and, in certain areas, guinea pigs and, rarely, other domestic animals, have been found to be naturally infected. It is suggested that an evaluation be made through xenodiagnosis of the importance of the first group as a source of infection and that the possible role of the rest be investigated.
2. In other areas where triatomes and non-domestic mammals have been found to be infected by trypanosomes with a morphology similar to *T. cruzi*, it is suggested that a study be made of the conditions of transmission of the infection among these animals. These parasites should be isolated in both vertebrates and triatomes for proper identification and study. This will be a vital element in understanding the conditions under which infection is maintained extradomiciliarily and under which it may be brought into the home.

3. In both areas of domiciliary endemia and others, the vectors and peridomestic reservoirs should be carefully investigated in order to assess their role as a link between extra-domiciliary and domiciliary infection.

In areas where *T. rangeli* has been found, and particularly in others where its natural vectors have been discovered, the use of methods leading to positive identification of this protozoa is recommended, together with more extensive studies permitting a better understanding of its role in human pathology.

Research to investigate possible relationships between the genetic constitution of the individuals and their behavior in relation to Chagas' disease is indicated.

In view of the fact that the influence of environmental temperature on parasitemia in laboratory animals infected with *T. cruzi* has been verified, it is suggested that studies be undertaken with a view to establishing possible correlations between environmental temperature and other climatic factors and the characteristics of infection by this protozoa.
9. VECTOR

In order to combat more effectively the triatome vectors of Chagas' disease, the intensification of studies on the ecology and physiology of these insects is considered to be of primary importance.

From the physiological viewpoint the following research is necessary:
1. A study of the metabolism of the insect.
2. Investigations designed to bring about a better understanding of the relations between this invertebrate and the protozoas it transmits, in order to obtain a more exact knowledge of the parasite-host relationship and of the factors influencing transmission.
3. Studies on the metabolization of insecticides in these triatomes, especially determination of the mechanisms of their action and the possible development of resistance.

Another point for investigation will be the variation in susceptibility of different species and of strains of the same species, including determination of the factors causing these differences in the insecticides.

With a view to a better description of strains that vary in behavior, it is recommended that biochemical studies be conducted such as those already carried out with amino acids in the contents of the alimentary canal of triatomas or other insects.

In the field of ecology, it is advisable that studies be made not only on auto-ecology but also on the ecology of triatome colonies, with special emphasis on regional, and possibly local, peculiarities.

In reference to auto-ecology, it is recommended that special attention be given to reproductive activity, metamorphosis, mobility, feeding frequency and feeding preferences and the influence of variations in environmental conditions on these aspects.
With regard to ecology, emphasis should be placed on factors associated with the dynamics of colonies not only with regard to geographical distribution, dispersion, and displacements, but also to the limiting factors regulating density, natality, longevity, and mortality of these insects.

An important aspect for investigation will be the interaction between these vectors and other populations in the environment.

In addition to the methods that have been used for such studies, it is recommended that research being carried out using radioactive substances be extended.

Other problems for investigation refer to aspects of the morphology of the triatomes through the use of modern microscopic techniques.

10. CONTROL

Results obtained to date over extensive areas of Latin America where residual insecticides have been used against the triatome vector of *T. cruzi* have resulted in a very sharp decrease in the population of these insects in homes, with a marked repercussion on the incidence of human infection.

Although it recognizes the important role that improvement of rural housing accompanied by a higher level of health education of the people could play in a more radical and permanent attack on Chagas' disease, the Group insists upon the urgency of extending and intensifying the use of insecticides because of the immediate results that may be obtained.

Nevertheless, despite the very favorable results obtained to date, chiefly by the application of insecticides in millions of houses in the poorest regions of Latin America, the Group recommended an intensification of studies aimed at a better selection of the insecticides to be used, and precise determination of the extent of the surface area to be sprayed, of the optimum concentrations to be used and, especially, of the intervals
between applications.

Again in relation to the control of vector triatomines, it is suggested that a study be made of the possibilities of applying the method of introducing sterile individuals or carriers of dysgenetic factors.

With regard to rural housing, improved construction represents a radical change of the biotope of the triatomines with a resulting reduction in their density, and thus facilitates their elimination. In addition, this measure contributes substantially to improving living conditions in the endemic areas.

In view of the technical difficulties still presented by the problem of improving rural housing, it is suggested that an effort be made to intensify studies designed either to improve the various types already existing or to obtain economical rural housing unfavorable to the proliferation of triatomines.

As to health education, research should be carried out with a view to elaborating techniques to enlist the population of the endemic areas in the fight against Chagas' disease.

With relation to blood transfusions, the difficulty of excluding donors infected with *T. cruzi* with any degree of certainty makes it advisable that studies on drugs already demonstrated to be effective against *T. cruzi in vitro* in the blood (tri-phenylmethanic dyes) be extended, and that new drugs with such action be procured.

The fact that the presence of *T. cruzi* in mother's milk has already been verified indicates a need for investigations designed to verify the factors involved in such transmission, as well as the frequency of this manifestation, particularly in the chronic form of the disease, and the prophylactic measures indicated.

11. COORDINATION OF ACTIVITIES FOR RESEARCH PROGRAMS

In various scientific fields, the coordination of activities has made
a substantial contribution to the advancement of knowledge either in basic research or applied research. In the particular case of Chagas' disease, the value of such cooperation is obvious not only for the clarification of the problems that have been raised with regard to possible regional differences in the pathology of this disease, but also for the benefits to be derived from a standardization of methods with a view to more widespread use of such methods and a more vigorous campaign against the disease in different areas or countries. The Group believes that PAHO/WHO could play an important part in cooperation by:

1. Contributing to the establishment of centers responsible for producing and controlling antigens for laboratory diagnosis. This task will be greatly facilitated by providing assistance for the development of centers already carrying out this work.

2. Promoting centers to maintain strains of trypanosomes under known conditions and facilitating their exchange.

3. Elaborating standardized diagnostic techniques with a view to comparing the results obtained.

4. Coordinating regional studies in order to obtain more economical and efficient spraying methods, taking into account local and regional peculiarities.

5. Promoting an exchange of information through the granting of fellowships and the organization of meetings of specialists actively interested in the various aspects of the disease.

6. Facilitating the training of technicians and auxiliary personnel to assist in research work.

7. Collecting, analyzing, and disseminating information.
8. Stimulating and supporting studies that require coordinated efforts of research workers in various centers or countries.

9. Cooperating with the several countries in preparing or carrying out research programs by providing specialized consultants.

10. Contributing to a better knowledge of the mortality from Chagas' disease through coordinated anato-pathological work and by placing greater stress on this disease in reporting on mortality data.

11. Assisting research projects on Chagas' disease with its own resources or with those obtained from other international organizations.

12. PRIORITIES

Considering that the establishment of an order of priority for the problems to be investigated will depend largely on local conditions regarding the personnel and material available, the Group lists the following fields of study that should be given preference:

a. Perfection and standardization of diagnostic procedures, primarily because of their importance in evaluating the magnitude of the problem.

b. A broad survey designed to evaluate the true extent and magnitude of the problem.

c. Ecology of vectors with a view to more radical control.

d. Chemotherapy, since to date no therapeutic agent has been found to be really effective against this protozoosis.

e. Prophylaxis, mainly envisaging:

1. perfection of methods of applying insecticides, chiefly designed to discover more economical techniques;

2. discovery of active substances to combat T. cruzi in blood
in vitro.

f. Basic research on correct identification of trypanosomes similar to *T. cruzi* and on the nutrition, metabolism, and immunological behavior of this parasite.