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Margaret J. Triffitt

"TRYPANOSOMA CRUZI"

In the vertebrate and invertebrate hosts,  
with special reference to the modes of transmission.

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## HISTORICAL.

(a) Discovery of the disease and first report of the parasite.

In 1909 Chagas, while organising prophylactic measures against malaria in the north of Minas Geraes State, Brazil, learnt of the occurrence of a biting insect known as Barbeiro, which inhabited human dwellings. He procured specimens of this insect, which he identified as belonging to the Hemiptera heteroptera, family Reduviidae, genus Conorrhinus, species probably megistus. It was found to occur in enormous numbers in the houses of the poor, living during the day in cracks in the walls and ceiling, and emerging after dark to feed upon the human inhabitants and domestic animals of the house. The usual part attacked seemed to be the face and lips of the sleeper, hence the name Barbeiro, meaning "the barber". Inhabited houses were found to be infested by all stages of the life history of the bug (adults, nymphs and larvae) which apparently bred freely, but even recently deserted houses were never found to contain the insect in any stage of development. This was probably due to migration in search of food, facilitated in the adult stage by the presence of well developed wings.

Chagas collected a number of these insects from houses, and, on examining the intestinal contents, discovered in the hind gut numerous flagellates of the crithidial type. He then carried

out a series of experiments to determine whether this parasite was capable of being transmitted to a vertebrate host. Infected bugs were allowed to bite small laboratory animals - guinea-pigs, rabbits, dogs and monkeys, all of which were subsequently found to harbour trypanosomes in their peripheral blood. Dr. Oswaldo Cruz, to whom some of the bugs had been sent, was successful in infecting, by the same means, a small monkey, Callithrix penicillata. To all the animals thus infected the trypanosome was found to be pathogenic, Callithrix and guinea-pigs being the most susceptible.

Chagas then commenced investigations to determine the usual vertebrate host of this parasite. The inmates of the houses from which the infected bugs had been obtained were examined, and trypanosomes were found to be present in their blood, and also in that of a cat from one of the houses. These trypanosomes were morphologically similar to those experimentally produced in animals by the bite of the bug.

Chagas' attention was then drawn to a complex of symptoms common in young children, associated with a disease to which the names Opilacao and Cauguary were given. The chief of these symptoms were, extreme anaemia, oedema, enlargement of all the peripheral glands, enlargement of the spleen and occasionally of the liver, and functional disturbances especially of the nervous system, accompanied by delayed development with infantilism and

frequently actual imbecility. The mortality was high. The blood of some of these cases was examined and was found to contain a trypanosome morphologically identical with that previously found in people inhabiting houses infested with Conorrhinus megistus, and in laboratory animals infected by the bite of this bug. Inoculations were made of the blood of patients into clean laboratory animals in which the disease was reproduced.

A series of observations were made on the development and morphology of the trypanosome in man, Conorrhinus megistus, and in animals artificially infected from both these sources. From the blood of vertebrates, Chagas described two phases of the parasite, one free in the plasma, the other enclosed in red blood corpuscles or attached thereto by their posterior ends. These latter being found chiefly in the early stages of an infection, he considered to be the first stage of the cycle in the vertebrate host, later being set free into the plasma. From the interglobular forms onwards Chagas distinguished a well-marked dimorphism which he interpreted as being sexual. The male forms described by him were long and slender, with a free flagellum variable in length, and a large egg-shaped blepharoplast with its largest diameter transversely arranged across the hind end of the body. The trophonucleus was oval or band-shaped, situated about the middle of the body, with its longest diameter arranged in the longitudinal body-axis, and containing a deeply staining corpuscle

corresponding to a centrosome. The female forms were much broader and contained a smaller rounded blepharoplast, and a round trophonucleus with the chromatin less condensed than in the former case. This dimorphism was found to be constant, both in the blood of man and of inoculated animals. No dividing forms were found in the blood, but multiplication of the parasite was described as taking place in the lungs by a process of shizogony. In the capillaries of the lungs the trypanosome lost its flagellum and undulating membrane, and in some cases its blepharoplast, the two ends then approached and merged and the blepharoplast, when persistent, blended with the trophonucleus. This composite nucleus then divided into eight portions, called by Chagas the merozoites, which were surrounded by a common membrane. When mature the merozoites worked their way through the membrane into the bloodstream and penetrated red blood corpuscles wherein they became flagellates. Chagas distinguished between the sexes in this schizogony according to the loss or retention of the blepharoplast. In the latter case each resulting merozoite contained a portion of the blepharoplast, and these constituted the male forms; in the former, the merozoites were smaller, lacking a blepharoplast and were considered to be female. The sex of the merozoites persisted, as a matter of course, through their subsequent development.

This peculiar process of schizogony led Chagas to create



a new genus for this trypanosome, which he called Schizotrypanum cruzi.

For the study of the development of S. cruzi in the invertebrate - Conorrhinus megistus, young larvae were used which were hatched in the laboratory and infected by being fed on an animal containing trypanosomes in its peripheral blood. These larvae, though hatched from the eggs of infected adults, were never found to be naturally infected. Six hours after the ingestion of infected blood, the gut contents of the bugs were examined. In the stomach trypanosomes were found in which the blepharoplast had moved forward close to the trophonucleus, with which in some cases it seemed to blend. In the majority of cases the flagellum and undulating membrane were lost, but occasionally the flagellum persisted and appeared longer than usual. The parasites then rounded up and multiplied rapidly by longitudinal division. In the chyle-gut similar organisms were found which had, however, more pear-shaped bodies, and in addition to these small oval forms occurred in groups. All these forms were found undergoing multiplication by longitudinal division. After twenty-five hours the parasites were found to have passed into the posterior, cylindrical part of the intestine, which contained a black granular fluid representing the last stages of digestion of the blood. In this they seemed to multiply rapidly and remain for a long time. All the forms found in this part of the gut were flagellated and most

were of the crithidial type, either true crithidia with the blepharoplast near, but just anterior to the trophonucleus, or intermediate between this and the trypanosome type with the blepharoplast lying alongside the trophonucleus. Chagas also described rounded organisms with a very large nucleus as occurring in the gut of larvae fed on long-infected Callithrix, and in examinations made 140 to 150 hours after the ingestion of blood, schizogonous forms in the forepart of the mid-gut. These latter consisted of groups of eight egg-shaped bodies, each with a long nucleus and what appeared to be a blepharoplast at one end, held together by a central mass of cytoplasm. In two cases Chagas found parasites in the body cavity of bugs which contained crithidial forms in the hind gut. These resembled salivary gland forms, and Chagas concluded, from the rarity of these cases, that the passage through the body cavity to the salivary glands was a rapid one. In three cases developmental stages were found in the salivary glands, one of these was an artificially infected bug, which had been fed twelve days previously on an infected Callithrix. The other two were bugs caught in infected houses. Assuming that infection was transmitted only by the bite of the insect, Chagas concluded from this data that only a very small percentage (probably about 1%) of infected bugs were themselves infective. Morphological study of the salivary gland parasites showed that these conformed to two types, long slim forms similar to those found in

the body cavity, with flagellum and undulating membrane, large blepharoplast and long band-like trophonucleus, and fewer broad forms with small blepharoplast and oval trophonucleus.

Artificial cultures were made of the trypanosome which grew readily on N.N.N. medium, and sub-inoculations were found to be generally successful up to and including the third generation. Round forms, pear-shaped forms and crithidia developed as in the gut of the bug. The forms with thickened membrane and schizogonous forms were never found, but on two occasions forms occurred corresponding in morphology with those found in the peripheral blood of vertebrates and salivary glands of the bug.

It is worthy of note that neither the bite of the bug nor inoculations of the contents of the mid-gut containing flagellates gave uniformly positive results, nor did inoculation of artificial cultures, from which some animals became infected, but not all.

Repeated sub-inoculation of animals of one species seemed to weaken the virus, and inoculation from one vertebrate to another did not produce so severe an infection as did the bite of the infective bug. Intraperitoneal inoculation was found to be more effective than sub-cutaneous inoculation.

From these observations and experiments Chagas drew the following conclusions: -

- (1) That Schizotrypanum cruzi develops in Conorrhinus along two lines, one a simple culture of the parasite, the other, probably preceded by sexual processes not yet observed, shows the cycle of development which occurs in the transmission from vertebrate to vertebrate.
- (2) That Conorrhinus is the true intermediate host in which the developmental cycle takes not less than eight days.
- (3) That the crithidia which occur in the free-living Conorrhinus may be culture stages of Schizotrypanum cruzi, or may be exclusively insect parasites.
- (4) That the occurrence of the sexual cycle of development in the organism of Conorrhinus depends on states, as yet unelucidated, of the flagellates in the blood of the vertebrate.

