Bulletin of the World Health Organization, 56 (3): 467-480 (1978)

Proposals for the nomenclature of salivarian trypanosomes and for the maintenance of reference collections*

Studies on the characterization of salivarian trypanosomes are at present developing rapidly and difficulties in communication tend to arise because of different procedures in the different laboratories concerned. Terms can be divided into two classes, those that are purely operational and simply describe the laboratory history of the organism, and those that imply characterization. Of the Linnaean taxa, the section Salivaria and the four subgenera thereof, Duttonella, Nannomonas, Pycnomonas, *and* Trypanozoon, *are useful at the present time but lower taxa are often unsatisfactorily defined. Characters such as the clinical course of infection, which have been used for subspeciation, are being found to correlate with such characters as isoenzyme patterns, antigenic make-up and DNA constitution.*

Serial passage of organisms has serious disadvantages and so reference collections are primarily of stabilates—cryopreserved suspensions of living organisms. Methods for the preparation of antigens, antisera, and isoenzymes are briefly noted.

Conventions for the documentation of reference collections are proposed, in particular that the designation of materials should consist of two components, one signifying the primary isolation and the other the particular derived material used. The requirements for rapid information retrieval and global cataloguing of materials are considered.

The rapid progress at present being made in the study of protozoal pathogens, particularly the trypanosomes, is posing problems of communication between workers, since many different systems of characterization, conventions of nomenclature, and procedures for cryobank operation and documentation are arising among the many research centres involved. For example, there are at least three terms—isolate, strain, and line—being used for trypanosome materials maintained in the laboratory by serial passage; some of these terms have different connotations in other fields. The " ETat " system of nomenclature for antigenic types of trypanosomes proposed by Lumsden et al. in 1967 (*1*) has been taken up by several other laboratories and has led to illuminating comparisons of antigenic type collections held in different laboratories (*2*, *3*); however, difficulties arise with this system of nomenclature when the same original material is worked on in different laboratories, and much further expansion of the system appears likely to become unmanageably complex. Similar problems will soon arise in the designation of trypanosome isoenzyme types (*4*, *5*, *6*). There are many collections of cryopreserved stabilates (*7*) but the full value of these collections for definitive and comparative purposes is not yet realized because of the multiplicity of different systems of isolation, stabilation, and recording. It is because of these difficulties that the following proposals for the nomenclature of salivarian trypanosomes and for the maintenance of reference collections have been drafted.

The genus *Trypanosoma* includes organisms parasitic over the entire range of vertebrate classes—amphibia, fish, reptiles, birds, and mammals—and taxonomic relationships over the whole genus have still not been resolved. For practical purposes, Hoare (*8*) proposed a classification restricted to those trypanosomes infecting mammals and therefore of medical and veterinary importance. He proposed two " sections " at a level between genus

* These proposals were prepared following a meeting at the London School of Hygiene and Tropical Medicine, London, England in September 1976, that was supported jointly by the Wellcome Trust and the Ministry of Overseas Development of the United Kingdom. The participants in the meeting are listed on page 478. The report of the discussions on which these proposals are based will be published in: LUMSDEN, W. H. R. & KETTERIDGE, D. S., ED. *Biology of the Kinetoplastida*, Vol. 2. London & New York, Academic Press (in press).

and subgenus: the Stercoraria, comprising species whose developmental cycle in the vector insect is typically completed in the rectum (or posterior station) and in which transmission is " contaminative ", i.e., by infective forms contained in the faeces of the vector invading the new host *via* skin abrasions or mucous membranes; and the Salivaria, those species whose developmental cycle is typically completed in the mouth parts (or anterior station) of the vector and whose transmission is " inoculative ", i.e., by infective forms being injected into the new host by a bite or in the saliva of the vector insect. The Salivaria is the more homogeneous of the two groups.

DEFINITIONS AND NOMENCLATURE

Besides the terms relating to Linnaean taxa (see below), the terms used to describe organisms or populations of organisms fall into two distinct classes. First, there are the purely operational terms describing the laboratory history of the materials and second, those terms that are related to recog-

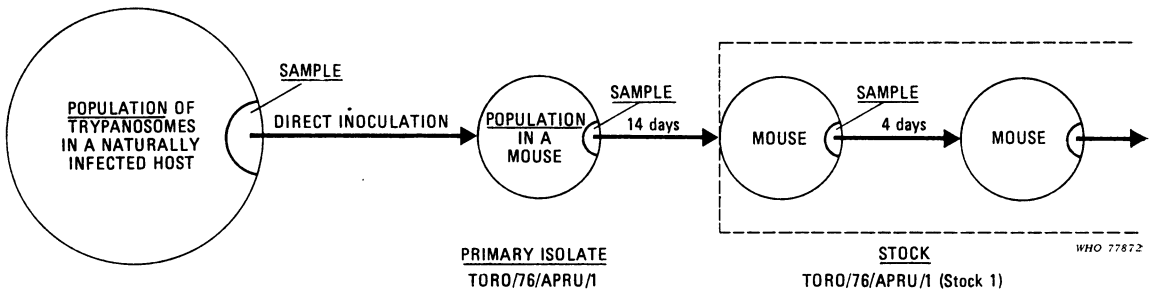

Fig. 1. Origin of a stock. *Population* may be qualified by an adjective, e.g., wild. The *primary isolate* is that resulting from the first passage. TORO = locality of sample collection; 76 = year; APRU = laboratory (African Protozoology Research Unit); 1 = laboratory number.

