Statements and Recommendations of the ICNB Subcommittee on the Taxonomy of *Leptospira*

22–28 July, 1966

Moscow, USSR

The following statements and recommendations have been approved by a majority of the ICNB Subcommittee on the Taxonomy of *Leptospira*, if not unanimously, by circular correspondence over a period of 2 years.

Definition of the genus *Leptospira*.

It was agreed that the definition of the genus *Leptospira* which was proposed at the Montreal meeting [see Int. Bull. Bacteriol. Nomencl. 13:162] should be emended.

The Subcommittee is aware that the terminology of the morphological features of spirochaetes in general, and of *Leptospira*, has not been standardized but needs to be standardized. Moreover, further data are required before the electronmicroscopic appearances—and therefore the details of the morphological features—can be agreed upon. Nevertheless, it is hoped that the definition which is now offered will be adequately descriptive and comprehensible.

Genus *Leptospira* Noguchi 1917.

Helicoidal organisms which may be a few micrometers to 40 μm or more in length, but usually 6 to 20 μm ; and about 0.1 μm in diameter. The coils measure about 0.2 to 0.3 μm in overall diameter and about 0.3 to 0.5 μm in wavelength. In liquid media both ends are usually hooked, but in some strains only one end is hooked and in other strains the ends are straight and show no hooks. In the living state the organism is not visible with ordinary illumination; it is observed clearly by dark-field and much less clearly by phase-contrast microscopy. Cells are not readily stained by aniline dyes, but they can be demonstrated by silver-impregnation techniques. The structure of *Leptospira* as revealed by electron microscopy consists of a helicoidal cellular corpus (body) wound round an axistyle with a common external sheath enveloping both these elements. The cellular corpus is approximately circular in transverse section. Nuclear material, cytoplasm, and a limiting cytoplasmic membrane can be differentiated. The axistyle is apparently a single structure, about 0.01 to 0.02 μm in diameter, which is inserted in the cellular corpus subterminally at each end; but there is purported evidence that the axistyle is comprised of two lesser components ("axial filaments") each of which is inserted by a

terminal disc or knob at opposite extremities of the cellular corpus with their free ends in the middle region of the organism where they may overlap. There is no free flagellum or undulating membrane. In liquid media the characteristic movements are rapid rotation around the longitudinal axis and translation without polar differentiation; in semisolid media, flexion, vermiform, and boring movements also occur. Can usually be grown in artificial media. Oxygen is required. This genus includes forms which are parasitic in man and various animals (some forms are pathogenic) as well as apparently free-living forms with no known animal maintenance host.

The Subcommittee recognizes the fact that different electron-microscopic techniques reveal different details (some of which are apparently contradictory) in the anatomical features of the protoplasmic cylinder, axial filament and enveloping sheath. It was therefore agreed (a) that the time has not yet been reached when standardization of techniques is desirable, and (b) that a subgroup be appointed to coordinate further electron-microscopic studies and to report the findings and conclusions.

Main infrageneric categories. It was agreed that there are not yet sufficient reliable data available for the circumscription of two species of *Leptospira*, such as had been recommended at the previous meeting in Montreal.

Some strains which have been isolated from man, domesticated animals, and from wild animals are reported to react in various in vitro, so-called differential, tests in the manner characteristic of biflexa strains. Yet some of these isolates appear to have been pathogenic in their hosts. Report of such strains have been received from various parts of the world.

Some of the so-called differentiating tests (for example, the copper sulfate resistance test) have given discrepant results in various laboratories. The reasons need to be defined by a collaborative investigation.

Further studies are required in connection with antigenic relationships and differences, nutritional requirements and other growth characteristics, tolerance of 8-azaguanine, differences in enzymic and other biological activities, and in DNA base composition.

After considerable discussion it was therefore agreed by a majority (seven out of eight; one abstention) to recommend that only one species be recognized until adequate data have been collected. (According to the Code a genus must have at least one species.)