In a critical survey of Chagas' results and deductions published a year later, Minchin pointed out that no sexual behaviour had been observed in the life cycle of Schizotrypanum cruzi to justify Chagas in interpreting the dimorphic blood forms and merozoites as being in any way sexual. Further, he considered Chagas' figures of the intracorpuseular blood parasite to be unconvincing. Whereas Chagas believed the crithidial forms in the intestine of Conorrhinus to be a mere culture phase, the end product of atavistic degeneration, Minchin considered that they formed a multiplicative phase intermediate between the rounded up

forms and the trypanosomes which formed the ultimate propagative phase in the invertebrate. In support of this supposition he draws attention to the fact that the three main phases in the invertebrate, viz. the rounded up forms, crithidia, and trypanosomes, also occurred in the artificial cultures, whereas the encapsuled forms (zygotes) and schizogonous forms, to which Chagas attached such importance, were lacking. Minchin therefore concluded that the "zygotes" of Chagas were merely resting stages of crithidia and that the Schizogonous forms were probably yeasts parasitic in the intestine of the bug.

The later work published by these and other authors is most easily dealt with under the headings of its various aspects.

The morphology of Schizotrypanum cruzi as it occurs in the vertebrate host, and the significance of its various forms. Max Hartmann, while making observations on a strain of S. cruzi sent to him by Chagas, discovered multiplication forms which had previously been overlooked, in the lungs of guinea-pigs. These he described as various stages of multiple division, taking place in greatly hypertrophied endothelial cells. The stage figured consisted of an endothelial cell densely packed with small aflagellate pear-shaped bodies, each containing a densely stained rod-shaped blepharoplast transversely situated, and a rather more diffuse trophonucleus. Earlier forms described in the text were stages in the same process, in which however the cytoplasm formed

a single mass, separation not yet being effected.

In 1911 Chagas described similar forms occurring in the tissues of a child dead of a Schizotrypanum cruzi infection. The tissues mainly affected were the heart, brain and spinal cord, and the striated muscle. In the heart Chagas found that a single trypanosome penetrated a muscle cell where it multiplied rapidly, giving rise to a multitude of these rounded forms. In the central nervous system scattered foci were found in the brain and spinal cord, and the striated muscles of the body were infested in much the same way as the cardiac muscle, but to a smaller extent. In laboratory animals similar forms were found in the testicle and suprarenal. These observations, while accounting for the discrepancy in number previously noted by Chagas between the trypanosomes in the peripheral blood and Schizogonous forms in the lungs, led him to suppose that the lung forms were gametogonous while the tissue forms were asexual, the former being an essential part of the life cycle, the latter merely multiplicative phases for establishing the infection in each individual vertebrate. In support of this theory he notes that in the blood of man the parasites are present in two forms sexual and asexual, whereas in the blood of artificially infected guinea-pigs only the asexual forms are usually present, further that a Conorrhinus fed on the blood of an infected man becomes infective to other vertebrates, while one fed on an artificially infected guinea-pig very rarely does so.

If the formation of gametes is a reaction on the part of the parasite to unfavourable conditions, then their absence in guinea-pigs is due to the greater degree of susceptibility to infection of these animals than is the case in man.

In 1912 Mayer and Rocha Lima found tissue parasites in the heart, striated muscle, smooth muscle, spinal cord, lymph glands, fat, and subcutaneous tissue of inoculated animals. In addition to the rounded leishmanial type previously described by Chagas, these authors describe spindle-shaped bodies occurring in foci in the various organs. A figure is given of these spindle-shaped forms which appear to be intermediate morphologically between the leishmania forms and the tissue trypanosome forms described three years later by Rosenbusch and Maggio. It is significant that in each focus all the forms are at approximately the same phase of development, thus they are all leishmania like, all spindle-shaped or all trypanosomes. Another slight morphological variation was pointed out by Novaes, who found that in dense tissue such as cardiac muscle the leishmania forms tended to be smaller and narrower than when they occurred in looser tissue such as fat. This he ascribed to the varying amount of room available for the parasite during its growth. Hartmann, in his first account of the tissue forms and in a subsequent paper published in 1917, asserted that multiplication of these forms took place by a process of true Schizogony, the trophonuclei and blepharoplasts first

multiplying in the common cytoplasm which later became segmented off into oval bodies. He also described a marked dimorphism of the parasites which were divisible into two forms, one with, the other lacking a blepharoplast.

Mayer and Rocha Lima found no dimorphism and stated that the tissue forms multiplied by repeated longitudinal binary fissions. Chagas supported this later view and considered that the products of these divisions were set free in the bloodstream as indifferent or asexual trypanosomes.

The Schizogonous forms in the lungs, described by Chagas as stages of gametogony, were discovered in 1913 by Aragao in the lungs of animals not infected with Schizotrypanum cruzi, and were subsequently given the generic title of Pneumatocystis by Delanoe, who confirmed Aragao's work, and found that these bodies were vegetable organisms. The genus Schizotrypanum thus ceased to exist, and the parasite became Trypanosoma cruzi.

All writers on the subject of the trypanosomes of the peripheral blood agreed in finding thin forms, broad forms and transitional forms described by Chagas as male, female and indifferent or asexual forms respectively. Hartmann supported Chagas in this theory of sexual dimorphism, but Brumpt, Nagler, Delanoe, Novaes and Blanchard agreed with Minchin that the "male" forms of Chagas were young trypanosomes newly released from the internal organs, which passed through the "indifferent" or



asexual stage during their circulation through the peripheral blood before becoming the adult blood forms, or "female" forms of Chagas. Nagler found two dividing trypanosomes, one in the blood of a mouse, the other in peritoneal fluid. These are the only dividing forms of *T. cruzi* in the peripheral circulation which have ever been observed. Occasionally, in particularly heavy infections, leishmania forms have been found in the blood, but these have been considered prematurely escaped tissue parasites which have not yet reached their full development into trypanosomes. Brumpt remarked a close relationship existing between the number of cysts in the internal organs and trypanosomes free in the peripheral blood. No intra-corpuseular stages have been observed since Chagas' first description of them, and Novaes stated that they were absent from the cases described by him.

(b) Morphology and physiology of the parasites in artificial cultures.

Chagas was successful in cultivating *T. cruzi* on N.N.N. (Novy, Mc.Neal, Nichol) medium and found that the three main vertebrate types, viz. leishmania forms, crithidia and trypanosomes grew readily. Sub-inoculations succeeded up to the fourth generation. Delance (1912) found first cultures easy to establish, but was unable to produce sub-inoculations. Bayma found that blood agar made up with rabbit's blood remained sterile.

Maggio and Rosenbusch made cultures on blood bouillon and blood agar from infected peripheral blood, heart muscle, striped muscle and spleen. These were easily kept alive by sub-inoculation and showed colonies of leishmania forms in their early stages, which later gave place to crithidia. Brumpt's attempts to cultivate the trypanosome were entirely negative. As recently as 1920 Noller has been successful for the first time in growing plate cultures at 30° C. Sub-inoculations of these cultures have proved virulent to laboratory animals, inoculations from the fifteenth sub-culture having reproduced the disease.

All the successful cultivation experiments have given rise to forms morphologically identical with those found in the invertebrate host and, moreover, these forms occur in the same sequence, which leads to the supposition that the cultivation of the parasite on artificial media is analogous to that which occurs in the gut of the bug.

(c) The Physiology of *T. cruzi* in the vertebrate host.

The results obtained by different workers in dealing with *T. cruzi* in laboratory animals differ to such an extent that the worker is forced to the conclusion that a tremendous variation occurs in the physiology of the various strains isolated primarily from different portions of the infected area, and secondarily in the laboratory by different investigators.

