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MEDICAL REVIEWS

Arenaviruses[1]

JORDI CASALS

Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510

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I. INTRODUCTION

Arenavirus is the proposed designation for a set of viruses which have a unique morphology (76). The virions are round, oval, or pleomorphic with diameters between 60 and 350 nm, an electron-dense membrane with spikes or projections, and a number of inclusion-like, dense particles that give the virion an aspect of having been sand sprinkled (*arenosus*). Arenaviruses have an RNA genome, are inactivated by lipid solvents, and share antigenic components. Since the first recognized member of this group was lymphocytic choriomeningitis (LCM) virus, it is considered the prototype.

II. HISTORICAL BACKGROUND

LCM virus has been known since 1934 and soon after it was etiologically associated with a disease syndrome of man, acute benign aseptic meningitis. Tacaribe virus, isolated from bats in Trinidad in 1956 and first described in 1963, has not been associated with human disease (23). The next virus to be isolated and characterized was Junin virus, in 1958; the virus was isolated from patients with Argentinian hemorrhagic fever (AHF). Five years later another virus, Machupo, was isolated from a fatal case of an illness, Bolivian hemorrhagic fever (BHF), clinically very similar to AHF. Other viruses, antigenically placed in the Tacaribe group, were discovered soon after: Amapari in Brazil, 1964 (71), Latino in Bolivia (93), Parana in Paraguay (92), Pichinde in Colombia (88), and Tamiami in USA (16), in 1965. None of these five is known to cause human illness. The last member of the group, Lassa virus, was recovered from patients suffering from a severe disease first seen in Nigeria.

A serological relationship among members of this set was first observed between Junin and Tacaribe viruses in 1963, resulting in the creation of the Tacaribe antigenic group (57); the other viruses, except LCM and Lassa, were easily shown to be related to Tacaribe and Junin and placed in the group. The similarity of morphology and morphogenesis between LCM and the Tacaribe group viruses was noted in 1969 (62) and soon after it was shown that there was an antigenic connection between

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them (77). Finally, it was observed in 1970 that Lassa virus was antigenically related to LCM and to some of the Tacaribe group agents (15) and that its morphology conformed to that described for LCM (86).

III. METHODOLOGY

A. Mortality

The case fatality rate of some members of this group is sufficiently high that deaths from the disease gives a good idea of the morbidity rate provided an accurate diagnosis can be made. For example, the mortality from Lassa fever ranges from 30–60%, Argentine hemorrhagic fever 3–15%, and Bolivian hemorrhagic fever 5–30%. However, deaths from lymphocytic choriomeningitis are rare. For the most part these diseases are rare, limited to localized outbreaks, and confined to a very few geographic areas. For those reasons, the official mortality records would be unlikely to reflect their occurrence in a country.

B. Morbidity

Epidemiological studies on arenavirus infections of man are hindered by the fact that disease reporting is uncertain and incomplete. The diagnosis of sporadic cases of LCM is, most likely, not made or is guesswork in nearly all instances, unless laboratory aid is sought. Diagnosis of AHF in the area and season where the disease is anticipated is confirmed by laboratory studies in about 70% of clinically diagnosed cases (54, 49); how many clinically undiagnosed infections go undetected is not known. It is doubtful whether an effective reporting system has been devised and implemented for these diseases, with the possible exception of AHF. Owing to the highly specialized type of diagnostic work required for identification of the viruses and antibodies resulting from infection, and due to the risk associated with certain aspects of the laboratory procedures, the knowledge of the prevalence of the infections and illnesses caused by the arenaviruses is limited.

C. Serological Surveys

Due to the difference in duration of different antibodies, seroepidemiological surveys are carried out mainly by means of the neutralization test (14, 34, 40) even though this is a more cumbersome test than complement fixation; it should be noted that not many extensive surveys have been carried out, except with LCM virus. The complement-fixation test is extremely useful as an aid to the diagnosis of a recent illness; the fact that it is less type specific than the neutralization test, to the point that it hardly differentiates between Junin and Machupo viruses infections of man (95), does not detract from its value due to the limited geographic distribution of these viruses.

D. Laboratory Diagnosis

While a clinical diagnosis of fully developed, typical cases of AHF and BHF can be made with considerable accuracy in the districts where the diseases are endemic at the time of year when they prevail, particularly if several similar cases appear simultaneously, it is most doubtful whether a sporadic case of LCM can be accurately diagnosed. A presumptive diagnosis of Lassa fever can be entertained in known endemic areas by experienced physicians faced with a severe or moderately severe case (59).

A specific diagnosis of these illnesses requires that either the virus be isolated and

identified from the patient's blood, excretions, or secretions, usually early during the disease; or that development of specific antibodies is shown to occur late in the disease or in convalescence.

The complement-fixation (CF) test has been most useful as a diagnostic aid with these illnesses; it is not, however, an early means of diagnosis since from 15 to 30 days from onset are required for it to become positive with most patients, perhaps a shorter time with LCM. The CF is not a specific test within the arenaviruses, certainly not between Machupo and Junin viruses (95, 47); but since these viruses occur in different areas a diagnostic error between the two is unlikely to occur. On the other hand, difficulties in the interpretation of CF results have arisen in an area where Junin and LCM viruses have infected man, simultaneously or sequentially (49, 50). In fact, in view of the known wide distribution of LCM virus, it is conceivable that diagnostic problems may arise when the CF test is used to diagnose Machupo or, if in Africa and if LCM virus is there, with Lassa viruses. Since LCM virus occurs in many European countries, introduction of an exotic arenavirus in that continent might result in diagnostic difficulties if the CF test alone were used.

The fluorescent antibody technique (FAT) has been used advantageously for an early diagnosis of LCM virus infection of man (7, 25). The neutralization test has been used less for diagnosis of current illnesses and serological surveys—LCM excepted—than for characterization of the viruses themselves; as will be seen in another section, it is sharply specific (93).

IV. THE VIRUSES

A. Biochemical and Physical Properties

The most extensive studies to determine biochemical and physical properties of the arenaviruses have been done with LCM (69) and Pichinde (74). The picture that emerges from these studies and from less complete investigations of the other viruses in the group is one of remarkable uniformity.

Through the use of various drugs that inhibit nucleic acid synthesis in infected cells in cultures, it has been indirectly shown that all arenaviruses—with the exception of Tacaribe, for which no studies appear to have been reported—contain RNA; compounds which preferentially inhibit DNA synthesis such as halodeoxyuridines (BUDR, FUDR), have no inhibitory effect on these viruses.

Studies with LCM and Pichinde viruses have directly shown the presence of single-stranded RNA in the virion. Analysis of the RNA by zonal centrifugation in 5–20% sucrose gradients and by electrophoresis in acrylamide gels showed, with each virus, the presence of five components of molecules with sedimentation values of 31S, 28S, 23S, (22S for Pichinde), 18S, and 4–6S. It has been concluded that, for both viruses, the 31S and 23 (or 22)S RNA's were virus specific; the 28S and 18S RNA's which account for 25–50% of the labeled RNA in the LCM virion, represented ribosomes from the host cell located inside the virion; and the 4–6S components were also from host cell origin (69, 74).

The base composition of the viral RNA was found to differ markedly from that of the host cells RNA. The RNA responsible for viral coded products would be about 3.2×10^6 daltons, with Pichinde virus (74).

Analysis of the viral proteins by electrophoresis in acrylamide gels has revealed, in Pichinde virus, the presence of four polypeptides coded for by the virus and incorporated in the virion; two of these are glycopolypeptides. A polypeptide and a

glycopolypeptide, both with molecular weights about 72,000 daltons, appear to be associated with the viral nucleic acid functioning as nucleoproteins; the other glycopolypeptide, molecular weight about 34,000 daltons, is an envelope constituent; and the second polypeptide, with a molecular weight of 12,000 daltons, has no assigned function (74).