It was also agreed that the emphasis previously given to parasitic (pathogenic) and saprophytic (free-living) divisions of *Leptospira* was not taxonomically justifiable (though permissible in classifications for special purposes). In this respect the Subcommittee concurs with the statement of G. C. Ainsworth (Symp. Soc. Gen. Microbiol. 12:249–269): “For microbial taxonomy in general a major current trend is the realization that rational and stable basic classifications result only by treating pathogens and non-pathogens, parasites and saprophytes, on an equal footing and assessing their overall similarities and dissimilarities.”

It was further agreed that, though there were inadequate data for circumscribing two species, two main groups in the genus were discernible. It was therefore agreed that these two groups, and any further major infrageneric groups which might become apparent in the future, be designated “complexes” (using the word in the sense of “groups”); but it should be clearly understood that such complexes have no official taxonomic or nomenclatural significance. In effect, the present situation would be that instead of recognizing two species (*L. interrogans* and *L. biflexa*) the epithets would be used to indicate two complexes (interrogans and biflexa), a temporary expedient pending the accumulation of adequate data of taxonomic value.

The Subcommittee also noted with interest the suggestion of Terskikh that a group of “sapronotic” leptospirae, intermediate between the pathogenic and saprophytic forms, be recognized. This proposal, which was presented only in summary, will be considered by the Subcommittee when a translation into English of the full text of Terskikh’s note is available.

The circumscription (definition) of the single species of *Leptospira* would therefore be identical with the circumscription of the genus with the addition of the following sentence: “The single species is comprised of serotypes which are distinguishable by their agglutinogenic structure and, in some instances, by various other characteristics.”

Nomenclature of the species. It was agreed unanimously that *Leptospira interrogans* be recommended for the species name. The following objections to this recommendation were

raised, but they were considered to be irrelevant for the reasons stated.

(1) Stimson did not isolate “? *Spirochaeta interrogans*.” Rule 9a (Code, 1958, p. 53) [Rule 9d (1), Note 1, of the 1966 version of the Code now applies] states that the nomenclatural type of a species may be a description, and the present edition (1966, Rule 14a[3]) states that names of species which cannot be grown in culture but whose description is based on morphology are validly published as long as the name is published as a binary combination.

(2) Is it certain that Stimson’s description pertained to microorganisms which are now known as leptospirae? Noguchi (Amer. J. Hyg. 1:127) and Sellards (Trans. R. Soc. Trop. Med. Hyg., 33:546), who examined Stimson’s original preparations, were convinced that the organisms described by Stimson belonged to the genus *Leptospira*. Later, Noguchi (1928) wrote “. . . the type species of the genus *Leptospira* automatically shifts back to Stimson’s *interrogans*, which antedated Inada and Ido’s *icterohaemorrhagiae*. . . .” (In The Newer Knowledge of Bacteriology and Immunology, E. O. Jordan and I. S. Falk (ed.), p. 454. University of Chicago Press, Chicago).

(3) The notation “? *Spirochaeta*” renders the name illegitimate. Seemingly, it does not. The Note to Rule 12c (Code, 1958, p. 65; 1966 English version, Int. J. Syst. Bacteriol. 16:470) states that the Rule does not apply to names or epithets published with a question mark or other indication of taxonomic doubt, yet published and accepted by the author. There is no suggestion that Stimson did not accept the name, and he did publish it [Publ. Hlth. Rep. (Wash.), 22:541; and also, verbatim, in Trans. R. Soc. Trop. Med. Hyg., 3:56–57].

Nomenclatural types. It was agreed that the recommended type of the genus *Leptospira* Noguchi 1917 [which, according to Rule 9a, Code 1958, p. 53; Int. J. Syst. Bacteriol. 16:467 is a species] should be *Leptospira interrogans* (Stimson 1907) Wenyon 1926. [The citation of authors is governed by Rules 15 and 16, Code 1958, p. 73–79.; Int. J. Syst. Bacteriol. 16:472–473.]

