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BIOLOGICAL VARIATIONS IN TRYPANOSOMES AND THEIR RELATION TO THE EPIDEMIOLOGY OF CHAGAS' DISEASE

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SUMMARY

A brief review of the literature indicates that *Trypanosoma* species are composed of several antigenic variants or that they become polygenic after exposure to certain chemical or other stimuli.

The concept of strain mixtures and variations is suggested to explain certain aspects of the epidemiology of Chagas' disease in the Americas. Strain mixtures, and therefore the etiology of *Trypanosoma cruzi* infections, seem to vary with their environment and their maintaining hosts and vectors. Man-to-man transmission of *T. cruzi* probably exist only under conditions of heavy infestation of domestic *Triatoma* species, but the main stream of transmission is mostly in wild animals, so that the strain characteristics are determined by the sylvatic rather than by the domestic cycle. In the United States *T. cruzi* infections in wild animals and in triatomid bugs are found as far north as Maryland. To date, the only two proven cases of Chagas' disease reported in the United States have been from Texas. Positive serological tests from a number of persons, however, show that clinically unrecognized Chagas' disease may exist in parts of the country where the population is exposed to possible infection from wild animals and from triatomid bugs. The relatively avirulent character of the *T. cruzi* strains found in the United States is probably due to weeding out of the more virulent strains by their passage through rodents, their maintaining hosts in most parts of the country, and by the selection of strains or degree of development in the local invertebrate vectors. Changes in the "natural" transmission cycle however may possibly produce more variants of greater virulence.

INTRODUCTION

Studies of parasitic protozoa, both in natural and experimental infections, reveal their biological, and sometimes morphological, variability. This paper reviews this subject in relation to trypanosomes and to the epidemiology of Chagas' disease in the Americas. It has generally been accepted that the reproduction of trypanosomes is solely by binary fission; the possibility of protoplasmic exchange between trypanosomes is therefore not considered here. In the absence of gene recombinations, the evolution of trypanosomes, as well as of other asexual unicellulars, would be a single line of unchanged organisms from their original stock, but for mutation and selection of those types best fitting environmental conditions at a given time. Mutation and selection constitute an ever-ongoing process; the strain variations in trypanosomes and other microorganisms examplify this process, which leads eventually to speciation when long isolation of the strain

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and other circumstances permit. In most metazoa genetic changes are often expressed in observable modification in the phenotypes, but eventual morphological changes resulting from genetic changes in microorganisms are usually masked by their relative minuteness and mainly revealed by changes in their physiology and, in the case of parasitic forms, by their action upon the host. The selection of parasitic strains may well be of major importance to the course of subsequent host-parasite relationships and of further transmissions and thus to the epidemiology of a disease in a given area. Numerous examples of "mild" and "severe" outbreaks of certain infectious diseases caused by the same organism can be explained by strain differences, in conjunction with the degree of individual resistance of the host. This selection is also observed in experimental *in vivo* work, while a decrease of virulence, to the extent of complete loss of infectivity, is often seen in pathogenic trypanosomes when they are maintained in culture. During prolonged subculturing, those variants survive which multiply best in the artificial medium, bringing about changes in the overall trypanosome population. It then assumes biological characteristics which may be quite different from those of the parent strain. Loss of the kinetoplast provides directly observable morphological evidence of changes in trypanosome populations. This occurs in varying percentages of several species (HOARE [10, 11], INOKI [13], KILLICK-KENDRICK [15], MUHLPFORDT [18, 19], TOBIE [25] and WERBITZKI [27]). The proportion of akinetoplastic individuals arising spontaneously in these trypanosome species (trypanosomes with atypical kinetoplast, as MUHLPFORDT prefers to call them), can be markedly increased by the use of certain organic dyes, such as Trypaflavin, Pararosaniline, Pyronin, Oxazin, and others, both in blood forms and in culture forms. This does not apply, however, for *Trypanosoma* (*Schizotrypanum*) *cruzi* *. Except for the blood forms of the *Trypanosoma brucei* group (*T. brucei*, *T. gambiense*, *T. rhodesiense*, *T. evansi*, *T. equinum* and *T. equiperdum*), none of the atypic forms reproduces under these experimental conditions. In *T. evansi* the atypical forms are predominant as explained by MUHLPFORDT [19], who found that the akinetoplastic form has a shorter generation time than the normal form. Generation time is shorter, however, in the normal strains of *T. gambiense*, *T. brucei* and *T. equiperdum*, so that for these species the typical form predominates. HOARE [10] pointed out the interesting selection factor in *T. brucei*, *T. rhodesiense* and *T. gambiense* where individuals showing atypical kinetoplast are eliminated during cyclic development in the tsetse fly, whereas the atypical forms of *T. evansi* and *T. equiperdum* survive their normally mechanical transmission that occurs in biting flies not belonging to the genus *Glossina*. This led HOARE [10] to postulate that *T. equinum*, causing the disease called "mal de caderas" in horses in South America, derived from an akinetoplastic *T. evansi* strain introduced with the first horses imported to the Island of Marajó, near the estuary of the Amazon. This example is a rare instance in which speciation through mutation, followed by geographic isolation, can be traced in recent history.