Buoyant density in sucrose gradients has been reported as 1.17 or 1.18 g/ml for LCM, Pichinde, Machupo, Junin, Tacaribe, and Amapari and between 1.18 and 1.2 g/ml for Parana virus in cesium chloride. A noninfectious CF antigen produced by these viruses has a buoyant density between 1.09 and 1.11 g/ml (40).

All arenaviruses are easily inactivated by ethyl ether, chloroform, and sodium deoxycholate. Thermal inactivation of LCM virus is accelerated by the presence of divalent cations in the suspending medium. The effect of betapropiolactone on Lassa virus has been reported; the virus infectivity is completely inactivated with a concentration of the drug between 0.1 and 0.15% with preservation of complement-fixing activity (15).

B. Morphology and Morphogenesis

The similarities in morphology and morphogenesis are so marked and distinctive that they were the basis for first associating the viruses in the present taxon (62, 76).

Thin-section electron microscopy of Vero cells infected with all 10 arenaviruses show them to be indistinguishable from each other. Coinciding with the highest infectious titers of the inoculated cultures there is a large number of particles seen in the cultures. The particles are round, oval, or pleomorphic, 60–280 nm in diameter, have a membranous envelope with surface projections or spikes approximately 6 nm long, and contain a variable number from 2–10, internal electron-dense granules, about 20 nm in diameter, strongly resembling ribosomes. No symmetry has been discerned with any of these viruses (63, 64).

The particles mature by budding from plasma membranes. Vero cells infected with each of the viruses contain distinctive intracytoplasmic inclusion bodies, consisting of a smooth matrix in which are embedded dense granules similar to those seen in the virions and indistinguishable from the host cell ribosomes. These inclusion bodies seem to match in size and location the cytoplasmic inclusions observed under light microscopy in cells infected with the viruses (63, 64).

Negative-contrast electron microscopy of virus particles sedimented from infected cell cultures has been reported with these viruses except Junin, Machupo, and Lassa; technical or hazard considerations prevented their study. The results with the remaining arenaviruses have, again, shown decided uniformity of the viruses, with pleomorphic particles slightly larger than in thin sections, from 90–350 nm, pronounced surface projections, and no resolution of internal structure (64).

Electron microscopy studies of whole animals infected with arenaviruses (64) have revealed the presence of particles similar to those described above in a number of tissues of *Calomys callosus* infected as newborn with Machupo and Latino viruses. No such particles have been seen in hamsters infected with Junin virus and only few in the salivary gland tissue of mice infected with Tacaribe virus. In general, only occasional virus particles have been observed in the brain tissue of mice infected with LCM, Tacaribe, Lassa, or Tamiami viruses, while parallel studies indicate the presence of specific antigen.

The particles associated with arenavirus infection of cells in culture have been shown to contain specific antigen material by labeling procedures, at least with

LCM virus; whether all size particles are equally infectious cannot be decided by electron microscopy alone (53). Estimates of infectious size particles by centrifugation or filtration have given sizes between 37 and 60 nm for LCM virus (43); by filtration, between 70 and 140 nm for Lassa virus (15).

C. Antigenic Properties

Early studies with LCM virus demonstrated the existence of a CF antigen distinct and separable from the infective particle by centrifugation; it was designated soluble antigen. The nature and properties of this antigen has been the subject of recent studies (33) which confirm that virion and CF antigen are distinct entities. The latter on inoculation to experimental animals induces formation of antibodies which react *in vitro* with the CF antigen but will not neutralize the virion; furthermore, repeated inoculations of CF antigen fail to induce any protection against subsequent challenge of guinea pigs. It appears that the CF antigen is not present on the surface of the virion; it has not been determined whether it is a structural component of the virion.

The humoral immune response with arenaviruses has certain characteristics mainly observed with LCM virus, but which may also appear with other agents: CF and neutralizing antibodies and antibodies detected by the fluorescent antibody technique (FAT) seem to be independent (35). CF antibodies against LCM in man appear relatively early in the disease, from 8 days to 2 mo; neutralizing antibodies are found later, usually not before 2 mo after onset, and FAT antibodies may be detected earlier than CF antibodies (35). It is easy to prepare immune sera in mice with Pichinde or Lassa viruses, which react with good titers by CF test; the same sera may have little neutralizing capacity when tested in a mouse neutralization test (88) or in a plaque-reduction test (15, 59).

Antigenic relationship among arenaviruses has been mainly detected by the CF test. Table 1 is a composite table incorporating results from several sources and it is an attempt to illustrate the relative position of the viruses in the taxon. Tacaribe, Junin, Machupo, Amapari, Parana, and Latino viruses are very closely related by

TABLE 1
Complement-Fixation Test with Arenaviruses[a]

	Serum											
		JUN		TCR								
Antigen	MAC	Ser. 1	Ser. 2	Ser. 1	Ser. 2	AMA	LAT	PAR	PIC	TAM	LAS	LCM
Machupo	128	64		128			64	32		0		
Junin	64	256	16	256		64	64	32		0	4	8
Tacaribe	64	64		512		32	32	32		0	4	8
Amapari		64		32	64	128	32	32		8	4	4
Latino	32	32			8	8	256	16	8	0		
Parana	32		16		8	16	16	512	16	0		
Pichinde							4	64	256	4	0	
Tamiami	32	8		8		32	0	32	64	128	0	
Lassa		0		0		0			0		256	4
LCM		0		0		4			4		16	256

[a]Composite table derived from various authors.
Reciprocal of serum titers; 0, no fixation at dilution 1:4 or 1:8.

CF with mouse hyperimmune sera; the available fragmentary evidence shows that Pichinde and Tamiami viruses are not closely related to the others nor to each other. LCM and Lassa viruses are very distantly related to the other agents; only when using the highest titered antisera can cross reactions be observed.

Results of neutralization tests, many of them done with samples of the same sera that showed markedly crossing by CF, are markedly specific. In comprehensive plaque-reduction tests (16, 37, 93) in which sera had homologous titers in the range from 1:32 to 1:2048, generally 1:128–1:512, no cross neutralizations have been noted even between viruses which are very close by CF, such as Machupo, Tacaribe, and Junin. The same marked specificity has been observed when constant serum was used with varying dilutions of virus. Studies with LCM and Lassa viruses are less extensive but they also show marked specificity in the N test.

Investigations with the FAT, especially the indirect rest, show this technique to be less specific than the neutralization test; in fact it was with this method that the serological association between LCM and the Tacaribe group viruses was first observed (77). Antisera to all members of the Tacaribe group reacted with LCM virus; LCM antisera reacted with Amapari virus but little with Tacaribe. Cross-reactions have also been observed between Lassa and LCM viruses (15) and between Latino antiserum and Pichinde antigen (93).

D. Biological Properties

The natural hosts and reservoirs of the arenaviruses that cause human disease are discussed in the corresponding sections. The remaining viruses have been isolated in nature from the following animals: Tacaribe from *Artibeus* bats; Amapari from *Oryzomys* and *Neacomys* rats; Pichinde from *Oryzomys* and *Thomasomys;* Parana from *Oryzomys;* Latino from *Calomys;* and Tamiami from *Sigmodon* (cotton rat) and *Oryzomys*. Attempts to isolate these viruses from other natural hosts, including arthropods, have been reported, largely with negative results: Pichinde virus has been isolated from ectoparasites taken off viremic hosts; Amapari has been isolated from mites (*Gamasidae*); Tacaribe was reported to have been isolated from a mixed mosquito pool.

Among experimental hosts, 1- to 4-day-old mice develop fatal illness after intracerebral inoculation of most, but not all, arenaviruses. Latino virus does not infect mice, and Parana inoculation results in illness but no death; LCM virus strains, in general, are lethal when inoculated to young adult mice but not to newborn mice. Newborn hamsters are lethally infected by Junin, Latino, Machupo, Parana, and Pichinde viruses; guinea pigs are susceptible to LCM and Junin viruses.