The original strain used by Chagas produced infections

in mice, rats, guinea-pigs, rabbits, monkeys and dogs, of which guinea-pigs and Callithrix penicillata proved particularly susceptible. Mayer and Rocha Lima on the other hand, using a strain isolated by Cruz, found that in guinea-pigs the virulence was slight, rare trypanosomes appearing after 14 days and disappearing during the course of the next 14 days. In mice, however, trypanosomes appeared on the 13th day, after inoculation direct from the invertebrate, but after 12 passages from mouse to mouse the virulence became markedly increased, trypanosomes appearing on the 6th day and death ensuing in from two to three weeks. In rabbits and rats the infection remained light and temporary, but three monkeys died in four, three, and four weeks respectively. Neumann found that guinea-pigs were particularly susceptible and that immunity to infection differed not only in different species, but in different individuals of the same species. Blanchard and Brumpt succeeded in infecting mice, rats, Cercopithecus ruber, marmosets, and newly-born guinea-pigs, with a virus obtained from Bahia, but newly-weaned and adult guinea-pigs proved refractory. This result, compared with those of Chagas emphasises the differences between the virus of Bahia and that isolated by Chagas only 800 kilometres distant from Bahia, to which adult guinea-pigs were particularly susceptible. A series of experiments conducted by Brumpt establish the connection between the two. Using mice as experimental animals, Brumpt proved that a

previous infection with the virus of Bahia conferred immunity from infection with Chagas' virus. He then infected bugs with Chagas' virus and inoculated mice with the cultures obtained from their intestines, thus producing a slight infection, never fatal but corresponding to the infections previously produced by the virus of Bahia. From these results Brumpt concluded that on passage through the intermediate host the exalted virus of Chagas falls to the level of the virus isolated from Bahia.

(d) The Invertebrate hosts of *T. cruzi*, their distribution and biology.

The natural host and transmitting agent of *Trypanosoma cruzi* in the State of Minas Geraes, locally known as the "Barbeiro" was first classified as *Conorrhinus megistus*. Later, however, it was found by Chagas to belong to the genus *Triatoma* and was subsequently known as *Triatoma megista*. The biology of this bug was worked out by Neiva and confirmed by Brumpt and da Silva. The main outline of the life history is as follows: -

The young larva emerges from the egg after a period of from twenty-five to forty days. The first moult takes place about the forty-fifth day, the second between the 60th and 90th day, and the third and fourth moults during the fourth, fifth, and sixth month, and about the 190th day respectively. The fourth moult ushers in the nymphal phase which lasts for not less than forty-two days when the fifth and last moult occurs, and the

wingless nymph becomes a winged imago. The female imago lays her eggs in batches of from eight to ten, and Brumpt found that a single femlae laid thirty-eight times, producing a total of two hundred and eighteen eggs between March 5th and June 30th. The life cycle from egg to egg takes about 324 days and the total life of the bug has been estimated at about two years. As previously stated, Triatoma megista infests inhabited houses of primitive construction, where it multiplies rapidly, living in cracks in the walls and ceiling during the day and emerging to feed upon the inhabitants during the night. Empty houses are quickly deserted by the bug, which migrates in search of food. The wings in the imaginal stage facilitate migration and render prophylactic measures difficult. All stages of the life cycle suck blood, and all are capable of transmitting the disease.

It is interesting to note that Triatoma has only been known to be parasitic upon man since the European occupation of Brazil, when house construction of wood and mud was introduced. Before this tents made of skins were generally in use, and these dwellings afforded no suitable breeding places for the bugs.

Triatoma megista occurs naturally infected in Minas Geraes, Gozaz and Matto Grosso, but Neiva found that in Gozaz while T. megista was fairly rare, another species, T. sordida occurred in great numbers, was naturally infected and formed the chief agent for the transmission of the disease in this state.

Neiva also discovered T. vitticeps in the country surrounding Rio, and T. demidiata (var. maculipennis) at San Salvador, both of which he proved to be natural infective agents.

Brumpt found T. infestans naturally infected, and was successful in producing artificial infections in Cimex lectularius, Cimex boueti, Cimex rotundatus (the common bed bug of Brazil) Rhodnius prolixus and Ornithodoros maubata. Neiva showed that Rhipicephalus sanguineus, a tick common in dogs in Brazil and stated by Neumann to be occasionally parasitic upon man, was capable of transmitting T. cruzi from dog to dog. Of other workers on the invertebrate hosts of T. cruzi, Torres denied that Cimex lectularius was capable of carrying the parasite, and Nagler, although unable to infect C. lectularius, thought that this was due to unsuitable temperature, as his experiments were carried out during the winter. Tegera found human cases in Venezuela where T. megista does not occur, and proved that Rhodnius prolixus was the natural transmitting agent. Brumpt attempted to infect Dog fleas, Rat lice, Argus persicus, Lealaps and Haemaphysalis inermis, by feeding them on infected vertebrates, but all of these forms proved refractory.

The first discovery of vertebrates other than man and domestic animals, infected with T. cruzi was made by Chagas in 1912. A specimen of Triatoma geniculata containing a natural

infection, was caught sufficiently far from human habitations to exclude the possibility of infection from this source. Investigations were made which led to the discovery of trypanosomes, morphologically identical with T. cruzi, in the blood of armadillos, amongst which T. geniculata was the transmitting agent. In 1918 Chagas stated that 45 to 50% of the armadillos found in States infested with T. cruzi were infected with the parasite. The chief species of armadillo were Dasypus novencinctus, D. sexicinctus, and D. unicinctus. In 1914 Brumpt and Gomes discovered a new species of Triatoma, T. chagasi living in the burrows of a rodent Kerodon rupestris. Both the bug and the rodent were infected with T. cruzi.

(e) The life cycle in the invertebrate and modes of transmission of the disease.

Chagas in his first description of the parasite (1909) described seven forms occurring in the invertebrate host. These were: -

- (1) Rounded up leishmanial forms in the mid-gut.
- (2) Crithidial forms in the hind gut.
- (3) Trypanosomes in the hind gut.
- (4) Forms with a thick membrane - "zygotes".
- (5) Schizogonous forms.
- (6) Trypanosomes in the body cavity.
- (7) Trypanosomes in the interstitial tissue of the salivary glands.

From these observations he deduced the following life cycle. Trypanosomes ingested in infected blood rounded up in the stomach of the bug and became leishmanial forms. Of these two

kinds were present, some developed from asexual trypanosomes and these multiplied rapidly and gave rise to crithidia non-infective to the vertebrate, others developed from sexual forms in the vertebrate blood. These conjugated, forming zygotes, which multiplied by a process of Schizogony, the products of which became trypanosomes in the hind part of the intestine, passed rapidly through the coelom and made their way into the salivary glands. This process took not less than eight days, when the bite of the bug became infective to vertebrates.

Brumpt and other workers found leishmanial, crithidial and trypanosome forms in the gut of T. megista, morphologically identical with those described by Chagas, but were unsuccessful in finding Chagas' sexual forms or any trypanosomes in the body cavity or salivary glands. The proportion of crithidial and trypanosome forms in the hind gut was proved to be influenced by the amount of food. The life cycle according to these workers was simpler than that described by Chagas. Trypanosomes ingested with infected blood rounded up to form leishmania-like organisms, these became crithidial forms which underwent rapid multiplication, finally giving rise to trypanosomes which were voided with the faeces, and were infective to vertebrates about twenty days after the ingestion of infected blood. Brumpt showed that the developmental cycle in Cimex lectularius was completed more rapidly than in Triatoma megista. Bugs fed on infected rats and kept at 25° C.



showed after six hours some normal trypanosomes and some in process of digestion in the stomach. Twenty-four hours later (30th hour) crithidial forms were found in the hind gut undergoing rapid multiplication. After 78 hours no trypanosomes were left in the stomach, but crithidia were very numerous in the hind gut and forms intermediate between crithidia and trypanosomes occurred on the 14th day when the faeces became infective to young rats. On the 17th day an almost pure culture of trypanosomes was found in the hind gut. Eighty per cent. of Cimex lectularius and C. rotundatus became infected after one feed, while Triatoma megista gave 100% success.

Torres repeated Chagas' experiments and found that out of thirty-five animals bitten by Triatoma megista nineteen gave a positive result, the remaining thirteen being negative. Precautions were taken against accidental contamination with the faeces by confining the bugs in narrow glass tubes with the mouths covered over by gauze through which the insects pushed their proboscis.

Blanchard and Brumpt did not succeed in infecting animals by the bite of either T. megista or C. lectularius, although faeces containing numerous infective forms were freely passed on to the punctured skin. They conclude therefore that the usual mode of infection in man is by means of faeces passed upon the mucous membrane of the eyes and mouth, and in domestic animals by

the ingestion of the whole insect, infection by the bite being due to abnormal localisation of the trypanosomes in the salivary glands.

This work was confirmed by Maggio and Rosenbusch who found the bite of T. megista uninfective and the faeces infective.

