Memoranda

Research needs in leptospirosis*

Leptospirosis is a widespread infection of man and animals, and locally it assumes considerable importance as a public health and economic problem. It is an important occupational infection among persons working with animals or in environments that may be contaminated by infected animals. In recent years, considerable attention has been devoted to this infection but efforts to control and eliminate it, especially from natural foci, are hindered by gaps in our knowledge. This memorandum is a review of recent progress and current problems in leptospirosis research, with special reference to taxonomy, epidemiological methods, and control measures. Certain aspects of the basic biology of leptospires that are relevant to these topics are also discussed.

BASIC BIOLOGY OF LEPTOSPIRES

Morphology [1]

Leptospires are Gram-negative, helicoidal microorganisms consisting of a cytoplasmic body with an " axistyle " inserted subterminally at each end, and a sheath or envelope that encloses both structures. The axistyle consists of two axial filaments which are inserted by terminal disks or knobs at the ends of the cytoplasmic body and whose free ends lie in the middle region of the organism. The axial filaments thus lie between the sheath and the cell wall.

By means of various physical and chemical treatments, highly purified preparations of axial filament have been obtained. Chang & Faine (1970) and Naumann et al. (1969) have pointed out the similarities between axial filaments and bacterial flagella in their general morphology, size, and physicochemical characteristics. These findings support the notion that the axistyle of leptospires is concerned with the motility of the organism and that the axial filaments are uniquely located counterparts of flagella.

Chang & Faine (1970) reported that axial filaments contain antigens that can be identified but whose serological patterns do not correlate completely with the classification of leptospires by standard agglutination and cross-agglutination reactions.

There is no general agreement about the structure of leptospires or the number of layers of membrane and body wall. The following separate concentric structures have been reported from the surface inwards: (1) an envelope or enveloping sheath; (2) a " perimural layer " in which the axistyle is embedded and from which the fibrils mentioned below appear to arise; (3) a cell wall that seems to be closely associated with the cytoplasmic membrane.

The nature and function of the enveloping sheath has not yet been resolved. It has been described in most reports as a triple-layered structure, but 5 layers have also been reported (Anderson & Johnson, 1968). The enveloping sheath is reported to be easily damaged or removed by treating leptospires with water or saline, ethanol, formol, thiomersal, phenol, or 2-oxetanone (β-propiolactone). The treatment of some strains with antibody and complement removes the sheath (Anderson & Johnson, 1968). Yanagawa & Faine (1966) reported that cells whose sheaths were partially removed by treatment with ethanol

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[1] Two important points should be emphasized. First, some investigators have been unable to demonstrate certain fine morphological features that have been described. Second, the terms used to describe some fine morphological features are confusing: some terms seem to be used to denote different structures while different terms may be used to denote the same structure. Thus, a critical appraisal of the literature and standardization of the nomenclature of these fine morphological features is needed. Such a review should should not be restricted to the genus *Leptospira* but should include other genera of the order Spirochaetales as well as " spiral microorganisms " that cannot yet be assigned to any recognized genus. The terms used in this memorandum are those conventionally used by various authors, and their use does not imply that they are recommended as a definitive nomenclature.

showed decreased agglutinability. Further studies on the sheath are required.

The perimural fibrillar layer described by Yanagawa & Faine (1966) was also observed by Yanagihara & Mifuchi (1968). An electron-dense layer between the enveloping sheath and the cell wall was observed by Nauman et al. (1969).

Several workers have shown that the cell wall of leptospires is structurally and chemically analogous to that of other Gram-negative microorganisms (Anderson & Johnson, 1968 ; Pillot & Ryter, 1965 ; Yanagawa & Faine (1966). The cell wall apparently has poor agglutinogenic characteristics (Yanagawa & Faine, 1966).

