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**COMPARATIVE STUDIES OF
AMERICAN AND AFRICAN
TRYPANOSOMIASIS**

Report of a WHO Scientific Group

WORLD HEALTH ORGANIZATION

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OF AMERICAN AND AFRICAN TRYPANOSOMIASIS

Washington, 11-16 December 1967

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COMPARATIVE STUDIES OF AMERICAN AND AFRICAN TRYPANOSOMIASIS

Report of a WHO Scientific Group

A WHO Scientific Group on Comparative Studies of American and African Trypanosomiasis met in Washington, D.C., from 11 to 16 December 1967. Dr F. C. Goble was elected Chairman, Professor R. Zeledón Vice-Chairman, and Dr M. P. Hutchinson Rapporteur. Dr C. L. Williams, Jr, Deputy Director of the Pan American Health Organization and of the WHO Regional Office for the Americas, opened the meeting on behalf of the Director-General of WHO.

INTRODUCTION

The control of American and African trypanosomiasis involves considerable difficulties, particularly the continental dimensions of the problem, the large numbers of people exposed to the risk of infection, and the lack of a single inexpensive method of mass control that is effective in all epidemiological conditions.

The areas principally affected by the diseases are the developing countries, which are unable to use some of the measures that have been developed for epidemiological investigation, control, and surveillance, since these often involve considerable expense, large numbers of trained field workers, and facilities that are not readily available. Progress in the control of American and African trypanosomiasis will, therefore, depend greatly on co-ordinated research to develop simple and inexpensive control methods.

It was the task of the Scientific Group to review the advances that have been made, to draw attention to techniques for the study of one disease that could be adapted for the study of the other, and to make recommendations for research, with particular attention to areas in which comparative study is possible. It is believed that a comparative approach will help to elucidate some of the aspects in which the two diseases differ, as well as those in which they are similar. Such clarification would be of great value in the development of improved methods of control.

1. GENERAL CONSIDERATIONS

In the Americas, there are two trypanosome species that are infective to man: (1) *Trypanosoma (Schizotrypanum) cruzi*, which is pathogenic to man, but which has also been recovered from a wide range of wild animals of different orders; and (2) *Trypanosoma rangeli*, one of the *Herpetosoma* or *lewisi* group of trypanosomes, which is nonpathogenic and has been recovered from man and several animals. Both these trypanosomes are members of the *Stercoraria* and are cyclically transmitted by triatomid bugs.

Although Chagas' disease, caused by infection with *T. (S.) cruzi*, was first described in 1909, comparatively few cases were reported for a considerable time. However, by 1936, the wider use of serological diagnosis and the recognition of the late clinicopathological effects of the disease had led to a realization of its importance and extensive distribution. The prevalence of Chagas' disease, particularly in South America, is far greater than was formerly supposed, and it has been estimated that up to 7 million people may harbour *T. (S.) cruzi* infections. Although the mortality resulting from the acute form of the disease may be less than 10%, the long-term social and economic effects of the chronic stages are incalculable. Furthermore, the distribution of the arthropod vectors and the occurrence of *T. (S.) cruzi* in animals are more widespread throughout the Americas than is the extent of human infection, presenting a further epidemiological threat.

In Africa, devastating epidemics of human trypanosomiasis have occurred in the past. The two trypanosome species infective to man are members of the *T. (Trypanozoon) brucei* group and are morphologically identical. Both belong to the *Salivaria* group of trypanosomes and are carried by certain species of *Glossina*, in whose salivary glands they complete their cycle of development to the metacyclic form. Throughout the years, control measures involving strict surveillance, early diagnosis, chemotherapy, chemoprophylaxis, and vector control were applied, and in most affected areas the disease was brought under control but not eradicated. However, there is a real danger of a recrudescence of the disease (as has already occurred in some areas) with the cessation or disruption of such control measures. Furthermore, as in America, the distribution of the arthropod vector is considerably wider than the known distribution of human infection.

2. MORPHOLOGICAL AND BIOLOGICAL VARIATIONS BETWEEN SPECIES AND STRAINS

Although some 70 years have passed since Bruce isolated the first of the African trypanosomes, and 60 years since Chagas identified the causative agent of American trypanosomiasis, the designation of species and strains

remains a basic problem. In man and domestic animals, *T. (S.) cruzi* can usually be identified by morphology alone. *T. (H.) rangeli* can be differentiated on morphological grounds and by its invasion of the haemolymph and salivary glands of the vector. In wild animals, however, it is difficult to identify organisms resembling *T. (S.) cruzi*. Host sensitivity, symptomatology, and drug sensitivity are still the guiding principles for the identification of African species.

2.1 Morphology

It is not difficult to differentiate *T. (S.) cruzi* from the African species on the basis of morphological characteristics.

T. (S.) cruzi

The blood-stream form of *T. (S.) cruzi* is smaller than the African species that are pathogenic for man, is nondividing, and is characterized by its very large kinetoplast. It is easy to differentiate *T. (S.) cruzi* and *T. (H.) rangeli* on morphological grounds; identification can also be made by xenodiagnosis.

T. (T.) gambiense and *T. (T.) rhodesiense*

T. (T.) gambiense and *T. (T.) rhodesiense* are polymorphic species of the *T. (T.) brucei* group that multiply in the blood. They can be distinguished from the morphologically identical species *T. (T.) brucei* only by the infection of human volunteers. Organisms of the *T. (T.) brucei* group can be distinguished morphologically from *T. (Duttonella) vivax* and *T. (Nannomonas) congolense*. Their smaller kinetoplast, longer body, and rounded posterior end differentiate them from *T. (S.) cruzi* and *T. (H.) rangeli*, whose large kinetoplast lies near the pointed posterior end.

Biometric studies of African and American trypanosomes may prove to be of value in distinguishing strains with different behavioural characteristics.

2.2 Ultrastructure

Studies of the fine structure of trypanosomes by electron-microscopy have shown that the basic organization is similar in all types studied. Such studies are at present of no help in identifying related species, but the introduction of more refined methods may make them of value in the future.

2.3 Immunology

Antigenic variation occurs so frequently and unpredictably in African species of trypanosome that immunological methods are as yet of no value in differentiating species and strains. Some antigenic differences have been observed between strains of *T. (S.) cruzi*.

2.4 Hosts

As already noted, host sensitivity is an important guide to species determination. Standard *Glossina* and reduviid vectors are not yet available for experimental studies. The application of insect genetics must be pursued to produce uniformly sensitive laboratory strains. Although mice are generally sensitive to *T. (S.) cruzi*, and many laboratories are moving towards the use of the C3H strain, further search for a standard test animal should be encouraged. Human experimentation and extensive animal breeding pose difficult problems for those working with the African organisms.

2.5 Comparative physiology, metabolism, and biochemistry

Metabolic studies have revealed striking differences in the oxidative pathways of blood-stream and culture forms of the *T. (T.) brucei* group of African trypanosomes. Cyanide does not affect the respiration of blood-stream forms, which lack some of the most important mitochondrial enzymes and can be further characterized by the presence of a reduced-NAD¹-linked glycerophosphate dehydrogenase. On the other hand, the respiration of culture forms is sensitive to cyanide, and such organisms contain cytochromes and the enzymes of the citric acid cycle. *T. (S.) cruzi* does not show any such dramatic changes in metabolism. More detailed studies of metabolism are urgently required and may reveal differences that will be of use in distinguishing species and strains.

Detailed study of the chemical composition of trypanosomes is also urgently necessary. There is already evidence that organisms differ in their phospholipid and sterol content; such variations may prove to be valuable for identifying species and strains.

A third means of identifying organisms may result from recent work on the composition of DNA. Variations in base composition that can be detected by buoyant density centrifugation and hybridization have been used to differentiate other micro-organisms and may prove valuable in the differentiation of trypanosome species.

As yet no defined media are available for the growth of trypanosomes, but detailed nutritional studies might reveal important differences between species.

Detailed histochemical studies of trypanosomes have not been made, but preliminary work has been promising. Tetrazolium staining methods may help to detect mitochondrial activity, and other methods that can detect specific enzymes may reveal differences between species. The quantitative determination of kinetoplast and nuclear DNA in single organisms by means of microdensitometry may also be of value.

¹ Nicotinamide-adenine dinucleotide.

Application of the above techniques to the systematic study of the haemoflagellates will necessitate the standardization of procedures. If each worker chooses entirely new conditions for his experiments and clinical studies, innumerable publications will result but advances will be insignificant. It is recommended that a "standard bank" of organisms and antisera be established (see section 9). Standards must also be established for cultures, animal transfers, growth stages, and other factors. The ultimate aim is to study molecular biology in the haemoflagellates by means of standard organisms under standard environmental conditions.

2.6 Trypanosome polymorphism

Trypanosome polymorphism may be defined as variation in the dimensions and shape of trypomastigote forms in the blood of the vertebrate host. It is always displayed by the African pathogenic trypanosomes of the *T. (Trypanozoon) brucei* group, but tends to be lost during extended laboratory passage. Polymorphism is a common but not invariable feature of *T. (S.) cruzi*. When present, however, it is usually maintained in spite of repeated passage in laboratory animals.