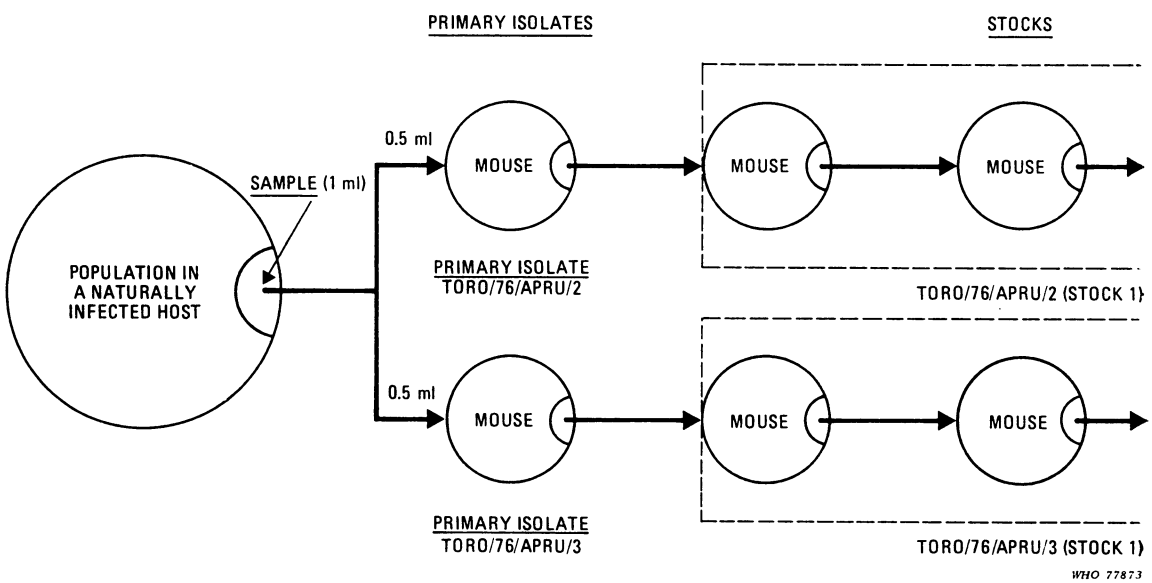

Fig. 2. Origin of two primary isolates from a single sample. If, as shown, a sample from a naturally infected host is divided, each part must be considered the equivalent of a separate sample. This is because organisms present in very low concentrations may be present in one part and absent from another, and primary isolates derived from them would therefore be different.

nizable characteristics of the organisms. Some terms, such as " strain ", carry both connotations and so lead to confusion of thought and communication.

It is recommended that the following terms, as defined, be used in the study of salivarian trypanosomes. Notes, where added, are intended to be of an explanatory nature only and are not part of the definitions. Fig. 1–6 exemplify the various concepts.

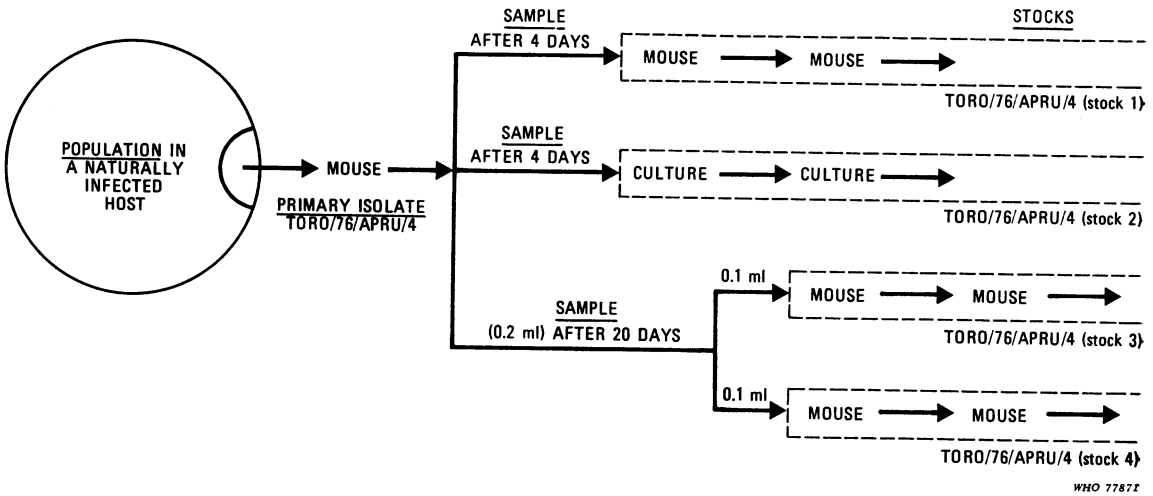

Fig. 3. Origin of four different stocks from a single primary isolate. The definitions require that each sample taken from the primary isolate originates a separate stock; thus, if a sample is divided, each part is the origin of a stock. Note that there is no implication of characterization.