Previously described intracytoplasmic components—e.g., nuclear regions and ribosomal and lamellar structures—have been demonstrated again in recent studies. Katz et al. (1968) have stated that some of the electron-dense cellular inclusions appear to be polysaccharides. Reproduction by transverse fission has been confirmed but the nature of cyst-like and organelle structures at the ends of the cells (Ritchie & Ellinghausen, 1965 ; Katz et al., 1968) is still obscure.

The prospects for defining the roles of cellular structures in antigenicity and virulence seem to be promising. The application of new staining methods, thin sectioning, and freeze-etching techniques in electron-microscope studies should elucidate the ultrastructure of some doubtful components. Suitable electron-microscopic histochemical techniques to clarify the chemical nature of structural components are required.

Immunochemical and antigenic characteristics

The immunochemical characteristics of antigenic components and their relationships to serological reactions and virulence are still puzzling. Preliminary findings (Chang & Faine, 1970 ; Chang et al., 1970) have shown the need for further studies of the antigenicity of some of the structural components. The occurrence of " serotype-specific " (e.g., agglutinogens) as well as more broadly reacting antigens (e.g., erythrocyte-sensitizing substances and cross-reacting antigens of some strains of the *biflexa* complex) in leptospiral cells is well known.

The agglutinogens in particular have been intensively studied in conventional agglutination and agglutinin-absorption procedures, which provide the basis for the current classification of leptospires. The chemical nature of these antigens is still unknown. Notable advances were made by Kmety (1967) in the serological analysis of agglutinogenic factors. The occurrence of a thermolabile agglutinogen that is rapidly destroyed at 56°C was reported recently by Borg-Petersen (1971); the possibility that this antigen may be subject to phase variation has not yet been investigated.

Generally, the biological stability of agglutinogens has not been found to vary significantly in cultures maintained under laboratory conditions. Apparent discontinuous variations in stock cultures have been observed infrequently and have usually entailed minor differences between parental and derived lines of strains. However, it has been demonstrated repeatedly that stable serological variants with major agglutinogenic differences may be obtained by growing strains in the presence of homologous and related heterologous antisera. Therefore, serological mutants seem to occur in leptospiral cultures and may be selected by culturing in the presence of antisera. Further studies of the biological and chemical properties of parental and derived strains would be valuable.

Variations in the agglutinability of cultures are known to occur. Stalheim (1966) demonstrated that optimum fatty acid requirements must be supplied for the strain to develop adequate agglutinability. When a virulent strain was grown in a synthetic medium containing limiting amounts of Tween 80, the cells were poorly agglutinable and agglutinogenic, and they incompletely absorbed homologous agglutinins. Full agglutinability and agglutinogenicity was restored after passages in a complete medium, but the virulence of the strain was lost. In current taxonomic studies Stalheim's (1966) report has led to greater attention being given to cultural conditions in the preparation of antigens for absorption and agglutination purposes, and in the preparation of rabbit immune sera. A more important application is that it serves to indicate new lines of study on the nature of agglutinating antigens.

The role of agglutinogens and other cellular factors in immunity should now be reexamined. For many years, on the basis of empirical evidence, agglutinogens were thought to be related to immunogens. It has been demonstrated repeatedly that infection with a particular serotype strain confers protection against reinfection with other strains of the same serotype but not against strains of serotypes unrelated on the basis of agglutino-

genic characteristics assessed by means of rabbit immune sera.

Cross-protection against infection in man and some animals has been observed between serologically heterologous, but related, serotypes (Babudieri, 1959). Until recently, the degree of cross-protection (i.e., in allaying symptoms) has not been seriously studied. Kemenes (1964) and more recently Pleško (1968) and Pleško & Lataste-Dorolle (1970a, 1970b) have demonstrated experimental cross-protection with some serotypes, more a less antigenically unrelated (*icterohaemorrhagiae, canicola, pomona, grippotyphosa, australis, bataviae*), but not with other unrelated serotypes (*sejroe* and *tarassovi*). It is interesting to note that differences in cross-protection after infection among serologically heterologous serotypes are consistent with their classification according to genetic and biological groups.