Drug resistance is still another biological character that discloses specific variations in strains (INOKI [12], INOKI et al.[14], OSAKI [22]). The stability of resistance to aromatic arsenicals of certain strains is so marked that it can be used as a tool for genetic identification; it explains the failure of these drugs to act on certain strains. The literature and new techniques related to this subject have been reviewed and discussed recently by WALKER [26]. CUNNINGHAM & VICKERMAN [4] demonstrated the varying antigenic characteristics of trypanosomes of the *T. brucei* group, freshly isolated from natural human and animal infections, and suggested two interpretations of their findings. One is based on the assumption that each individual organism contains a number of antigens, of which only one at a time is capable of "surface expression" while others remain masked according to the host's immune response. The other interpretation is based on the possibility of the selection of mixed antigenic

* *T. cruzi* differs from other trypanosomes in other respects; many authorities consider this organism to belong to a separate genus: *Schizotrypanum*. One of the supporting factors for this separation is its multiplication in the vertebrate host, where *T. cruzi* develops intracellulary in the tissues in the leishmania form; for other *Trypanosoma* species it occurs in the bloodstream by binary fission in the trypanosome stage.

populations through the agency of drugs, antibodies or physiological idiosyncrasies of the host. Such selection would result in the dominance of one population at a given time, which would be the one detected by the agglutination reaction. From his own experiments and review of the findings of others, GRAY [8] concluded that each trypanosome species has its own basic and characteristic antigen but also contains variations having secondary antigenic characters. Antigenically identifiable variants in trypanosomes have also been reported by other Authors (RITZ [23], LOURIE & O'CONNOR [16], OSAKI [22]). HAWKING & WALKER [9] comment on differences of arsenical resistance between old and new trypanosome strains. They hypothesize that for each character (viz. virulence, morphology, biochemistry, antigenicity, etc.) a number of possible populations exists, and the change from one to another is discontinuous. "The apparent characters of the complete strain would then depend upon the relative proportions of the different populations present at some particular moment". The specificity of an artificially selected trypanosome strain, as well as its equally specific immune response in the vertebrate host, is well exemplified in a series of experiments by CANTRELL & BETTS [3]. They noted that rats infected with *T. equiperdum* and treated with oxophenarsine acquired a rapidly developing and highly specific immunity. When large amounts of heavily infected blood were injected intravenously in these exposed animals, the injected trypanosomes quickly disappeared. After an interval, varying from one experiment to the other but constant within a given experiment, the parasites reappeared in the blood and caused death within a few days. From data gathered from numerous experiments these Authors concluded that the trypanosome population is composed of a major antigenic type and one or more secondary types. Their experiments suggested that the period of protection may depend upon the prevalence (and reproductivity) of the secondary genotypes that failed to act as an immunization stimulus. Evidence of the presence of these antigenic variants was derived from cross-immunity experiments. From the period of protection and the generation time of the strains producing the relapses, the Authors calculated the proportion of the secondary antigenic variant, but admitted that these figures are highly sensitive to generation times and are only rough estimates. In a later article examining the origin of antigenic variants and their rate of occurrence, CANTRELL [2] concluded that they are produced by mutation, the rate being two mutants per million trypanosomes per generation. Two variants per million would certainly find no expression in the biological characteristics of a strain. Should the normal trypanosome population disappear or greatly decrease, however, then the mutants would become predominant and find expression in the overall characteristics of the strain; if the generation time between normal and mutant types is the same, then the circulation in nature of strains of varied composition is to be expected. But even should strains revert to the parent type, perhaps because of better survival values or shorter generation time of the dominant type, occasional modified types would still be produced by the chance exposure favoring certain mutant types. The polygenetic composition of populations of parasitic protozoa would seem to result in only partial antibody formation in the host, i.e., only against antigens of the predominant type. This might cause relapses and parasitemia-crisis alternations, as each population would be in a position to multiply without challenge until specific antibodies were again produced by the host.