There is some evidence that each such variant has a particular biological function. For example, it has been suggested that the short, stumpy form of African trypanosomes is adapted to development in the insect vector. The broad form of *T. (S.) cruzi* may be similarly adapted. Although polymorphism is probably a cyclical response to environmental conditions and not the expression of genetic differences, methods of obtaining clonal populations would be a great aid in settling this controversial question. Once a means of selecting or producing the different forms has been found, biochemical and fine structural studies can be undertaken.

Although it has been suggested that polymorphism may be "triggered" by a variety of factors (e.g., temperature, urea, and special nutrients), no basic biochemical mechanism is apparent; indeed, it is not even known whether the mechanisms involved *in vitro* and *in vivo* are the same. A series of biochemical alterations in the oxidative system (particularly the glycerophosphate dehydrogenase system) of the African trypanosomes has been described.

There is evidence that in *T. (S.) cruzi* the chondriome decreases in size in the intracellular stage, but further comparative enzymological investigations are recommended. Experiments should be based on the working hypothesis that the kinetoplast is an essential factor in cyclical transformation; gene repression may be considered to be the main regulatory process involved.

Ambient temperature also affects the level of parasitaemia, but it is not known whether this occurs through modification of the morphogenesis of a particular form (and thus of its penetrability and infectivity) or

simply through inactivation or alteration of the host response. The role of antibody systems in the development of polymorphism is also obscure since radiation, cortisone treatment, and splenectomy of infected animals do not radically alter the pattern of such development.

3. IMMUNOLOGY

The immunological reactions of a host to invasion by foreign organisms are highly complex. Both humoral (antibody) and cellular (delayed hypersensitivity) aspects of the immune response are recognized but so far only the antibody aspect has been studied in trypanosomiasis. Delayed hypersensitivity in this disease should also be studied. Invading organisms comprise a mixture of complex antigens that elicit a wide variety of antibodies differing in important features, such as their ability to activate complement or to cross barriers between body compartments. The combination of these antibodies with their antigens *in vivo* may have beneficial effects (e.g., elimination of the infective agent) or harmful effects (see section 3.1) on the host.

Immunological investigation is important since it may lead to (1) fuller understanding of certain aspects of the pathogenesis of African and American trypanosomiasis; (2) the development of methods for diagnosing these infections and assessing cures; and (3) the development of effective methods for immunoprophylaxis and/or therapy.

3.1 Immunopathology

Several hypotheses have been advanced to explain some of the more common features of the pathogenesis of trypanosome infections. The recognition of antimyocardial antibodies in some cases of Chagas' heart disease, and of antibodies against myelin in some forms of demyelination resulting from African trypanosomiasis, has led to the suggestion that these conditions may be caused by autoimmune reactions. However, there is no conclusive evidence that typical lesions can be induced in experimental animals by these antibodies, either following artificial immunization or following passive transfer in animals that have not previously been infected. Similarly, the recovery of kinin in cases of trypanosomiasis provides no direct evidence of the occurrence of immediate hypersensitivity phenomena. Attempts should be made to determine whether passive cutaneous anaphylactic antibodies, or histamine and other pharmacologically active substances, are present in infected humans and animals following challenge with homologous antigen. If such substances are found to be present, their natural history should be studied.

It has been postulated that inflammatory phenomena such as arteritis may be initiated by antigen-antibody complexes. Experiments using fluorescein-tagged antigens, antibodies, and complement should be undertaken to determine whether such complexes are found in cases of arteritis resulting from trypanosome infections. In addition, complement levels in serum should be studied, and a thorough search should be made in infected humans and animals for antigen-antibody complexes in the basal membranes of glomeruli, and for the possible presence of glomerular nephritis resulting from prolonged infections in which an excess of antigen can be demonstrated or is suspected.

3.2 Immunodiagnosis

Various tests are available for the detection of antibodies in the serum of man and animals infected with trypanosomes, but tests for the detection of trypanosome antigens are lacking. If such tests could be developed, they might make rapid microscopic diagnosis possible through the use of tagged antisera that combine specifically with the parasites; they might also permit indirect measurement of the intensity of infection. Adaptations of existing fluorescence techniques, of tests using radioactive tracers (e.g., the Farr test), and of scanners and other mechanical devices (e.g., the Coulter counter) might be ideally suited for such purposes.

3.3 Characteristics of antigens

Two different types of trypanosome antigen are now recognized: those that are stable through many waves of parasitaemia, and those that vary during the course of an infection. The stable antigens may be characteristic of certain species, or the same antigen may be found in a number of species. Variable antigens have so far been recognized only in some of the *Salivaria*.

A large number of variable antigens are developed following infection with a trypanosome population, but it is not known whether a single organism can cause the sequential production of different antigens. Despite the wide range of antigens that can be produced, repetition frequently occurs, particularly following transmission to new hosts either by insect or syringe or in field conditions. The mechanism of such variation and the possibility that *T. (S.) cruzi* may show similar variation require further study.

Under some circumstances the variable antigens are found *in vivo* in the serum of infected animals or *in vitro* in a culture medium. Such antigens have been fractionated by salt precipitation into those that elicit the formation of protective antibodies and those that elicit the formation of apparently nonprotective precipitating antibodies. The number of antigens involved,

their location in the parasite, and the immunoglobulin class of the antibodies produced need further study. It has been suggested that the release of antigens into the surrounding body fluids is an important factor in antigenic stimulation in the host, but it is considered that there is no conclusive proof of this hypothesis. Studies should be made to determine whether these characteristics of the *Salivaria* are also found in *T. (S.) cruzi*.

3.4 Serological tests in the host

When only a few parasites are present in the body of the host, or when the parasites are confined to some inaccessible tissue, particularly in the chronic form of the disease, diagnosis can be made more readily by means of serological tests; indeed, such tests may offer the only possible means of diagnosis. Many different serological procedures are used to detect specific antibodies. In selecting procedures to be used, consideration should be given to their sensitivity, simplicity, rapidity, cost, and usefulness for epidemiological surveys, clinical diagnosis, checking the results of chemotherapy, and scientific purposes.

The following are particularly useful for diagnosing the early stage of Chagas' disease: (1) the indirect immunofluorescence test with dead integral culture forms of trypanosomes used as antigen; (2) the tube precipitation test with polysaccharide fractions of culture forms of trypanosomes used as antigen; and (3) the direct agglutination test with living culture forms used as antigen.

In the later stages of Chagas' disease, when it is very difficult to demonstrate the parasite, reliable serodiagnostic tests are particularly necessary. The most widely used and best-studied technique is the complement-fixation test using soluble trypanosome antigen prepared from culture forms. This test has been particularly recommended¹ for use in epidemiological studies and for the detection of infected blood-bank donors in endemic areas.

Low concentrations of antibodies can be detected by means of (1) indirect haemagglutination tests with tanned red cells, sensitized with soluble antigen prepared from culture forms; and (2) indirect immunofluorescence slide tests, using integral culture forms of trypanosomes.

Owing to the wide variety of antigen types in African trypanosomiasis, tests based on variable antigens are informative even if they give negative results. Serological tests based on the stable antigens are of limited value in individual cases, since a high degree of correspondence

¹ Pan American Health Organization, Study Group on Chagas' Disease, San Juan, Puerto Rico, 6-8 November 1966 (mimeographed report).

has not yet been obtained between immunological and parasitological tests. However, such tests are valuable for detecting disease in a group or herd.

A further notable feature of the changes that occur in the plasma of man and animals suffering from African trypanosomiasis is a major increase in the concentration of immunoglobulin M (IgM). This increase can easily be detected by the use of specific antisera to IgM in gel diffusion tests. Determination of IgM levels is a valuable screening test that can considerably increase the effectiveness of diagnostic surveys by focusing attention on individuals suspected of the disease.

However, major increases of plasma IgM are not specific to trypanosomiasis. In apparently uninfected Africans, high levels have been found with increasing frequency in older age groups, and some forms of liver disease and a number of infections can also result in increased IgM levels. It has been shown that measurement of the IgM level in cerebrospinal fluid is a more specific diagnostic test. Plasma IgM levels alone do not provide specific diagnostic indications, and might be of greater significance if related to the levels found in uninfected individuals of the same age as patients in the same community.

The most widely used technique for IgM determination is that of single radial diffusion using specific anti-IgM serum incorporated in agar plates, the concentration in the serum being compared with that of a standard IgM preparation. Tests have usually been performed on separated serum but it has recently been shown that blood dried on filter paper may also be used. Since the latter technique is more convenient for field work, it should be further investigated.

Owing to the difficulty of obtaining standard IgM preparations, it is difficult to compare the IgM levels observed in surveys carried out to date. A standard for IgM is under investigation by the WHO International Reference Centre for Immunoglobulins, and it is recommended that in future surveys IgM levels be related to this standard so that results may be comparable.¹

It has been shown that in cases of trypanosomiasis IgM may include specific anti-trypanosome antibody, a cross-reacting (heterophile) antibody to sheep red cells, and antiglobulin "rheumatoid" factors. It is not known whether such antibodies entirely account for the increased IgM concentration, and the extent to which "protective" antibodies occur in IgM as compared with other immunoglobulins is also unknown. The

¹ Advice on techniques and on the standard IgM preparation are available on request from : Centre international OMS de référence pour les immunoglobulines, Institut de Biochimie, 21 rue du Bugnon, 1000 Lausanne, Switzerland ; and from WHO Regional Center for Immunoglobulins, National Cancer Institute, Immunoglobulin Center, 6715 Electronic Drive, Springfield, Va., USA.

factors that give rise to increased synthesis of IgM are not understood, but could be related to the continued stimulation provided by the succession of antigenic variants of the parasite.