Mayer and Rocha Lima found that mice became infected both by inoculation of blood containing trypanosomes, and by placing infected vertebrate blood on the mucous membrane of the mouth, but that the trypanosomes were unable to penetrate undamaged skin. Similar results were obtained by Brumpt with infective faeces, which only once produced an infection when placed upon healthy skin, but gave uniformly positive results when placed upon the mucous membrane of the eye, mouth or rectum.

Using infected Cimex lectularius, Blacklock found that faeces dropped upon healthy skin gave only one positive result in 28 cases. Inoculations of the faeces into mice and guinea-pigs gave only 9 positive results in 32 attempts. Of these 9, 7 were mice and 2 were guinea-pigs, the incubation period in the former being 22 days and in the latter 25 days. Blacklock showed that the faeces of Cimex might be infective as early as 21 hours after the infective feed and remain infective up to 77 days.

Maggio and Rosenbusch found that in naturally infected T. infestans when first caught the trypanosomes were half as numerous again as the crithidia, but when kept in the laboratory

the trypanosomes rapidly dwindled in number and the crithidia increased proportionately.

Hereditary infection of the young was never reported in Triatoma or Cimex and Mayer found that although Ornithodoros moubata retained the infection for 5 years, young hatched from eggs laid during this time were not naturally infected.

Other methods of infection passing from invertebrate to invertebrate were noted by Brumpt. In studying a strain of T. megista kept in the laboratory he found that a form of cannibalism was frequent among young larvae which sucked blood from one another's bodies which had previously been ingested from vertebrates. Coprophagy also occurred, the larvae displaying a marked taste for freshly passed dejecta. Both these habits decreased with age, but Brumpt thought it probable that to a certain extent at least they might be responsible for the spread of infection from bug to bug. To prove that passage through the vertebrate need not necessarily occur before a fresh generation of invertebrates can become infected, defibrinated blood of an ox mixed with infected dejecta of T. megista was placed in the fresh skin of a mouse and offered to adults and larvae of Cimex lectularius. Three out of 14 of these bugs which sucked blood became infected.

Basing his deductions on these observations, Brumpt suggested the hypothesis that T. cruzi was originally an insect

parasite exclusively, but by the frequent occurrence of coprophagy and cannibalism it became progressively adapted to living in blood during the course of digestion, and this may have formed the first stage in the final adaptation to parasitism in the blood of vertebrates.

Torres maintained that the only natural means of bugs becoming infected was by sucking the blood of human beings and domestic animals, and that when cannibalism did occur only coelomic fluid was withdrawn which contained no parasites and was non-infective. He also denied that the faeces of Triatoma formed a means of spreading the disease.

Triatoma megista was proved to retain an infection for five months, and Brumpt and Gonzalez-Tugo found that Rhodnius prolixus, the natural transmitting agent in Venezuela, remained infective for more than two months. If the infection is retained indefinitely these authors consider that R. prolixus is probably a more important agent in the transmission of the disease to man than is T. megista. This hypothesis is based on the fact that while Triatoma defecates about half a minute after feeding, Rhodnius defecates immediately upon withdrawing its proboscis, thus greatly increasing the probability of infected dejecta falling upon the mucous membrane of the eyes and mouth.

Although Chagas' disease chiefly attacks young children, it may persist in a chronic form into the adult state, when hereditary transmission sometimes occurs from mother to child. Chagas

found trypanosomes in the blood of newly-born children, and in one case in a foetus.

Nayler, working with laboratory animals, was unable to find any trace of hereditary transmission, but, more recently, Nattan Larrier has shown that this may take place either by the trypanosome passing directly into the foetus in utero through the maternal blood-stream and amniotic fluid, or the newly-born young may become infected through the mother's milk.

Vianna reported the discovery of T. cruzi in the semen of two infested guinea-pigs, and Nattan Larrier (1922) conducted experiments with mice, to determine whether infection could be transmitted from male to female during coitus. A small quantity of a dilute suspension of T. cruzi was introduced into the vagina of three mice, all precautions being taken to avoid any erosion of the vaginal mucosa. Infection ensued in each case.

Xenodiagnosis is the name given to a natural culture of parasites in a suitable host. Brumpt found that where parasites were scanty in the blood of a vertebrate, examination of peripheral blood frequently failed to reveal any parasites and a false diagnosis resulted. He found that the surest means of detecting the presence of blood parasites was by feeding a suitable invertebrate host on the blood of the suspected vertebrate, by which means a culture of the parasite was obtained. This method was found to give 100% of successful diagnoses in the case of trypanosomes of fishes when leeches were used, and in cases of

T. cruzi by using T. megista as the invertebrate host. Infections in guinea-pigs which did not show by direct examinations were easily demonstrated by this method. Brumpt pointed out that from 10 to 500 times more blood was ingested by a single bug than could be examined in one blood film, while when one vertebrate was inoculated with infected blood from another, if the parasites were scanty they might be destroyed by leucocytes before the infection could be demonstrated in the second animal. Larvae and nymphs of T. infestans, T. megista, T. chagasi, T. sordida and Rhodnius prolixus were found to give 100% success in the use of this method.

(f) The Distribution of Chagas' Disease, actual and possible.

The area of distribution of the genus Triatoma is a wide one, extending from 41° N. to 41° S. in the New World, although only in certain regions of this area are bugs infected with T. cruzi found, and the infection is limited to comparatively few species. The genus Rhodnius prolixus occurs naturally infected in Venezuela and Triatoma is found infected in the States of Minas Geraes and Gozaz, Brazil. In Argentina infected Triatoma have been found in the provinces of Salta, Tucuman, Santiago del Estero, La Rioja, Catamarca, Cordoba, Santa Fe, Bensos Curis and La Pampas.

In the more mountainous regions of Argentina, although infected bugs abound, Chagas' disease is unknown, while in the southern regions no infections have been found in Man, Triatoma

or undomesticated animals.

Kraus and Rosenbusch suggested that the peculiar distribution of Chagas' disease in the Argentine is due to modification of the trypanosomes by climatic influence. Brumpt and Gomes attributed the distribution of the disease to various factors, the chief of which are the relation of Triatoma to man, and the effect of geographical and climatic factors on the evolution and survival of the trypanosome in the invertebrate host. These authors consider T. cruzi to be an infection of virgin regions which man contracts and of which he then becomes the most important reservoir. They also point out that Triatoma has only become domesticated since European occupation. The capacity of each species to conserve and inoculate the virus seems to vary, while temperature has been proved to form one controlling factor, as in bugs kept at 0° C. the trypanosome fails to develop.

It seems probable, in view of these facts, that in the mountainous regions of Argentina the temperature is so low as to modify the development in the invertebrate sufficiently to make transmission to man impossible, while it does not occlude the transmission of the virus from bug to bug or from undomesticated animals to the bug. In the same way, the complete absence of the infection in any form in the Southern parts of Argentina, may be attributed to the still lower temperature prevailing in these parts.

ORIGINAL WORK.

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(a) Life History of *Rhodnius prolixus*.

A number of *Rhodnius prolixus* infected with a strain of *Trypanosoma cruzi* were obtained from Professor Brumpt of Paris in 1921. These were kept in the laboratory where they bred freely and observations were made on their biology and on the morphology and physiology of the parasites in both vertebrate and invertebrate hosts, special attention being given to the modes of transmission.

The bugs were kept in glass tubes covered in with gauze and were fed upon uninfected pigeons. Eggs were laid in batches of 6 to 15, and were oval, about 2 mm. long by 1 mm. broad, of a coral pink colour and with a conspicuous operculum at one end. They were firmly attached, usually in a regular double row, to a piece of Bristol board left in each tube for this purpose.

After from 10 to 14 days the young larvae emerged, which, when one to two days old, were ready to suck blood. Four moults occurred before the nymphal stage was reached, each one taking place shortly after a feed. The time elapsing between the moults was found to vary with the amount of food. Thus the larval stage could be greatly prolonged by semi-starving the bug. The average time taken for the nymphal stage to develop was found to be four to seven months. The larva was wingless, but in the nymph rudimentary wings were present, extending over the metathorax and the first two mm. of the abdomen. The fifth moult gave rise to the



winged adult, which measured about 2 cms. in length, excluding the proboscis. This was about 5 mm. long and was carried, except while the bug was feeding, bent underneath and closely applied to the ventral surface of the head and thorax. All stages of the life cycle fed readily on the blood of pigeons, rats, or man. In the latter case the bites were found to be extremely irritative, causing painful swellings, although the actual formation of the wound by the proboscis was painless. When ready to feed the abdomen of the bug was quite flat and wafer-like, but when the bug was gorged, the abdomen became so swollen as to be almost spherical. Within 30 seconds of the withdrawal of the proboscis a clear colourless fluid was excreted in large drops, being probably the secretion of the coxal glands. About five minutes after the first drop of this fluid was excreted a thick, black granular fluid was passed. Both of these forms of dejecta were collected in capillary tubes, blown upon slides and examined microscopically, and both were found to contain flagellates, although these were very much more numerous in the clear fluid than in the granular excretion. These specimens fixed wet in osmic acid vapour, allowed to dry, fixed again in methyl alcohol, were stained with Giemsa's stain. The morphology of the parasites was then studied.

