THE PRESENT STATUS OF CHAGAS´ DISEASE IN THE UNITED STATES

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This study was partially supported by a grant from the U. S. Navy, Office of Naval Research.

In 1907 Carlos Chagas discovered in Brazil a new disease that now bears his name. Since that date many investigators all over the world have been looking for evidence of this new disease in human beings and for vectors and reservoirs of its etiological agent. As a result of this intensive and diligent search during the last forty-five years, today it is known that Chagas' disease exists in all South and Central American countries as well as in Mexico (1, 2, 3, 4, 20, 36).

In recent years references to the disease have appeared in the literature in the United States and Canada, but no naturally occurring endogenous human cases have been reported in these countries. Generally speaking, the medical profession of North America is largely unacquainted with the clinical picture of Chagas' disease and with the possibility of its existence in the United States and Canada (3, 16, 26, 30, 36).

The object of the present communication is to present the status of Chagas' disease in the United States and review briefly some of the more important contributions wich have come from the United States for a better understanding of this disease.

VECTORS OF CHAGAS' DISEASE IN THE UNITED STATES

It is a well-known fact that many years before the discovery of Chagas' disease and its insect vectors in Brazil, Charles Darwin in 1835, in Mendoza, Argentina, described colorfully the habits of *Triatoma* (wich he called "Benchuca") and the manner in which they attack and feed on man (5). A similar observation was made in the State of Georgia in the United States in 1859 by Stal who not only described *Triatomla sanguisuga* (Leconte), but also stated that persons bitten bv these insects were ill for nearly a year (35). This important observation suggesting the possibility of a disease transmission by these insects was buried in entomological literature in 1916 Kofoid and McCulloch found that *Triatoma protracta* (Uhler) in California was naturally infected with a flagellate which they regerded as a new species and which they named *Trypanosoma triatomae* (14). Later Kofoid and Donat (1933) recognized that this flagellate was identical with *Trypanosoma cruzi* (12,13). A second species of reduviid bug, *Triatoma uhleri* (Neiva), was found also naturally infected with *Trypanosoma cruzi* by Kofoid and Whitaker during 1936 in Arizona (16).

A third species of reduviid bug, *Triatoma sanguisuga* (Leconte), from Temple, Texas, was found by Packchanian in 1938 to be naturally infected with *Tripanosoma cruzi* (25).

In 1939, Packchanian found that *Triatoma gerstaeckeri (* Stal) was also naturally infected with *Tr. cruzi.* These blood-sucking insects attack man and animals very readily. The distribution of *T. gerstaeckeri* in South Texas is a wide one, particularly on ranches, among wood rat dens, in cactus, and among mesquite trees and represents an important public health problem (26).

Again in the State of Texas in 1940 Packchanian found an additional species of blood-sucking insect, *Triatoma heidemanni*, naturally infected with *Trypanosoma cruzi.* This species of *Triatoma* has become a household pest in certain localities and certainly is well established in chicken huts as well as among armadillo and opossum nests

(27).

During 1940 a few live specimens of *Triatoma longipes (Barber)* were received from the mountains of Arizona and found naturally infected with *Tr. cruzi* by Packchanian (Unpublished data). In 1941 independently, Wood also found *Triatoma longipes* naturally infected with *Tr. cruzi* in Arizona (43).

Wood in 1941 reported *Triatoma rubida* (Uhler) naturally infected with *Tr. cruzi* in Texas (44).

Triatoma sanguisuga ambigua (Neiva) naturally infected with *Tr. cruzi* in Texas was reported by Davis, McGregor and de Shazo during 1943 (8).

Thus, so far at least eight species of *Triatoma* are known to be naturally infected with *Tr. cruzi* in Southern and Western parts of the United States. Seven additional species of *Triatoma*, namely *T. indictiva Neiva, T.maxima (*Uhler), *T. neotomae (*Neiva*), T. occulta (Neiva), T. occellata* (Neiva), *T. phyllosoma* (Burmeister), and *T. rubrofasciata* (De Geer) found in the United States are not known to be naturally infected with *Tr. cruzi* (10, 18, 35, 39).

During 1936 Packchanian experimentally infected *Triatoma sanguisuga ambigna* with *Tr. cruzi,* and proved once more that the bite of the infected insects were not infectious to experimental animals (28). Also, *Triatoma indictiva,* was experimentally infected with *Tr. cruzi* by Wood in 1941 (45).