There is no evidence of an increase in IgM levels in Chagas' disease; however, owing to the paucity of evidence that is available, further studies of IgM concentration in all phases of the disease are warranted.

3.5 Application of tests in diagnosis

Tests for use in epidemiological surveys should be simple and inexpensive, and it should be possible for small laboratories in rural areas to apply them to large numbers of people. Alternatively, blood samples can be shipped to a central laboratory, where complex immunoserological tests can be performed. Air shipment of dried blood or serum on filter-paper is now common. Highly sensitive immunological methods with a low degree of species specificity (e.g., the immunofluorescence antibody test) are useful for diagnosing trypanosomiasis (particularly the African form).

Large-scale use of more complex procedures, such as the complement-fixation test, may be feasible if automatic pipetting machines are used for conventional tube techniques or if a micro system is employed (using pipette droppers, loops, and transparent plastic plates with shallow depressions). Immunofluorescence techniques using whole blood dried on filter-paper are recommended for field use, as are new flocculation techniques such as the "rapid card test".

For the clinical diagnosis of individual cases, in which it is sometimes essential to establish the etiology of the infection, it is necessary to use a highly sensitive procedure (e.g., the indirect haemagglutination test) and the most specific antigen possible (i.e., pure protein antigen).

For the evaluation of chemotherapeutic effects, it is necessary to use the most sensitive antigen and serological procedure, or a series of quantitative serological tests, to determine the development of antibody titres during and long after treatment. Serum samples must be stored under optimum conditions (deep frozen or lyophilized) and examined together under standard conditions.

The diagnosis of congenital *T. (S.) cruzi* infections involves the difficulty of distinguishing between antibodies transferred from the mother and those produced by the foetus in response to infection. In this connexion, it has been established that congenital toxoplasmosis and rubella stimulate an increase in foetal immunoglobulins. It is possible that increased IgM levels also result from congenital *T. (S.) cruzi* infection. Since IgM is not normally transferred to the foetus, increased IgM levels and IgM antibodies specific for *T. (S.) cruzi* in cord or neonatal blood may be indicative of congenital infection.

3.6 Preparation of antigens

In the study of trypanosomiasis and other infectious diseases, the need for purified antigens depends to a great extent upon the purpose for which they are to be used. It is obvious that reproducible results can be obtained more easily when purified and standardized antigens are used. Furthermore, in order to avoid nonspecific reactions, it is essential that all antigens be as free as possible of factors derived from hosts and culture media. On the other hand, the more numerous and complex the fractionation procedures employed, the greater is the likelihood that labile proteins will be denatured, with consequent loss of sensitivity and possibly specificity.

Trypanosome antigens are usually required for one of the following purposes :

(a) *Immunization.* Attempts to use parasite materials have indicated that relatively crude preparations, such as irradiation-attenuated or inactivated whole organisms (see section 3.7), provide protection to a challenging infection. Since it is not clear which trypanosome antigens stimulate the production of protective antibodies and which are stage-specific, the use of whole organisms (possibly including all stages of the life cycle of the parasite), should be used for optimum results. Whenever possible, attempts should be made to determine the relative degree of protection induced by antigens from different stages. Selective absorption studies should be carried out in order to resolve this question.

(b) *Immunodiagnosis.* To be of practical value, serological tests must have a high degree of sensitivity, specificity, and reproducibility. Sometimes it may be desirable to sacrifice some degree of sensitivity in order to attain a high level of specificity ; on other occasions, the converse may be true. For example, in screening blood-bank donors for the possible presence of *T. (S.) cruzi* infections, sensitivity is of paramount importance, whereas high specificity is required for the differential diagnosis of individual cases of Chagas' disease. For the diagnosis of African trypanosomiasis in man, specificity is desirable, but for the diagnosis of trypanosomiasis in cattle it is preferable to use a test that will react positively with all the major species.

Antigens can be purified by means of one or more of the commonly used fractionation techniques (e.g., electrophoresis in agar, starch, or acrilamide gel ; molecular sieving ; ion-exchange chromatography ; density-gradient analysis in the ultracentrifuge ; and the removal of lipids by fat solvents).

Reproducibility of results within a given laboratory and in different laboratories depends to a large extent on standardization of antigens. Whenever possible, the following should be specified : (1) the stage of the

stabilate¹ strain ; (2) the source of the trypanosome species used ; (3) the fractionation methods used ; and (4) the protein or polysaccharide content. Antigen preparations should always be tested against a number of reference standard antisera. If possible, the number of reacting components that they contain, as determined in agar gel and by other methods, should be specified. However, since even the most highly " purified " trypanosome antigen consists of a heterogeneous mixture of components, each of which is probably an antigenic mosaic, its reactivity may frequently be unrelated to its protein or polysaccharide content. Therefore, reactivity should be measured against a predetermined reference standard serum in comparing results.

3.7 Active immunization with dead and living vaccines

The possibility of developing methods of active immunization against protozoa seems to be most promising for the haemoflagellates. However, it is difficult to compare the different methods of immunization in experimental use owing to the lack of standardization. Immunization and challenge inocula must be derived from standard stabilates and the amount of antigen contained in immunization inocula and the infectivity of challenge inocula should be measured and specified. Furthermore, the breed and age of the experimental animals used, and the conditions under which they are maintained, may also be important and should be standardized.

Animals may be rendered immune to *T. (S.) cruzi* with live organisms of natural low-virulence strains or with disintegrated dead parasites, but not with organisms that have been killed by chemical treatment without disintegration. The way in which disintegration is carried out may have important effects on the quality of the antigen ; the method used, the maintenance of low temperatures, and the use of so-called " inert " gases during the preparation are crucial factors. Such antigens are stable at -70°C for months ; however, they lose their immunogenic capability in a few days at 4°C . Studies of the following should be carried out : (1) the effect of adjuvants, (2) the relation between the immunization schedule used and the rise and fall of immunity, (3) the use of irradiation for the attenuation of organisms, and (4) the use of " released " antigens.

Immunity to the *Salivaria* can be produced in the same way as to *T. (S.) cruzi*. Immunity to the homologous antigenic type can also be produced by the method of infection and drug treatment. In most immunization schedules, multiple inocula have been used, but immunization can be attained by the use of single vaccine doses of organisms inactivated by β -propiolactone or formol.

¹ A stabilate is defined as a trypanosome population in viable condition preserved so that it cannot undergo modification.

The use of killed antigens should be investigated, with particular attention to the conditions under which they should be stored and to the development of single-dosage immunization. Active immunization should be studied in relation to different methods of stimulating the reticuloendothelial system and to other methods for augmenting the immune response.

The practical application of immunization against the *Salivaria* seems to depend on the following factors: (1) the number of antigenic types of trypanosome circulating in the field and capable of being developed by a given trypanosome population; (2) the possibility of inducing immunity to several types by a single inoculation; (3) the occurrence of cross-immunity between types, and (4) the development of methods for the cultivation of blood-form antigen, or the extended use of antigen grown in animals by adjuvant procedures.

4. EPIDEMIOLOGY

Although both American and African trypanosomiasis are principally transmitted by arthropod vectors, the epidemiological features of the two types of disease differ in many ways. For example, infections with African trypanosomes are normally acquired by human beings whose activities bring them into contact with the tsetse vector during the day; *Trypanosoma (T.) rhodesiense* infections are usually acquired at a greater distance from centres of domestic activity than are *T. (T.) gambiense* infections. On the other hand, humans most commonly acquire *T. (S.) cruzi* infections in the home while asleep at night.

Congenital infection and accidental infection by blood transfusion are considerably more common with American trypanosomiasis than with the African form of the disease. In recent observations in Chile and elsewhere, many cases of congenitally acquired Chagas' disease have been noted, and the possibility of transmission through the milk of an infected mother cannot be excluded. Although there is incontrovertible evidence of congenital transmission of African trypanosomiasis, it occurs relatively rarely, possibly owing to the well-known effect of this disease in reducing fertility in the female or causing abortion or miscarriage. The possibility that Chagas' disease may also cause abortion or miscarriage requires further investigation.

Another striking difference between the two types of disease is the fact that the risk of accidental infection in the laboratory is high with *T. (S.) cruzi* but relatively low with African trypanosomes.

4.1 Reservoir hosts

There is a wide range of potential reservoir hosts, including many orders of animals, of American trypanosomes. It also appears that many wild animals are potential reservoir hosts of African trypanosomes of the *T. (T.) rhodesiense* type, although few different orders are involved. *T. (T.) rhodesiense* has also been isolated from cattle. It is uncertain whether there are animal reservoir hosts of *T. (T.) gambiense*, although there is evidence that the domestic pig may act as such a host.