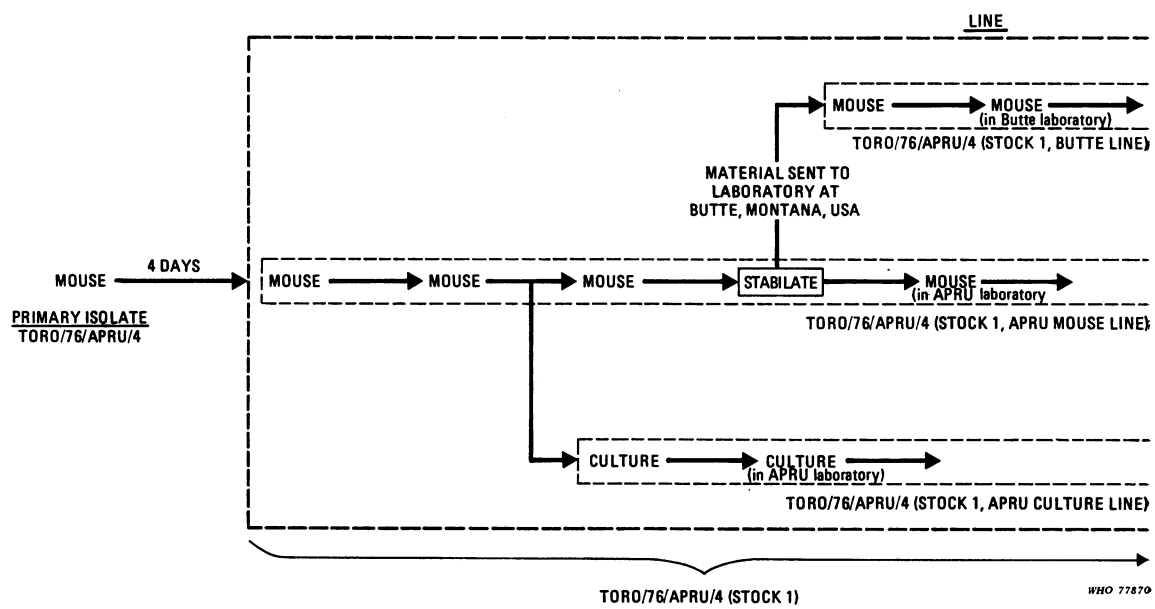

Fig. 4. Concept of lines.

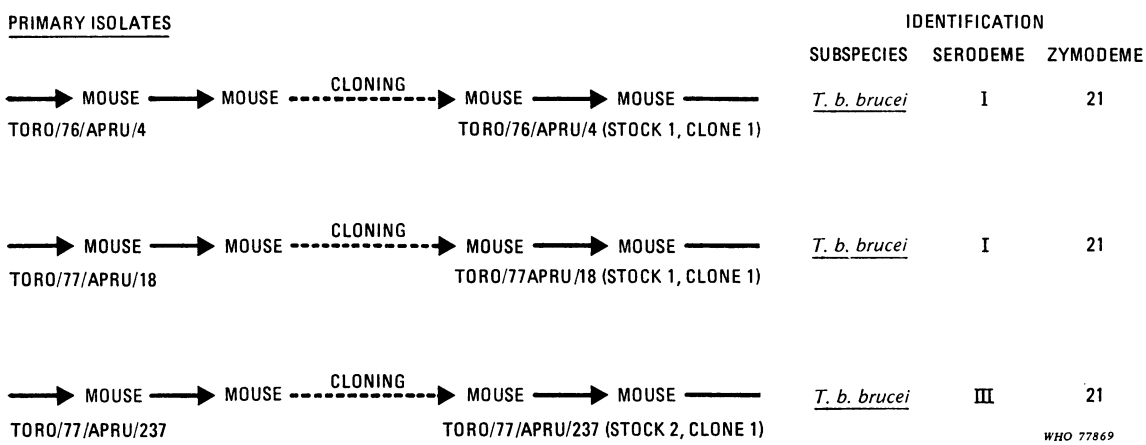

Fig. 5. Concept of demes. On the information presented here, the two clones TORO/76/APRU/4 (Stock 1, Clone 1) and TORO/77/APRU/18 (Stock 1, Clone 1) are of the same deme, whilst clone TORO/77/APRU/237 (Stock 2, Clone 1) is of a different deme.

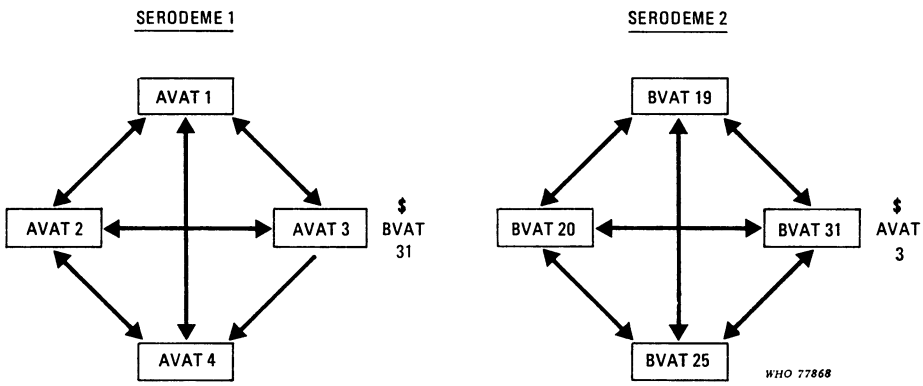

Fig. 6. Concept of serodeme. A serodeme consists of a group of variable antigen types, each of which can be derived from and give rise to (by antigenic variation) all the other variable antigen types within that serodeme. Some variable antigen types, present in different serodemes, are immunologically similar (e.g., BVAT 31 and AVAT 3, as shown); however, the present evidence is that BVAT 31 will give rise only to variable antigen types in serodeme 2, and AVAT 3 only to variable antigen types in serodeme 1. $ = immunologically similar to.

TERMS RELATING TO LINNAEAN TAXA

Salivarian trypanosome species

An assemblage of organisms that can be distinguished from other species by one or more stable, discontinuous morphological characters.

Salivarian trypanosome subspecies

Assemblages of organisms within a species that cannot be separated from each other by morphological characters but only by other stable characters.

Note: When material is defined at the level of subspecies, living type-material should be deposited in a named stabilate collection for future reference.

OPERATIONAL TERMS WITHOUT IMPLICATION OF CHARACTERIZATION

The terms in this section do not *of themselves* imply any characterization; however, some of them may, of course, also be used to denote characterized materials.