The habits of *Triatoma sanguisuga ambigua,* as found near the Miakka River at Sarasota Florida, by Packchanian, are rather unusual and interesting. These small-sized *Triatoma* were found living among decayed leaf-stalks of cabbage palmetto trees *(Sabal palmetto),* and apparently were feeding on tree toads (genus *Hyla sp.* ?). No wood rat dens were found in this area which consisted mostly of swamps. Careful examination of many palmetto trees in other parts of Florida, Georgia, and Texas revealed no *Triatoma,* this finding may possibly be correlated with the absence of tree toads on palmetto trees in these regions (28).

The 15 species of *Triatoma* that are found thus far in the United States all fall in the area between 25° and 42° latitude. Since eight species are known to be naturally infected with *Tr. cruzi,* and inasmuch as other species represent potential vectors, the question of human cases of American trypanosomiasis in the United States is quite pertinent.

THE RESERVOIR HOSTS OF *TR. CRUZI* IN THE UNITED STATES

The first species of animals naturally infected with *Tr. cruzi* in the United States was reported in 1934 by Wood, who demostrated a trypanosome morphologically identical with *Tr. cruzi* in one wood rat *(Neotoma fuscipes macrotis*) in California. This finding is probably correct; but proof of the identity of the flagellate is not conclusive (16, 42).

The next important discovery of reservoir hosts was by Packchanian in 1942, who found for the first time in the United States the following species of animals naturally infected with *Tr. cruzi:*

(a) armadillo (*Dasypus novemcinctus), (b)* opossum *(Didelphys virginiana*), *(c)* house mouse *(Mus musculus),* and *(d)* wood rat *(Neotoma micropus micropus).* The trypanosomes were demonstrated in the peripheral blood of these animals by direct microscopic examination and were cultured *in vitro.* Animal inoculation tests, xenodiagnosis, and histopathoological studies left no doubt that these animals were naturally infected with *Tr. cruzi (30*).

In Texas from 1939 to 1946 Packchanian has examined many wood rat dens which were found among cactus and mesquite trees. From most of these rat dens, large numbers of *Triatoma* in all stages of development, were collected during all four seasons of the year. Many *Triatoma* collected were found to have blood in their intestines, indicating that they had fed on some animal in or around the rat dens. Whan the farmers in certain localities were informed of the potential dangers existing in the wood rat dens, an effort was made to remove this menace by burning all brush heaps and rat dens and by removal of mesquite trees with a special tractor (“bull-dozer”). As a result of these efforts, the number of *Triatoma* in these areas have been greatly reduced during the last two years, according to farmers and local observers.

CULTIVATION OF *TRYPANOSOMA CRUZI* IN VITRO

Various strains of *Trypanosoma cruzi* from North, South, and Central America have been successfully grown on several culture media. While Novy and MacNeal's (N.N.) medium or Novy-MacNeal-Nicolle's (N.N.N.) medium are ideal media on which to grow *Trypanosoma cruzi,* in the writer's experience *Tr. cruzi* grows readily on plain infusion agar with about 15% to 20% defibrinated rabbit blood. One of the essential factors is to have sufficient quantity of water of condensation in the culture tubes. The tubes should be sealed with rubber caps and incubated at a temperature of about 25ºC. (3, 4, 22, 23, 26, 29, 36, 41).

Packchanian cultured a strain of *Trypanosoma cruzi* in 1932, and maintained it *in vitro* on N.N. medium up to the present time by monthly subcultivation. This culture was still infective to experimental animals after 13 years of cultivation *in vitro* (33).

While it is desirable to subculture *Tr. cruzi* monthly, the flagellates remain viable, and will give rise to subcultures without being transferred for two, three and even four months. In a few instances Packchanian has obtained subcultures from tubes as old as six years. This, however, is a rare finding (32).

