AKINETOPLASTIC STRAINS OF *Trypanosoma evansi* AND THE STATUS OF ALLIED TRYPANOSOMES IN AMERICA

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INTRODUCTION

Trypanosoma evansi is one of the most widely distributed trypano somes affecting domestic ungulates and other mammals, in which it causes a disease known as *Surra* and under various local names. This trypanosome is closely related to members of the *brucei* subgroup (*T. brucei, T. gambiense* and *T. rhodesiense)* (Hoare, 1949a, 1949b), and, as I have suggested elsewhere (Hoare, 1940a, 1948), it has probably originated from *T. brucei* in tropical Africa. Thance it was spread by camels into northern Africa and adapted itself to mechanical transmission by bloodsucking Diptera (especially Tabanidae) after losing the power to develop in tsetse-flies. Since the camel has for centuries been the chief transport animal in the vast region of Africa lying north of 15-16 N. Lat. and in countries of the Middle East, it is conceivable that the disease was gradually disseminated by caravans to camels in other parts of Africa, later extending to Asia, and eventually reaching the Americas. In some countries the original cameline strain probably grave rise to secondary strains, adapted to other mammalian hosts, such as bovines and equines. At present *T. evansi* occurs in the Old World practically all over southan Asia, on some islands of the Pacific and Indian Oceans; in Asia Minor, parts of south-eastern Europe; and in Africa (Mediterranean coasts of North Africa, Egypt, Anglo-Egyptian Sudan; in countries bordering on the Red Sea and the Indian Ocean, and in some parts of West Africa). Strains identical with or closely related to *T. evansi* are also found in the New World, mainly in Central and South America.

T. evansi comprises geographical and hostal races which differ from each other in their effect upon various mammals (Hoare. 1943). In some localities (Indo-China, Java, Central and South America) horses suffer more than cattle, the disease in the former being acute and in the latter mild or chronic. In other countries (Anglo-Egyptian Sudan, Somaliland), on the contrary, horses are refractory to infection, cattle acquire mild infections, but camels are highly susceptible hosts. Conditions in India vary, for in some parts of the country the effect of *Sierra* upon equines and bovines is similar to that in Indo-China and Java, while in the Punjab the infection in camels, horses and cattle shows the same differences as in the Sudan and Somaliland. On the other hand, in North Africa horses and camels are equally affected. The biological differences between local races of *T. evansi* are thus manifested in the degree at their pathogenecity to various mammalian hosts. Because of the existence of numerous geographical and hostel races and because of the importance formely attached to cross-inmunity tests in the differentiation of such races many of these have been separated from *T. evansi* under a number of specific names, the chief of which are: *T. soundanense, T. marocanum, T. aegytum, T. annamense, T. ninae kohl-yakimov, T. su-auru, T. hippicum, T. venezuelense, T. equinum.* With the exception of the last-named, all these species are morphologically identical with *T. evansi* and, since the type of disease produced by them is also similar, many authorities rightly recognize *T. evansi* as the only valid species. As regards T. e*quinum,* it differs cytologically from *T. evansi* in the absence of a kinetoplast.

LOSS OF THE KINETOPLAST

One of the most characteristic cytological features of the flagellate family Trypanosomidae is the presence at the starting point of the flagellum, in addition to the basal granule, or blepharoplast, of a prominent structure known as the *kinetoplast* (sometimes described as kinetonucleus, centrosome, blepharoplast, parabasal: cf. Hoare, 1938). The kinetoplast appears to have very much in common with the plastids present in some other flagellates and in plant cells. Like the plastids it is a cytoplasmic structure of constant form, possessing the power of independent reproduction, each kinetoplast being derived from a pre-existing one by bipartition, and being handed over to each of the dauhter-individuals whan the flagellate reproducers by binary fission.

Though typically a permanent structure in trypanosomes, the kinetoplast may be absent in some or all individuals of certain strains and races.

In trypanosomes devoid of the kinetoplast all the vital functions remain unimpaired, aberrant strains behaving in every respect like normal ones. It is therefore evident that the kinetoplast —whatever its physiological rôle may be— is not indispensable to the existence of these flagellates.