Population

The group of trypanosomes present at a given time in a given host or culture.

Notes: (1) The group may consist of a mixture of several species and subspecies. (2) The term may be qualified by an adjective if further description is required.

Sample

That part of a trypanosome population collected on a single occasion.

Primary isolate

The viable organisms present in a culture or in an experimental animal host following the introduction of a sample, or part of a sample, from a naturally infected host.

Notes: (1) The primary isolate may consist of a mixture of several species and subspecies. (2) The use of the term " isolate ", unqualified, for this should be discontinued.

Stock

The population derived by serial passage *in vivo* and *in vitro* from a primary isolate without any implication of homogeneity or characterization.

Note: Stocks derived at different times from a single primary isolate may differ.

Line

A laboratory derivative of a stock maintained in different physical conditions or in different geographical locations.

Note: " Different physical conditions " includes different animal species as well as cultures.

Stabilate

A sample of organisms preserved alive, usually in replicate, on a single occasion.

Note: The term " stabilation " is used to describe the process of preparing a stabilate.

Clone

The trypanosomes derived from a single individual by binary fission.

Note: The genetic uniformity of a clone is not to be expected to be conserved by continuous passage *in vitro* or *in vivo*.

TERMS IMPLYING CHARACTERIZATION

Deme

Trypanosome populations that differ from others of the same species or subspecies in a specified property or set of properties.

Notes: (1) Prefixes may be given to the term to designate the general grounds on which the deme is defined, e.g., serodeme, zymodeme. (2) There is no certain implication of common ancestry for demes displaying similar characters.

Strain

A set of populations originating from a group of trypanosomes of a given species or subspecies present at a given time in a given host or culture and defined by the possession of one or more designated characters.

Notes: (1) A strain is obtained by characterization of a subdivision of a stock; unambiguous characterization can be ensured only if such a subdivision is initiated by a single organism. (2) The term " strain ", as generally used till now, has carried a dual implication: first, the purely operational implication of its maintenance by serial passage in the laboratory; second, an implication of characterization, i.e., it is a hybrid of " stock " or " line " on the one hand and of " deme " on the other, as now defined.

Variable antigen type (VAT)

The identity of a single trypanosome as determined by the variable antigen expressed at its surface.

Note: Certain VATs, which tend to arise first or early in the course of infection, have been termed " basic ", " parent ", or " predominant " VATs. The main points of Gray's descriptions of these (*9*) [a] are as follows:

Basic or parent antigen type. In addition to a great capacity for antigenic variation, each trypanosomal

[a] GRAY, A. R. Notes on immunology. WHO unpublished document PD/68.12 (1965).

strain seems to have an innate tendency to develop in one relatively stable antigenic form variously called the " parent " or " basic " antigenic type of the strain. The facts in support of this finding may be summarized as follows:

1. When a variant of a strain is maintained for a long time by repeated sub-passage at intervals of a few days in laboratory animals, it tends to revert to one serotype which has been called the parent antigenic type of the strain (*10*).

2. Several authors have shown that the trypanosomes that develop at the first relapse when animals are infected with different serological variants of a strain are often of one similar predominant antigen type (*11*, *12*).

3. When a tsetse fly ingests trypanosomes with variant antigens of a strain, there is a tendency during cyclical development for such trypanosomes to be displaced wholly or partly by organisms producing one particular antigen (*13*). This antigen has been described as the basic strain antigen.

Predominant antigen type. In addition to the " parent " or " basic " strain antigen, certain other antigens of each strain of trypanosomes tend to develop during the first few weeks of infection whenever a strain is introduced into a new host by syringe passage (*11*, *14*). These antigens have recently been called predominant strain antigens to distinguish them from numerous other less prominent variant antigens that appear during the late chronic stages of infection.

Homotype (*VA homotype*)

A VAT expressed by the majority of trypanosomes in a clone and against which monospecific antiserum can be prepared.

Heterotype (*VA heterotype*)

A heterologous VAT that arises within a clone of presumed homologous VAT.

Reference VAT

The first example of a VAT to be characterized and used as a reference point.

Note : As a result of the methodology, the reference VAT material will be a cryopreserved stabilate predominantly of VA homotypic organisms.

VAT repertoire

All the VATs that can be expressed by a clone.

Serodeme

Populations of trypanosomes each of which can express the same VAT repertoire.

Note : Comprises all the trypanosomes in the world that are able to express the same VAT repertoire.

NOMENCLATURE OF VARIABLE ANTIGEN TYPES

Variable antigen types have hitherto been designated by a system peculiar to the worker concerned or by the system proposed by Lumsden et al. (*1*). In the latter system, VATs are designated by a pronounceable code composed of letters indicating the laboratory, a letter indicating the subgenus, and two letters as an abbreviation of the words " antigenic type ", followed by a number, e.g., ETat (Edinburgh *Trypanozoon* antigen type) 17, BoTat (Bordeaux *Trypanozoon* antigen type) 34, AnTat (Antwerp *Trypanozoon* antigen type) 12, etc. This has greatly facilitated the interchange of information between laboratories. However, this very interchange has enabled discoveries to be made that have revealed deficiencies in the present system. For instance, some VATs have been found to be shared by two serodemes (e.g., AnTat 11 is indistinguishable from ETat 7). Also, more than one serodeme may be under examination in any one laboratory, and further VATs may be isolated from a serodeme originally examined at some other place (e.g., Antwerp has produced new VATs from the " ETat " serodeme; to give them the prefix " AnTat " would be confusing).