All arenaviruses, except LCM, replicate with production of plaques under agar overlay in Vero cell monolayers; some have marked cytopathic effect (CPE) in cells in fluid cultures. Other cells, $LLCMK_2$ and HeLa, are susceptible to some of the viruses with CPE development. LCM virus multiplies reaching high titers in nearly all cells in culture that have been tried but CPE, including plaque formation, is not a feature of the multiplication of this virus, except in rare circumstances and particular systems (70).

A special property of arenaviruses that cause disease of man, repeatedly described with LCM and Machupo, is their capacity to induce persistent tolerant infection in their natural hosts with no ill effects to the host and in the absence of an immune response; the epidemiological implications of this fact are evident.

V. PATHOGENESIS AND IMMUNITY

LCM virus infection of the adult mouse is the classical example of virus-induced immunopathological disease. Intracerebral inoculation of the adult mouse results in manifest disease and death while infection before or soon after birth leads to a nonpathogenic lifelong carrier state. In the neonatal mouse during the period of immunological immaturity the virus does not stimulate an immune response; since the virus is, presumably, harmless for the mouse, no ill effects result. In the adult mouse which has reached immunological maturity, the virus incorporated in the cells stimulates an immune response from the host; it is this conflict between the host and the virus which results in disease, a fatal choriomeningitis with no evidence of neuronal destruction. Numerous observations (35, 43) by many workers have firmly established the above pathogenetic mechanism for the disease; it is, furthermore, observed that immunosuppressants protect adult mice against death due to LCM virus infection. Since among the immunosuppressing treatments, antilymphocytic serum and neonatal thymectomy are effective, it appears that the immune disease is cell mediated rather than caused by antibodies.

Studies with Tamiami virus (32) showed that suckling mice after intracerebral inoculation of the virus develop an acute CNS disease with cerebellar ataxia and less frequently paralysis, convulsions, and death. Neonatal thymectomy totally prevented the disease in spite of the presence of virus in high titer in the brain tissue; it was suggested that the acute disease caused by the virus is immunomediated.

With the present state of knowledge no definite statement can be made concerning the mechanism through which the arenaviruses cause disease in man. There is nothing to indicate that lesions and disease are immunopathological or allergic phenomena, as is so clearly the case in adult mice infected with LCM virus; clinical and pathological observations rather favor the view that direct damage to cells by the virus best explain the disease.

Little is known about the type and localization of lesions in man after LCM virus infection and about the multiplication and distribution of the virus, duration of viremia, and persistence of antibodies. With other arenaviruses, autopsy of 10 patients who died of AHF consistently revealed generalized lymphadenopathy on gross examination; microscopically, endothelial swelling in capillaries and arterioles of all organs examined was seen without exception and lymphocyte depletion in the spleen was generally observed (30). In another series (24) it was stressed that lesions in several organs and tissues were probably caused by direct cytotoxic action of the virus on cells, in the absence of conspicuous cellular infiltration. In connection with the diffuse involvement of capillary and arteriolar endothelium (30) it is of interest to mention that disseminated intravascular coagulation has been described in one case of AHF and suggested as a pathogenetic factor in the disease (5).

The result of a liver biopsy in a fatal case of Lassa fever is highly pertinent to the pathogenesis of the disease (96). Diffuse hepatocellular damage was evident, with focal necroses; by electron microscopy was observed a clear association between damaged liver cells and virus particle formation and maturation, while inflammatory response was minimal. These findings suggested that a direct cytopathic effect was responsible for, at least, the hepatic lesions in Lassa fever and that cell-mediated or humoral immunologic damage is not a major factor.

The most constantly reported lesion in AHF, probably also in BHF, is a diffuse swelling of the endothelium of capillaries and arterioles in the absence of inflamma-

tory reaction; clinically, there is in nearly all patients an adenopathy, local or generalized; there is also leukopenia, late appearance of antibodies, and the fact that recovery occurs at the time when antibodies begin to build up; biphasic patterns of clinical disease are all but unknown with AHF and BHF. Finally, cell damage, when it occurs in specific organs, appears to be associated with a direct cytopathic action of the virus on the cell, with no cellular infiltration. Based on these observations, the following pathogenesis has been suggested (40) for arenavirus infections of man: the virus gains entry by the upper respiratory or alimentary route, is caught in the local lymphoid tissue or lymph nodes where it first replicates; it then invades the cells of the reticuloendothelical system including all the cells involved in the immune and cellular immune responses, whose functions are, therefore, inhibited at this time. The virus causes, directly or indirectly, extensive capillary damage resulting in capillary fragility, hemorrhagic tendency, and hypovolemic shock; malfunction of the various organs is due probably to capillary damage and edema of the parenchyma rather than actual cell damage. When the disease regresses, no permanent damage ensues, since there has been little cell damage; antibodies develop to a high titer. In progressive cases, a direct cytopathic action of the virus on the cells follows; coagulopathy can occur but immunopathology is at no time evident.

After overt infection of man with arenaviruses, antibodies develop which have been detected by complement-fixation and neutralization tests and, recently, by the fluorescent-antibody technique; no hemagglutination-inhibition test is available for these viruses. With LCM, AHF, and BHF complement-fixing antibodies are generally short lived, with their titers diminishing rapidly between 6 and 12 mo from onset at the end of which period most sera are negative; on the other hand, neutralizing antibodies remain detectable for years (40, 43). Lassa fever having been recognized only within the last few years, no information is available on antibody persistence.

Antibodies have been detected in persons in whom no specific diagnosis had been made, perhaps having had only a subclinical infection, particularly with LCM (1, 14); the current view is that clinically inapparent infection is rare with Junin and Machupo viruses (40).

VI. LYMPHOCYTIC CHORIOMENINGITIS

LCM virus was first isolated in 1933 in the course of investigations on the etiology of an epidemic of encephalitis in St. Louis, MO (8); the virus may have been present in the CNS tissue of a patient who died of that illness or, more likely, derived from monkeys inoculated during the study. An etiological association between the virus and a disease of man, acute aseptic meningitis, was established (75, 83) by isolation of the agent and demonstration of development of antibodies. While at an early period it was assumed that LCM virus was the exclusive etiological agent of acute aseptic meningitis, or Wallgren's disease, it soon became apparent that the virus caused only a small proportion of the cases. Traub (89) reported that a colony of laboratory albino mice was chronically infected with a virus subsequently identified as LCM; this finding was the beginning of a new concept, persistent tolerant virus infections, which has considerable epidemiological implications on LCM virus and other arenavirus infections of man.

A. Descriptive Epidemiology

1. Incidence and prevalence. Determination of infection or illness caused by LCM virus requires a laboratory-confirmed specific diagnosis; in general, this is not attempted since the required laboratories are not always available. Efforts to obtain a specific diagnosis usually require special circumstances, such as a large number of clinically suspect cases appearing simultaneously (7); or the continuing interest of groups of investigators (Adair *et al.* (4); Meyer *et al.* (58); Blumenthal *et al.* (14)).

Soon after the discovery of the virus and its association with cases of aseptic meningitis it became clearly apparent that clinical infection of man due to LCM virus was a rare event; later surveys supported the view.

One of the most extensive surveys to determine the prevalence of clinical LCM virus infection in man was conducted among USA military personnel and dependents over an 18-yr period, from 1943 to 1960 (Adair *et al.* (4); Meyer *et al.* (58)). Examination of nearly 1600 CNS illnesses revealed that only 8% were specifically diagnosed as LCM infections; on the average seven cases a year occurred during the entire period. No estimate can be made of undiagnosed cases or, if they existed, of subclinical infections; a study in the USA (97) showed that 5% of about 1200 sera from residents of various areas had neutralizing antibodies; it is conceivable that a certain degree of nonspecific inhibition of virus may have occurred in that study (43) so that the results may not be specific.