In addition to the cytological and physiological interest attached to the kinetoplast, the loss of this organ in individual trypanosomes and the production of entire strains devoid of it have a bearing on the genetics and evolution of these flagellates. It has long been known that in strains of different species of mammalian trypanosomes there commonly occurs a certain proportion of individuals lacking the kinetoplast (cf. Hoare & Bennett, 1937). Such *akinetoplastic* forms are the least prevalent in members of the *vivax* and *congolense* groups, their percentage in *T. congolense* and *T. simiae* varying from 0 to 0.8, and in *T. vivax* from 0.1 to 0.2. In the *brucei-evansi* group the number of akinetoplastic forms reaches a much higher figure, their proportion usually varying from 0.01 to 10.0%, though in some strains their number is subject to wider fluctuations. Thus, in *T. brucei* Curasson and Adjovi (1940) recorded anything up to 77.7% akinetoplastic forms, while in *T. evansi* and allied forms (including *T. hippicum* and *T.venezuelense)* their proportion may rise to 30.2%, the culminating point being reached by *T. equinum*, which is totally akinetoplastic. The fluctuation in the number of akinetoplastic trypanosomes has been more fully studied by me in a strain (NS) of *T. evansi* which was isolated from a camel in the Anglo-Egyptian Sudan in January 1938 (Hoare & Bennett, 1939) and has been under continuous observation for five years. At first it contained 1% akinetoplastic trypanosomes but in subsequent passages through rodents the percentage of these forms underwent considerable fluctuation in the course of one year (1938-1939), rising and falling irregularly, with a maximum of 71% and a minimum of 1%. From 1939 onwards the fluctuation gradually diminished in amplitude and frequency, and by 1943 —when regular observations were discontinued— this strain became to all intents and purposes "normal", with the number of akinetoplastic forms only occasionally rising to 20 or 25%.

AKINETOPLASTIC STRAINS

In addition to individual variation in the number of akinetoplastic forms naturally occurring in various species and races of trypanosomes, it is well known that totally akinetoplastic strains can be produced *artificially,* by treating infected animals with certain organic dyestuffs (for critical review of this work see Hoare & Bennett, 1937). It has been demonstrated that strains (including *T. evansi* in which all the trypanosomes had been thus deprived of the kinetoplast never recovered this organ, remaining completely akinetoplastic for years. The continued conservation of the aberrant strains indicates that a trypanosome, once deprived of the kinetoplast, tends to propagate similar individuals, or to "breed true".

During the first quarter of this century the only example of the *natural* occurrence of a strain or race of trypanosomes totally devoid of the kinetoplast was *T. equinum,* a South American species indistinguishable from. *T. evansi* except in this particular. In view of the successful artificial production and prolonged maintenance of akinetoplastic strains of the last-named species, it was thought that *T. equinum* might likewise have originated from it through the loss of the kinetoplast. Further light on this problem was thrown by the *spontaneous* transformation of a normal strain of *T. evansi* into an akinetoplastic strain, and by the discovery of such aberrant strains appearing spontaneously in individual hosts naturally infected with this trypanosome. In the first case, a normal North African equine strain of *T. evansi ("T. marocanum")* had been maintained in laboratory rodents for 5 years, after which it suddenly became totally akinetoplastic (Wenyon, 1928; Hoare & Bennett, 1937, 1939) and retained this peculiarity for 17 years (1928-1945), after which the strain was accidentally lost. The other cases relate to *T. evansi* of camels from the Anglo-Egyptian Sudan, which have been described in detail by Hoare and Bennett (1937, 1939) and Hoare (1940b). In the course of examination of the blood of more than one hundred camels suffering from *Surra*, over a period of about 3 years, five of these animals were found to be infected with akinetoplastic forms exclusively, while the trypanosomes in all the other camels were normal as far as this organ was concerned. One of the akinetoplastic cameline strains (SAK) was isolated into laboratory rodents and has been kept by me under continuous observation for the last 12½ years (January, 1937; October, 1949), in the course of which the aberrant condition has remained unchanged. It is thus seen that both induced and spontaneous akinetoplastic strains of *T. evansi* have become permanently fixed and breed true for an indefinite period. The loss of the kinetoplast in this species therefore represents a heritable variation leading to the formation of new strains and races characterized by the permanent absence of the organ in question.

NATURE OF THE AKINETOPLASTIC CONDITION

We are still in ignorance concerning the primary cause of the disappearance of the kinetoplast in trypanosomes. As regards the mechanism by which the aberrant condition is perpetuated, it would seem that failure of the kinetoplast to divide is mainly responsible for this phenomenon. In “normal" strains of trypanosomes, containing a small proportion of akinetoplastic forms, there are always present some dividing forms in which the kinetoplast has failed to divide, with the result that after binary fission one daughter-trypanosome possesses a kinetoplast and one lacks it (cf. Hoare & Bennett, 1937). Since all evidence—especially the prolonged maintenance of akinetoplastic strains— indicates that the kinetoplast once lost does not arise *de novo*, a trypanosome deprived of this organ will continue to propagate similar individuals, or breed true. The rôle of such irregular divisions in the production of akinetoplastic trypanosomes was clearly demonstrated for the fluctuating strain (NS) of *T*. *evansi* mentioned above, in which a rise or fall in the proportion of such forms was invariably preceded by a corresponding increase or decrease in the number of trypanosomes dividing irregularly (Hoare & Bennett, 1939). Other factors responsible for the fluctuation might be a variation in the rate of reproduction of normal and akinetoplastic individuals, and variation in the mortality rate of the different types of trypanosomes.