Attempts to improve and simplify the methods for culturing *Tr. cruzi* have been made by various investigators. In 1943 an autoclaved medium containing blood was used by Tom for culturing *Tr. cruzi* (38). Recently Little alla Subbarrow (1945) described a “liquid medium”, wich is autoclaved and in which *Tr. cruzi* grows readily. This, however, is hardly liquid medium because it contains a suspension of coagulated red blood cells (19)

The advantages that cultural methods have over xenodiagnosis are as follows:

1. Culture media is easy to prepare and more readily available than laboratory raised *Ttiatoma.*

2. The amount of 10 c.c. of the patient's bood can be examined by inoculating up to 10-12 culture tubes.

3. Allergic skin reactions after the insect bite, and the objection and discomfort of the patient may be minimized by replacing xenodiagnosis with cultural tests.

The use of cultural tests for the diagnosis of Chagas' disease, both in natural and experimental infections, has been found to be very valuable. Often positive cultures have been obtained when no trypanosomes could be demonstrated by microscopic examination of blood (23, 26, 27, 28, 30, 31, 32). It is recommended that cultures be used more often for the diagnosis of Chagas' disease. The medium is rather simple to prepare, and positive results may be obtained in two or three weeks.

SEROLOGICAL STUDIES

One of the most important contributions for studing Chagas' disease probably was the growth of *Trypanosoma cruzi in vitro* and the use of such cultures for serological and inmunological studies (24). Although serolocal studies such as complement fixation tests were performed as early as 1913 by Guerreiro and Machado (9) and later by others (37, 40), all used organs of intected animals, usually puppies, to prepare the antigen.

Packchanian in 1935 was the first to utilize *Tr. cruzi* cultures as antigens for serological and inmunological studies (24). Whan sufficient growth of *Tr. cruzi* was obtained after one to three weeks on blood agar slants, the growth was suspended in a physiological salt solution (Tyrode's or Ringer's) and washed three times be centrifugation and resuspension. The final sediment of washed trypanosomes was used to prepare the antigens for agglutination and precipitation tests and for inmunological studies (24).

Antigen for the precipitation test consisted of one volume of packed, washed *Tr. cruzi* with nine volumes of sterile distilled water. Cytolysis of the trypanosomes was accelerated somewhat by slow freezing and thawing of the suspension. Alcoholic and ether extracts of antigen for precipitation test were likewise prepared. The precipitation titer was low with all of these antigens, but the test is specific and of diagnostic value (24).*

The antigen used in the agglutination test was a suspension of live washed *Tr. cruzi* in Tyrode's or Ringer's solution. The turbidity of the suspension was about 1.75 on the McFarland nephelometer scales, or about the same as that of a 48-hour culture of *E. typhosa* in broth. On rare occasions formalin was added to the antigen for agglutination test. Antigen prepared in such a manner always gave a positive macroscopic agglutination reaction with serum samples from animals infected with *tr. cruzi* in dilutions of 1:256 to 1:1024. The reaction is definitely specific and diagnostic (24, 29).

In 1936, Kelser performed complement fixation tests for Chagas' disease using an antigen prepared essentially as Packchanian's antigen except Kelser added two volumes of 50% glycerine in physiological salt solution to the washed, packed trypanosomes (11).

Davis in 1943 reported a method of making *Tr. cruzi* antigen, which differs from Packchanian's original aqueous antigen mainly in that he uses merthiolate as a preservative and that he uses more rapid freezing in an attempt to liberate the antigenic substances. He used this antigen in complement-fixation tests on over 1,000 samples of humans sera with negative results (6, 8).

A macroscopic slide agglutination test for Chagas' disease was reported by Senekjie in 1943 to be valuable for diagnosis of infections in animals (34).

INMUNOLOGICAL STUDIES

A torough, systematic study of the inmunologic properties of *Tr. cruzi* has not been made, and most of what has been done has been from a diagnostic point of view. However, a few additional facts may be worthy of mention here. By repeated intravenous injection of washed trypanosome cultures into a rabbit, Packchanian succeeded in producing an antiserum that agglutinates *Tr. cruzi* antigen in a dilution as high as 1:260,000. This titer appears to be one of the highest ever obtained in any, protozoan infection. It was not until 43 injections at semi-weekly intervals had been completed that the titer rose to this height. The first seven injections consisted of formalized antigen, while the remaining ones were suspensions of live organisms. The relatively large number of inoculations at frequent intervals is apparently an important factor in the production of *Tr. cruzi* antiserum (24) .

Flagellar and somatic antigens, designated “H" and “O” as for bacterial antigens, have been demonstrated by Senekjie (34); the importance of this discovery has not yet been recognized.