Investigations in West Germany since 1960 (Ackerman *et al.* (2); Scheidt *et al.* (81); Blumenthal *et al.* (14)) indicate the extent of the distribution of LCM virus in that country and the close association between the incidence of infection of man and the presence of virus in the mouse. Early observations by these investigators had shown the rarity of the disease in a number of large hospitals in the country; furthermore, antibody surveys with sera from selected individuals revealed only about 1% of positives (1). In a subsequent survey (14) done after the distribution of the virus in mice had been investigated, sera from about 2000 persons from rural districts were tested for neutralizing antibodies; 68 of these sera, or 3.4% were positive; on the basis of this survey Ackerman (1) estimated that as many as 1000 new infections per year may occur in a population of about 6 million persons in rural German areas; since only a minute fraction of this number are clinically recognized, the inference is that most LCM virus infections go undiagnosed or are subclinical.

2. Geographic distribution. The virus of LCM may well have worldwide distribution, being present in all parts of the world where the house mouse is found. Well-documented proof of the virus presence has been given for European countries and North and South America; its presence has also been reported in Asia, less convincingly in Africa, and not in Australia (43).

3. Age, sex, and occupation. Since LCM is not usually reported, the effect of a number of variables in its spread and prevalence is difficult to appraise. A seroepidemiological report from West Germany (14) indicated that the distribution of antibodies was not influenced by sex or occupation—whether farm work or professional or office work; in that survey hardly any positives were found among persons under 20 yr of age. On the other hand, no influence of age was seen in hamster-related outbreaks (3, 7). It is possible that mouse-associated infections are more common in rural populations or in lower socioeconomic urban groups and that hamster-associated cases are found, principally, in urban centers. Further, a seasonal fluctuation of cases has been suggested in man, more in winter than in summer. Perhaps

this is associated with migratory habits of the house mouse (43) and possibly with closer contact with mice in the cold months in the temperate zone.

Special attention should be given to LCM as an occupational disease in laboratory personnel, either in individuals who work with the virus or who work in other problems but who use animals—mice, hamsters, possibly monkeys—that may be infected. Reported laboratory accidents may well represent only a fraction of all the occurrences; in the period between 1952 and 1966, 45 laboratory infections with five deaths have been documented (87).

B. Mechanism and Route of Transmission

1. Spread of virus. The only known true carrier of LCM virus is the mouse from which man becomes infected. Probably other species are important, particularly the hamster, from which man also becomes infected. Man-to-man transmission seems unlikely.

The mechanism of transmission from mouse to man cannot be stated with certainty. It would appear that either the airborne route through household dust contaminated with mouse urine and other excretions and secretions, or the contamination of food and drink by the mouse excretions are the most likely sources of human disease. The portal of entry in these instances would be the upper respiratory or, possibly, the upper digestive tracts; the possibility of transmission through skin abrasions has also been considered.

While airborne transmission appears the logical mode of human infection, acceptance of this hypothesis runs into problems represented by the known lability of LCM virus under unfavorable conditions; however, no other explanation has been put forward to replace airborne or food contamination. Transmission by an arthropod vector has been investigated but the evidence is against it; it is worthwhile to mention, however, that *Aedes aegypti* has transmitted the infection by bite to monkeys 15 days after feeding on infected guinea pigs (20).

2. Reservoir. In nature, the virus in addition to man who is most likely a dead end, has been isolated from various animal hosts. Chief among these, for epidemiological implications including maintenance of the virus in nature, is the house mouse (*Mus musculus*). In recent years, the Syrian hamster (*Mesocricetus auratus*) is gaining importance as a source of infection of man, if not as a true reservoir.

From the first demonstration of LCM virus in house mice trapped in the home of two persons suffering from nonbacterial meningitis, Armstrong and Sweet (9), the abundance of isolations has left no doubt about the close association in nature between the virus and this rodent. Furthermore, it has been shown that experimental mouse colonies can be chronically infected (90). Studies on the nature of the infection of laboratory mice by LCM virus extending over a period of over 30 yr, have clearly shown that the mouse infected *in utero* or within a few hours after birth develops a tolerant persistent infection; mice thus infected circulate virus in their blood, develop no antibodies, and they maintain an active and relatively normal health condition for a period of time representing a good fraction of a normal mouse's life span. The epidemiologically important feature of the tolerant persistent infection is that wild mice so infected shed virus continuously for the duration of their lives, by way of urine, feces, nasal and mouth secretions and excretions. The virus thus excreted will contaminate the ambient in households, food, drink, dust, fomites, from which, and by ways as yet undetermined, man becomes infected. In

addition, new generations of mice become tolerantly infected at birth or *in utero,* thus maintaining the carrier status of the mouse populations; and, in addition, mice so infected may become the source from which other species—hamsters, guinea pigs, monkeys—are infected and they, in turn may infect man.

The studies of Ackerman *et al.* (2) and Blumenthal *et al.* (14) are particularly illustrative of the association between infection of mice and infection of man. Sixty-five mice of 1795 trapped between 1960 and 1962 in 44 of 376 areas in West Germany were LCM carriers, as shown by virus isolation; nearly all positive trapping areas were in North and Northwest Germany, none in South Germany. Serological surveys done at about the same time in which 1371 persons from rural districts were tested by neutralization test showed that, of 511 sera from persons in North and Northwest Germany, in or near the places where LCM virus had been isolated from mice, 9.1% had antibodies. The second set of sera from 811 persons were from South Germany where no LCM virus had been isolated from mice; only 5, or 0.6%, were positive.

In recent years, the Syrian hamster has emerged as an important source of human infection and illness caused by LCM virus. Small outbreaks had occurred in the past involving persons participating in biomedical research work in which hamsters were used (Lewis *et al.* (45); Armstrong *et al.* (10)). Between 1968 and 1971, 47 LCM infections were described in West Germany. These were specifically diagnosed by antibody detection as caused by LCM virus in persons who shortly before their illness had been in contact with pet hamsters; 45 of these infections were clinical, mainly influenza-like or aseptic meningitis, and two had no clinical manifestations (3). Subsequent investigations in West Germany (26) on commercial breeding colonies showed that of 598 animals examined, representing 11 different breeders, LCM virus was isolated from animals of six different colonies. According to the authors of that study, it is estimated that close to 1 million hamsters are sold annually as pets in West Germany; it is obvious that the importance of this animal as a source of infection of man cannot be overlooked.

Two outbreaks of LCM in man, associated with hamsters, have been recently observed in the USA. Early in 1973 an episode occurred in a laboratory where hamsters were used for cancer work (7). The investigation of the outbreak revealed several interesting points. In all 21 persons became ill with a severe influenza-like illness, of which 14 had occurred before LCM infection was suspected. In addition to the 21 clinical cases, confirmed by FAT antibodies, there were an additional 17 persons who had antibodies but no illness; in other words, inapparent infections occurred. The association with hamsters was clearly seen in that 75% of 20 persons admitting to have touched the hamsters were seropositive, while only 17% of 61 persons who had no contact other than entering the premises were positive; the latter may have been instances of airborne infections. LCM virus was isolated from 11 of 24 hamsters tested. The animals appear to have been infected from virus present in the tumor line with which they had been inoculated.

In another extended episode, 93 human cases in seven states were diagnosed, and specifically confirmed, between December 1973 and April 1974 (25). The association with pet hamsters was established in every instance; in some instances, two or three cases occurred in a family. The diagnoses were established by FAT and CF tests, in man and hamsters; it appears that of several breeders whose animals were tested, only one had an infected colony.

The episodes in the USA and Germany point to the importance of hamsters as a source of human infection with LCM virus; while this animal is not a true life-long carrier, it can circulate and excrete virus for periods of 2 or 3 mo after its infection.

There are additional animal species from which the virus has reportedly been isolated in nature such as monkeys, guinea pigs, and dogs. The role that these species play in virus dissemination to man is undetermined but it appears to be unimportant.