A knowledge of the immunoglobulin classes of antibody in the immune response would be valuable in understanding the nature of antigens, antigen–antibody reactions, immunity, and persistence of infections. From a practical point of view, the immunoglobulin components in man and various animals infected with different serotypes, and the specific immunoglobulin reactivity in various serological procedures, should be known. The types of immunoglobulin elicited in hyperimmunized rabbits by different serotypes when different immunizing regimens are used, and the homologous and cross-reactivity of the immunological components and their bearing on agglutinin-absorption techniques, should be determined. This information is required for a rational standardization of procedures for typing strains. Any interpretation and evaluation of studies of experimental immunization and infection should also take into account known and potential differences of immunoglobulins and immune reactions to infection between different strains of experimental animal. Some studies of the immunoglobulin classes of antibody response have been made but further research is needed.

Studies of the lysis of leptospires by factors in normal serum have failed to elucidate their significance, particularly in relation to virulence and the site of action of specific and non-specific serum factors. Again, further research is required. In view of the findings of Hoeden (1966) the comparative immunology of antileptospiral substances in non-mammalian animals should be studied. Reports from different laboratories on the lytic effects of antibody and complement on leptospires are not necessarily contradictory since the experimental conditions were not comparable. The lysis by antibody in the presence of complement demonstrated by Johnson & Harris (1968) occurred under conditions not used in current routine microscopic agglutination tests.

Metabolism and other chemical activities

The importance of lipids as a source of energy for leptospires has been well established. A knowledge of lipid utilization made possible the development of albumin–Tween-80 media for the cultivation of both pathogenic and saprophytic leptospires (Ellinghausen & McCullough, 1965), and a chemically defined medium (Shenberg, 1967) that supports the growth of some strains. There has been some progress in defining the fatty acid requirements for growth (Johnson et al., 1969, 1970) but further work is required. Differences have been reported between strains in their sensitivity to free fatty acids in culture media. Such findings may be important for an understanding of leptospire metabolism and in attempts to cultivate leptospires, particularly in synthetic media. For the routine isolation and cultivation of leptospires it may be necessary to use a series of synthetic culture media, each containing different amounts of various fatty acids.

There is no confirmed information about the role of any metabolites other than fatty acids in the energy cycle of leptospires. Evidence now exists (Green et al., 1967; Baseman & Cox, 1969a) that leptospires have citric acid, glycolytic, and pentose pathways. Various enzymes have been studied and attempts made to correlate their presence with taxonomic or pathogenic properties, but the results have been inconclusive. The possible association between cytochrome-*a* content and pathogenicity (Baseman & Cox, 1969b) merits further study.

The high lipid level of leptospiral cells is well established. Some of the component fatty acids of the cell have been determined (Johnson et al., 1970; Livermore et al., 1969; Stern et al., 1969). An unusual positional isomer of hexadecenoic acid was found to be a major lipid component of a *ballum* strain (Livermore et al., 1969), and the same unusual isomer was found to be a major lipid component of a *canicola* strain when this

strain was grown on oleate (Stern et al., 1969). The fatty acid composition of leptospires may be useful in taxonomy.

The lipolytic activity of various pathogenic and saprophytic strains of leptospire has been extensively studied by many investigators, whose findings have been remarkably consistent. The saprophytic strains invariably show lipase activity whereas pathogenic leptospires comprise both lipase-negative and lipase-positive strains. Interesting and significant correlations have been discerned between lipase activity and other attributes; Kemenes (1964) drew attention to a possible relationship between lipolytic activity and cross-immunity, and antigenic differences in lipases have been reported by Bakoss & Chorvath (1965). The lipolytic activity of all parasitic strains tested was completely or partially neutralized by antiserum against the whole culture of a parasitic strain, but the enzymes of parasitic strains were antigenically distinct from those of saprophytic strains; there was no cross-neutralization. These preliminary studies further suggested that the lipases of saprophytic strains include at least two antigenically different types. It would be interesting to determine whether the serological specificity of lipase is correlated with observed genetic differences between saprophytic strains.