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* Protein and polysaccharide complex extracted from *Tr. cruzi* by various methods all gave strong positive precipitation reaction with *Tr. cruzi* antisera and with the serum of known n positive cases of human Chagas' disease in high dilution. (Unpublished data of the writer.)

HISTOPATHOLOGY AND CHEMOTHERAPY

Amog other scientific contributions of value from the United States pertaining to Chagas' disease was one in 1940 by Packchanian, who found aflagellar or leishmania-like segmenting forms of *Tr. cruzi* in fat cells of an experimentally infected animal (27). Kofoid and his co-workers have reported studies of *Tr. cruzi* in tissue culture and the effects of arsenicals on *Tr. cruzi* in tissue culture (15, 17).

SEARCH FOR HUMAN CASES OF CHAGAS' DISEASE IN THE UNITED STATES

Since 1933 Kofoid and his co-workers, particularly Wood and Wood, in California have made diligent search to find the first case of Chagas' disease in the United States; they have obtained negative results so far (12, 14, 38, 41, 42).

During 1936 Packchanian was given an opportunity to initiate a research unit with the National Institute of Health of U. S. Public Health Service for studynlg Chagas' disease. Several field studies were conducted by Packchanian in various parts of Texas in an effort to locate persons with American trypanosomiasis. Many persons that had been bitten by *Triatoma* were interviewed; blood samples collected from over 75 such persons from 1937 to 1946 have been inoculated into N-N medium without success (25, 26, 27, 28, 30, 31). Since 1941, Davis at the National Institute of Health has also been interested in locating a human case of Chagas' disease in Texas, so far with negative results (7, 8).

There was a time when questions were raised by many people wheter *Tr. cruzi* as found in *Triatoma* in the United States was infective to man, and some went so far as to ridicule the idea of Chagas' disease in this country. All doubt as to the virulence of the Texas strain of *Tr. cruzi* for man was dispelled whan the intestinal contents of a naturally infected *Triatoma* indigenous to Texas were introduced into the conjunctival sac of a human volunteer. A

typical case of Chagas' disease resulted; various laboratory procedures confirmed the clinical findings. And thus, the Texas strain of *Tr. cruzi* proved to be pathogenic and identical with that of the South American strains (31).

It has also been established that strains of *Tr. cruzi* found in *Triatoma* and reservoir animals in California, Arizona and Texas produce infection in experimental animals, including monkeys (13, 26, 27).

Failure to find Chagas' disease in man in the United States may be attributed to the following factors:

(1) The reservoir hosts of Chagas' disease, chiefly wood rats, opossums, and armadillos are usually limited to uninhabited areas or areas near farms and ranches.

(2) The mejority of homes in rural areas have screened windows and doors, thus reducing massive invasions of homes by these insects. *Triatoma* wich lay egss in or near homes rarely are allowed by the inhabitants to propagate themselves for more than one generation. Since apparently no reservoir host exists in homes, the young nymphs hatched in the homes have been found to be free from infection.

(3) Human cases of Chagas' disease may occur in people living on ranches and farms; however, because the disease is mild in its manifestation, medical counsel usually is not solicited.

(4) Most physicians in the United States are not on the alert for this disease. They are not, as yet, well acquianted with the clinical characteristics of American trypanosomiasis and whit the possibilites of its presence in the United States.

(5) For absolute laboratory diagnosis of Chagas' disease one has to utilize cultural, animal inoculation, and serological tests. The facilities available to the average physician in the rural areas, where cases are more ap to occur, do not permit such studies.

It is interesting to note that investigators in Uruguay searched for nearly 16 years before the first human case of Chagas' disease was diagnosed. After that, the diagnosis of this disease was made with increasing frequency and is now quite commonplace in that country. It is the opinion of the writer that it is only a question of time before authantic cases of Chagas' disease will be reported from some of the southern and southwestern states of the United States.

REFERENCES

1. BRUMPT, E., MAZZOTTI. L., and BRUMPT, L. (1939). Enquetes Epidemiologiques Sur La Maladie de C. Chagas au Mexique (1). Reduvides Vecteurs. Animaux Reservoirs de Virus. Cas Humains. Ann. de Parasitol., 17:299.