(b) Morphology of the parasite in the invertebrate host.

Critithidial forms were very rarely present in the faeces and were probably due, when they did occur, to the accidental washing away of forms from the anterior region of the hind gut.

The true critithidia were found to vary in length between 15 $\mu$  and 45 $\mu$ . The broadest portion of the body, usually just posterior to the trophonucleus in the posterior third of the body, varied in diameter from 2 $\mu$  to 3 $\mu$ . The undulating membrane was very narrow and the free flagellum either very short or entirely wanting. The trophonucleus, usually situated at the junction of the middle and posterior thirds of the body, was large, oval, with diffuse chromatin showing no distinct karyosome. The kinetonucleus, situated either just anterior to the trophonucleus or separated from the latter by a space of as much as 2 $\mu$  showed as a very distinct, deeply staining rod-shaped body. The anterior end of the body tapered gradually to a fine point, while the posterior end tapered more abruptly to form a more obtuse angle. In some of the largest forms, the portion of the body behind the trophonucleus was very greatly elongated, so that the trophonucleus was situated centrally, or even in the anterior half of the body.

The trypanosomes in the dejecta varied in length from 15 $\mu$  to 25 $\mu$  and in breadth from 1.5 $\mu$  to 2 $\mu$  in their greatest diameter. The undulating membrane was fairly well marked and the free flagellum short. The trophonucleus was situated centrally,

or in a slightly posterior position, and was diffuse, no karyosome being made out. The kinetonucleus was very characteristic, being large, egg-shaped and very densely staining. Situated either at the extreme tip of the pointed posterior end or as much as 5 $\mu$  anterior to it, the kinetonucleus gave the appearance of projecting laterally beyond the cytoplasm.

Early transition stages, between crithidial and trypanosome types varied in length up to 40. In these forms the kinetonucleus lay alongside the anterior portion of the trophonucleus or more frequently, overlapping it. The band shape of the kinetonucleus, typical of the crithidia was retained in these forms. Later transition stages approximated more in length to the trypanosome type, although some forms of over 25 $\mu$  long still occurred. The kinetonucleus of these forms was placed alongside or overlapping the posterior portion of the trophonucleus, and was broader and larger than in the true crithidia.

Young trypanosomes were found in which the kinetonucleus had reached a point posterior to the trophonucleus, but had not yet attained the extreme posterior position characteristic of adult trypanosomes. These forms were rare. Leishmanial forms never occurred in the faeces.

Careful examinations were made of stained preparations of the salivary glands of infected Rhodnius prolixus, both smears and sections, but no forms of the parasite were ever found. These

observations tend to confirm the life history of the parasite as described by Brumpt.

(c) Methods by which the invertebrate becomes infected.

Although all stages of the life history of Rhodnius prolixus have been under observation for a period of nearly three years, cannibalism and coprophagy have never been observed, and young larvae hatched and kept for some time with infected adults have never become infected from the infected adults.

Specimens of R. prolixus infected by being fed on animals containing trypanosomes in their peripheral blood, have been proved to remain infected and infective to vertebrates during the remainder of their life, a period of about nine months.

(d) Infection in Cimex lectularius.

A number of Cimex lectularius hatched in the laboratory were fed upon the blood of an infected rat during the first fortnight of larval life. After the next feed upon the blood of an uninfected pigeon, the faeces were collected and 50% of the larvae were found to be infected. Eight months later, although no subsequent infective feed had been given, the bugs, now adults and beginning to die off, were found to contain a very heavy infection in the hind gut, which, when inoculated into young rats produced an infection. Cimex lectularius, therefore, retains an infection of T. cruzi throughout life and remains infective to the vertebrate for an indefinite period.

It is worthy of note that the infection in one of these

adult lungs was so heavy that parasites were voided with a single drop of clear fluid. Of these the proportion of trypanosomes to crithidia was at least 20 to 1. This leads to the supposition that the crithidial stock in Cimex lectularius is maintained in the fore-part of the hind gut, and is continually augmented throughout life, thus the infective trypanosomes become produced in ever increasing numbers, and thus the infectivity of the bug increases with the amount of time which has elapsed since the first infective feed.

A second generation of Cimex lectularius bred from these infected adults also became infected in the proportion of 50% at the first infective feed. A second attempt at infection only raised this figure to about 70%, the remainder proving refractory. The salivary glands of these bugs were examined, but no trypanosomes were found.

Hereditary infections in the invertebrate. Experiments were performed to establish whether hereditary transmission took place in R. prolixus or C. lectularius. Inoculations of the faeces and gut contents were made into susceptible animals, and corresponding specimens were examined microscopically. The salivary glands of six individuals of each species bred from infected adults were extracted, macerated in normal saline, and inoculated intraperitoneally into susceptible animals. No infections were produced by either of these means, and examinations

of smears and sections of the intestines and salivary glands of these bugs proved negative. (Vertebrates.)

(e) Infection in Vertebrates.

Rats of ages varying from three weeks onwards were inoculated with the faeces of Rhodnius prolixus proved to be infected. Infections were only produced in young rats of three to four weeks old. After this age the rats seemed to become immune, even large quantities of infective faeces inoculated intraperitoneally produced no effect.

The following table shows the results of inoculation of these young animals.

No.	Date of inoc.	First appear. of tryps.	Incubation period in days.	Date of last appear. of tryps.	Date of death.	No. of days tryps. persisted.	Remarks.
1.	9.11.21.	14.12.23	35		no record		
2.	20. 2.22.	-	-	-	-		immune
3	22. 3.22.	(reinoculation)					to in- fection
3.	22. 3.22	7. 4.22.	16	24.4.22	24.4.22	17	
4.	22. 3.22	8. 4.22	15	20.4.22		12	killed
5.	28. 3.22	5. 4.22	8	12.4.22	12.4.22	7	
6.	9. 5.22	23.5.22	12	1.6.22	1.6.22	8	
7.	15. 6.22				26.6.22		killed
8.	13. 9.22	25.9.22	12	7.10.22	7.10.22	12	
9.	7.11.22	21.11.22	14	24.11.22.			killed
10.	7.11.22	27.11.22	20	29.11.22			killed
11.	9. 1.23	20. 1.23	11	30. 1.23	30.1.23	10	
12.	28. 2.23	13. 3.23	13				
13.	2. 3.23	26. 3.23	24	2. 4.23	2.4.23	6.	

The resistance to the disease varied even in young rats, as shown by the above table. Numbers 2 and 7, although of susceptible age, did not become infected.

In cases where infections were produced, the incubation period varied from 8 to 35 days, the average time being 16 days for trypanosomes to appear in the peripheral blood. An infection being established, the disease either proved fatal or the parasites increased for three or four days, and then gradually died out when the rat became immune to further infection. In the former case death ensued in from 6 to 17 days, the average time being about 10 days. These observations show that the power of resistance to the virus varies in different individuals, not only of different ages, but of the same age.

Blood films were taken daily of inoculated rats and the morphology of the parasites in the peripheral blood was studied. Infected rats were killed in various stages of the disease and portions of every tissue in the body were fixed, embedded and sectioned. The distribution and morphology of the tissue forms of the parasite were studied in material thus obtained.

(f) Morphology of the parasite in the vertebrate host.

In the peripheral blood free trypanosomes circulating in the plasma were found. These varied in length from 14 to 20 $\mu$ . The smaller of these forms were narrow, only about 1.5 $\mu$  to 2 $\mu$  across their greatest diameter, and corresponded morphologically with those found in the gut of the invertebrate hosts. These corresponded to the male forms of Chagas. The larger trypanosomes were as much as 4.5 $\mu$  across their broadest diameter, and

probably corresponded to Chagas' female forms. Intermediate types were found between these two and were more numerous than either of the former. The length of the free flagellum was found to vary in different individuals, never being long. The two kinetonucleus in all of these forms was found to be egg-shaped and bulging outwards at the sides of the body in a similar manner to those of the insect forms. The trophonucleus was band-shaped to oval, situated about the middle of the body, with diffuse chromatin. The diversity in form of the trophonucleus was probably due to accidental distortion caused by the making of the blood films.