It was also agreed that the original description of *Spirochaeta interrogans* (Stimson 1907) be recommended as the nomenclatural type of the species *Leptospira interrogans* (Note 1 in Rule 9d amended 1963–Int. Bull. Bacteriol. Nomencl. 13:9, refers [Note 1 in Rule 9d (1), 1966 English edition of the Code, Int. J. Syst. Bacteriol., 16:468 now refers]) until a neotype strain has been designated.

It was agreed that a neotype strain would be

required for the species *Leptospira interrogans* and that strain RGA, which is maintained in the collection of the Leptospira Laboratory of the Instituut voor Tropische Hygiene at Amsterdam, be recommended. Strain RGA (originally isolated by Uhlenhuth and Fromme in 1915) was unanimously accepted as authentic and well documented. It was preferred to the Japanese strain "smp No. 1" (splenectomized mouse passage strain No. 1) originally isolated by Inada and his colleagues, because the authenticity of this strain is now doubtful (see Report for 1962-1966 and "Review of type strains." (Since the meetings were held, research into the history and the serological identity of the Japanese strain "Ictero No. 1" has raised further problems. As a result, the Subcommittee now withdraws this recommendation pending further discussion.)

Infrasubspecific categories. It was agreed unanimously that the category hitherto designated subserotype was needed for individual epidemiological purposes to detect chains of infection. However, it was also unanimously agreed that the designation subserotype should be abandoned and that the former subserotypes be regarded as serotypes, because it has been shown that they are distinguishable by their possession of at least one main agglutinogenic factor. It was therefore agreed that the definition of serotype (Int. Bull. Bacteriol. Nomencl., 13:163) be emended so that it reads as follows:

"*Serotype.* Two strains are considered to belong to different serotypes if, after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titer regularly remains in *at least* one of the two antisera in repeated tests. This definition is based on antisera prepared in rabbits."

The definition of serotype contains a reference to a technique (absorption), and it is a fact that different workers sometimes obtain different results when they examine different cultures labeled with the same name. It was therefore agreed that a subgroup be appointed to investigate the causes of such variations and the possibilities of standardizing the relevant procedures.

Meanwhile it was agreed that: (i) The media customarily used should continue to be used. (ii) The same end point should be used by all workers (that is, the 50% end point previously recommended). (iii) The other four items (conduct of agglutinin-absorption tests, arbitrary arithmetic criteria, agglutinability of strains, and pure-culture techniques) should also be investigated by the subgroup. (iv) Authors should indicate in detail the exact method used.

It was agreed that the agglutinogenic characteristics of strains of *Leptospira* maintained in conventional media are generally sufficiently stable for practical purposes under normal laboratory conditions. In exceptional instances, agglutinogenic variants may appear. The nature of those variations, whether they reflect a mixed culture, a transient growth phase, or mutation, is not known.

A stock culture (strain Djasiman) in one laboratory has been found to undergo a minor, but stable, agglutinogenic change when compared with cultures of the same strain which were maintained in two other laboratories.

There is no evidence that antigenic (agglutinogenic) changes occur *in vivo* under natural conditions, but the possibility will be investigated by long-term cross-infection studies.

It was agreed that freezing cultures at -79°C or lower probably offers the most effective means of preserving strains of *Leptospira*. A subgroup will be appointed to investigate materials (glassware or other materials for containers, freezing-protectants, media, etc.) and methods (rate of freezing, holding temperature, etc.) as well as the problem of whether deep-freezing and/or thawing result in any sort of selection.

Alternative serological criteria. Factor analysis has already produced useful results. The sharing of common main antigenic factors may enable serogroups to be defined and is useful for determining the most suitable serotypes for use in screening tests of unknown sera. Factor sera, prepared by absorbing antisera with various related serotypes, are useful for the definitive identification (typing) of numbers of strains which have been shown to be closely related in cross-agglutination tests. This method is an advance over the orthodox method in which isolates and their antisera must be compared in a series of cross-absorption tests with recognized serotypes and their antisera.