In the case of akinetoplastic strains induced by the action of chemicals the same mechanism is involved. The effect of the chemical on a trypanosome appears to be twofold. On the one hand, it may destroy the kinetoplast directly and cause it to disappear; on the other hand, it may deprive the kinetoplast of the power to divide. If it is assumed that this disability affects the kinetoplast of all the trypanosomes thus treated, together with their progeny, there will be a progresive increase of akinetoplastic forms in successive generations, the ratio of aberrant individuals to normal ones being as $(2^n-1):1$, where n = number of divisions. It is obvious that the two factors, acting singly or combined, would be capable of producing an akinetoplastic strain of trypanosomes (Hoare & Bennett 1937; Hoare, 1940b).

The loss of the kinetoplast in trypanosomes under natural conditions has all the attributes of a *mutation,* for variants possessing the new character (absence of a kinetoplast) appear suddenly, breed true from the beginning, and give rise to a new strain or race, which —in the absence of sexual reproduction— becomes permanently fixed (Hoare, 1940b). The stability of this character is evident from the prolonged maintenance of the akinetoplastic strains of *T. evansi* described in this and in previous papers, and from the existence of *T. equinum,* in which this condition has been known for half a century.

Though the elimination of the kinetoplast in trypanosomes has all the qualities of a mutation, the factors governing this phenomenon remain obscure, but the production of akinetoplastic strains by the action of chemicals can be regarded as a form of induced mutation. As regards other conditions, Curasson and Adjovi (1940) believe that variation in the proportion of akinetoplastic forms occurring in different strains of the same species of trypanosome depends upon the species of mammalian host harbouring the strain. However, Hoare and Bennett (1939) have shown, in the case of *T. evansi,* that the type of host does not appear to play any part in the production or fluctuation of the akinetoplastic condition.

Nothing definite is known about the manner in which totally akinetoplastic strains of *T. evansi* arise in nature. It is conceivable, however, that such an aberrant strain might originate in one of the following ways. First, indirectly, through the chance inoculation by the insect-vector of a single trypanosome devoid of the kinetoplast into a new host, in which it continues to breed true. Secondly, directly, if, in a strain subject to wide fluctuation of the proportion of akinetoplastic forms, the amplitude reaches 100%, involving all the trypanosomes present. Normally, in strains of *T. evansi* containing up to 10% akinetoplastic forms, the chances of a totally akinetoplastic strain arising from aberrant individuals, as suggested in the first hypothesis, are very remote. However, the occurrence of a strain (NS), in which the fluctuation of akinetoplastic forms may exceed 70%, lends considerable support to this contention, for in such a strain there occur favourable periods, when the chances of an akinetoplastic trypanosome being inoculated into its mammalian host by the vector are actually higher than the chances of introducing a normal individual. From the genetic point of view, therefore, the production of an akinetoplastic strain by this method is due to the accidental selection from a mixed trypanosome population of a mutant individual devoid of a kinetoplast. The high degree of fluctuation in the proportion of akinetoplastic forms attained by this strain of *T*. *evansi* also substantiates the second hypothesis. In fact, it would be difficult to explain the transformation of the normal strain of *T. evansi (= T. marocanum*) mentioned above in any other manner.

As regards the significance of the loss of the kinetoplast in trypanosomes—apart from its rôle in the evolution of new races—we can only speculate. Reichnow (1940) has correlated its absence with the loss of cyclical development in the intermediate host of the trypanosome. As already noted above, akinetoplastic individuals and

strain are particularly common in members of the *brucei-evansi* group, but are scarce in the *congolense* and *vivax* groups. Reichenow points out that trypanosomes of the two last-named groups also show a higher degree of infectivity for their insect-vectors than members of the *brucei-evansi* group. Among these *T. evansi* and *T. equninum* do not undergo any development whatever in the vector, and this is correlated. in the former species, with a strong tendency to produce strains lacking a kinetoplast, and in the latter species, with the permanent absence of this organ. Reichanow accordingly suggests that the presence of a kinetoplast might he in some way connected with the power of the trypanosomes to develop in the insect-host, and, conversely, that the loss of infectivity for the vector favours the disappearance of the kinetoplast. In support of these views Reichanow mentions an experiment with *T. gambiense,* in which 70%, of the flagellates had been artificially deprived of the kinetoplast. Whan this strain was cultivated, only normal trypanosomes were found to be present, indicating that they alone were capable of developing in culture. Since the conditions under which this trypanosome grows in culture correspond to those under which it develops in the tsetse-fly, it was inferred that the akinetoplastic forms would likewise be unable to develop in the insect.