STRAIN VARIATION AND THE EPIDEMIOLOGY OF CHAGAS' DISEASE

In the cyclic transmission of protozoa by an intermediate host, such as trypanosomes carried by insect vectors, the organism is exposed to the vicissitudes of cyclic development in one or both hosts and of transfers from host to intermediate hosts. Under natural conditions of a given biotope, the trypanosome strain is transmitted by the same vector species into the same host species, resulting in little change in strain composition. The introduction of different hosts or vectors, or the migration of the trypanosome into another area with different hosts and vectors, may result in the selection of a different strain composition, giving rise to other overall biological characteristics. The epidemiology of parasitic diseases usually involves

exceedingly complex processes of interaction of many factors. This is true for Chagas' disease, which involves the transmission of *Trypanosoma cruzi* by hemiptera of the subfamily Triatominae. The disease occurs from southern Argentina to the southern United States in a maze of transmission patterns related to local conditions. Chagas' disease is a zoonosis. The parasite is maintained in a wild (sylvatic) environment in a transmission cycle between "wild" triatomid bugs and wild animals. In the urban habitat, domestic animals (dogs, cats, pigs, etc.) may serve as domestic reservoirs, and "urbanite" animals, such as rats, mice, opossum, etc., occasional residents of man's environment, link the sylvatic and the domestic transmission cycles. The degree of transmission of Chagas' disease is influenced by certain factors in man's habitat, such as: (a) type of habitation; (b) number and kind of domestic animals and their location; (c) type of immediate environment (urban or rural); (d) altitude and life-zone; (e) presence and type of wild vertebrate reservoirs; (f) abundance and kind of vector species. Transmission rates and other epidemiological factors are also related to the opportunity of circulation of the parasite provided by number of host and vector species. In the list that follows, that aspect is compared for three broad areas in the Americas:

	South America	Central America	North America
Number of proven vector species	27	10	8
Number of unproven vector species	26	13	6
Number of (genera of) proven hosts ...	36*	5	5

* Including 4 primate species belonging to 4 genera, but excluding bats.

The greater number of species in South America is related to the greater diversity of ecological niches found in the tropical environment and, of course, to that of great land masses and altitudes. All the environmental gradients discussed so far would seem to explain the distribution and severity patterns of Chagas' disease. The fact that *T. cruzi* strains isolated at the northern edge of its area of distribution are of a much lower virulence than those found in the area of great endemicity in South America suggests that there is also a qualitative factor involved, e.g., strain variation in *T. cruzi*. The large variety of host and vector species in South America provides favorable conditions for the development and maintenance of a broad spectrum of strain mixtures. In the northern half of the area of distribution of *T. cruzi* the choice of hosts and vectors narrows and the possibilities for broad strain mixtures to pass unchanged decrease. Strains are weeded out in the process. In addition to the number of host and vector species, their behavior in relation to contact with man and their physiological influence upon the development of the trypanosome strains should be considered. Except for relatively rare instances of direct man-to-man transmission in some areas of South America (GÓMEZ-NUÑEZ [7]), *T. cruzi* is principally maintained in wild-animal reservoirs. As such, the animal reservoir hosts are the determinant sieves, and the strains isolated in nature are those that emerge from the sylvatic environment. In the United States *T. cruzi* circulates in only a few animal species, principally rodents, and strains that survive are of low virulence for those animals. When such strains are tested in mice, they usually prove to be avirulent. Strains maintained in laboratory rodents, which retain their virulence because they are passed serially before the previous animal dies, should be distinguished from those strains isolated from natural infections in wild rodents, where they have survived in an avirulent form by natural selection. Virulent strains could conceivably be introduced through the importation of infected South American animals, such as monkeys (DUNN et al.[5]).

The only indigenous proven cases of Chagas' disease found so far in the United States come from Texas, although *T. cruzi* organisms have been isolated from various animals and triatomid bugs in all other southern States and as far north as Maryland. But serological evidence of human infections elsewhere in

the United States indicates occasional human exposure to the parasite. WOODY et al.[28] have reported that nine out of 500 persons living in the vicinty of Corpus Christi, Texas showed significant levels of complement-fixing antibodies to *T. cruzi*. FARRAR JR. et al.[6] tested sera from three groups of persons living in Georgia. A total of 523 from two groups of unselected persons showed four positives; 28 from the third group came from persons selected for "diffuse myocardial disease" and showed two positives, a significantly higher proportion than the unselected group, indicating that certain unexplained myocardial diseases in the southern United States might well have their origin in unrecognized Chagas' disease. NORMAN & KACAN [20] reported that the seven strains they had isolated from opossum (5), skunk (1) and raccoon (1) in Georgia and Florida were less virulent in mice than the *T. cruzi* strain from Corpus Christi in Texas where the two human cases came from. Therefore this information has some significance. The armadillo, an important South American *T. cruzi* reservoir, extends its range into Texas and parts of Louisiana. Can the presence of this animal be correlated with the more virulent Texas strains?