C. *Patterns of Host Response*

1. Clinical aspects. Infection of man by LCM virus presents different clinical forms and there may be inapparent infections. Three major clinical forms seem to prevail: aseptic meningitis, influenza-like or non-nervous system type, and meningoencephalomyelitic type (43). The influenza-like and meningeal are the most frequent types. The incubation period is believed to be from 6 to 13 days. In the influenza-like (or grippal) type there are fever, malaise, muscular pains, coryza, and bronchitis; in the meningeal type, which is the commonest, there is a "grippe"-like beginning followed by definite signs and symptoms of meningitis, with stiff neck, headache, and nausea, which may remain mild and of short duration or can be pronounced, last for 2 wk or longer, and lead to considerable prostration.

The great majority of specifically diagnosed, clinical infections follow a benign course; only a few fatal cases have been reported, either after CNS involvement (4) or after systemic generalized illness with hemorrhagic manifestations (84). Chronic sequelae, although rare, have been reported, including paralyses, headaches, personality changes; it appears that the documentation of most such cases is ambiguous (43).

2. Diagnosis. Clinical and routine laboratory analyses are indicative of only aseptic meningitis: the CSF is under increased pressure, with slightly increased protein, normal or slightly reduced sugar, and moderate number of cells, from 150–400/mm^3.

The virus in man can be isolated from blood, CSF, and, in fatal cases, from brain tissue. The best sources for isolation are blood during the febrile period and CSF during the period of meningeal manifestations; virus can be isolated from the blood and CSF from experimentally infected man for 20–25 days (13).

The animal of choice for virus isolation is the laboratory albino mouse, 3–5 wk old; after intracerebral inoculation the incubation period and signs of illness are nearly pathognomonic. Care must be taken to use mice from a colony known to be free from the virus. Inoculation of cells in cultures could be used since most cells support replication of LCM virus; however, since virus replication in general does not cause CPE or plaques, detection of viral antigen in the cells must be made by CF or FAT tests.

The techniques employed for detection of antibody development are the CF and the indirect FAT tests; it has been reported that the latter appears earlier than CF antibody (35). In recent investigations of antibody determination in man and hamsters, there has been nearly complete agreement between the results of these two tests (Woodall, personal communication, 1974). Neutralizing antibodies appear much later after onset; therefore, the neutralization test is not helpful for an early diagnosis; it is most profitably used in serum surveys (14).

D. Treatment and Prevention

There is no specific treatment advocated; in view of the definite association with mice, it would appear that rodent control may minimize the risk. Monitoring of hamster colonies for presence of virus and/or antibodies would be indicated.

VII. ARGENTINIAN HEMORRHAGIC FEVER (AHF)

A disease resembling AHF and with the same geographical location seems to have been first recognized in 1943 (72). Arribalzaga (11) gave the first detailed account of the disease and considered it a new nosologic entity; his description included extremely accurate clinical and epidemiological observations. The causal agent, Junin virus, was isolated in 1958 (66, 73). Annual outbreaks of the disease have occurred since 1958 and the endemic zone has been progressively increasing in area.

A. Descriptive Epidemiology

Collection of data concerning clinically diagnosed cases of the disease and laboratory efforts to confirm the clinical diagnosis, appear to be efficiently done through local, provincial, and national Public Health centers in Argentina (21, 72).

1. Prevalence in man. AHF is predominantly a rural disease that affects adult males with agricultural occupations, particularly harvesting of maize; 80% of nearly 1000 cases recently analyzed were in males and 63% of the total number were in the age group between 20 and 49 yr (Maiztegui (49); and personal communication).

2. Geographic and seasonal distribution. The endemoepidemic zone was first recognized in the northwest of Buenos Aires province; by 1958 its area was estimated at 16,000 km^2. Since that year the zone has spread west and north, to include additional localities in Buenos Aires as well as sections of two adjacent provinces, Cordoba and Santa Fe; the affected area was estimated in 1970 to be 80,000 km^2 in which lived a population of 800,000 persons (79). The total number of cases reported from 1958 to 1972 is about 13,000, with annual fluctuations between 100 and 3500; the mortality among laboratory-confirmed cases studied at Pergamino has been from 10 to 20% (Maiztegui (49); and personal communication).

The disease is sharply seasonal with the outbreaks beginning late in summer (February), reaching a peak in autumn (May), and ending early in winter. The seasonal distribution coincides with the intensification of agricultural labors, particularly harvest of maize, and with an influx of transient farm workers; at the same time there is an increase in the population of wild rodents, which are considered the principal reservoir of the virus. While overwhelmingly a rural disease, cases of AHF have been observed in an urban setting in the near absence of the main reservoir (*Calomys*) of the virus (Maiztegui *et al.* (51); Sabattini and Maiztegui (79)); however, the simultaneous existence of LCM and Junin virus in an area may create diagnostic problems (50).

B. Mechanism and Route of Transmission

1. Spread to man. Chronic infection of rodents with associated viruria is the basic mechanism of transmission of the virus to man; there is no evidence implicating arthropod transmission (40, 79). The mode of transmission from wild-infected rodents to man has not been definitely established. It may be airborne from dust contaminated by the excretions or secretions from rodents; or by the oral route through ingestion of food and drink equally contaminated. Since the disease has been transmitted to human volunteers by injection (73), it may be possible that the

disease is also acquired through skin abrasions in the course of farm work while handling materials contaminated with rodent's excreta.

Although the virus has been isolated from throat swabs and urine from patients, contact transmission between individuals is exceptional (78).

2. Reservoir. The possible connection between wild rodents and AHF was first stated by Arribalzaga (11). Accumulated observations beginning in 1958 support the close association between disease and rodents in the endemic areas. The main reservoir are two species of *Cricetidae, Calomys laucha* and *C. musculinus,* which are present in farm fields and along hedgerows, the latter species predominating in Cordoba province (79); Junin virus has also been isolated from *Akodon, Azarae,* and, rarely, from *Mus musculus* (21). Field and laboratory investigations show that Junin virus causes a chronic tolerant infection in *C. musculinus* with persistent viremia and viruria and no development of antibodies (79); most likely, the virus is maintained in nature by infection of the rodents at birth. Although the virus has been isolated from mites (68, 73), it has not been established that they play a role in transmission between rodents or to man.

C. Patterns of Host Response

1. Clinical features. The disease presents a syndrome that includes manifestations of renal, cardiovascular, and hematic involvement; pronounced neurological manifestations are also described, but not frequently. The disease lasts from 7 to 14 days and terminates with either complete recovery with no sequelae or with death. After an incubation period estimated at from 7 to 16 days there is an insidious and gradual onset with chills, asthenia, malaise, headache, retro-ocular pain, muscular pains, often pronounced in the costovertebral angle, anorexia, nausea, and vomiting. The most prevalent signs at the outset are fever with temperatures up to 102–104° F, conjunctival injection, enanthem, exanthem on face, neck, and upper thorax, a few petechiae particularly in the axilla, polyadenopathy, and muscular tenderness at the thigh. Three to five days after onset the signs and symptoms become more pronounced in the severe cases, with dry tongue, dehydration, oliguria, hypotension, relative bradycardia and, in the worse cases, hemorrhages from the gums, nasal cavities, also hematemesis, hematuria, and melena; oliguria may develop into anuria. In the severe cases there are psychosensorial and motor alterations. Death is due to uremic coma or hypotension and hypovolemic shock due to plasma leakage, not whole blood loss. In nonfatal cases the fever diminishes by lysis, there is marked diuresis and rapid improvement within days; however, convalescence is prolonged. The case fatality rate has been as high as 20%; usually it is between 3 and 15% in different outbreaks. Clinically inapparent infections appear to be very rare (55, 79, 37).