From a taxonomic point of view, the lipase activity of pathogenic strains was well correlated with the initial sensitivity of the strains to growth-inhibiting effects of 2,6-diaminopurine (DAP). On the basis of trioleinase activity and sensitivity to the purine analogues DAP and 8-azaguanine, Johnson & Harris (1968) differentiated 3 groups of leptospire. Groups 1 and 2 comprised strains of the parasitic complex while group 3 contained strains of the saprophytic complex. Strains in group 1 produced trioleinase and were sensitive to DAP (initially at least) and 8-azaguanine (e.g., strains of serotypes *icterohaemorrhagiae, canicola,* and *pomona*). Group 2 comprised strains that were trioleinase-negative, resistant to DAP, and sensitive to 8-azaguanine (e.g., strains of serotypes *javanica, ballum,* and *tarassovi*). The strains in group 3 were trioleinase-positive, and resistant to both DAP and 8-azaguanine. One strain (Kabura) of the parasitic complex was trioleinase-negative and sensitive to both DAP and 8-azaguanine; this strain may represent a fourth group. The three groups of Johnson & Harris (*op. cit.*) could be associated with the four genetic groups disclosed by Haapala et al. (1969). It would be valuable to determine the difference in metabolic pathway reflected by these observations.

Investigations by Kasarov & Addamiano (1969) of the lipolytic activity of leptospires on rabbit serum lipoproteins have provided additional evidence of basic differences between parasitic and saprophytic leptospires. The triglycerides of serum lipoproteins were hydrolysed completely or almost completely by all 6 saprophytic strains that were investigated, whereas 15 out of 16 parasitic strains affected the serum triglycerides only slightly or not at all. The single exceptional prasitic strain was lipase-positive when tested on animal depot fat. When serum phospholipids were used as substrates, differences in the lipolytic activity could be demonstrated not only between the saprophytic and parasitic strains but also within the parasitic group. Superficially, the two groups (groups B and C) of parasitic strains did not appear to correspond with the groups of parasitic strains described by Johnson & Harris (1967) or Haapala et al. (1969). The saprophytic strains (group A) acted on lecithin but not on sphingomyelin, parasitic strains of group B acted on both lecithin and sphingomyelin, and parasitic strains of group C did not affect either of those phospholipids. Kasarov (1970) found no basic difference between the degradation of phospholipids bound in serum lipoproteins and that of phospholipids bound in the membranes of erythrocytes. Because of the known qualitative and quantitative differences in phospholipids of the membranes of erythrocytes in various species of mammal, these observations again raise questions about a possible relationship between leptospiral phospholipases and pathogenicity.

Genetics

Before 1969 there was little published information on the genetics of leptospires. The apparent selection of mutants by growing various strains in homologous sera has already been mentioned. Faine & Hoeden (1964) reported the separation of an avirulent mutant of *icterohaemorrhagiae*. The change in virulence was linked with colonial and cellular morphology. Successful *in vivo* transformation of virulence and antigenic characteristics, or both, of homologous or closely related serotypes has been reported (Bazovska & Kmety, 1968; Riel, 1964).

Using DNA base composition and DNA duplex formation, Haapala et al. (1969) described four

distinct genetic groups of leptospires among selected parasitic and saprophytic strains of various serotypes. Pathogenic leptospires could be divided into two groups on the basis of the percentage of guanine plus cytosine (G+C) in their DNA; one group had 36% and the other had 39%. The saprophytic strains had 39% G+C but were further separated into two groups on the basis of DNA annealing tests. Strains within groups had a high degree of specific duplex formation (75% or more binding). There was little or no genetic relatedness between strains of the four groups (less than 15% homology). The strains within groups were not necessarily related on the basis of their agglutinogenic characteristics; however, strains of the same serotype or very closely related serotypes were in the same genetic group.