No interglobular forms were found, and no dividing forms were ever observed in the peripheral blood.

Post mortem examinations of the internal organs were made by means of sections and smears. Cardiac muscle, striated muscle, smooth muscle, brain, spleen, liver, kidneys, testis, supra-renals, lymph glands, thyroid, thymus, stomach, intestine and lung were examined in this manner. Foci of rounded leishmanial forms were found in every tissue except the lungs, from which they seemed to be completely absent. Particularly heavy infections were found in the cardiac and striated muscle and in the brain. In muscular tissue the foci were found within greatly distended fibres. In some cases cardiac muscle was so heavily infected that about one-third of the muscle fibres were attacked.



In striated muscle the infection was usually rather more slight. In the brain foci occurred inside the neuroglia cells which were situated near to capillaries. The neuroglia cells attacked were, with the exception of the nucleus, completely packed with parasites, but no pathological changes were observed in neighbouring non-infected cells. Of other tissues spleen was the next most heavily infected, then unstriped muscle and the remainder to a much smaller extent.

Morphologically the tissue parasites may be divided into three types, namely leishmanial forms, spindle-shaped forms and small trypanosomes. Usually these forms were found occurring each in separate foci, but occasionally two or more forms occurred together in a single focus.

The rounded up leishmanial forms were oval in shape, about  $\frac{3}{4}$  in the long diameter and  $\frac{1}{4}$  in the short diameter. There was no trace of flagellum or undulating membrane. The kinetonucleus was rod-shaped and stained a very deep purple with Giemsa's stain. The trophonucleus was more diffuse, oval or rounded in shape, and stained a fainter purple. The cytoplasm enclosing these two bodies stained a faint blue with Giemsa's stain. The arrangement of these two nuclei varied in different individuals, but the kinetonucleus was usually arranged longitudinally parallel to the long axis of the trophonucleus. Dividing forms of these parasites were found in different stages. The kinetonucleus was

found to divide first, the two halves then travelling apart. This was followed by division of the trophonucleus, and ultimately of the cytoplasm resulting in two similar bodies. The leishmanial forms were by far the most numerous in the tissues. No dimorphism was found between the parasites in the muscular tissues and those of softer constituency as described by Novæes.

The spindle-shaped bodies were similar to the leishmanial types, but were slightly larger and drawn-out to a point at either end. They were intermediate in size and form between the leishmanial and trypanosome types.

Trypanosomes were comparatively rare in the tissues, but when found they were morphologically identical with the smallest forms found circulating in the peripheral blood.

From these observations the life cycle in the vertebrate seems to be as follows: - Trypanosomes injected into the blood stream make their way immediately into the tissues of the internal organs, where they round up, losing their flagellum and undulating membrane. Here they undergo multiplication by a series of binary fissions, the kinetonucleus dividing first, then the trophonucleus and subsequently the cytoplasm. Foci of leishmanial forms are thus developed which, when ready to be set free into the blood stream develop very rapidly into spindle-shaped bodies and then into trypanosomes which as soon as they are formed make their way into the blood stream. Here they are found

as very thin forms - Chagas' males, but during the course of circulation they increase in breadth passing through the intermediate stage to become broad forms - Chagas' female forms. These broad forms are infective to susceptible invertebrates if ingested, but some of them repenetrate the tissues and repeat this cycle, thus increasing the infection in the single vertebrate.

No connection was observed between the number of trypanosomes circulating in the peripheral blood and the number of foci of developmental forms in the internal organs. Frequently trypanosomes were scantiest in those cases which on post mortem examination revealed the heaviest infection of the internal organs.

(g) Physiology in the vertebrate.

Attempts were made to keep the strain of T. cruzi passing through rats by means of sub-inoculations of infected blood in order to study any changes of virulence which might occur. The first rat was inoculated from the faeces of infected bugs and sub-inoculations were made as soon as trypanosomes appeared in the peripheral blood. In the first sub-inoculations infections were established with difficulty, trypanosomes appearing in the blood of about every 5th rat inoculated. Second sub-inoculations made from the blood of these rats invariably gave negative results.

Attempts were then made to keep the strain in rats by inoculating emulsions of the internal organs of freshly killed infected rats intraperitoneally into uninfected rats of a suitable age. Heart and spleen pulp were used for this purpose and by this

means one strain was successfully passed through three generations, two of which were sub-inoculations. After the third generation rats proved refractory to this method of infection.

These experiments lead to the conclusion that this strain of T. cruzi decreases rapidly in virulence on passage through<sup>1</sup> laboratory animals. This is directly contrary to the findings of Mayer and Rocha Lima, Blanchard and Brumpt, who found that passages through susceptible animals were easily obtained by means of sub-inoculations.

Cultivation of the parasite on N.N.N. (Novy, Mc.Neal, Nichol) medium was attempted, heart blood, cardiac muscle and spleen tissue being used from freshly-killed rats, but these were never successful. It is worthy of note that Brumpt, from whom this strain was obtained, was also unable to obtain cultures upon artificial media.

(h) Modes of transmission to the vertebrate.

A series of experiments were made to determine the various ways in which infection could be transmitted from the invertebrate to the vertebrate host. A number of infected bugs both R. prolixus and C. lectularius were allowed to feed upon uninfected young rats. In order to avoid accidental faecal contamination the tubes containing the bugs were removed before any faeces had been passed. Daily examination of the blood of some of these rats was made, and others were killed after 16 days, (the normal incubation period) and a careful post mortem examination was made of the tissues. No forms of the parasite were ever

found. Similar examinations were made of rats which had been inoculated intraperitoneally with the macerated salivary glands of Rhodnius prolixus and Cimex lectularius. These experiments also gave negative results, thus proving that except in rare cases the salivary glands can take no part in the infection of the vertebrate.

Inoculations of the faeces of infected R. prolixus both sub-cutaneous and intraperitoneal, generally proved infective to young rats, the incubation period being about 16 days. Inoculations of the faeces of Cimex lectularius produced infections of the peripheral blood after an average of 12 days incubation. This difference was probably due to the heavier infection which usually prevailed in Cimex.

Experiments to demonstrate the possibility of infection taking place through healthy mucous membrane were carried out, using the faeces of infected Rhodnius as the transmitting agent. Faeces placed upon the mucous membrane of the mouth of a young rat produced infections of the peripheral blood after only 9 days of incubation. Faeces dropped into the eye produced an infection after 22 days. These results confirm the work of Mayer and Rocha Lima, who also succeeded in producing an infection through the mucous membrane of the rectum.

In view of the conflicting results of other workers on the transmission of the parasite through the salivary glands, an

experiment was performed to determine whether infective faeces dropped upon skin previously wounded by the proboscis of insects were capable of producing an infection. A young rat was fastened down so as to be unable either to lick or to scratch its abdomen. The fur of the abdomen was then cut short, precautions being taken against any abrasions of the skin. Six infected nymphs of R. prolixus were then allowed to feed upon this surface, an area of about one square inch. About twelve punctures were made by these bugs in feeding. The faeces passed were scanty, but all the black granular fluid was collected and spread over as much of the wounded area as they would cover, about one-third of the whole. They were then left until quite dry, no attempt being made to keep them moist by artificial means. After the faeces were completely dried up, the rat was released and daily examinations were made of its peripheral blood. After 15 days trypanosomes were found in the blood, and 13 days later the rat succumbed to the infection.

With regard to this experiment it must be pointed out that only a small amount of the semi-solid faeces (usually poor in infective forms were used) and that the possibility of infection through the mouth by licking was excluded by the faeces being thoroughly dried up before the rat was released. In view of these facts it seems probable that the saliva acts upon the blood of the vertebrate in such a way as to prevent clotting of the blood from taking place before the infective trypanosomes have had time to penetrate the wounds. Thus it seems probable that a very small

drop of the highly infective clear excretion might, by running down the side of the tube in which the bugs were kept, be sufficient to contaminate the wounds and yet be overlooked by the observer. This would naturally lead to the belief of the infectivity of the salivary glands.

(k) Conclusions.