2. CHAGAS, C. (1909). Uber eine neue Trypanosomiasis des Menschan. Mem. Inst. Oswaldo Cruz, 1:158.

3. CHANDLER, ASA C. (1940) . Introduction to Parasitology. Sixth Edition. John Wiley and Sons, Inc., New York.

4. CRAIG, C. P., and FAUST, E. C. (1943). Clinical Parasitology. Third Edition, Lea and Febiger, Philadelphia.

5. DARWIN, C. (1835). Journal of Researches into the Natural History and Geology of the Countries Visited During the Voyage of H. M. S. Beagle Round the World, under the Command of Capt. Fitz Roy, R. N., D. Appleton and Company, 1896, pp. 330.

6. DAVIS, D. J. (1943). An Improved Antigen for Complement Fixation in American Trypanosomiasis. U. S. Pub. Health Rep., 58:775.

7. DAVIS, D. J., McGREGOR, T., and DE SHAZO, T. (1943). *Triatoma sanguisuga* (Leconte) and *Triatoma ambigua* Neiva as Natural Carriers of *Trypanosoma cruzi* in Texas. U. S. Pub. Health Rep. 58:353.

8. DAVIS, D. J., and SULLIVAN, T. (1946). Complement Fixation Tests for American Trypanosomiasis in Texas. U. S. Pub. Health Rep., 61:1083.

9. GUERREIRO, C., and MACHADO, A. (1913) . A reaccal de Bordet e Gengow na Molestia de Chagas como elemento de Diagnostico, Brazil Medico, 23:225.

10. HUSSEY, R. F. (1922). A Bibliographical Notice on the Reduviid genus *Triatoma,* Psyche, 29:109.

11. KELSER, R. A. (1936). Complement-Fixation Test for Chagas' Disease Employing Artificial Culture Antigen, Am. J. Trop. Med., 16:405.

12. KOFOID, C. A., and DONAT, F. (1933). Experimental Infection with *Tr. cruzl* from Intestine of Cone-nose Bug. *T. protracta.* Proc. Soc. Exp. Biol. d Med., 30:459.

13. KOFOID, C. A., and DONAT, F. (1933). The Occurrence of South American Trypanosomiasis of the Human Type in Mammals in the United States. Calif. and Western Med., 38:245.

14. KOFOID, C. A., and MCCULLOCH, I. (1916) . On *Trypanosoma triatomae,* a new flagellate from a hempteran bug from th eneste of the wood rat, Neotoma fuscipes, Univ. Calif. Publ. Zool., 16:113.

15. KOFOID, C. A., and MCNEIL, E., and WOOD, F. D. (1937). Effects of Arsenicals on *Trypanosoma cruzi* in Tissue Cultures. J. Pharm. d Exp. Ther., 59:424.

16. KOFOID, C. A., and WHITAKER, B. C. (1936). Natural Infection of American Human Trypanosomiasis in Two Species of Cone-nosed Bugs. *Triatoma protracta* and Triatoma *uhleri* Neiva in Western United States. J. Parasitol., 22:259.

17. KOFOID, C. A., WOOD, F. D., and MCNEIL, E. (1933) . The Cycle of *Trypanosoma cruzi* in Tissue Culture of Embryonic Heart Muscle. Univ. of Cal. Pub. Zoo., 41:23.

18. LECONTE, J. (1855). Remarkes on Two Species of American Cirmex, Proc. Acad. Nat. Sc., 7:404.

19. LITTLE, P. A., and SUBBARROW, Y. (1945) . A Practical Liquid Medium for Cultivation of *Trypanosoma cruzi* in Large Volumes. J. Bact., 50:57.

20. MAZZA, S., MIYARA, S., and JÖRG, M. E. (1944). Investigaciones sobre Enfermedad de Chagas. Universidad de Buenos Aires Pub. Nº 68, Misión de Estudios de Patologia Regional Argentina.

21. MAZZOTI, L. (1937). Infección natural de *Trypanosoma cruzi* de Chagas en *Triatoma phyllosoma*, Burmeister y Triatoma pallidipennis de la Costa del Pacifico de México. Medicina, México, 17:161.