It was pointed out above that the two genetic groups of pathogenic strains could be distinguished from each other and from the two genetic groups of saprophytic strains by a number of phenotypic characters. More strains should be examined by genetic grouping methods. The existence of other genetically heterologous and intermediate groups of leptospires can be predicted with reasonable confidence.

It is essential to use pure clones for genetic studies and cloning techniques should therefore be improved.

At present, on the basis of what is known about phylogenetic relatedness and DNA characteristics in other bacteria, there seems to be some justification for taxonomists to consider assigning the rank of species to the genetic groups of leptospires.

It is now opportune to think about the classification of leptospires in terms of both genotypic and phenotypic characteristics. Genetic techniques are not yet sufficiently developed to be used routinely in taxonomy, and the differentiation of organisms is still contingent on easily discernible phenotype characters and regulatory mechanisms. However, the demonstration of diverse genetic groups of leptospires and the association of several phenotypic characters with some of the groups is an encouraging beginning for the development of a natural and working taxonomic scheme. The phenotypic attributes that are now available or show promise for differentiating groups of leptospires include antigens; growth in the presence of selected concentrations of 2,6-diaminopurine, 8-azaguanine, copper sulfate, and sodium hydrogen carbonate; growth at 13°C; utilization of various fatty acids for growth; lipase (e.g., trioleinase) activity and its serological specificity; relative oxidase activity; production of cytopathogenic effects in tissue culture; lecithinase and sphingomyelinase activity; and sensitivity to serum or serum factors. The possibility of using antibiotic sensitivity patterns for taxonomic purposes should be explored. Some of the phenotypic differences are quantitative but that does not necessarily preclude their usefulness.

The information on markers and DNA base ratios is potentially applicable to studies of genetic changes occurring under natural conditions. Further work is required on the mechanisms of genetic change that could operate under natural circumstances. The discovery of leptospiral phages [1] or other transfection mechanisms could help to elucidate these problems.

TAXONOMY

Species

The *Index Bergeyana* (Buchanan et al., 1966) lists 40 species and 5 varieties of *Leptospira* as validly published and legitimate, and 53 serotypes without species designation. Most of these named taxa were distinguished on serological grounds and would now be regarded as serotypes, i.e., as infrasubspecific forms. More serotypes have since been reported (see WHO Expert Group on Current Problems in Leptospirosis Research, 1967).

The signatories of this memorandum endorse the recommandation (WHO Expert Group on Current Problems in Leptospirosis Research 1967) that the genus *Leptospira* should be considered as monospecific until it is possible to circumscribe species confidently. Meanwhile, the specific epithet " interrogans " may continue to be used because it is the earliest and, more important, it cannot be confused with epithets applied to other groupings of *Leptospira* such as serogroup and serotype, which are still those of practical importance to clinicians and epidemiologists.

Recent studies have revealed that the genus *Leptospira* comprises several biological groups distinguishable by phenotypic or genetic characteristics. Neither the genetic nor the other biological groups fit neatly into the current scheme of classification based on agglutinogens.

[1] The association of a bacteriophage-like entity with *Leptospira* was reported by Ritchie & Ellinghausen (1969).

It is suggested that the Taxonomic Subcommittee on Leptospira should consider the proposal made by Haapala et al. (1969) that the genetic groups be assigned the taxonomic rank of species; those authors did not propose names. The Subcommittee could also consider the problems of nomenclature that might be involved.

Serotype

The serotype remains the basic taxon. Kmety (1970) proposed 5 conditions that should be met in the typing of strains. If these conditions were adopted the number of discrepancies in the findings of different laboratories might be reduced. It is suggested that the Taxonomic Subcommittee might consider whether these conditions should be incorporated in the definition of serotype. The Subcommittee might also consider whether the occurrence of a thermolabile agglutinogen in some strains calls for revision of the definition of "serotype".