2. Simultaneous occurrence of AHF and LCM. Investigations in areas of the AHF endemic zone to determine the source of virus in an urban setting (52) led to the finding of antibodies against LCM virus in mice (*M. musculus*); at about the same time a strain of LCM virus was isolated from that species (80). A re-examination of antibodies in acute and convalescent sera from nearly 3000 cases of AHF, using LCM and Junin antigens, revealed that in a substantial number of instances, AHF occurred in persons who showed evidence of previous infection with LCM virus (49). Furthermore, there were a few cases previously diagnosed as AHF in which the serologic diagnosis was changed to LCM (49). Additional evidence of the activity of LCM virus in the endemic AHF area is given by the simultaneous ad-

mission to a hospital of two agricultural workers with clinical diagnosis of AHF, but with specific serological conversions to Junin virus in one, to LCM virus in the other (50).

Since only between 60 and 70% of patients clinically diagnosed as having AHF are generally serologically confirmed, it had previously been suspected that other agents were active in the endemic area in epidemic times; serological evidence of infection with group B arboviruses has been reported (54) in a number of persons diagnosed clinically as AHF cases and from one, St. Louis encephalitis virus was isolated (56).

3. Diagnosis. Clinical diagnosis of AHF has been confirmed either serologically or by virus isolation in from 60 to 70% of reported cases (55). In a thorough study involving 2249 reported cases over the period 1965–1972, in the city of Perganino, the diagnosis was confirmed in 62% and was doubtful in 11% of cases (Maiztegui (48, 49)).

During the epidemic season of AHF, there appear to occur in the same areas other diseases of viral etiology which at the early phase present clinical manifestations similar to AHF (Schwarz *et al.* (82); Mettler (54)). A study of signs and symptoms in a number of patients clinically diagnosed as having AHF, in whom laboratory confirmation was sought, showed that during the first week of illness a combination of asthenia, dizziness, petechiae in the axillary region or anterior chest wall and conjunctival congestion was present in 71% of confirmed cases, only in 3.5% of nonconfirmed ones. When in addition are found leukopenia, thrombocytopenia, and casts in the urine, the diagnostic accuracy is further increased (82).

Specific diagnosis is based on isolation of virus or demonstration of serological conversion. The virus is isolated from the blood during the acute period, probably from the 3rd to the 10th day after onset; and, in fatal cases, from liver, spleen, kidney and clotted blood. The materials are intracerebrally inoculated to newborn mice, 1–3 days old; or to guinea pigs by peripheral or intracerebral route. While Junin virus replicates with CPE in several cells in culture, little has been reported on the use of tissue cultures for isolation of the virus from nature (79).

The test of choice for demonstration of antibodies is the complement-fixation test; AHF shares with other diseases caused by arenaviruses the characteristic that complement-fixing antibodies develop relatively late after onset; an early sample and a second one taken not before 30 days after onset seem to offer the best possibility to detect serological conversion.

Recent developments (Oro *et al.* (65); Maiztegui (49)) have shown the simultaneous development of CF antibodies against Junin and LCM virus antigens in patients in the AHF endemic zone, clinically diagnosed as cases of AHF, including some from whom Junin virus was isolated from acute-phase blood. This fact creates serious difficulties for reaching a definite diagnosis, not heretofore encountered with other arenavirus infections of man; as a result, epidemiologic evaluation of data may be faulty.

D. Treatment, Control, and Prevention

There is no recommended specific treatment; administration of serum from recovered individuals has not been generally used. Vaccination of the exposed population has been advocated; however, there is no available vaccine. Efforts to develop a vaccine employing an attenuated strain of Junin virus have been reported; administration of the preparation to a small number of persons on an experimental basis led to the development of antibodies with only relatively minor febrile reactions (67).

Ecological control to reduce the number of rodents and exposure of man to them appears difficult to implement. The circumstances under which the bulk of the exposed population live and work at the time of the harvest will not be likely to change in the near future; increased mechanization of farm labors and improvement of housing conditions would, undoubtedly, reduce morbidity (21).

VIII. BOLIVIAN HEMORRHAGIC FEVER (BHF)

The disease was first recognized in 1959 in two rural areas in the northeastern part of Bolivia, Department of Beni. Late in 1962 and early 1963 cases began to appear in a nearby town, San Joaquin, developing into a large outbreak that continued until the middle of 1964; nearly 700 persons were ill with a mortality of 18%. The disease has continued to appear in the Department of Beni more or less annually in sharply localized outbreaks (37, 40, 46). An outbreak which occurred in 1971 in Cochabamba, Bolivia (40) differed ecologically from previous ones and represented an extension of the virus to a new area.

A. Descriptive Epidemiology

1. Prevalence in man. Before 1962 the small outbreaks affected mainly adult males and most cases occurred from April to September, which is the time of highest agricultural labor. From 1963, when the disease appeared in towns and villages and larger numbers of persons sickened, the pattern changed. Adult males still presented somewhat higher rates of morbidity, but all persons were affected with little relation to sex, age, and occupation; it was soon apparent that the disease was "house-associated" with the lower socioeconomic groups experiencing the highest incidence of disease. Although a seasonal pattern is evident, with the highest incidence from February to September, cases occur in each month (46, 37).

2. Geographic distribution. The main epidemic centers, San Joaquin and Orobayaya, are located in the Department of Beni, in the northeastern section of Bolivia; these centers are on an immense flat plain east of the Andes. The prevailing vegetation type is that of a grassland broken with "islands" of forest and numerous tree-lined rivers and streams (Kuns (42)). The human settlements where cases have occurred in the past were on slightly elevated sites that generally escape flooding during the heavy rains; the houses are on the edge of the forest, overlooking the grass-covered marsh lands. These villages and settlements are heavily infested with *Calomys callosus,* a mouse-like rodent which, although pastoral, readily invades and lives in houses in a manner similar to the house mouse, *Mus musculus* (42).

B. Mechanism and Route of Transmission

1. Spread to man. Transmission from rodent to man is probably by contamination of food, water, or air with infected rodent urine or by inoculation through skin abrasions (40). Human-to-human transmission can occur in rare cases of close contact (22), but it is not considered important in the spread of the natural infection. There may be, however, circumstances that promote such type of transmission, as shown in a relatively recent small outbreak in Cochabamba, Bolivia, which is outside the habitat of *Calomys callosus;* from an index case, who had acquired the infection in the endemic area, five secondary cases developed including family and medical personnel; all were fatal except one (40).

2. Reservoir. The distribution of cases in a town in the form of clusters definitely associated with certain houses, the absence of evidence of human-to-human trans-

mission and the equally negative evidence of an arthropod vector, led to the inference that a reservoir may be involved that lived in or near households; soon the association of disease with a rodent, *Calomys callosus* was firmly established. This rodent has been trapped in all households where cases have occurred; houses which were located in sites which did not favor the presence of this rodent, were spared; finally, the dramatic termination of the epidemic in San Joaquin in June 1964, 2 wk after continuous trapping of *C. callosus* had been implemented left little doubt about the association (42, 46). Fifty percent of *C. callosus* caught wild at the time of that epidemic were infected with the virus (38); experimental studies with colonized *Calomys* show that on inoculation of Machupo virus the rodent develops a tolerant infection with persistent viremia and viruria and no development of antibodies (41). *Calomys* are easily infected by oral and nasal routes, and also by contact with infected cage mates; about 50% develop viremia and viruria for life (39).

All efforts to isolate the virus from arthropods caught in the epidemic area at the epidemic time have been negative (42).

C. Patterns of Host Response

1. Clinical features. The disease is clinically so similar to AHF that a joint description is often given (36, 37). The incubation period is estimated at from 7 to 14 days. The onset is insidious, and the fever, which has been carefully monitored in numerous etiologically confirmed cases, reaches a temperature between 102 and 105° F, with little diurnal variation, remaining at that level for at least 5 days. About 30% of the patients present hemorrhagic manifestations consisting of petechiae on the upper part of the trunk and oral mucous membranes and on occasion, bleeding from gums, nose, stomach, intestines, and uterus; blood loss is, however, not a threat to life (40). Nearly half the patients exhibit a fine intention tremor of tongue and hands beginning 4 or 6 days from onset; about one-fourth of these may develop a frank and extensive neurological course. Somnolence and coma are hardly ever seen, except in very young children. The acute disease can last for 2–3 wk; convalescence is long, with complaints of severe generalized weakness and manifestations of autonomic dysfunction. Probably owing to the continuously elevated temperature, loss of hair and transverse grooving of the nails is common. The mortality has varied depending on outbreaks from 5 to 30%; clinically inapparent infection is very rare.

