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 which 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 (which 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 Triatoma sanguisuga (Leconte), but also stated that persons bitten by 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 regarded 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 uhlern (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 Trypanosoma 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 heidemann, 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.
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 ambigua* 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 demonstrated a trypanosome morphologically identical with *Tr. cruzi* in one wood rat (*Neotoma fuscipes macrots*) 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 histopathological 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. When 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", which 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 blood 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 studying Chagas' disease probably was the growth of *Trypanosoma cruzi* *in vitro* and the use of such cultures for serological and immunological 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 infected animals, usually puppies, to prepare the antigen.

Packchanian in 1935 was the first to utilize *Tr. cruzi* cultures as antigens for serological and immunological studies (24). When 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 by centrifugation and resuspension. The final sediment of washed trypanosomes was used to prepare the antigens for agglutination and precipitation tests and for immunological 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 thorough, systematic study of the immunologic 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.

* 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 positive cases of human Chagas' disease in high dilution. (Unpublished data of the writer.)

**HISTOPATHOLOGY AND CHEMOTHERAPY**

Among 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 studying 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 whether 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 when the intestinal contents of a naturally infected Triatoma indigenous to Texas were introduced into the conjunctival sac of a human volunteer. A
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 authentic cases of Chagas' disease will be reported from some of the southern and southwestern states of the United States.

REFERENCES


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