With both types of trypanosomiasis, it may be difficult to ascertain the exact status of trypanosome strains isolated from animals or vectors. Human-infective strains of African trypanosome can be identified only by a direct test of their ability to infect man, a test that is sometimes justifiable if it is shown beforehand that the resulting infection can be cured with certainty. Such tests can be undertaken more readily with *T. (T.) rhodesiense* than with *T. (T.) gambiense* because of the rapidity with which the former manifests itself and the reliability of treatment of early cases with suramin. Since *T. (S.) cruzi* infections are at present incurable, such tests cannot be used with isolates presumed to be this organism; however, presumptive identification can be made on the basis of criteria such as morphology, cultivability, production of tissue leishmanial (amastigote) forms, development in reduviid bugs, and cross-resistance to superinfection in laboratory animals. The standardization of criteria and techniques for the identification of American and African trypanosome isolates is urgently necessary, as is a method of distinguishing strains that are infective to man.

The importance of reservoirs for both types of disease depends greatly upon ecological conditions. If it is concentrated in a wild reservoir, the disease may occur only sporadically, but if it transferred to areas of human habitation direct transmission from man to man through the vector may result in focal or even widespread outbreaks.

There is one marked difference in the way in which reservoir hosts are infected with African and American trypanosomes. Infection with African trypanosomes can be acquired only by the bite of an infected tsetse fly or by the passive transfer of infected blood by another biting fly. However, it is possible that some of the animals most commonly infected with American trypanosomes (e.g., opossums, dogs, and cats) may acquire the infection by eating reduviid bugs or other infected animals.

4.2 Man-vector contact and its effects

The habitats and behaviour of the vectors of American and African trypanosomes are so different that the causes and results of man-vector contact bear virtually no resemblance in the two diseases. As already

noted, there is a tendency for African trypanosomiasis to be acquired outside human habitations by day-time contact with tsetse flies, and for American trypanosomiasis to be acquired inside the house by night-time bites of reduviid bugs. Furthermore, reduviid bugs may be attracted to light at night and so bring the infection into areas that are normally free from it, whereas tsetse flies are very rarely attracted to light and this factor plays no part in the spread of African trypanosomiasis. Reduviid bugs may also be passively introduced into domestic surroundings (e.g., on construction materials, firewood, or animals); they can also move out of houses, to distances exceeding 50 m, where they can come into contact with wild reservoir hosts.

The conditions of man-vector contact not only govern the total incidence of African trypanosomiasis, but also its distribution by age and sex. They may also determine whether the disease is acute or chronic.

The evidence of a number of workers has explained why the total incidence of Gambian sleeping sickness is not related to tsetse density but is often very high at the fringe of the distribution of some riverine species. The chance of infection is increased by the intimate man-fly contact brought about by climatic conditions, and possibly by the effects of temperature and desiccation in both accelerating the development of infection in tsetse flies and causing them to feed at an earlier time after emergence.

Much of the incidence of Rhodesian sleeping sickness is attributable to human contact, brought about by certain occupations, with a vector infected from an animal reservoir. Epidemic outbreaks may occur if close and continuous man-vector contact, or perhaps sustained contact between man, vector, and reservoir host, gives rise to man-fly-man transmission.

Most of the occupations that lead to contact between man and the vectors of African trypanosomiasis are those of the male sex and tend to be pursued at a distance from centres of domestic activity. Consequently, the incidence of the disease by sex and age commonly provides an indication of the distance of the source of infection from the principal human habitations. If the infection occurs predominantly in mature males, its source is usually distant; under these conditions, people such as travellers, hunters, and fishermen are particularly affected. A high or rising rate of infection in women and children usually indicates that the source is relatively close to their habitations. However, it may be necessary to modify these generalizations depending upon local conditions and customs.

It has been postulated that the apparent differences between *T. gambiense* and *T. rhodesiense* sleeping sickness, and their association with riverine and savannah tsetse, respectively, may result from the conditions of transmission of the parasite and the degree of man-vector contact. When man-fly-man transmission predominates, as with riverine tsetse, there will be a greater chance of transmission of the less virulent strains, which survive in man for longer periods. On the other hand, when the

fly feeds mainly on animals, as do the savannah tsetse, only the more virulent strains that are capable of infecting the animals (which are relatively resistant) can survive to be transmitted to man.

Such a concept is consistent with the following known features of African trypanosomiasis: (a) the normal association of *T. gambiense* with riverine tsetse and of *T. rhodesiense* with savannah tsetse; (b) the fact that either group of tsetse can transmit either form of the disease; (c) the fact that the incidence of Gambian sleeping sickness is generally higher than that of the Rhodesian form; and (d) the apparently identical virulence of *T. brucei* and *T. rhodesiense* to laboratory animals (the former, being non-infective to man, is dependent on transmission through animals).

Chagas' disease, like African trypanosomiasis, occurs mainly in rural areas, but is associated with poor socio-economic and sanitary conditions. Dirty and dark houses made of materials such as mud, grass, and cane, and accumulated rubbish, offer excellent breeding places for *Triatoma* and *Panstrongylus*. The eggs of *Rhodnius* are often laid on the leaves of palm trees, which are used for thatching huts; consequently, huts are often already infested when they are ready for human occupation. It has been shown that the incidence of infection is closely related to methods and materials used in house construction.

The finding of *Rhodnius prolixus* eggs in the feathers of migratory birds in Venezuela may explain the peculiar discontinuous distribution of this species in America.

In Costa Rica, dirty floors are good breeding places for *Triatoma dimidiata*, since the nymphs of this species can cover themselves with dust so that they become practically invisible to predators.

Not all species of triatomid show the same efficiency in transmitting *T. (S.) cruzi*. Some South American species defecate more rapidly and more frequently than some North American ones. The number of metacyclic trypanosomes produced by different species of bug can also vary. Since the better adaptation of a local strain to a local insect in a given area has an important effect on the sensitivity of xenodiagnosis, it seems advisable to use a local vector in this test.

Further work on different vectors of both American and African trypanosomiasis is needed to determine their relative efficiency as transmitters and their relative infectibility with species or strains of trypanosomes. The readiness with which they bite man and the number of metacyclic trypanosomes produced in them should be studied. Some such work has been done on tsetse, but not on reduviid bugs. In the tsetse, study of the relationship of the development and biochemistry of the peritrophic membrane to infection with trypanosomes might be profitable; however, this does not apply to reduviids.

There is a need for further knowledge of insect behaviour and of host preferences, not merely of the vectors of American and African trypano-

somiasis as a group, but of different insect species. Much work has been done on the behaviour and host-preferences of tsetse, the results being of great value in the planning of control measures. On the other hand, precipitin tests performed with the blood found in the stomach of reduviid bugs have given contradictory results with respect to host preferences. A new approach is necessary to determine whether such preferences are real and whether the insects, once they have found a host, tend to remain close to it.

Physiological studies of tsetse have also yielded information of great practical value and should be continued. Similar work should be undertaken on reduviid bugs, which have not so far been studied in this way.

Recent developments in genetics, irradiation, chemosterilants, electronics, and gas-liquid chromatography have brought new prospects of understanding the behaviour of vectors and of developing methods for their control. The use of males sterilized by irradiation is practicable in the control of tsetse flies, whose first mating is the effective one; however, it is less practicable for the control of *Rhodnius*, whose subsequent matings are effective.

The means by which blood-sucking vectors navigate, seek their hosts, and take a blood meal are at present a matter of supposition. It is likely that many senses are involved in seeking a host. Electronics has revolutionized studies of insect behaviour, and may be expected to lead to some clarification of these problems.

Chemical attractants probably play some part in insect navigation, but the chemical analysis of trace substances such as flavours, although theoretically easy by gas-liquid chromatography, is in practice complex, and must be done in conjunction with laboratory investigations of insect physiology. Such work has only just been started on tsetse, and could be carried out on the vectors of *T. (S.) cruzi*. The use of chemosterilants together with attractants will not be practicable with any precision until the behaviour of the tsetse is better understood.

4.3 Epidemiological effects of the environment

The principal environmental factor that influences the epidemiology of American and African trypanosomiasis is temperature. In South American countries where there are marked seasonal temperature changes, the acute disease is associated with summer conditions.

There is some evidence that, in America, high temperatures tend to reduce the level of parasitaemia in animals — and, consequently, the proportion of naturally infected reduviid bugs and the general incidence of the disease. A survey in El Salvador, carried out at altitudes between sea-level and 900 m, appears to support this view; however, two different species of reduviid occur at different altitudes in this country. A comparative study

should be carried out in an area where a single species is found in a wide altitude range (e.g., Peru, where the same species occurs from sea-level to 3000 m). Such a pilot project is necessary to obtain base-line data on epidemiological and other aspects of Chagas' disease, which should be of the utmost value in the planning of future control operations. Socio-anthropological studies should be included in any such project.

In Africa, the distribution of the vector species of tsetse is determined by temperature or by the desiccation that frequently results from high temperatures. At certain seasons, intimate man-fly contact may be brought about if the tsetse are confined to areas around water on which the local human population are entirely dependent. High temperature and desiccation may also, as noted above, tend to increase both the rapidity of development and the proportion of trypanosome infections in tsetse.

Little is known of the effects of climate in determining the distribution of vectors of American trypanosomiasis, and much remains to be done in this field.