The results of these experiments lead to the following conclusions: -

1. That Rhodnius prolixus retains an infection of T. cruzi throughout life, and from 14 days onwards after the infective feed it remains infective to vertebrates.
2. That the majority of these bugs found infected in nature, only become so by feeding upon the blood of infected vertebrates.
3. That Cimex lectularius becomes infected in the proportion of 50% only, after one infective feed, and that 30% of these bugs are consistently immune to infection.
4. That Cimex lectularius, when once infected, remains infective to vertebrates throughout the remainder of its life, the infectivity increasing, proportionately with the amount of time elapsing since the infective feed.
5. That no hereditary transmission takes place either in Rhodnius prolixus or in Cimex lectularius.
6. That the salivary glands in these species remain uninfected, and that the bite is not infective to vertebrates.

7. That the chief methods of infection in vertebrates, apart from hereditary transmission, are by no means of the infected faeces falling upon the mucous membranes of the body, or upon those portions of the skin previously punctured by the probosces of the insects while feeding.

8. That immunity to infection in rats varies according to age and to other factors unknown, and that one temporary infection, when outgrown, increases the immunity to further infection.

9. That the life cycle in the vertebrate consists of two phases of the parasite, one multiplicative in the internal organs, the other infective to the invertebrate and occurring in the peripheral blood. The former phase, which serves to establish the infection in the single infected vertebrate, is generally much more numerous than the latter, there being no correlation between the numbers of the two types of parasite present.

10. That T. cruzi is pathogenic in young rats, frequently causing death after a period of from six to seventeen days.

11. That passage through animals of one species tends to decrease the virulence of this strain so rapidly that after two sub-inoculations it becomes non-infective to animals of this species.

12. That the life cycle in the invertebrate host is similar to that described by Brumpt and that passage through the invertebrate forms a very essential part of the whole life cycle since the strain cannot be kept alive by passage through vertebrates alone.



A general survey of the results of other workers leads to the conclusion that T. cruzi has only recently become parasitic upon man, and that, through modification to this new habitat, it is passing through a condition of extreme physiological instability. This condition is emphasised by the modifications in virulence, etc brought about by isolation of different strains in the laboratory, and by the number of invertebrate hosts which, when artificially infected, are found to be suitable for the development of the parasite.

Climatic influence seems to be the chief controlling factor of the spread of the disease, which is at present confined to South America, but in view of the number of insect species capable of transmitting the infection, it seems probable that if introduced into other countries, it might have an almost world-wide distribution.

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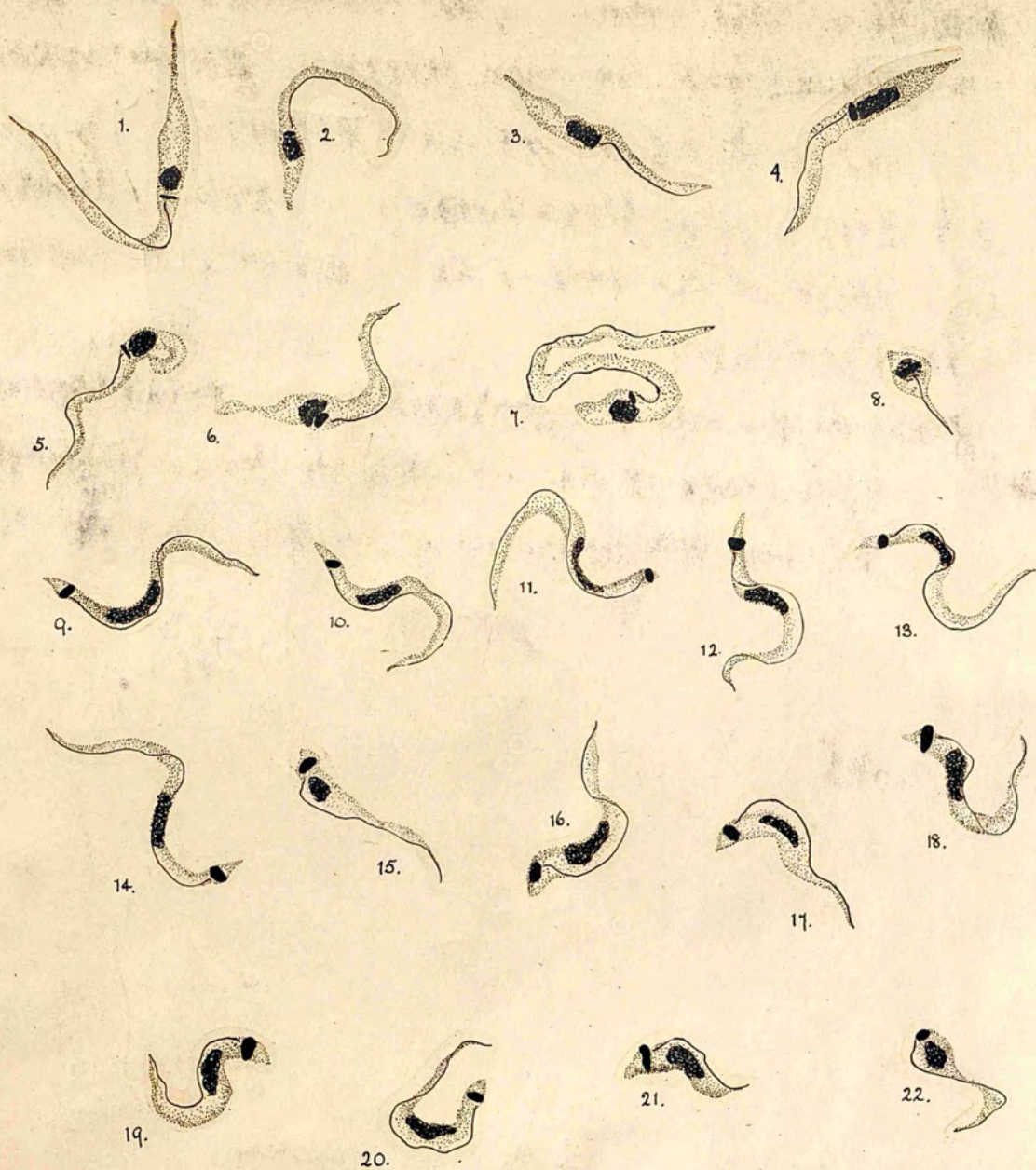
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Plate 1.

Faeces of Cimex.

- 1-5. Crithidial forms. These show the typical band-shaped kinetonucleus.
- 6-8. Transition forms. The kinetonucleus is passing posteriorly, is lying alongside the trophonucleus and is becoming more oval in shape.
- 9-18. Long slender trypanosomes. This is the next phase in the development, and follows the crithidial stage.
- 19-22. Short stumpy trypanosomes. These represent the last stage in development in the invertebrate host, and are the infective or so-called metacyclic forms.



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x 2200 1750

M. J. T.

Plate 2.

Faeces of Rhodnius.