Serogroup

The principle is retained of grouping serotypes on the basis of close agglutinogenic relationships, as disclosed by cross-agglutination reactions at high titres with antisera prepared in rabbits. However, there have been proposals that the large Hebdomadis serogroup should be subdivided into small serogroups because the strains in the current list do not cross-agglutinate with all the other antisera at high titres. It has also been proposed that the Autumnalis serogroup should be subdivided. The Taxonomic Subcommittee could usefully consider these proposals.

DIAGNOSTIC PROCEDURES

Microscopic examinations

Immunofluorescence techniques have been used by various workers for the detection of leptospires in samples of tissues and body fluids, particularly from animals. None of the described techniques has been adequately evaluated; consequently, no particular procedure can be recommended for routine use at present.

A suitable immune serum for the immunofluorescence test should permit the recognition of a leptospire irrespective of the serotype of the infecting strain. Research should continue on the potential usefulness of one antiserum, or of a combination of antisera, against broadly reactive strains of certain serotypes, e.g., strain Patoc 1 (*patoc*), Andamana CH 11 (*andamana*), Jez Bratislava (*bratislava*), Poi (*poi*).

It is emphasized that immunofluorescence methods for the detection of leptospires have limitations that, although less acute, are similar to those affecting other microscopic methods of detection in requiring a suitable concentration of the organism. All diagnoses based on immunofluorescence methods should be confirmed whenever possible by cultural and serological methods.

Isolation by direct culture

Media containing 5-fluorouracil have been successfully used by various workers to inhibit contaminating bacteria in cultures and in the primary isolation of leptospires from specimens that are not obtained aseptically. In some laboratories, however, 5-fluorouracil media completely inhibited leptospiral growth. Difficulties connected with the solubility of 5-fluorouracil may be avoided by using commercially produced sterile solutions that can be further diluted aseptically and stored at 4°C. A procedure for preparing solutions of 5-fluorouracil has been described by Turner (1970).

Solid media containing 1% agar and dispensed in plates (Petri dishes) have been useful for the purification of contaminated cultures, for the direct isolation of saprophytic leptospires from water, and in some instances for isolating pathogenic strains from samples of blood, urine, and various tissues. Not all pathogenic strains can be easily cultivated on these solid media. For direct cultivation of pathogenic leptospires from aseptically derived specimens, conventional (i.e., serum-containing) semisolid and liquid media are recommended.

Microscopic agglutination test

The microscopic agglutination test continues to be the standard reference procedure for the serological diagnosis of leptospirosis in man and animals and for the serological classification of leptospires. When used with a judicious selection of serotypes for the antigen suspensions, the microscopic agglutination test has excellent sensitivity and specificity, not only for detecting current infections in individual patients but also for epidemiological surveys.

The variable factors known to affect the sensitivity and specificity of microscopic agglutination reactions were described by the WHO Expert

Group on Current Problems in Leptospirosis Research (1967). Attention has already been drawn to observations that lipids and possibly other nutrients in culture media may significantly affect the quality and quantity of agglutinogens elaborated by leptospires.

On conducting agglutination tests, it is important to use well grown cultures of morphologically typical leptospires, and it is desirable to check the agglutinability of test antigens by using an appropriate dilution of homologous rabbit antiserum as a control for each test. The selection of antigens suggested by the WHO Expert Group on Current Problems in Leptospirosis Research (1967) for use in the microscopic agglutination test has been a useful guide for many laboratories but it may not provide complete coverage of the serotypes found in some countries. A revised list based on further experience is therefore given in Annex 1.