LUCENA [17] in 1952, reviewing his observations and those of other Authors in the States of Pernambuco, Paraíba and Ceará in Northern Brasil, commented that, despite the presence of infected vectors and numerous patients with symptoms of the chronic form of Chagas' disease, not a single acute case was found in that area, in contrast to other areas in Brasil and elsewhere in South America. He suggested that this situation is somewhat similar to parts of the United States, where subclinical cases seem to exist and where the environment is similar to the semiarid parts of Northern Brasil, and that this type of environment may be responsible for strains of low pathogenicity.

Detailed studies of the antigenic constitution of South American *T. cruzi* strains isolated in areas in Brasil led NUSSENZWEIG et al.[21] to divide them into two immunologically distinct types. Type A includes two human strains, in addition to a strain isolated from a *Triatoma* and a strain from a bat from areas endemic for Chagas' disease. Type B includes trypanosomes isolated from two species of opossum and from a water rat. Type-specific antigens from types A and B cross-react, indicating their structural similarity. These studies led the Authors to suggest that the existence of distinct immunological types of *T. cruzi* might explain the clinical diversity observed in Chagas' disease. BRENER [1] undertook the morphological study of different *T. cruzi* strains isolated in South America and has described three morphological groups. Interestingly enough, he found them related to certain biological characteristics of the groups. A recent publication by RYCKMAN [24] on the host specificity between *T. cruzi* and triatomid vectors reports some noteworthy results. When North American species of triatomid bugs (*T. protracta*) were infected with South American strains of *T. cruzi*, a much lower metacyclic density was found in the hindgut than when the same North American *Triatoma* was infected with a North American *T. cruzi* strain. When South American triatomids (*T. megistus* and *T. infestans*) were infected with *T. cruzi* strains from North America, few metacyclic forms could be found. The same low metacyclic development occurred when the Mexican Sonoran strain was used. These experiments, which should be extended to include other triatomid bugs and trypanosome strains, indicate that the vector species may significantly influence the natural selection of trypanosome strains and that they may be at least partially responsible for filtering out certain South American trypanosome strains when they arrive in Central and North American *Triatoma* species areas. RYCKMAN's studies show the influence of *Triatoma* species upon the development of the trypanosome due to differentiated metacyclic output, but it is not clear whether this is merely quantitative or related to specific biological changes within the flagellate. It would be interesting to submit the trypanosomes, as they emerge from one or the other *Triatoma* species, to antigenic tests. From the development within the vertebrate host, RYCKMAN concludes, however, that the passage of the North American *T. cruzi* strains through South American *Triatoma* vectors does not increase the virulence of the strain.

RESUMO

Variações biológicas em tripanossomos e sua relação com a epidemiologia da moléstia de Chagas

Breve revisão da literatura indica que as espécies do gênero *Trypanosoma* compõem-se de diferentes variantes antigênicas ou se tornam poligênicas após exposição a certos quimioterápicos ou outros estímulos. O conceito de variações e misturas de cepas é proposto para explicar certos aspectos da epidemiologia da moléstia de Chagas nas Américas. Misturas de linhagens e, pois, a etiologia das infecções pelo *Trypanosoma cruzi*, parecem variar com o ambiente e seus reservatórios e vectores. Transmissões interumanas do *T. cruzi* provàvelmente só prevalecem em condições de alta infestação de espécies domésticas de *Triatoma*, mas a principal via de transmissão é representada por animais silvestres, de modo que as características da cepa são determinadas mais pelo seu ciclo silvático do que pelo seu ciclo doméstico.

Nos Estados Unidos da América, infecções de animais silvestres e de triatomíneos pelo *T. cruzi* só ocorrem, setentrionalmente, até Maryland. Até hoje, os dois únicos casos comprovados da moléstia de Chagas registrados nos Estados Unidos, são do Texas. Entretanto, a positividade de numerosos testes sorológicos humanos indica que a moléstia de Chagas, clìnicamente não evidenciada, pode ocorrer em regiões do país onde a população está exposta a possível infecção por animais silvestres e triatomíneos. O caráter relativamente avirulento das linhagens de *T. cruzi* encontradas nos Estados Unidos se deve, provàvelmente, à atenuação das cepas mais virulentas através sucessivas passagens por roedores, seus reservatórios mais freqüentes no país, e pela seleção de linhagens ou de diferentes estágios de desenvolvimento nos vectores invertebrados locais. Entretanto, alterações no ciclo "natural" de transmissão produziriam, possìvelmente, variantes de maior virulência.

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