22. NOVY, F. G., and MACNEAL, W. J. (1904). On the Cultivation of *Trypanosoma brucei.* J. Infect. Dis., 1:1.

23. PACKCHANIAN, A. (1934). On the Cultivation of Seven Species of *Trypanosomes* in vitro. Science, 80:407.

24. — (1935). Agglutination and Precipitation Tests for the Diagnosis of *Trypanosoma cruzi* infection (Chagas' Disease) . J. Inmunol., 29:84.

25. — (1938). Chagas' Disease. Ann. Rep. Surg. Gen., U. S. Pub. Health Service, Government Printing Office, Washington, D. C.

26. — (1939). Natural Infection of Triatoma Gerstakeri with *Trypanosoma cruzi* in Texas. U. S. Pub. Health Rep., 54:1547.

27. — (1940). Natural Infection of *Triatoma heidemanni* with *Trypanosoma cruzi* in Texas U. S. Pub. Health Rep., 55: 1300.

28. — (1940). Experimental Transmission of *Trypanosoma cruzi* Infection in Animals by *Triatoma sanguisuga ambigua.* U. S. Pub. Health Rep., 55: 1526.

29. — (1940). Eperimental Production of Agglutinins for *Trypanosoma cruzi.* U. S. Pub. Health Rep., 55: 2116.

30. — (1942). Reservoir Hoses of Chagas' Disease in the State of Texas. Am. J. Trop. Med., 22:623.

31. — (1943). Infectivity of the Texas Strain of *Trypanosoma cruzi* to Man. Am. J. Trop. Med., 23:309.

32. — (1943), On the Viability of Various species of *Trypanosoma* and *Leishmania* Cultures. J. Parasitol., 29:275.

33. PACKCHANIAN, A., and SWEETS, H. (1946). Infectivity of *Trypanosoma cruzi* after Cultivation for Thirteen

Years *in vitro* without Animal Passage. Proc. Soc. Exptl. Biol. d Med., 64:169.

34. SENEKJIE, H. A. (1943) . Inmunologic Studies in Experimental *Trypanosoma cruzi* Infections: Slide Agglutination and Interdermal Tests. Proc. Soc. Exp. Biol. and Med., 52:56.

35. STAL. C. (1859). Monographie der Gattung Conorhinus and Verwandten. Berl. Ent. Zeit., 3: 99.

36. STRONG, R. P. (1942). Stitt's Diagnosis, Prevention, and Treatment of Tropical Diseases, Vol. I. The Blakiston Co., Philadelpia.

37. TALIAFERRO, W. H. (1942). The Inmunology of Parasitic Infections. The Century Co., New York.

38. TOM, N. (1943). A Modification of N-N Medium for Cultivating *Trypanosoma cruzi*. Am. J. Trop. Med., 23: 615.

39. USINGER, R. L. (1944). The Triatominac of North and Central America and the West Indies and their Public Health Significance. U. S. Pub. Health Bull. N° 288, Governement Printing Office, Washington, D. C.

40. VILLOLA, E., and CHAGAS, B. (1923). As pesquisas de laboratorio no diagnostico da Molestia de Chagas. Mem. Inst. Oswaldo Cruz, 16:13-29.

41. WENYON, C. M. (1926). Protozoology, Vol. I, William Wood and Co., New York.

42. WOOD, F. D. (1934): Natural and Experimental Infection of *Triatoma protracta* Uhler and Mammals in California with American Human Tripanosomiasis. Am. J. Trop. Med., 14: 510.

43. WOOD, S. F. (1941). Chagas' Disease. Southwestern Med., 25: 112.

44. — (1941). Notes on the Distribution and Habits of Reduviid Vectores of Chagas' Disease in the Southwestern United States, I and II. Pan-Pac. Ent., 17: 85-115.

45. — (1941). New Localities for *Trypanosoma cruzi* Chagas in the Southwestern United States. Am. J. Hygs., 34:1.

46. — (1942). Observations on Vectors of Ckagas' Disease in the United States. I. California, Bull. Southern Calif. Acad. Sc., 41: 61.

47. — (1942). Reactions of Man to Feeding of Reduviid Bugs. J. Parasitol., 28: 43.

48. WOOD, S. F., and WOOD, F. D. (1941). Present Knowledge of the Distribution of *Trypanosoma cruzi* in Reservoir Animals and Vectors. Am. J. *Trop*. Med., 21: 335.

49. YORK, W. (1937). Chagas' Disease. A Critical, Review. Trop. Dis. Bull. 34: 275.