However, E. Kmety and his colleagues at Bratislava, Czechoslovakia, are unable to handle the remaining work unaided, and other laboratories should therefore assist in these studies. Mrs. Galton has agreed to cooperate.

Modifications of the current serological criteria will be included in the studies of the subgroup mentioned above.

As mentioned above, there was unanimous agreement that the category subserotype be abolished and be promoted to serotype by emending the current definition of serotype (*see above*).

Concerning the potential usefulness of other

(i.e., nonserological) criteria for the differentiation of strains, it was noted that:

(1) Biochemical characteristics require further study. This implies a wider application of the tests which are supposed to distinguish the interrogans and biflexa complexes (resistance to copper sulfate, hydrolysis of egg yolk, oxidase activity, hemolysis of mouse erythrocytes, and growth in the presence of 8-azaguanine). More strains should be tested, and all recognized serotypes should be included in the study.

The essential nature of each test reaction needs to be elucidated.

Further investigations of enzyme activity are needed.

(2) Some strains do not grow as readily as others in various media. The reasons are not clear, and further study of metabolic processes and of growth requirements should be encouraged.

(3) The doubts which have arisen from occasionally reported discrepancies in the results obtained at different laboratories have been mentioned. These discrepancies may have been due to differences in materials or methods, and further collaborative investigation may reveal that the importance of the apparent aberrations has been unduly exaggerated.

Nomenclature of infrasubspecific taxa. The paper by R. E. Buchanan on this subject (Int. Bull. Bacteriol. Nomencl., 8:97–109) was noted.

Turner drew attention to the "Draft Proposal for Emendation of Rule 8. Names of infrasubspecific subdivisions and infrasubspecific forms" which was published under Minute 33 (Int. Bull. Bacteriol. Nomencl. 13:5–8). He informed the Subcommittee that he had written to H. P. R. Seeliger enquiring whether the Draft had been approved and stating various objections which he believed would be shared by other members. Seeliger's reply indicated that no action had been taken. [A slightly changed version of the "Draft Proposal for Emendation of Rule 8" was approved by the IXth International Congress for Microbiology, Moscow 1966 (Int. J. Syst. Bacteriol. 16:465).]

The Subcommittee noted with disapproval those parts of the Draft Proposal which were indicated as "(From Bradley draft)." It was agreed that, while these sections might be applicable to zoological nomenclatural practice, they were directly opposed to the opinions and policy of the Subcommittee. Thus the Subcommittee favors the retention of serotype as the basic taxon within the genus *Leptospira* until

such time as further studies may reveal the advisability of up-grading such taxa. Moreover, the Subcommittee also favors safe-guarding the names of serotypes (and the rights of their authors) at the infrasubspecific level where so much essential differentiation of microorganisms is detected.

There was unanimous agreement that new serotypes should be reviewed annually and that a list of those recognized by the Subcommittee be submitted for publication in the International Journal of Systematic Bacteriology (which is the new name for the International Bulletin of Bacteriological Nomenclature and Taxonomy). The closing dates for such annual reviews should be 31 December.

Grouping (serogroups). There was unanimous agreement that the prefix "sero-" be reintroduced to indicate that the groups of *Leptospira* are differentiated by serological means. This decision accords with the advice given in the 1966 English edition of the Code [Recommendation 8a (7)–Int. J. Syst. Bacteriol. 16:466–467].

Because factor analyses have yet to be completed, it is still not possible to define (circumscribe) serogroups. The Subcommittee therefore reemphasizes that the term "serogroup" does not denote a taxon within the genus *Leptospira*. It is agreed that the following statements express the concept and purpose of serogroups at this time. At present the term serogroup is used to denote those serotypes which cross-react to high titers in agglutination tests performed with their antisera (which are prepared in rabbits). Although these serogroups lack taxonomic definition and validity, they do serve practical purposes: (i) they enable a strain to be referred to a narrow range of serotypes within the genus (and "complex"); and (ii) they provide a basis for the selection of strains to be used in the screening of sera for diagnostic and survey purposes and of sera for the preliminary screening process of typing (identifying) newly isolated strains. Future studies by a subgroup of the Subcommittee will, it is hoped, enable the analysis of agglutinogenic factors of serotypes of *Leptospira* to be extended. The results of such studies may enable the serogroups to be more accurately constituted; but this is likely to involve the subdivision of some of the currently recognized serogroups, and even then some serotypes may not fit neatly into one serogroup.