There is need for increased exchange of information and of reagents for comparative purposes, to obtain uniformity of treatment. Such exchange will be necessary if VA typing is to be applied epidemiologically. Work on VA typing should be particularly towards defining the minimum number of VATs necessary to identify serodemes. It is therefore recommended that workers actively engaged in the isolation and identification of VATs should jointly recommend and publish an improved system of nomenclature consistent with the terms defined above and with recent advances in knowledge of their interrelationships. It would be a part of the function of the reference laboratories to hold and compare stabilates of and antisera against VATs, as well as to communicate with involved workers, perhaps by means of a newsletter.

RELATIONSHIP OF CHARACTERIZED POPULATIONS TO LINNAEAN TAXA

Genus

Since these proposals cover only species of a single genus, *Trypanosoma* Gruby, 1843, definition at the generic level is not required.

Section

The terms " Stercoraria " and " Salivaria " were introduced by Hoare (*8*) as the names of two groups described as " sections " at a level between genus and subgenus, and referring only to trypanosomes of mammals. Article 42 (d) of the International Code of Zoological Nomenclature (1964) states that " a uninominal name proposed for a primary subdivision of a genus, even if the subdivision is designated by a term such as " section " or " division ", has the status in nomenclature of a subgeneric name . . . ", so that strictly no such category is permissible. However, the terms have proved so useful in a descriptive sense that they should be provisionally retained, though not as formal taxa (which Hoare had not intended them to be); in the future their status will have to be revised. There is much evidence (morphological, behavioural, geographical, and from antigen composition) that the Salivaria are a homogeneous group of species, though this is not true of the Stercoraria.

Subgenus

A subgenus of the genus *Trypanosoma* is defined as a group of species having common morphological characters. This definition places the emphasis on morphology. It does not exclude the possibility of subgenera also possessing common behavioural or other characters (e.g., site of development in the vector) and of such characters forming a part of a definition, but they are not an essential part of it.

Introduction of the four subgenera *Duttonella*, *Nannomonas*, *Trypanozoon*, and *Pycnomonas* by Hoare (*8*) materially clarified evolutionary relationships within the salivarian trypanosomes and substantially simplified description and discussion, so they should not at present be abandoned. However, the considerations outlined here make it likely that their use will have to be reconsidered in the near future since, if the sectional group Salivaria is treated as a subgenus as the International Code requires and if, as is proposed below, each of the existing subgenera becomes unispecific, they may well be considered redundant. As it is, they probably represent a lower level of taxonomic separation than that existing between them and the other subgenera of *Trypanosoma*. If this action were ultimately taken, the name Salivaria might not be available for the new subgenus since it was not proposed in conjunction with the designation of a type species, as required by the International Code (Article 12 (b)).

It has been pointed out by Hoare that the citation of subgeneric names is not necessary in publications dealing only with species of a single subgenus but only when comparison is being made with members of another subgenus; the specific binominal or subspecific trinominal is adequate identification.

Species and subspecies

In considering the validity of taxa of salivarian trypanosomes at this level, and taking into account the definition of species proposed above, it can be seen that more convincing evidence is required for the differentiation of many of the commonly accepted taxa; purely mensural characterization of species is often inadequate unless a thorough statistical treatment of data from a wide range of materials is available and, in the absence of other morphological criteria, supporting evidence of other kinds (e.g., macromolecular and immunological) should be sought. Also, many of the differential morphological characters currently used are clinal rather than discontinuous in geographical distribution (e.g., length in the subgenus *Nannomonas* and incidence of dyskinetoplasty in *Trypanozoon*). Because of this it is suggested that, for the present, each salivarian subgenus should be regarded as unispecific unless and until further definite evidence to the contrary is forthcoming.

In *Duttonella*, the nominate subspecies would be *T. vivax vivax* Ziemann, 1905; the marked behavioural difference (absence of cyclical development) and geographical separation warrant retention of *T. v. viennei* Lavier, 1921 as a distinct subspecies, and the mensural differences cited by Hoare (*8*, pp. 405–407) justify separation of *T. v. uniforme* Bruce et al., 1911 at the subspecific but not, perhaps, at the specific level. The status of *T. v. ellipsiprymni* Keymer, 1969 may require further investigation.

In *Nannomonas*, the morphological and behavioural differences between *T. congolense* and *T. simiae* (see Hoare, *8*, pp. 436–437 and 458) intergrade

to such an extent that they can only be regarded as a cline of a single species (*T. congolense* Broden, 1904) and not as warranting even subspecific distinction (*15*).

In *Trypanozoon*, it is proposed that only a single species (*T. brucei* Plimmer and Bradford, 1899) be accepted at present; it would comprise the following five subspecies:

T. b. brucei Plimmer and Bradford, 1899

T. b. rhodesiense Stephens and Fantham, 1910

T. b. gambiense Dutton, 1902

T. b. evansi Steel, 1885

T. b. equiperdum Doflein, 1901

Inability to infect man and resistance to human serum *in vitro* under certain conditions (*16*) distinguish *T. b. brucei* from both *T. b. gambiense* and *T. b. rhodesiense*. Differences in the clinical course of infection in man generally separate *T. b. gambiense* from *T. b. rhodesiense* (*8*, pp. 520–525). Differences in antigens (*17*), isoenzymes (*18*), and DNA constitution (the possession of an additional heavy satellite component revealed in centrifugation in cesium chloride) (*19*) distinguish *T. b. gambiense* from both *T. b. rhodesiense* and *T. b. brucei*. All three should therefore be retained as valid subspecies.

The inclusion of *T. b. evansi* and *T. b. equiperdum* along with other subspecies in the species *T. brucei* was suggested by Hoare (*8*, p. 484). They are separated from the other subspecies and from each other by differences in behaviour (absence of cyclical development in both, and by venereal transmission in *T. b. equiperdum*).