2. Diagnosis. The clinical diagnosis by an experienced physician, in the endemic area and in moderately severe or severe cases, is fairly accurate; because of the toxic condition of the patient, BHF may resemble typhus or typhoid fever. Certain clinical laboratory data help the clinician: leukopenia, thrombocytopenia, and increased hematocrit; the latter indicates a poor prognosis (40).

A specific diagnosis is based on virus isolation or development of antibodies. The most successful animal for virus isolation has been the newborn hamster, inoculated by the intracerebral or intraperitoneal routes. Recovery of Machupo virus from acutely ill patients is, however, quite difficult; only one in five samples, mostly sera, from serologically confirmed cases yielded virus, most frequently between the 7th and 12th day after onset. Virus is rarely isolated from urine or throat swabs. In fatal cases, on the other hand, virus is easily and generally isolated from spleen and lymph nodes (37).

The most convenient and efficient means for establishing a specific diagnosis is the CF test. While this test is group specific rather than type specific, no diagnostic

problems have arisen with Machupo virus due to its sharply localized geographical location. As with other diseases in this group of viruses, CF antibodies are relatively late in appearing; although they have been found on the 14th day after onset, it is advisable to test a sample of serum between 40 and 60 days after onset (46, 47).

D. Treatment, Prevention, and Control

Administration of convalescent plasma has been advocated and used. In spite of some impressions of favorable clinical responses, its efficacy has not been established or denied, owing to insufficient observations (36).

Rodent control by continued trapping has been the most effective single means for preventing human infection and for terminating an epidemic; this approach, however, seems unlikely to be the long-term solution of the problem. Given the sharply localized geographical distribution of the disease, it would seem that vaccination of exposed populations would be the effective answer, but no vaccine is available. Attenuation of a strain of Machupo virus by serial intracerebral passage in suckling mice has been reported and its possible use as source for a vaccine suggested (40).

IX. LASSA FEVER

The disease was first observed in 1969, in a missionary nurse stationed at a locality in northeastern Nigeria; after her admission to a hospital in Jos, Nigeria, two contact cases developed in nurses at that hospital (27). Owing to the circumstances surrounding this outbreak and to the fact that two of the three persons affected died, the disease acquired from the outset a reputation for severity which subsequent events amply justified. In addition to the initial episode, four more outbreaks have occurred between 1970 and 1974 in Nigeria, Liberia, and Sierra Leone (17, 28, 61); furthermore, laboratory accidents have occurred in the USA (12, 44).

1. Geographic distribution. The disease has been observed in several localities in Nigeria (Lassa, 1969; Jos, 1970; Onitsha, 1974[2]), in Liberia (1972), and Sierra Leone (1970–72). The number of cases seen, or retrospectively diagnosed, in the outbreaks has varied from three to slightly over 60. Retrospective serological surveys have demonstrated the existence of Lassa virus infection in Guinea (34) and Central African Republic (Frame and Casals, unpublished).

2. Epidemic types: Season, age, and sex distribution. Two types of outbreaks have been observed. The first type, hospital associated, develops as a result of exposure and spread from a hospitalized index case to other patients, visitors, and medical staff. The index case usually acquires the infection in the nearby community; between 10 and 20 days after admission to the hospital a cluster of secondary cases develops (17, 27, 61). This type of outbreak has been the rule with one exception.

The second type of outbreak of which there is at present only one example (Sierra Leone, 1970–72), occurs in the community at large. Patients acquire their infection at home or other community surroundings, rather than by exposure or contact in the hospital with another patient. However, there is also possibility of nosocomial transmission in this type of outbreak, particularly to the hospital staff (28).

Tertiary cases have been recorded but with a few exceptions, notably by transmission to medical staff, they have been milder. No evidence has been reported of further propagation of the disease (17).

[2]Information relative to the episode in Onitsha was supplied by Dr. E. A. Smith, Federal Ministry of Health, Lagos, Nigeria and Dr. Allan S. Noonan, Smallpox Measles Program/CDC, Lagos, Nigeria.

The mortality in hospitalized cases has been between 30 and 66% in different outbreaks, with an average of 36%. The mortality after infection, however, appears to be much lower. In the Sierra Leone focus many persons were found with antibodies who either had not been ill or possibly remembered a mild disease; the over-all mortality from Lassa virus infection in that area may have been only 3–5% (28).

There is a seasonal distribution of hospital-associated outbreaks, which have occurred from January to April, during the dry season; the Sierra Leone cases occurred through the year with somewhat higher incidence in the wet season.

With respect to age and sex distribution, the Sierra Leone outbreak with its pattern of transmission in the villages revealed no important predilection in morbidity or in case-fatality rates (28). In the hospital-associated outbreaks, the distribution, by sex and age, was determined largely by the characteristics of the exposed population; physicians and nurses have been particularly affected.

B. Mechanism and Route of Transmission

1. Spread of virus. The transmission in a hospital setting is undoubtedly from person-to-person by either the contact or the airborne routes, including direct contact, droplet spread, sharing of drink, food, clinical instruments, objects, and utensils. The same mode of transmission may be at work in contact infections acquired in the home.

In the community-centered outbreak at Sierra Leone there was definite clustering of illness and seropositivity without illness in certain households; this could be explained either by multiple instances of human infection from the same natural source, or by person-to-person transmission after a house index case who had acquired the infection from the reservoir (28).

The mode of transmission to man from the natural source or reservoir—a rodent—is still unknown: it may include direct contact with the rodent and its excretions and secretions; eating of uncooked rodent's flesh; contact with food and drink contaminated by the rodent; and it could also be airborne. The possibility of an arthropod vector appears extremely remote to explain transmission to man. Whether ectoparasites can transmit the infection between rodents is not known. Penetration through a cut while performing an autopsy appears to have been the mode of infection of a physician (94); and infection through a cut on a finger may have occurred in a nurse (27). Most medical and nursing personnel probably acquire the disease by droplet infection, since it has been established that the virus is present in the throat washings of patients for several days (15, 44, 60).

2. Reservoir. Certain similarities between Lassa fever and the diseases caused by other arenaviruses, as well as the observation that Lassa virus persists for months in the urine of experimentally infected laboratory mice which appear otherwise healthy (15), support the view that this virus may have a rodent reservoir in nature.

Small wild vertebrates collected in the course of investigating the Sierra Leone outbreak, 1970–72, focused attention on rodents and bats, since these hosts are implicated in the ecology of other arenaviruses. Pooled tissues of heart, lung, spleen, and kidney from 325 field specimens tested for the presence of virus revealed only one infected species, *Mastomys natalensis.* This yielded 10 viral isolations in 46 specimens tested (6). This species is common and widely distributed in sub-Saharan Africa. In addition to its wild habitat, it is often peridomestic, entering houses and other buildings; the potential for human contact is considerable.

Additional information is required in order to settle the question of the reservoir of Lassa fever virus, particularly whether other vertebrate species are involved in the

natural cycle. More study is also needed on the dynamics of *Mastomys natalensis* population and its ecology.

C. *Patterns of Host Response*

1. Clinical features. Lassa fever is a disease with generalized organ involvement manifested in severe cases by pharyngitis, pneumonitis, myositis, myocarditis, encephalopathy, nephropathy, and hemorrhagic diathesis. The over-all spectrum of infection of man is not yet fully known; therefore, it is not possible to estimate the risk of severe illness after infection with the virus. All earlier reports (17, 27, 61, 94) stressed the severity of the disease; however, there is evidence of milder forms, perhaps even inapparent infections (28).