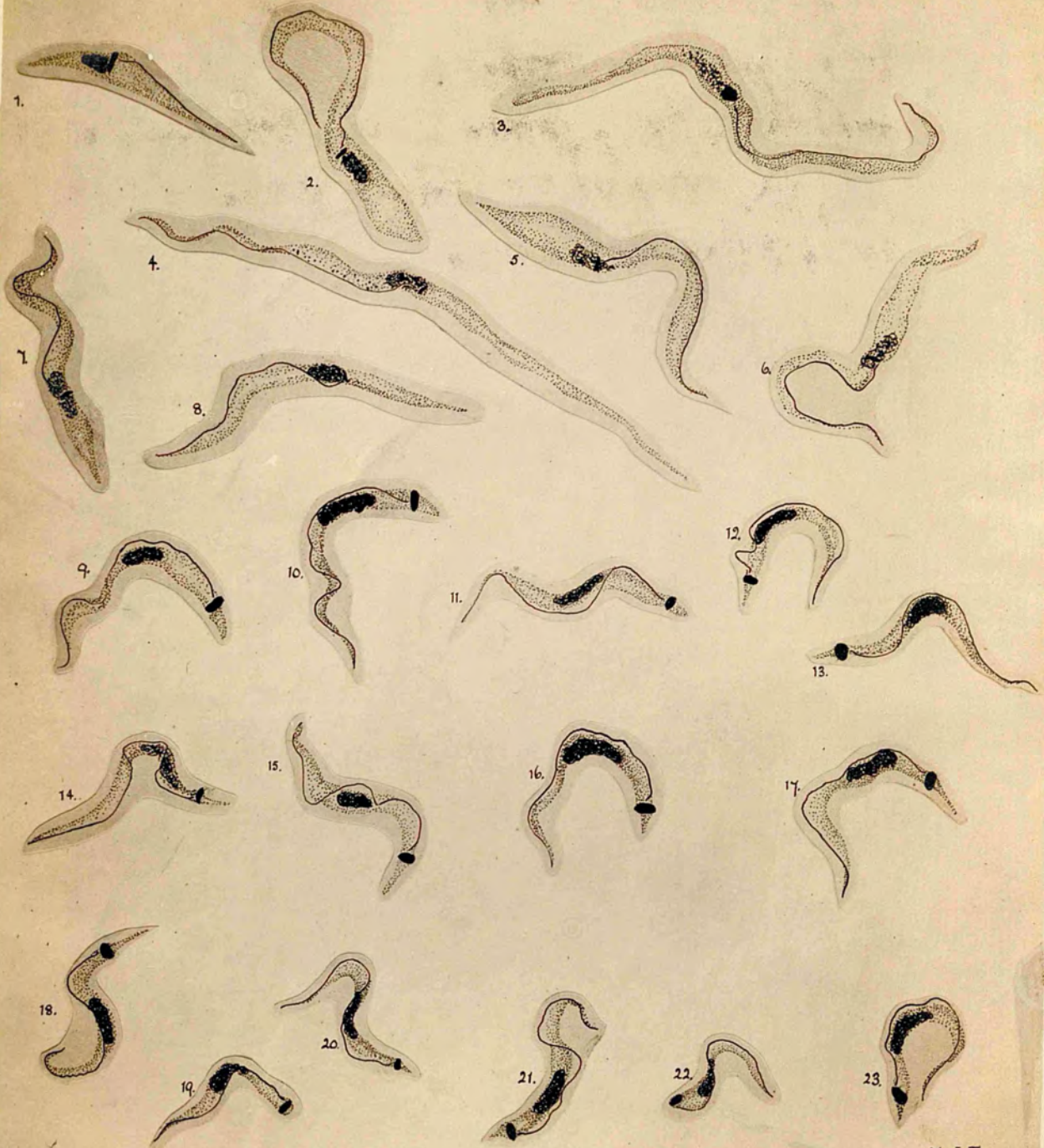
1-4. Crithidial forms.

5-7. Transition forms.

8-. Young trypanosome. The kinetonucleus lies just posterior to the trophonucleus.

9-23. Trypanosome forms.





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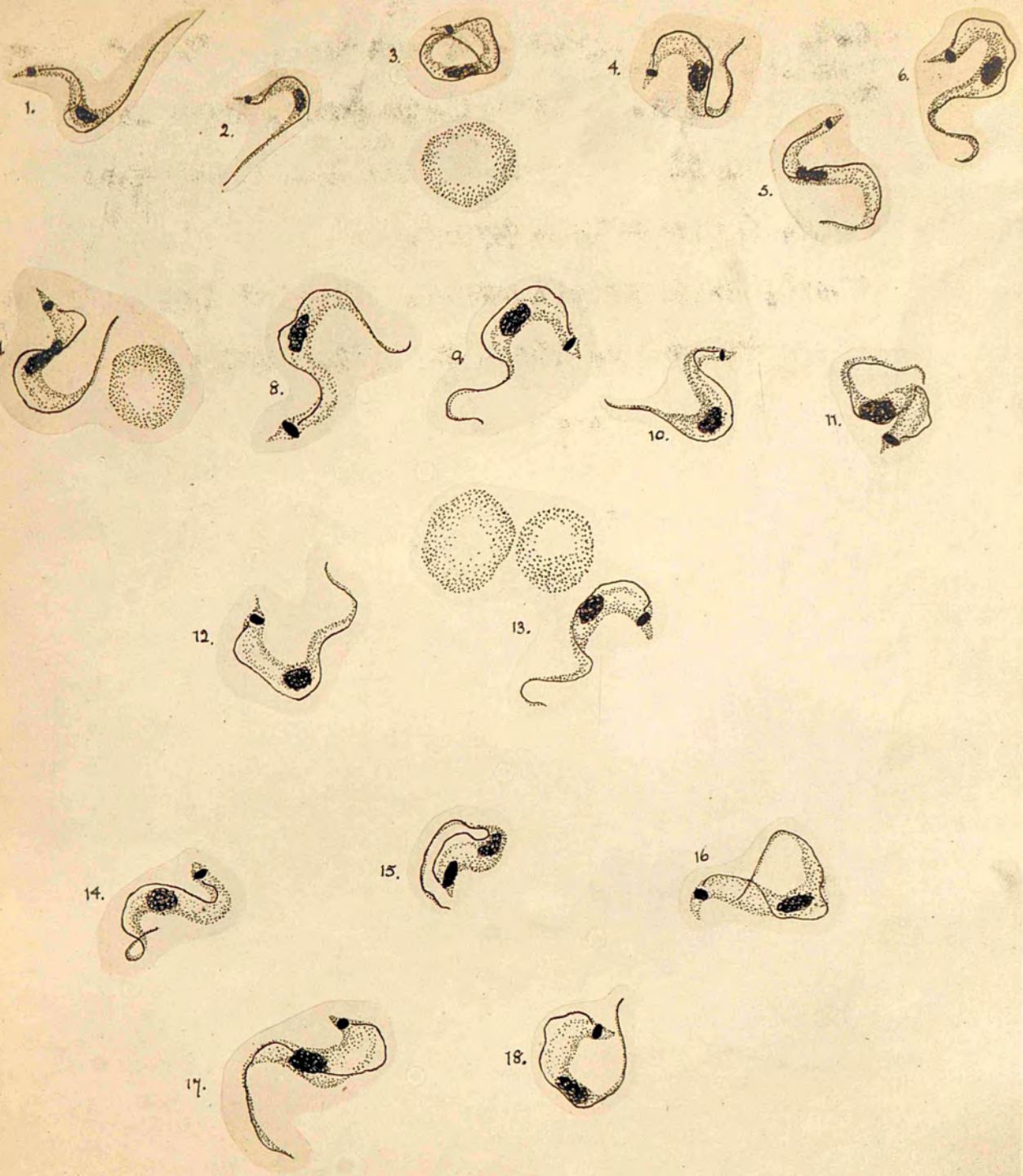
Plate 3.

Blood of Infected Rat.

1-6. Long slender trypanosomes. "Male" forms of Chagas. These are young trypanosomes recently liberated into the blood stream.

7-13. Intermediate types.

14-18. Broad trypanosomes. "Female" forms of Chagas. These are adult forms infective to the invertebrate.



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Plate 4.

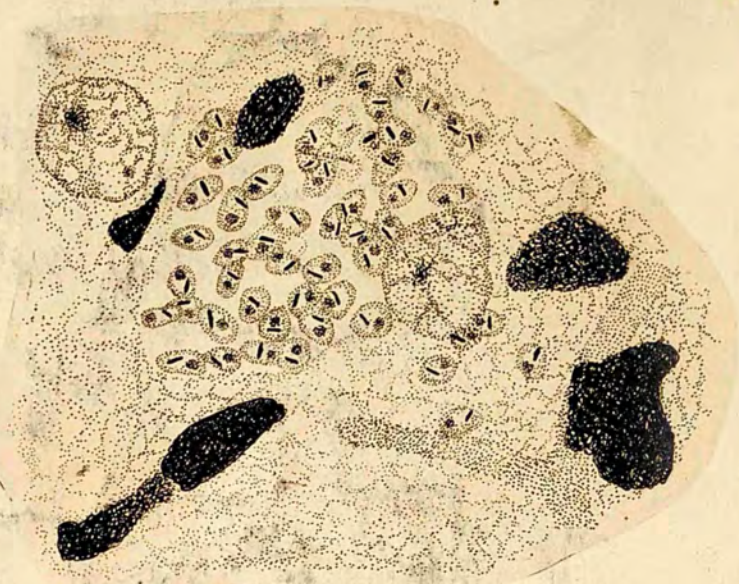
Tissue Parasites of the Vertebrate.

1. Section of cardiac muscle from an infected rat. Shows a muscle fibre invaded by rounded up or leishmania-like forms.
2. Section of brain. Also from a young rat. The cell which contains the parasites - in the leishmania-like stage - is probably a neuroglia cell. Several of the rounded or oval bodies lie in the surrounding tissue.
- 3-6. Early division stage of the leishmania-like forms. The kinetonucleus has divided.
- 7-8. Later stage in division showing the trophonucleus in process of division.
9. Late division stage. The kineto- and tropho-nucleus are divided. This is followed by division of the cytoplasm.
- 10-16. Trypanosomes from foci in the cardiac muscle. These are about to be liberated into the peripheral blood.

1.



2.



10 μ  
x 1750

M. J. T.