Morphological differences between *T. b. evansi* and *T. b. equiperdum*, e.g., reduction of the proportion of stumpy trypomastigotes, sometimes to zero, are too inconsistent as criteria for separation at species level. Dyskinetoplasty, which occurs in proportions ranging from zero to 100% in all the subspecies of *T. brucei* under certain conditions, does not provide adequate grounds even for subspecific differentiation. Hence, following Hoare (*8*, pp. 483–484), *T. equinum* should be regarded as a synonym for *T. b. evansi*. *T. b. elephantis* Bruce et al., 1909 is too inadequately characterized at present to merit subspecific status.

Recommendations

When insufficient evidence is available to determine to which subspecies a given material should be assigned, it is correct to use the designation *T. brucei* ssp. indet. The adoption of this convention routinely to indicate absence of information regarding infectivity and pathogenicity to man is strongly urged, so as to draw attention to the need for safety precautions when handling such uncharacterized material.

Characterization of the factor or factors governing infectivity or non-infectivity to man of *T. brucei* subspecies should be urgently pursued.

In *Pycnomonas*, the single member of the subgenus, *T. suis* Ochmann, 1905, is provisionally retained; further investigation to establish its status is recommended.

MAINTENANCE OF REFERENCE COLLECTIONS

LIVING ORGANISMS

The maintenance of salivarian trypanosome stocks in temperate countries is limited to those organisms adapted to laboratory conditions and, for most requirements, infective to laboratory animals. It therefore continues to be necessary to refer to tropical sources and collections for those organisms more fastidious in their requirements of host species.

Serial passage

Serial passage *in vitro* and *in vivo* has severe limitations for the maintenance of more than a few trypanosome stocks and strains, both logistically and because of the changes in the biological character of the populations following artificial selection under the special conditions of the passage. Serial passage is invalid for the maintenance of clones since homogeneity would not be conserved.

Stabilation

For the above reasons, reference collections are necessarily of stabilates—cryopreserved suspensions of trypanosomes in appropriate media. Most Protozoa have not been successfully lyophilized, desiccated, or preserved in any other way so as to remain viable. Efficient methods of cryopreservation using heat-sealed glass capillary tubes have been widely used, but the tubes may shatter if imperfectly sealed and stored in liquid nitrogen. Plastic capillary mate-

rials have advantages in this respect. Vapour-phase storage in liquid nitrogen containers offers considerable improvement in safety.

Recommendations

Lyophilization. Cryopreservation is at present the only practical method, but it has the disadvantages of vulnerability to the interruption of refrigerant supplies or of power supply, when the collection would be lost. As far as possible, materials should be maintained in more than one centre as a precaution against loss. Recent experimentation has indicated that viable preservation of eukaryote cells by lyophilization may be possible (*20*); research should be carried out to investigate this possibility for trypanosomes.

Reference stabilates. Reference stabilates to represent new isolations should be made at the earliest possible passage level.

Working stabilates. Reference stabilates should be used as a seed for setting up working stocks and working stabilates in order to conserve the original reference stabilates. When working stabilates are exhausted they should be re-established from the original reference stabilate rather than by serial stabilate passage.

Materials difficult to cryopreserve. It is known that some trypanosome stocks, as well as some trypanosome species, survive cryopreservation much better than others. Techniques that have been designed and extensively proved for *T. brucei* subspecies may not always be found applicable in the cryopreservation of trypanosome materials that have received little previous attention.

Studies are required on tailoring the techniques of operation (rates of cooling, use of cryopreservatives, etc.) to suit difficult material in order to improve viability and retrieval. This experimentation should include comparisons of viability (e.g., by suitable quantitative infectivity tests).

Metacyclic trypanosomes. To date, collections are almost entirely of non-cyclically transmitted organisms. Special efforts should be made to expand holdings of metacyclic trypanosomes, both by field collection and by cyclical transmission of populations in the laboratory as soon as possible after primary isolation.

ANTIGENS

The organisms used as reference material for immunological tests should consist of clones available as stabilates. These organisms should be characterized and typed by direct agglutination, lysis, immunofluorescence, and/or immunoelectrophoresis, using reference antisera (see below) before preservation as well as before actual use.

Agglutination, lysis, and neutralization tests

These tests are carried out with living organisms from a stabilate or from the first usable populations grown from a stabilate, as described above, and maintained, if necessary, by rapid passage. In the latter case, the number of passages and days between stabilation and use should be recorded. For lysis and neutralization techniques see Van Meirvenne et al. (*2, 3*).

Immunofluorescent antibody test

Van Meirvenne et al. (*2, 3*) use organisms air-dried on slides at 37°C for 1 hour, fixed in acetone for 15 min, shaken-off, wrapped in soft absorbent tissue, and stored on silica gel in sealed plastic bags. Such preparations are stable for 2 days at room temperature and for many weeks at —20°C.

Immunoelectrophoretic analysis

Organisms should be studied at the first usable passage from a stabilate, although this may be less essential for culture forms (stabilation of culture forms is more difficult than for blood forms). For preparing water-soluble extracts, Le Ray (*21*) collected organisms which were then washed in buffer (pH 7.2–7.4) at 2°C, disrupted in 0.017 mol/litre NaCl at —25°C to —35°C by repeated shearing, and centrifuged. The supernatant was dialysed, freeze-dried, and stored in inert gas in sealed vials.

Separated pure antigens

Recent advances in biochemistry and immunochemistry offer a potential for separating putative antigens. When applied to trypanosomes, as elsewhere, strict descriptions of the criteria of immunological purity should be given.