5. CLINICOPATHOLOGICAL ASPECTS

5.1 Distribution of trypanosomes in host tissues

5.1.1 *American trypanosomiasis*

The penetration of *T. (S.) cruzi* through the skin and mucous membrane is followed by an acute inflammatory reaction manifested clinically by a chagoma or by Romana's sign. Subsequently, the parasites reach the blood stream and become localized in different visceral tissues. In the acute disease they change to the leishmanial (amastigote) form, multiply by binary fission, and transform the host cells into pseudocysts. The most commonly parasitized tissues are the heart, skeletal muscle, smooth muscle, and glia, but parasites have also been observed in uncommon sites such as epithelial cells of skin, subcutaneous and adipose tissues, gastric submucosa, testes, transitional epithelium, and chorionic villi in both man and animals. It has been demonstrated that the duration of the endogenous cycle is 3-6 days in the acute disease, but the nature of the endogenous cycle in the chronic disease is not well understood. Studies of the distribution or localization of parasites in experimental animals have been unsatisfactory in that consecutive experiments with the same strain have shown extreme variation.

Trypanosomes and pseudocysts are very rarely found in chronic Chagas' disease. Pseudocysts have been found in the heart, which has been examined more intensively than any other organ, and in the oesophagus, large intestine, sympathetic ganglia, and skeletal muscle. There is no correlation between the extent and severity of inflammatory lesions and the location

and number of pseudocysts, although granulomatous inflammatory lesions are sometimes observed in association with ruptured pseudocysts.

It is the consensus of opinion that the principal organ involved in acute Chagas' disease is the heart. Parasites are usually present in the peripheral circulation. Other organs and systems are parasitized to a lesser extent; in descending order of frequency, these include the central nervous system, the digestive system, and the respiratory system. The reasons for preferential selection of the myocardium are not understood. The same distribution occurs in chronic Chagas' disease, but the parasite density is much lower, and it is often impossible to find any parasites.

5.1.2 *African trypanosomiasis*

Metacyclic trypanosomes may reach the blood of the host directly if they are introduced into or close to a capillary vessel by the bite of an infective tsetse. However, if deposited in the dermis, the trypanosomes may multiply locally and eventually produce, after a number of days, the typical initial lesion or chancre; from this point they rapidly spill over into the blood and lymphatic systems. Trypanosomes can be easily recovered from serum obtained by puncture of the lesion; following general dissemination, they can be isolated from blood or from the fluid obtained by puncture of a lymph node. Even at this early stage of infection, trypanosomes may invade the central nervous system. They have been detected in the cerebrospinal fluid obtained by suboccipital puncture, and the appearance of immunoglobulin M in this fluid (and a subsequent rise in its level) has been observed well before the appearance of other changes (e.g., increased leucocyte count and total protein content and altered electroencephalographic tracings).

As the disease develops, the effects of the trypanosomes seem to be most marked in the brain, as evidenced by the degree of diffuse mesenchymatous perivascular inflammatory changes that may be encountered. The condition later becomes a demyelinating encephalitis, which appears to progress even in the absence of the parasite; there is only one reliable record of the finding of the parasite in the parenchymatous tissue.

A number of tissues and organs may show evidence of nonspecific diffuse inflammatory changes, but the parasites are difficult to detect. In cases of *T. (T.) rhodesiense* infection, trypanosomes have been found in serous exudates such as pericardial or peritoneal effusions.

5.1.3 *Comparison of distribution in tissues*

The distribution of different forms of the African and American parasites in host tissues is compared in Table 1, and the sites of the principal lesions are compared in Table 2.

TABLE 1
DISTRIBUTION OF PARASITES IN HOST TISSUES

Form of parasite	Tissues in which the following parasites occur:	
	<i>T. (S.) cruzi</i>	<i>T. (T.) gambiense</i> , <i>T. (T.) rhodesiense</i>
trypanosomal (trypomastigote) form	blood	blood, lymph nodes, cerebrospinal fluid
leishmanial (amastigote) form	Intracellular, in tissues	does not occur

TABLE 2
SITES OF PRINCIPAL LESIONS

<i>T. (S.) cruzi</i>	<i>T. (T.) gambiense</i> , <i>T. (T.) rhodesiense</i>
heart (in certain geographical areas, digestive, respiratory, and other systems)	principally the nervous system (also liver, spleen, and other viscera and lymph nodes and bone marrow)

5.2 Site-of-entry lesions

In the acute stage of Chagas' disease the onset of the clinical symptoms may be preceded by the appearance of a lesion, called a chagoma, at the site of entry of the parasite. The typical chagoma is an area of inflammatory necrosis and fat necrosis of the skin and subcutaneous tissue. Romaña's sign, another type of site-of-entry lesion, consists of unilateral palpebral œdema, conjunctivitis, and lymphadenopathy, and is the result of contamination of the conjunctival sac by infected faecal material from the vector. Both types of lesion are of diagnostic value, and neither plays a significant role in the subsequent development of the disease. The lesions may persist for as long as 2 months, during which period parasites can be demonstrated in the cells of the skin and underlying tissues.

The frequency of occurrence of the "typical" chagoma is not well known. Romaña's sign is seen frequently, perhaps because the conjunctival sac is the most frequent portal of entry of the parasite, or perhaps because infection of the eye is a more compelling reason to seek medical care.

In African trypanosomiasis an initial chancre may be produced at the site of entry of the trypanosomes; the neck, limbs, and face are the common sites. The lesion appears a few days after the bite (lesions are sometimes observed within a few hours, but these seem to be reactions to the bite rather than to trypanosomes). It begins as an erythematous

nodule that grows in size and may be centrally vesiculated; a "pseudo-boil" appearance is often described. Puncture of the lesion gives evidence of trypanosomes — within hours in some cases, but more frequently on the second day. If treatment is not given, the lesion may persist for only a few days; more commonly, however, it disappears gradually over a period of several weeks. The regional nodes are enlarged and tender.

It is probable that the appearance of a chancre, and the subsequent course of events, depend on (1) whether the feeding tsetse is heavily infected and (2) whether the fly happens to puncture a large vessel and inject all its saliva and trypanosomes into the general circulation or whether, in its search for blood, it probes in many directions, distributing metacyclic trypanosomes widely in the tissues. Multiplication proceeds independently in the general circulation and the tissues, but profusely in the latter. Eventually, the chancre diffuses and large numbers of trypanosomes "overflow", via the lymphatic drainage, into the general circulation. This overflow raises the number of trypanosomes circulating in the blood to microscopically detectable levels, and also causes the onset of pyrexia. Consequently, symptoms of the febrile condition usually appear within a few days of the appearance of the chancre.

The lesion is observed more often with *T. (T.) rhodesiense* than with *T. (T.) gambiense* infections, perhaps as a result of the slower development of the Gambian disease or of the different factors involved in transmission. The initial lesion is observed in 92–100 % of experimental infections with *T. (T.) rhodesiense*, whether the organisms are introduced intradermally or subcutaneously.

5.3 Cardiac lesions

5.3.1 Chagas' disease

Cardiomyopathy is the most important and frequent manifestation of acute and chronic Chagas' disease, and it leads to arrhythmias, congestive failure, and thromboembolic phenomena. Acute Chagas' cardiomyopathy is characterized by severe and widespread interstitial inflammation and the presence of leishmanial (amastigote) forms of the parasite within myocardial fibres. The cellular infiltrates may be composed of polymorphonuclear neutrophils, monocytes, and lymphocytes and are associated with appreciable oedema. The inflammatory process may extend into the endocardium and pericardium, and the myocardium adjacent to the infiltrates may undergo degeneration.

Parasitized myocardial fibres do not give rise to inflammation until after they rupture. It has been shown that the cardiac lesions of acute Chagas' disease in animals sometimes become almost completely healed. The pathologic changes that occur in the heart during the latent period preceding chronic Chagas' disease in man are not well known.

The principal clinical manifestations of acute Chagas' disease are cardiac enlargement, with or without cardiac failure, and/or variable and transient changes in the ECG — namely, alterations of repolarization and, less frequently, conduction disturbances. Recovery occurs in the majority of cases.

In the chronic disease, the principal clinical findings are cardiac enlargement and failure, arrhythmias and conduction disturbances, and thromboembolic phenomena, any of which may cause sudden death.

It has been observed clinically that occasional sudden death from Chagas' cardiomyopathy may occur in the absence of demonstrable parasitism of the myocardium and without previous clinical manifestations of the acute or chronic disease. The characteristic macroscopic findings are hypertrophy; dilatation and thinning of the apices of the ventricles with formation of apical aneurysms, with or without thrombosis; and protrusion of the pulmonary conus.

The histopathological changes in chronic Chagas' cardiomyopathy include focal myocarditis, fibrosis, and extensive myocytolysis. These changes usually occur in the absence of demonstrable parasitism of myocardial fibres. The interstitial inflammatory reaction may be dense and polymorphous: monocytes, histiocytes, lymphocytes, and a few plasma cells and eosinophils may be present. The fibroblastic reaction, which varies in extent and intensity, is usually located within the inner two-thirds of the ventricles; it frequently occurs near the apex of the heart, where it may be associated with aneurysm.