The incubation period is ordinarily between 6 and 14 days. The disease has an insidious onset; in variable order appear malaise, asthenia, lassitude, headache, sore throat, muscular aches, abdominal pains, loss of appetite, nausea, vomiting, and diarrhea. Fever appears early with somnolence, indifference, and blurred vision; the temperature is in the range of 101–104° F, and may reach 107° F. Petechiae may be seen, although the disease is not severely hemorrhagic. There is marked pharyngitis with firmly adherent white patches on the soft palate, pharynx, and pillars. In severe, progressive forms signs of increase in capillary fragility appear with a suffusion or flush on the skin of face and upper thorax, puffed face, swollen neck, markedly blurred vision. There is increased oliguria and dysuria. Additional petechiae appear and sometimes larger subcutaneous hemorrhages on the arms, legs, and abdominal walls. Pleural effusions may also occur. The acute febrile stage lasts from 7 to 21 days; in fatal cases death occurs usually during the course of the second week, its immediate cause being sudden cardiovascular collapse. The death rate in hospitalized cases has been from 30 to 66%, over-all about 36% (17, 27, 44, 61, 94).

2. Diagnosis. Lassa fever, unlike the other arenavirus infections, has a marked tendency to spread by human-to-human contagion. Under these circumstances prompt diagnosis is essential in order to implement strict isolation measures. In the affected geographic areas a clinical diagnosis is complicated by the occurrence of other diseases which may resemble Lassa fever. These include malaria, yellow fever, and especially typhoid fever. The lack of local laboratory facilities makes a specific diagnosis unavailable. In endemic areas an illness characterized by unremitting fever with temperatures of 100° F or higher, persisting for 5–7 days or more, unresponsive to antibiotics and antimalarial drugs, accompanied by pharyngitis, malaise, toxic appearance, leukopenia and later, by facial edema, must give rise to a strong suspicion of Lassa fever (56).

A specific diagnosis is achieved by isolation of the virus, demonstration of antibody development, or both. Virus is isolated from the blood between the 3rd and 14th day of illness (15, 60), less frequently from throat washings, pleural effusions, and urine. Due to the recognized danger inherent in handling the virus, work with it including attempts to isolate it are restricted to laboratories with special high containment facilities. Development of CF antibodies between early and late samples of sera is a useful diagnostic procedure which can be done with inactivated noninfective antigens (15); but it is no help for an early diagnosis since the antibodies do not usually appear until 18 or 20 days after onset.

D. Treatment and Disposition of Patients

Plasma from recovered cases has been used in a limited number of instances, perhaps five or six. The number is not large enough to support any conclusions; nevertheless, it appears that its effect has been favorable (44, 60), although in one instance it failed to prevent a fatal outcome (94).

In view of the frequency with which the Lassa virus is propagated from person to person in a hospital setting, strict measures must be instituted in order to isolate patients and suspected cases. This includes the use of gloves, masks, and gowns by the staff, individual rooms for suspected cases, thorough decontamination of excretions and secretions, and sterilization of instruments, bedpans, and personal utensils (59).

Evacuation and international transportation of patients suspected of having Lassa fever presents a serious problem to public health officials, which is still largely unresolved.

E. Prevention and Control

Prevention at the individual level is based on strict sanitary precautions; no vaccine is available. Since *M. natalensis* is, thus far, the only known reservoir, measures that minimize contact of this rodent with man and his habitat will be helpful. However, it is unrealistic at this time to base too much hope on the effectiveness of the control of this rodent in the endemic areas.

X. UNRESOLVED QUESTIONS

A. Vaccines

With the exception of LCM virus which appears to have world-wide distribution, the other arenavirus diseases of man have been found restricted to definite geographic areas which, although showing a tendency to increase, are still easily identifiable. In the case of Lassa virus, although the areas are multiple and possibly more extensive than now known, they are confined to one continent. These geographic considerations added to the fact that some of the more exposed groups—maize harvesters for AHF and hospital personnel for Lassa fever—are well defined, make these diseases an excellent target for preventive vaccination. Control of the reservoirs would undoubtedly be effective in preventing disease or reducing its incidence. At the moment, however, it does not appear to be a realistic solution.

Investigations to develop a vaccine for AHF have been carried out to the point where a large number of volunteers have been inoculated with an experimental vaccine. Efforts to develop an attenuated strain of Machupo virus have been initiated. No attempts to develop a vaccine against Lassa fever virus have been thus far reported. In view of the severity of these diseases, continued efforts to develop safe and effective vaccines are desirable.

B. Early Diagnosis of Lassa Fever and Evacuation of Patients

The frequency with which hospital-centered outbreaks have followed admission of an undiagnosed Lassa fever case is a paramount reason for an early diagnosis; no such urgency generally applies to the other arenaviruses. Detection of the development of antibodies is not the answer since positive CF tests are exceptional before the end of the third week of illness. Currently, the fastest way of establishing a specific diagnosis is isolation of the virus in Vero cells in culture and identification by

the CF test. At the very best 6 days are required from the moment the specimen reaches the laboratory. Furthermore, there is only one laboratory at this time with the safe facility in which to conduct isolation attempts: The Center for Disease Control, Atlanta, Georgia. Even under optimal circumstances, by the time a presumptive diagnosis is confirmed the index case may have already infected a number of persons. The possibility of applying the fluorescent antibody technique to throat washings' smears or to smears of pelleted virus in a sample of serum should be investigated.

Transportation of suspected Lassa fever cases should be reduced to a minimum compatible with good medical and nursing care. Evacuation and international transportation of expatriate personnel suspected of having Lassa fever cannot be denied to the individual. However, it presents a serious problem to national and international health authorities whose responsibility is to localize the infection and prevent its spread to other areas.

C. Hemorrhagic Fever with Renal Syndrome

This disease is known by numerous synonyms among which are hemorrhagic nephroso nephritis and Korean hemorrhagic fever. It has a number of clinical and epidemiological characteristics similar to those seen with arenavirus infections. It appears to be caused by a virus, on the basis of investigations carried out with human volunteers by Smorodintsev and associates in 1940–41 (85) in the Soviet Union, and by Kitano and associates in Manchuria at about the same time (29). The etiological agent has not been clearly maintained in a laboratory host, although its propagation in tissue cultures and identification by the fluorescent antibody technique has been reported (31).

The disease is prevalent in several sections of the USSR, principally in the Far East and in the middle Volga region, Bashkiria; between several hundreds and several thousands of cases occur annually (18, 19). The disease also occurs in Korea, where in the period 1950–52 it affected several thousands of United Nations military personnel, particularly Americans; the same, or very similar syndrome, occurs in Sweden and has been reported in Hungary.

Clinically the disease has a prodromal stage, followed by sudden onset with chills, fever, lethargy, frontal or retro-orbital headache, myalgias, costovertebral pain, suffusion of face and upper part of thorax, petechiae or larger skin hemorrhages and pronounced renal involvement with proteinuria, oliguria, that may end up in anuria, and low fixed specific gravity of the urine; there is leukopenia and thrombocytopenia. Death is associated with shock and occurs in a variable proportion of cases, from 1–2 to 25–30%; the death rate is higher in the Far East than in the European part of the USSR (18, 19, 29).

Extensive epidemiological investigations in the USSR appear to have established a definite link—including season, place, occupation, and exposure—between disease and contact of man with various rodents, chief among them being *Clethrionomys glareolus, Apodemus sylvaticus, A. agrarius* and *Microtus fortis* (18). Soviet investigators consider that the rodents suffer a tolerant, persistent infection, excrete virus in the urine, and thus contaminate the human habitat. Support for the view that rodents are a reservoir of the disease agent is given by outbreaks in laboratory personnel who were in contact with collections of wild-caught rodents (91).

Only when the etiological agent of this disease is established in a laboratory host or system will it be possible to investigate its properties and determine its relationship with the arenaviruses.

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