ANTISERA

The optimal procedures to be followed for raising reference antisera against trypanosomes depend on the purpose in view, i.e., whether it be the characterization of whole organisms, in suspension or in films, or of the total antigenic makeup of the organisms, or of particular purified antigens. The antisera may be raised either by infection with living organisms or by inoculation of organism extracts. The organisms

against which the antibodies are raised should be clones. In practice, the organisms used will be grown from characterized stabilates; their antigenic identity with that of the stabilate should be checked again at the actual time of their use. The procedures in use have been published (*2, 21, 22*) and are summarized below.

Preparation by short-term infection

Infection of a rabbit is by the intravenous inoculation of antilog 6 (1 million) living organisms and bleeding on day 6 post inoculation. The method is most suitable for producing antisera that are VAT specific and usable in direct agglutination, lysis, neutralization, and immunofluorescence tests.

Preparation by infection and cure

In some circumstances, the multiplication of a VA homotype has to be stopped in order to avoid its being overgrown by more virulent VA heterotypes and consequent loss of VAT specificity of the antiserum. This can be achieved in mice, rats, and rabbits by chemotherapy on day 3 after inoculation and bleeding on day 8. Possible cross-reacting antibodies against common antigens can be removed by cross absorption with appropriate heterologous antigens before VAT-specific immunofluorescence tests are applied.

Preparation by hyperimmunization with adjuvant

Immunoelectrophoretic characterization of the antigenic structure of organisms requires that at least 20 different antigenic components are recognized. Hyperimmune sera for this purpose are obtained by long-term immunization of rabbits by weekly subscapular injection of 2 mg of water-soluble cell extracts mixed with Freund's complete adjuvant and by bleeding about every 14 days until immunoelectrophoresis shows the optimal number of precipitation lines. Such antisera are also usable in lysis, neutralization, immunofluorescence, and gel precipitation tests as well as for monitoring the precipitation of purified antigens.

Preparation of multiple-site inoculation with adjuvant

When only minute amounts of antigens are available they are given by multiple-site intradermal inoculation, with adjuvant, into a rabbit on a single occasion. The development of the antibodies is monitored by weekly bleeding for 4–6 weeks. The antisera are suitable for agglutination, lysis, neutralization, and gel precipitation tests (*22*).

Preservation and storage of antisera

Addition of metabolic inhibitors as preservatives to the antisera should be avoided for agglutination, lysis, and neutralization tests. Storage at 4°C and repeated freezing and thawing provoke antibody alteration, and some reduction in titre happens during storage at temperatures above —70°C. The antisera should be dispensed in small volumes and stored below —70°C or freeze-dried and stored in inert gas in sealed ampoules.

BIOCHEMICAL PRODUCTS

Isoenzymes

The requirements for the preservation and storage of trypanosome materials for isoenzyme studies are essentially the same as those for antigen preparations listed above. Efficient separation from host-cell components and/or the inclusion of host-cell preparations as controls is essential. Reference facilities for control of the conditions of separation and preparation of isoenzymes will be needed; a designated reference centre should be established as soon as possible.

DNA characterization

Many different techniques are used for the preparation of DNA. The fact that some techniques are not suitable for a quantitative recovery of kDNA or nuclear satellites should be considered. It is highly desirable that the trypanosomes used in all future studies should be prepared as far as possible in the same way as for immunological and isoenzyme studies. The level of discrimination at which DNA characterization is likely to be most useful requires experimental investigation before standardized preparative methods can be developed. Although DNA is a stable macromolecule, it should be extracted with precautions to ensure rapid nuclease inactivation and the prevention of denaturation by excessive shearing. The extracted DNA is probably best precipitated with isopropanol and stored in ethanol at —20°C or lower.

DOCUMENTATION OF REFERENCE COLLECTIONS

DESIGNATION OF MATERIALS

Designation of primary isolate

It is recommended that the description of any primary isolate should consist of a list of the following components:

1. Locality of the primary isolation: one non-hyphenated word of up to ten roman capitals.

2. The year of isolation: two arabic digits.

3. Laboratory code: not to exceed five roman capitals.

4. Number, which is the specific number of that isolate, in arabic digits.

In a written description, the four elements are separated from each other by an oblique stroke to allow for computer programming, e.g., SERENGETI/58/EATRO/1716.

Designation of derived materials

Any other designation used, e.g., of stock, line, strain, or stabilate, should be given in square brackets after the primary isolate designation, quoting a unique coding for the material designated, e.g. [Stabilate TREU 386].

Recommendation

It is recommended that materials should be designated in both ways, e.g., *Trypanosoma brucei* SERENGETI/58/EATRO/1716 [Stabilate TREU 386].

A primary isolate code and number should be given only by the laboratory making the isolation. This presents no problem for the future but for materials in current use it will be necessary, as a temporary measure until they are primary-isolate-coded, to omit any elements that are deficient and to define the material used by citation of a unique reference-derived material, which will usually be a stabilate, e.g,, *Trypanosoma brucei* LUGALA/59/-/- [Stabilate TREU 1811].

INFORMATION RETRIEVAL

Facilities needed for information retrieval and analysis

The need is seen for databases (and their appropriate maintenance and service facilities) to be established at two different levels:

1. A global catalogue of available material is required. This would allow individual researchers to enquire where particular types of material are available, and would also allow catalogues of the material available at particular centres to be produced. These databases would contain information about the reference material, primary isolates, stocks, lines, and strains existing at various laboratories, but would not contain extensive information about the experimental characteristics of the material.