The apical aneurysm is frequently observed in patients with chronic Chagas' disease in regional clinics and pathological laboratories, and is considered to be characteristic of the disease. Although many hypotheses have been advanced, the pathogenesis of the lesion is not understood.

In both acute and chronic Chagas' disease there is damage to the ganglia of the heart, with or without inflammation, and with a reduction of the number of ganglion cells.

Although the pathogenesis of Chagas' disease has been extensively studied, it is far from being completely understood. It is difficult to identify the mechanisms by which the different local effects of the parasite are produced. It has been postulated that, in addition to the mechanical factor related to the intracellular multiplication of the parasite, there are toxic and enzymatic factors that bring about rupture of the myocardial fibre, with resultant inflammatory response. However, no specific toxins have been identified. The importance of enzymatic factors has recently been emphasized by the demonstration of a lysosomic enzymatic system in the parasite, and of severe structural changes in the myocardial fibre, with concomitant inhibition of mitochondrial and myofibrillar enzymatic activity.

5.3.2 *African trypanosomiasis*

The literature on clinical and pathological studies of heart disease in African trypanosomiasis is meagre in comparison with that on Chagas' cardiomyopathy.

Cardiovascular signs are frequent but minor manifestations of African trypanosomiasis. Clinical cardiac insufficiency is exceptional in Gambian trypanosomiasis, but is more frequent in Rhodesian trypanosomiasis. The abnormalities are usually functional and clinical (e.g., muffled cardiac sounds and diminished and labile pulse pressure); they are often such as to be visible upon radiological examination (e.g., a more or less enlarged and hyposthenic heart). Most often, the only abnormality is found in the ECG, particularly the T wave. Extrasystoles and blocks are much less frequent than in Chagas' disease. Macroscopically the size of the heart may be normal or enlarged; enlargement is caused more by dilatation than by hypertrophy. No lesion comparable to the apical aneurysm of Chagas' disease has been seen.

The histopathologic lesions that have been described are mainly inflammatory changes, consisting of (1) infiltrates of lymphocytes, histiocytes, and plasma cells (sometimes forming true granulomas) and (2) oedema. These changes can progress to fibrosis. The lesions always involve the myocardium and may also involve the endocardium and pericardium. The infiltrates are most often observed in perivascular locations, but may also be seen around nerve fibres. In one case they were present in the right branch of His's bundle. The muscle fibres are rarely altered by the interstitial inflammatory infiltrates. As far as is known, parasites have been demonstrated in only one case (a patient with *T. (T.) rhodesiense* infection).¹ To summarize, the lesions are usually interstitial and inflammatory, and represent only a diffuse cardiac localization of an inflammatory disease; they cannot be considered to be specific for trypanosomiasis.

The fibrotic development of the inflammatory lesions may cause a chronic cardiomyopathy. Thus, cardiac insufficiency of undetermined origin, which is so frequent in Africa, may be caused (although rarely) by trypanosomiasis.

The cardiac lesion of African trypanosomiasis may be likened to a more or less localized version of the diffuse mesenchymal inflammatory changes that occur in Chagas' disease; however, the cardiac lesion is characteristic in the latter disease. In African trypanosomiasis, clinical cardiac findings are often minor; radiological signs and ECG changes are much more common, but the abnormalities characteristic of Chagas' disease are not found. The histological lesions in the heart are entirely inflammatory and nonspecific in the African disease; the integrity of the

¹ Hawking, F. & Greenfield, J. G. (1941) *Trans. roy. Soc. trop. Med. Hyg.*, **35**, 155.

cardiac fibres is largely maintained, and parasites are absent. In Chagas' disease, as already noted, the integrity of the fibres is not maintained and they are parasitized.

The principal cardiac manifestations of the two forms of trypanosomiasis are compared in Table 3.

TABLE 3
CARDIAC MANIFESTATIONS OF TRYPANOSOMIASIS

Manifestation	American trypanosomiasis	African trypanosomiasis
cardiac enlargement	very common	common
cardiac failure	very common	very rare
arrhythmias	very common	rare
conduction defects	very common	rare
thromboembolism	common	exceptional
ECG ST-T changes	common	common

5.4 Neurological lesions

5.4.1 *Chagas' disease*

Lesions of the ganglion cells of the autonomic nervous system occur in both acute and chronic Chagas' disease; their cause is unknown, although it has been suggested that they result from the action of the products of parasite degeneration.

Variation in the degree of involvement of the autonomic ganglia has been observed in different areas of South America. Consequently, it is difficult to evaluate the role of such lesions in the pathogenesis of the chronic phase of Chagas' disease. Comparative quantitative studies are necessary, and the Group recommends that such studies be undertaken, using standard techniques and uniform criteria. Studies should also be undertaken to clarify the functional disturbances of the various organ systems, resulting from nervous involvement, in patients with chronic Chagas' disease.

In acute Chagas' disease, severe lesions of an inflammatory and degenerative nature may occur in the central nervous system, and may cause death. Acute fatal cases with neurological involvement were frequently found by Carlos Chagas between 1909 and 1912, but such cases appear to be very rare today, which may be one reason for the decline in mortality.

5.4.2 *African trypanosomiasis*

The neurological lesions caused by African trypanosomiasis occur principally in the central nervous system. They are characteristic of the disease and, to varying degrees, are nearly always present.

The stage of dissemination and that of elective localization in the brain are not necessarily sequential, but may occur simultaneously. Therefore, neurological lesions may be observed at any time during the infection, but they are always essentially of the same type.

‡ Localization in the brain is first manifested as diffuse mesenchymal inflammatory lesions. As a rule, the parasite is not found in the parenchyma of the brain, but is present in the cerebrospinal fluid. The inflammatory infiltrates are predominantly perivascular and consist of lymphocytes, histiocytes, and plasma cells. These infiltrates first affect the choroid plexuses and the meninges, and subsequently appear at various points in the cerebral cortex and in the subcortical regions. Such inflammatory lesions are probably reversible if treated in time.

The appearance of the lesions changes during the course of the disease. Neuropathological alterations occur, principally affecting the basal nuclei and the cerebral axis, particularly the mesencephalon and diencephalon. Demyelination occurs in the white matter near Virchow-Robin spaces, whose perivenous extension is limited prior to this change.

A pan-encephalitis develops, simultaneously affecting the grey and white matter of the neuro-axis, but predominantly the white matter (demyelinating leucoencephalitis). At this stage, trypanosomes may not be found in the cerebrospinal fluid. The disease apparently evolves auto-progressively as a demyelinating immunological reaction. The change from the inflammatory to the demyelinating phase is critical, but the reasons for its occurrence are not precisely understood.

5.4.3 *Correlations between clinical manifestations and pathology*

Subcortical atrophy of the brain, which can often be demonstrated by gas encephalography, occurs as a result of the demyelination. The diencephalic and hypothalamic distribution of lesions accounts for certain important symptoms, such as misbehaviour, instinctive behaviour, endocrinopathies, and heightened sensitivity to stimuli. The progressive damage to the systems that control muscle tone accounts for the wide variety of motor signs that are observed.

The classical symptom of the disease, interference with sleep and wakefulness, can be explained only incompletely. The EEG of the patient when awake has a suggestive appearance. There is a slowing of rhythms, leading to disorganization of the tracing, together with other abnormalities that change from one day to the next, generally appearing in bisynchronous bursts and sometimes showing periodicity. The EEG of the sleeping patient is even more characteristic: there is a change in the cyclic and morphologic appearance of the pattern in every phase, with longer maintenance of the phase for eye movements. These findings seem to indicate that trypanosomiasis is not a simple hypersomnolence, but a loss of the pattern of sleep.

However, this symptomatology is poorly explained by the histopathological lesions that can be demonstrated.

Whereas the central nervous system is involved in African trypanosomiasis, cerebral damage is rare in Chagas' disease. Lesions occur principally, if not exclusively, in the peripheral nervous system (e.g., in the heart, digestive tract, respiratory system, and hypogastric plexus).

The Group recommends that further studies be undertaken to determine whether lesions occur in the peripheral nervous system in African trypanosomiasis and whether cerebral lesions are present in Chagas' disease.

5.5 Clinical manifestations caused by different species and strains

5.5.1 Chagas' disease

In central Brazil, the clinical and pathological manifestations of chronic Chagas' disease include involvement of the heart, digestive tract, and respiratory system. In southern South America, megacolon is found quite frequently and mega-oesophagus less frequently. Mega-organs apparently do not occur in northern South America or Central America. Experiments have demonstrated the predominance of lesions of the reticulo-endothelial system in infections with a Colombian strain and of lesions of the digestive tract in infections with a Brazilian strain. Such comparative studies of the relationship between different strains and different visceral tropisms should be extended.

5.5.2 African trypanosomiasis

The clinical manifestations of African trypanosomiasis have certain features in common, regardless of the type of parasite. These features include a lesion at the site of entry, a period of dissemination, and a stage of cerebral localization, all of which are found to varying degrees. However, the clinical features of the disease depend upon the rate at which it develops, the number of periods of subacute activity, and the degree of overlapping between the stage of generalized activity and that of cerebral localization.