Review of type strains. The status of serotype *icterohaemorrhagiae* was discussed at length during informal meetings. The decisions

reached during the last formal session were as follows:

(1) The strain at present maintained by Professor Yamamoto and designated by him "smp No. 1" is not considered to be an authentic culture of the original Japanese strain No. 1. The recovery of this strain, smp No. 1, from a contaminated culture by passage through splenectomized white mice, as well as other accidents in its history and the paucity of agglutinin-absorption studies which have been carried out with the original strain, render the strain smp No. 1 unacceptable to the Subcommittee as being truly representative of the original strain No. 1 of Inada and Ido.

(2) Therefore it was agreed that strain RGA (Uhlenhuth and Fromme, 1915) should be recommended as the nomenclatural type (neotype) of serotype *icterohaemorrhagiae*. Strain RGA is considered to be authentic, and it is well documented.

The original specific epithet, *icterogenes*, applied by Uhlenhuth and Fromme in 1916 to the spirochaete isolated by them in 1915 and represented by strain RGA is considered to be illegitimate if the spirit of the Code is applied at the infrasubspecific level: the epithet was used to denote an organism which, at the time, was not differentiated from the organism to which the epithet *icterohaemorrhagiae* had already been applied. It was, in effect, a synonym of *icterohaemorrhagiae*.

(3) Strain M 20 should represent serotype *copenhageni*, a new name proposed by E. Kmety (Ann. Soc. Belge Med. Trop. 46:103-108) for those strains previously known as the complete, or AB, type as distinct from RGA and others which were known as the incomplete, or A, type. The differentiation of the two types was achieved by Borg-Petersen in 1938.

Priority claims. The Subcommittee agreed to recommend the acceptance of serotype epithet *tarassovi* in place of serotype epithet *hyos*

because it had been applied (Terskikh, 1951; Varfolomeeva, 1958) as a replacement for the epithet DV-A which was used in the naming of a new species, *Leptospira DV-A*, by Kiktenko and Ananyin (1941). Strain Perepelicin, which Varfolomeeva suggests should be the reference strain of serotype *tarassovi* (syn. *DV-A*), was shown to be serologically identical with strain Mitis Johnson, and this has been identified as serologically identical with strain 300 (serotype *hyos*). (This subject, which presents many problems, is under further discussion.)

The claim that serotype *sorex* (Varfolomeeva and Nikiforova, 1948) is a heterologous serotype was not upheld. The Subcommittee considers that the strain representing the proposed serotype is serologically identical with strain Poi (serotype *poi*) named by Mino (1941); the epithet *poi* therefore has priority and should be conserved.

The claim of Ananyin that the serotype epithet *erinacei-europaei* (strain Yozh 1) has priority over the epithet *bratislava* (strain Jež Bratislava), which was proposed by Kmety, poses special problems. It was agreed that this matter should be carefully investigated and be discussed by correspondence.

Concerning the agenda item "Review of status of recognized and provisionally recognized serotypes," the eight members (out of nine) who were present noted that, while serving as members of the WHO Scientific Group at the meetings which preceded those of the Subcommittee, they had already agreed on a revision of the list which will be published. [Wld. Hlth. Org. Techn. Rep. Ser., 1967, 380. Note: there were some errors in the Appendixes, which deal with names of serotypes and their reference strains. WHO has issued a printed Corrigenda sheet.]

Annual reviews and notification were mentioned above.

L. H. Turner,
Secretary