Results of microscopic agglutination tests often provide clues to the identity of the infecting serotype. However, definitive identification of the infecting strain can be established only by recovering and typing the organisms. Good results have been reported from the typing of strains of certain serogroups by the use of factor sera, but a definite determination of the serotype status of a strain still requires the use of cross-absorption procedures.

Other serological procedures

Macroscopic slide agglutination tests conducted with pools of formolized antigens have been found useful for diagnosing current infections in man and animals. However, nonspecific clumping may occur in some prepared lots of antigen; this is liable to lead to "false positive" interpretations. Vigorous shaking of the antigen before use may eliminate nonspecific clumping. The inclusion of suitable positive and negative controls should be mandatory in the conduct of these tests.

Other proposed serological procedures, such as the haemolytic test or the complement-fixation test with the broadly reacting saprophytic strain Patoc 1 as antigen, have been used advantageously to detect antibodies in man, but have only limited application to sera from domestic animals. The differences in the immunoglobulin components of antibody provoked in animals and man may relate significantly to the sensitivity of serological procedures. Additional studies of the immunoglobulin response to infections in different species of animal by various leptospire serotypes should help to explain the difficulties met with in serological tests conducted with complement, and should provide new indications for the development and improvement of serological diagnostic reagents.

Laboratories competent in complement-fixation testing, but with limited capacity for carrying out microscopic agglutination tests, could easily apply their routine method for the serological diagnosis of leptospirosis in man by using the saprophytic strain Patoc 1 as antigen. If comparisons are to be made between the findings of different laboratories, a standard method must be adopted.

Reference reagents and standardization

The following additional criterion for the acceptance of new standard anti-*Leptospira* sera is suggested: the absorption of proposed anti-*Leptospira* sera with homologous antigens should remove detectable homologous and cross-reacting agglutinins.

PATHOGENESIS

Cytopathogenic effects on various types of cell and tissue culture have been reported. The significance of these observations lies mainly in the possible use of cultured tissue cells as indicator systems in studies of pathogenesis or virulence under conditions independent of host immune factors, for research on mechanisms and factors in the leptospires that are associated with virulence, and for research on the nature and cytopathology of changes in infected cells, which may be similar to pathological effects found in infection *in vivo*.

More attention should be paid to possible qualitative and quantitative differences in the pathology of infection in animals by different serotypes. In addition to factors in the leptospires, host factors should also be considered. For example, the following host factors may singly or together affect the pathogenesis and manifestations of the disease: the age and nutritional state, the species, and the genetic potential for expression of an immune response.

Several groups of workers have produced evidence for pathogenic activities of leptospiral cell-free extracts or fractions resembling those of the endotoxins of Gram-negative bacteria. Fur-

ther detailed studies of the chemical and serological nature and effects of purified preparations are necessary to determine their roles.

The possibility cannot be discounted that products of *in vivo* leptospiral metabolism, possibly lipids, may be toxic.

EPIDEMIOLOGY

Geographical distribution

Continued epidemiological studies have extended our knowledge of the occurrence of leptospirosis in different parts of the world, but epidemiological data are still lacking from many areas. Information on the occurrence of different serotypes all over the world should be systematically collected and kept up to date.[1]

Animal reservoirs

Except for the continued disclosure of new animal hosts, no new data have been recorded since the report of the WHO Expert Group on Current Problems in Leptospirosis Research (1967). The problems and questions posed in that report are still appropriate.

Epidemic cycles and surveys

Ecological investigations in endemic areas have provided interesting information about the dynamics of the epizootic activity of natural foci. Local conditions, such as flowing or stagnant water, humid or dry land, and frequency of contacts between wild carrier animals, domestic animals, and man, are of great significance in this process.

In many enzootic areas the chains of infection spread from the maintenance host to other species of animal living in the same biocenose—the so-called radiation phenomenon. The infectivity rate of the principal host and that of the whole animal population provides a good indication of the activity of a focus. The systematic collection of such data may form a basis for leptospirosis surveillance in selected areas and for efforts to prevent epidemic outbreaks. Because of dynamic changes in epidemiological conditions, only systematic surveillance can be expected to provide useful data on the factors involved in epidemiological cycles and on the need to introduce reasonable preventive measures.