THE ORIGIN OF *T. EQUINUM*

Among the mammalian trypanosomes *T. equinum* occupies an exceptional position, in that it is the only species devoid of a kinetoplast, where as in other respects it is indistinguishable from normal trypanosomes of the *evansi* subgroup, to which it naturally belongs. The ease with which akinetoplastic strains of *T. evansi* can be produced artificially has led a number of observers to regard *T. equinum* as a race or variety of the former species (Wenyon, 1926; Lavier, 1929, 1933). However, the manner in which *T. equinum* became differentiated from *T. evansi was* not clear till the discovery of the akinetoplastic strains arising spontaneously in the last-named species (Hoare & Bennett, 1937, 1939). There can now be no reasonable doubt that *T. equinum* has originated from an akinetoplastic strain of *T. evansi*, which had established itself in South America and has continued to breed true as a mutant race for at least fifty years since its discovery in 1901.

Regarding the earlier history of *T. equinum* little is known, except that (according to Laveran & Mesnil, 1912), horses suffering from *Mal de Caderas* were first imported to the island of Marajo, near the estuary of the Amazons, whance the disease spread over Brazil as far as Matto Grosso, giving rise, in 1860 and later, to severe epizootic outbreaks among horses. It is possible that the original *T. equinum* introduced to Marajo, was a strain of *T. evansi,* which had spontaneously become akinetoplastic in an individual equine host, like the cameline strains in the Sudan. At present the distribution of *Mal de Caderas* extends over a great part of tropical South America, in British Guiana. Brazil, Paraguay, Uruguay, Argentina and Bolivia.

The presence of a constant cytological difference between *T. equinum* and *T. evansi* entitles the former to an independent specific status. If the arguments advanced by me are correct, this case provides a unique example among the protozoa of the formation under natural conditions of a new species (*T. equinum),* the origin of which by mutation from the parent-species (*T. evansi)* can be traced.

AMERICAN RACES OF *T. EVANSI*

The position regarding the other two species of the *evansi* subgroup in the New World is not so clear. There appears to be a certain gradation in their distribution, the akinetoplastic condition becoming more pronounced as one proceeds from north to south. Thus *T. hippicum*, which causes *Murrina* in equines of Central America and Colombia, has about 4% akinetoplastic forms. In Venezuela this species is replaced by *T. venezuelense,* the causative agent of *Peste Boba or Desrengadera,* in which the proportion of akinetoplastic forms may reach 30%. Further south, the prevalent species is the totally akinetoplastic *T. equinum, T. hippicum* is thus indistinguishable from “normal” ra ces of the Old World *T. evansi*, while the high proportion of akinetoplastic forms in *T. venezuelense* is comparable to that in an equine strain of *“T. evansi var. sudanense”* described by Curasson and Adjovi (1940) from French West Africa. It is thus seen that, both in its geographical distribution and in the proportion of akinetoplastic forms, *T. venezuelense* occupies an intermediate position between *T. hippicum* and *T. equinum* Regarding the significance of this gradation nothing is known, but it is conceivable that *T. venezuelense* might represent a mixed infection of the two other species. In view of the absence of sharp distinguishing characters between *T. hippicum* and *T. venezuelens,* on the one hand, and *T. evansi* on the other, there are no valid grounds for separating the first two from the last one as independent species.

Since in Central and South America the hourse is the chief host suffering from diseases caused by

trypanosomes of the *evansi* subgroup, there can be no doubt that equine trypanosomiasis in the New World was absent before the introduction of the horse into the Western hemisphere by the Spanish conquerors during the 16th century, the history of wich is fully described by Piétrement (1883*)* and by Ridgesway (1905). Horses were originally brought into that continent in relatively small numbers, and, as they were exclusively cavalry mounts, it is highly improbable that any of them were already infected with trypanosomes. It is more likely, that —as in the case of *Mal de Caderas* mentioned above— trypanosomiasis was imported to the New World much later and at different periods with individual infected animals, the disease finding highly susceptible hosts among the domesticated and wild horses, which had spread widely over most of Central and South America after the Spanish conquests.

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