There are certain clinical features that are more or less associated with Rhodesian and Gambian trypanosomiasis, the two principal types of the disease. Rhodesian trypanosomiasis evolves more acutely; its reticulo-endothelial manifestations are more prominent, its neurological effects less characteristic and its cardiovascular manifestations more clear than those of Gambian trypanosomiasis. Owing to the acute nature of the disease, the initial chancre is recognized more frequently than in cases of Gambian trypanosomiasis, although this may not result from any property of the parasite itself. Gambian trypanosomiasis is often subacute in its early stages, and develops in successive outbursts over a number of years with periods of clinical and/or biological latency. The initial stages are

often unrecognized, the disease being more often detected when it affects the brain. However, although the two forms of the disease may vary in clinical features, there are no absolute differences between them. Many intermediate stages are seen; and the development of a Gambian chancre may resemble that of a Rhodesian chancre, and vice versa.

Atypical clinical features observed in some areas and in certain epidemics have been thought to result from infection with particular strains. For example, clinical features observed in northern Nigeria, Gambia, and Sierra Leone raised the possibility that hepatotropic strains might be involved, but the existence of such strains could not be proved. Such clinical differences may result from a special reaction of the host, or even from some characteristic of the fly. There is no clear explanation of the fact that similar trypanosomes may cause severe illness in one patient and a carrier state in another.

5.6 Congenital trypanosomiasis

Although it has been established that Chagas' disease may be acquired congenitally, the frequency and importance of transplacental transmission has not been extensively studied. However, well documented cases that have been reported indicate that it may well be important. There have been no systematic studies of the transplacental transmission of African trypanosomiasis, but congenitally acquired cases of both Rhodesian and Gambian disease have been reported.

The Group recommends that systematic prenatal and postnatal studies be carried out to determine the frequency and importance of transplacental transmission of pathogenic trypanosomes.

5.7 The role of antigen-antibody reactions in pathogenesis

5.7.1 Chagas' disease

It has been postulated that antigen-antibody reactions are responsible for some of the pathological alterations that occur in chronic Chagas' cardiomyopathy. This postulate is based on the following findings:

(a) Arteritis of the allergic type has been observed, although it is extremely rare in both humans and experimental animals and cannot be considered to be a fundamental or general mechanism.

(b) There is some experimental evidence that an allergic mechanism plays a role in the production of Chagas' myocardiopathy. However, it is considered that experiments of this type reproduce only some of the features of the disease, and not its chronic form.

The occurrence of an autoimmune reaction has also been postulated. Anti-heart antibodies have been demonstrated in both acute and chronic Chagas' disease, but their significance has not been determined.

5.7.2 *African trypanosomiasis*

As previously noted, African trypanosomiasis gives rise to a generalized histiocytic-lymphocytic-plasmocytic inflammation, with the production of immunoglobulins. Although the specificity of these immunoglobulins has not been demonstrated, their relationship to the development of the disease should be considered. The demyelinating encephalitis observed in the terminal stage of the disease is frequently attributed to the production of antibodies against nerve cells or myelin. Many workers consider that dissemination and cerebral localization are diffuse reactions that become localized; however, these workers have not explained the way in which such localization occurs.

Such descriptions are highly imprecise, but cannot be improved until the nature of immunoglobulins is more precisely understood and antibodies against nerve cells are discovered.

6. CHEMOTHERAPEUTIC PROBLEMS

One of the outstanding characteristics of *T. (S.) cruzi* infections is their resistance to drugs that are effective against African trypanosomes and to other chemotherapeutic agents. Although this resistance has been recognized for over 50 years, it has not yet been satisfactorily explained. A partial explanation may be found in the fact that a vital stage of the development of *T. (S.) cruzi*, including cell division, takes place intracellularly. Since there is no effective drug for the treatment of Chagas' disease, chemotherapy of the two forms of the disease cannot be compared.

6.1 Mechanism of drug action

It is not possible to explain precisely either the mechanism of action of any trypanocidal drug or the nature of drug resistance, owing to ignorance of the biochemistry and physiology of trypanosomes. The principal reason for this lack of understanding is the inability to grow blood-stream and tissue forms of the parasite *in vitro*. All the available information on mechanisms of drug action has been obtained from the study of "model systems". Any micro-organism that can be grown in controlled conditions and that is sensitive to a drug can yield useful information on the mechanism of drug action. At present it is important to obtain as much information as possible about the action of trypanocidal drugs on any biological system. Such studies should be conducted at the "whole cell" and "cell-free" levels, using isolated organelles (e.g., nuclei, mitochondria, and kinetoplasts), purified enzymes, and isolated cell constituents such as nucleic acids, lipids, and proteins. However, the limitations of such studies must be borne in mind, and any hypothesis of drug action developed from them

must be tested on viable blood-stream forms of trypanosome. Such studies have revealed the striking fact that, with the exception of arsenicals and suramin, most of the trypanocides used to treat African trypanosomiasis are potent inhibitors of nucleic-acid or protein synthesis. This finding directs attention to the reason for the effectiveness of such trypanocides. Studies of "cell-free" systems have given no evidence that specificity lies at the site of drug action. Such drugs inhibit the synthesis of proteins and nucleic acids in all types of cell, and the reason for their specific action against trypanosomes is not clear. The evidence suggests that the membranes and osmotic barriers of trypanosomes are involved, and that the selective activity of these compounds may depend on the varying permeability of different cells. More information is urgently needed on (1) the interaction of drugs with the lipoprotein structures of cell surfaces and (2) the factors that influence the penetration of drug molecules through such structures.

6.2 Future development of chemotherapy

The development of a more rational approach to chemotherapy requires more detailed knowledge of the structure and biochemistry of trypanosomes. The outlook seems promising; there is already evidence that trypanosome and mammalian cells show major differences that might form the basis of a new approach. The glycerophosphate dehydrogenase system of the blood-stream form of African trypanosomes appears to be unique. The kinetoplast, which plays an important role in polymorphism, contains DNA, whose replication can be selectively inhibited by phenanthridine and aromatic diamidines. Such inhibition gives rise to "akinetoplasic" organisms that are unable to infect the insect vector. Compounds that act in this way have long been known, but their ability to break the natural life cycle of trypanosomes has not been exploited. Compounds that act selectively on the kinetoplast may prove of value in controlling the spread of infections and, if used in conjunction with other drugs, may help to curb the spread of drug resistance.

In recent years biochemists have become aware of the existence of isoenzymes. It is now known that some enzymes in schistosomes and trypanosomes are much more sensitive to certain drugs than are those that catalyse the same reactions in mammalian cells. It is important that trypanosome enzymes be purified and that detailed comparative studies be made of the kinetics of enzymes from parasite and host cells.

6.3 Drug resistance

There are three areas in which further knowledge is needed if a full understanding of drug resistance is to be attained: (1) the way in which a cell becomes resistant (whether by mutation or selection); (2) the differ-

ences between resistant and sensitive cells ; and (3) whether transfer of drug resistance, of the type now known to occur in bacteria, occurs in trypanosomes (the occurrence of such transference has been reported,¹ but has not yet been confirmed). Knowledge is lacking in all these areas ; it is not even known whether drug molecules penetrate resistant trypanosomes. Early work on arsenical drugs suggested that the development of resistance is accompanied by a decrease in the permeability of the cell to the drug. More critical studies, using more sensitive methods to measure drug uptake, are required. Such studies would be greatly facilitated if isotopically labelled drugs of high specific activity were available. There is also an urgent need for further studies of the comparative biochemistry of host and parasite cells.

7. METHODS

7.1 Measurement of infectivity and virulence

It may be necessary to measure the infectivity of a trypanosome suspension in relation to a mammalian host, a culture, or an insect vector. Since the infectivity of individual trypanosomes varies, a statement of the concentration of organisms in a suspension is, by itself, inadequate as a measure of the infectivity of the suspension. However, the infectivity of many trypanosome suspensions may be measured experimentally by serial dilution and by the inoculation of animals or cultures with standard quantities of each dilution. The infectivity of the original suspension can be estimated from the number of recipients becoming infected at each dilution ; it is conveniently expressed in terms of 63% infective doses (ID_{63}). By comparison of this estimate with an estimate of the concentration of organisms in the suspension, the number of organisms constituting an infective dose may be deduced. This number sometimes approaches 1, but varies widely, and is usually much greater.

The above method can be used when an infection can be readily diagnosed by microscopic demonstration of the parasite. It can be used to measure infectivity by injection (by any route) and possibly also by penetration of intact body surfaces. When low levels of parasitaemia make microscopic identification of infection difficult, serological methods may be used.

For measuring the infectivity of trypanosome suspensions to the insect vector, it appears to be essential to use standard vector populations derived from laboratory colonies, and to use standard methods for maintaining them during cyclical development.

¹ Inoki, S. et al., *Biken's J.*, 1960, 3, 101 ; 1961, 4, 111.

Virulence can be assessed by observation of a given response (e.g., the time that elapses before death) in standard hosts following challenge with doses of known infectivity.

7.2 Maintenance of trypanosomes

7.2.1 *In vitro maintenance*

Maintenance *in vitro* may be regarded as a simulation of the propagation of the organisms in the insect host. Cultivation is usually undertaken on various media of the blood agar type, the trypanosomes growing in the "condensation water", which contains nutrients and salts from the agar mass with which it is in equilibrium.