2. Each laboratory will require a system for maintaining information about the nature, location, use, and properties of its own material. Such databases would be used not only for reference purposes but also for analysis of the collected information. This system should be designed in such a way as to permit its use in a standard form at all relevant laboratories, as it would be essential to be able to combine information from different laboratories for analytical purposes.

A team would be required to investigate in detail the requirements of such a system and to implement at least the laboratory databases for the host institution. The team would be headed by an information scientist, whose task would be to investigate the current use of databases in associated fields (e.g., the World Data Centre for Micro-organisms in Brisbane), to survey the available database software, to investigate in detail the requirements of the proposed system, and to design and supervise the implementation of an actual system. A technician, familiar with the work of the laboratory and prepared to learn about computing, would be required to extract the information from the existing system and prepare it for input to the database.

Computer-programming and data-preparation staff would also be required, though probably not full time. Three years should be sufficient to establish a first operational database, and the host laboratory should be aware that the team will make considerable demands on the time of its staff when designing and implementing the system.

Recommendations

The problem is to provide a documentation system sufficiently flexible to refer to the widely different kinds of material that will be included in the collection, such as uncharacterized stocks and sta-

bilates and characterized demes, strains, and stabilates, together with their pedigrees. A distinction must be made between purely operational data, such as the origin and passage history of the material, and characterization data, whether morphological, biochemical, or immunological. Systems require to be developed so that they can be assimilated into more comprehensive systems covering a wider range of micro-organisms.

It is recommended, therefore, that liaison be established with the World Data Centre for Microorganisms of the World Federation for Culture Collections at Brisbane, Australia so that the data capture systems used by reference trypanosome collections should be as compatible as possible with those used by the World Data Centre. Funds should be made available for the establishment of centres specifically for the maintenance and documentation of reference collections, preferably as extensions of existing centres with special expertise.

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RÉSUMÉ

PROPOSITIONS POUR LA NOMENCLATURE DES TRYPANOSOMES SALIVARIA ET POUR L'ENTRETIEN DE COLLECTIONS DE RÉFÉRENCE

Des progrès rapides sont actuellement enregistrés dans la caractérisation des trypanosomes Salivaria importants en médecine humaine et vétérinaire, qui a déjà donné lieu à de nombreux accords de nomenclature, ainsi que dans les procédures intéressant le fonctionnement des cryothèques et la documentation. Si la communication et l'échange de matériel entre laboratoires suscite néanmoins encore des difficultés, ceci est dû à l'emploi de méthodes non uniformes par les divers laboratoires. Il a donc été jugé utile de réunir les principaux chercheurs concernés, d'analyser leurs méthodes, de comparer celles-ci avec la pratique dans d'autres domaines comparables de la microbiologie et de proposer des systèmes provisoires pour l'avenir immédiat. La réunion a eu lieu à Londres en septembre 1976. L'article qui précède expose les conclusions et recommandations qui en sont issues. L'ensemble des discussions sera publié dans un autre contexte.

En dehors de ceux qui figurent dans la nomenclature linéenne, les termes utilisés pour décrire les trypanosomes se répartissent en deux catégories: ceux qui se réfèrent aux opérations en laboratoire sur le matériel objet d'étude, et ceux qui désignent des caractères déterminés et reconnaissables des organismes. Ainsi, les termes échantillon, isolat primitif, stock, lignée, stabilat et clône sont purement « opérationnels », alors que des termes tels que zymodème ou sérodème (formés à partir de la « dème » ou race physiologique), souche, type antigénique variable (VAT) visent à la caractérisation des organismes.

Les termes « Stercoraria » et « Salivaria » se sont révélés utiles, et leur emploi devrait être maintenu pour le moment, en dépit du fait qu'ils ne sont pas strictement conformes au Code international de Nomenclature zoologique. Leur statut tout comme celui des quatre sous-genres de Salivaria — *Duttonella*, *Nannomonas*, *Pycnomonas* et *Trypanozoon* — devra sans doute être revu. Au cours des discussions, les caractères présentés comme permettant de différencier les trypanosomes Salivaria au niveau de l'espèce et de la sous-espèce n'ont pas tous été jugés convaincants. Il a été suggéré de considérer pour le moment chacun des sous-genres de Salivaria comme étant unispécifique. On a toutefois constaté que des différences caractérisant l'évolution de l'infection clinique par diverses sous-espèces étaient en corrélation avec le diagramme isoenzymatique, le profil antigénique ou la constitution ADN.

L'entretien des stocks dans des laboratoires situés principalement en zone tempérée ne peut porter que sur des organismes capables d'infecter les animaux de laboratoire. Le passage mécanique pour la conservation des stocks est d'une utilité extrêmement limitée, non seulement sur le plan logistique, mais aussi en raison des modifications que subissent les caractéristiques biologiques des populations de trypanosomes lors du passage. Dans ces conditions, les collections de référence se composent essentiellement de stabilats — ou suspensions de trypanosomes conservées à basse température ((cryoconservation). On a défini différentes catégories de stabilats: stabilats de référence, stabilats d'expérience et stabilats métacycliques. Les méthodes appliquées à la préparation des antigènes et des antisérums nécessaires aux divers tests immunologiques et biochimiques ont été notées.

Il a été recommandé que tout matériel trypanosomique soit caractérisé par deux types d'indications se référant: *a*) à l'isolement primitif, avec mention du lieu d'origine, de l'année et du sigle du laboratoire, et *b*) au matériel dérivé de l'isolat qui est utilisé dans l'expérience (stock, lignée ou stabilat). On a pris note de la nécessité, aux fins opérationnelles et biologiques, d'une extraction rapide de l'information et de l'établissement d'un catalogue de tout le matériel trypanosomique Salivaria disponible.

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