Surveillance procedures should be developed at the national level as a basis for international activity in this field. Only surveys conducted under adequate statistical control can yield reliable information about leptospirosis in any area and during any period.[1]

[1] Information about the identification of new serotypes or new hosts in any country should be submitted to: Mrs Catherine Sulzer, Leptospirosis Unit, Center for Disease Control, Atlanta, Ga., USA, for inclusion in revisions of the leptospiral serotype distribution lists, which are arranged by countries and by hosts.

IMMUNIZATION AND THERAPY

Vaccines

Attempts have been made to inactivate leptospires either by gamma-irradiation or by adding streptomycin to well-developed cultures. Inactivated leptospires are still motile but are no longer infective to animals or able to multiply. Vaccines prepared from inactivated leptospires have been tested in laboratory animals and, on a very small scale, in cattle and pigs. The vaccines were found to be safe but their effectiveness remains doubtful. There is no evidence at present that these live vaccines are more effective than formolized vaccines in conferring protection against the development of the carrier state.

The use of live, avirulent vaccine obtained from leptospires cultivated in special media has been suggested (Stahlheim, 1968), but the effectiveness of such vaccine needs further study. It would also be useful to know if, and to what extent, the effectiveness of live vaccines depends on growth of the leptospires *in vivo*. Live vaccines have the disadvantage that they cannot be kept for any length of time and must be used soon after being prepared.

The identity of the protective antigen in vaccines should, if possible, be established, and the amount of antigen and the potency of vaccines should be measured by recognized standard methods so that vaccines prepared in different laboratories could be compared and evaluated.

Cross-immunity and multivalent vaccine

In areas where multiple leptospiral infections occur, efforts should be continued to develop multivalent vaccines containing as few serotypes as possible and having a broad antigenic spectrum.

[1] Statistical advice on the conduct of surveys is freely available from WHO.

Therapy

The use of peritoneal- or haemo-dialysis has proved to be useful in treating patients with renal failure. Continuing research on the therapy of leptospirosis is needed in order to profit from new developments in antibiotics.

TOPICS FOR RESEARCH

The following topics are suggested for further study:

(1) the ultrastructure of leptospires and the chemical nature of the various structural components;

(2) the immunological characteristics of antigenic components of leptospires;

(3) the development of culture media;

(4) the cross-protection afforded by natural infections and by vaccines;

(5) immunoglobulin classes of leptospiral antibodies in man and various animals;

(6) the lysis of leptospires by factors in normal and immune sera;

(7) metabolic processes, especially those of pathogenic strains;

(8) the lipolytic activities of strains;

(9) the genetics of leptospires;

(10) improvements in immunofluorescence techniques and materials;

(11) the development and evaluation of serological tests, including screening tests for use with sera from domestic livestock and other animals;

(12) the roles of leptospiral and host factors in the pathogenesis and manifestations of leptospiral infection;

(13) the epidemiology of leptospirosis;

(14) the development of surveillance programmes;

(15) the preparation and assay of vaccines; and

(16) the effectiveness of new antibiotics.

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Annex 1

ANTIGENS FOR USE IN THE MICROSCOPIC AGGLUTINATION TEST FOR PRELIMINARY SCREENING PURPOSES

Reference laboratories may recommend other serotypes than those listed below to represent certain serogroups, and they may advise the use of strains other than the reference strains of a particular serotype.

copenhageni	grippotyphosa
poi	wolffi
canicola	borincana
castellonis	szwajizak
pyrogenes	djatzi
autumnalis	tarassovi
bratislava	patoc
pomona	

Supplementary serotypes that may be used include the following:

shermani	djasiman
panama	cynopteri
celledoni	louisiana