T. (S.) cruzi grows readily in a number of diphasic and liquid media at temperatures of 25–28°C, but grows less readily, or may die, at 37°C. Metacyclic forms tend to appear in old cultures. Repeated passage leads to a loss of infectivity of the culture forms, although this is seldom complete; however, animals may have only cryptic infections. Cultures of *T. (H.) rangeli* lose virulence rapidly and metacyclic forms never appear.

The *Salivaria* are more difficult to grow on artificial media than *T. (S.) cruzi* and require greater concentrations of blood, which must be either human or blood in which complement has been inactivated. So far, they have been cultivated only at vector temperatures, usually below 28°C. The culture forms are typically noninfective to mammalian hosts. However, infectivity is sometimes reacquired, the important factors apparently being the age of the culture (maximum infectivity occurs after about 18 days) and the erythrocyte component of the blood used. Cultivated trypanosomes also differ in antigenic type from the blood forms from which they are derived.

7.2.2 *In vivo maintenance*

T. (S.) cruzi has been cultivated without difficulty in whole chick embryo and in tissue preparations; many different tissues and cells have been readily infected. The complete life cycle of *T. (S.) cruzi*, similar to that which occurs in the vertebrate host, can be observed in infected tissue cultures; such cultures are, therefore, extremely useful for the study of morphogenesis and chemotherapy.

Similar success has not been achieved with *T. (T.) rhodesiense* and *T. (T.) gambiense*. A limited study has been made, using the chick embryo, but the changes in the resulting trypanosome population have not been adequately assessed, although maintenance of infectivity to the mammalian host has been reported. *T. (D.) vivax*, which is not normally infective to laboratory animals, has been established in a tissue culture containing material derived from the insect vector, and stages infective to vertebrates

have apparently been produced. Further investigation of this type is warranted.

T. (S.) cruzi, unlike most other trypanosomes of the *Stercoraria* group, shows little host specificity, and infects a wide variety of wild and laboratory animals as well as man. The course of infection in experimental animals depends on the strain, the animals either dying of an acute infection or showing a chronic infection with prolonged parasitaemia. The pattern of parasitaemia and the biological behaviour of a given strain tend to remain constant in *T. (S.) cruzi* infections.

T. (T.) rhodesiense and *T. (T.) gambiense* are also maintained in small laboratory animals. Infection with *T. (T.) rhodesiense* is typically acute. *T. (T.) gambiense* may be difficult to establish in small rodents, and preliminary passage in other animals, such as young *Cercopithecus* monkeys or *Cricetomys* (the giant rat) may be necessary. The maintenance of these trypanosomes by serial passage in such experimental hosts is accompanied by profound changes in the characteristics of the strains (e.g., infectivity to the fly vector, virulence, sensitivity to drugs, and antigenic type).

Trypanosomes may also be maintained by cyclic transmission through the appropriate insect vector. This type of maintenance, which resembles the natural process, avoids some of the changes in characteristics that occur in serial syringe passage.

7.2.3 Preservation at low temperature

Low-temperature preservation has proved to be of great value in that it avoids the instability shown by the African pathogenic trypanosomes during strain maintenance in laboratory animals. Furthermore, large numbers of stabulates can be stored in this way, and their transport to laboratories in any part of the world presents few problems. Several collections of trypanosomes preserved at low temperatures have been established in Africa and Europe.

Established methods could probably be used for the preservation of *T. (S.) cruzi*. Although this organism appears to be much more stable than the African trypanosomes during strain maintenance, the establishment of standard reference material in central banks would offer several advantages.

Experience indicates that storage in liquid nitrogen or solid carbon dioxide is preferable to the use of mechanical refrigerators.

It is most important that trypanosomes stored in banks be carefully identified, since such identification is essential for laboratory work with the material.

If standard methods and stabulates are used in investigations, the results obtained in different laboratories at different times will probably be comparable.

8. RECOMMENDATIONS FOR RESEARCH

Immunobiological studies

The Group recommends that research and development be carried out in the following areas.

(1) The careful definition of antigenic types and the production of homologous antisera, necessary for systematic study of antigenic variation in the *Salivaria*. The work now in progress should be extended and improved, possibly by the use of immunologically impaired mice for the raising of clones.

(2) Study of the possibility of antigenic variation and its relationship to protective antibody in *T. (S.) cruzi* infections.

(3) A search for antigen-antibody complexes in the body of the mammalian host.

(4) The development of tests for the detection of trypanosomal antigens in infected hosts.

(5) Investigation of the different antigens elaborated by trypanosomes and the immunoglobulin class of their corresponding antibodies.

(6) Further study of the use of blood dried on filter paper for immunochemical tests, particularly for the estimation of IgM levels in trypanosomiasis.

(7) Study of the mechanism by which IgM synthesis is stimulated in African trypanosome infections.

(8) Investigation of IgM levels in Chagas' disease.

(9) The use of serological tests for the diagnosis of Chagas' disease as a part of the antenatal care of women from endemic areas.

(10) The performance of serological tests for Chagas' disease on serum, pericardial exudate, and spinal fluid when chronic cardiomyopathy is found at autopsy.

(11) Studies to determine whether or not Chagas' heart disease and demyelination are manifestations of autoimmunity.

Clinicopathological and epidemiological studies

(1) Further study of the acute and chronic stages of both American and African trypanosomiasis, using standard procedures and standard criteria, is necessary.

Studies are required of the functional and pathological changes occurring in the latent phase of *T. (S.) cruzi* infection. Comparative studies of

neural lesions and their role in Chagas' disease in different areas of South America should be carried out, and the possible part played by antigen-antibody reactions in their pathogenesis should be investigated. Further studies of the occurrence and importance of peripheral nervous system lesions in African trypanosomiasis are necessary.

(2) Particular attention should be given to clarification of the pathogenesis of the apical cardiac lesion, which appears to be characteristic of chronic Chagas' cardiomyopathy and is not found in other cardiomyopathies. The incidence and importance of cardiac lesions in African trypanosomiasis should be given further attention, as should the part that the disease may play in cases of cardiac insufficiency of unknown origin. More information is required on the clinical, radiological and electrocardiographic changes that occur in African trypanosomiasis, with particular attention to the effect of specific therapy on such symptomatology.

In general, there is a need for further comparative studies of the pathogenesis of cardiac lesions in African trypanosomiasis, in relation to other cardiomyopathies that occur in Africa and particularly in relation to Chagas' cardiomyopathy in America.

(3) Surveys to determine the existence and frequency of the extra-cardiac manifestations of Chagas' disease (e.g., the megasyndrome) should be undertaken in different areas of Latin America.

(4) There is a need for wider systematic prenatal and postnatal studies to determine the frequency of congenital transmission of both American and African trypanosomiasis and to assess the parasitological and immunological problems involved. The possibility that *T. (S.) cruzi* infections may cause abortion or miscarriage, as commonly occurs in infections with the African pathogenic trypanosomes, should be investigated.

(5) Further physiological studies of tsetse are required and similar studies of the reduviid bugs should be undertaken. Further study is needed of the factors that affect the efficiency of both vectors as transmitters and their relative infectibility with different strains of trypanosome. The importance of host preference in relation to epidemiological control needs further investigation.

(6) A pilot longitudinal study of Chagas' disease should be carried out to provide baseline data on epidemiological and other factors. In any such project, social and anthropological considerations must be taken into account.

(7) There is a need for a pilot field study of the effect of environmental temperature on parasitaemia in mammals with Chagas' disease. In the area selected for the study, the same naturally infected reduviid vector should occur in a wide altitude range.

9. GENERAL RECOMMENDATIONS

(1) It is recommended that WHO consider the establishment of at least two banks for the maintenance, in duplicate, of important frozen stabilates. Both African and American trypanosomes should be preserved in this way so as to be available for distribution as required. Collections of stabilates should be built up from widely separated geographical areas and a variety of hosts, both humans and domestic and wild animals.

Standard procedures should be established for the isolation of African pathogenic trypanosomes and for the isolation and cultivation of *T. (S.) cruzi*. Maintenance procedures should be specified, and should cover details such as inoculum size, the in-bred strain of mouse to be used, the method of freezing, the storage temperature, and, for *T. (S.) cruzi*, the source of blood for media. Consideration should be given to the clonal selection of organisms.

Stabilate banks could also serve as reference centres for antigenic types and specific antisera, which are essential for comparative studies undertaken in different laboratories.

(2) In view of the increasing importance of immunological methods in both diagnosis and research, it is recommended that WHO assist in establishing (a) standard techniques for immunological tests; (b) standards for all necessary reagents (e.g., antigens, antisera, specific antisera to immunoglobulins, and reagents for use in immunofluorescence tests); (c) a means for the exchange and analysis of information on tests carried out in different centres; and (d) arrangements for the training of technicians in established laboratories.

(3) The development of standard, genetically defined insect vectors is of the utmost importance and should be encouraged by WHO.

(4) It is recommended that WHO encourage the development of isotopically labelled trypanocidal drugs to be made available to research workers studying the chemotherapy of trypanosomiasis.

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