ARGENTINE HEMORRHAGIC FEVER: CURRENT KNOWLEDGE

by

Norma E. Mettler, M.D.

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NOTE

One of the commitments of the Pan American Health Organization, carried out through its Department of Research Development and Coordination, is to improve communication among biomedical scientists. In view of the scientific and public health importance of Argentine hemorrhagic fever—a disease that has been under study for over ten years by different teams of investigators—it seemed that a summary of current knowledge in this field would be of considerable interest to physicians and epidemiologists throughout the Americas. The present monograph, which is completed by an exhaustive bibliography, was prepared by Dr. Norma E. Mettler, formerly of the Department of Microbiology and Parasitology of the School of Medicine, University of Buenos Aires, Argentina, and at present at the Department of Epidemiology and Public Health of the Yale University School of Medicine, New Haven, Connecticut, U.S.A.

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1. HISTORICAL BACKGROUND

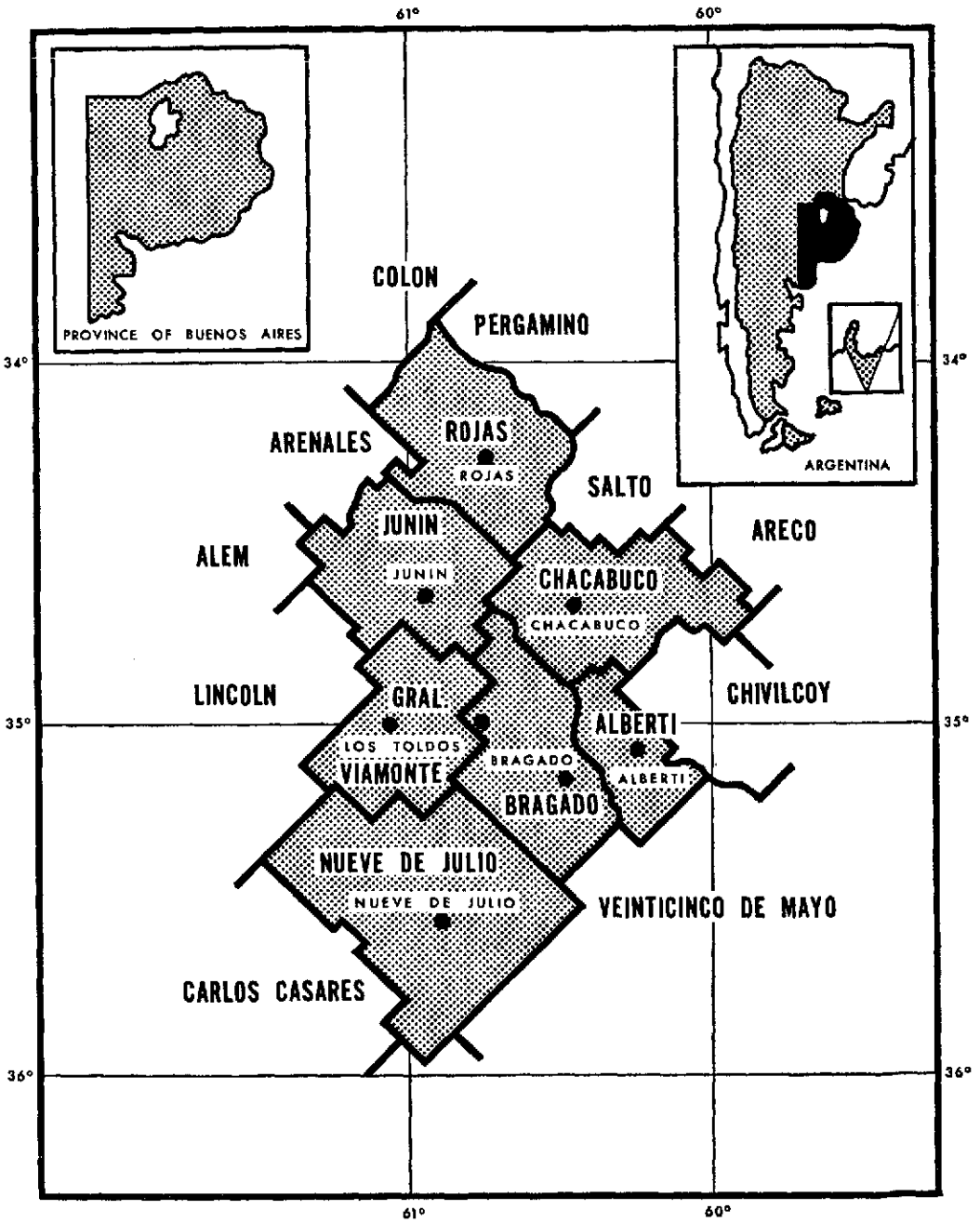
The endemo-epidemic disease that is the subject of this essay has been observed for a great many years in Argentina, in the northwestern part of the Province of Buenos Aires. Currently named Argentine hemorrhagic fever (AHF), after the country in which it was first described, it has been known in the past by a variety of other names—*enfermedad del sello, mal de los rastrojos* ("stubble disease"), *mal de O'Higgins* ("O'Higgins' disease"), *enfermedad de Junín* ("Junín disease"), *gripón* ("severe flu"), and so on.

It is difficult if not impossible to determine the exact year in which this malady was first observed. According to the data collected by Martínez Pintos (*54*), epidemics of variable intensity, reaching their peak in the fall of the year (April and May) and sometimes showing a high rate of mortality, have been recorded since 1943. Preliminary observations were made at the Julio de Vedia Hospital in Nueve de Julio Parish, Province of Buenos Aires (Fig. 1), where the clinical symptoms were thought to be due to "malignant grippe."

The first mention of the disease in the literature comes from Arribalzaga (*5*), who reported "a new epidemic disease of unknown etiology: nephrotoxic, leukopenic, and [characterized by] enanthematous hyperthermia." The first cases were observed in the city of Bragado, Province of Buenos Aires, in 1953 and 1954. The incidence was highest among rural laborers, and the first patients were potato harvesters who had been working in the outskirts of Bragado and Alberti. Later the epidemics spread to the urban areas. The 1953 epidemic started with sporadic cases early in the year, rapidly came to a peak in April and May, and tapered off slowly, to disappear after August. The following year morbidity was lower but the characteristics were basically the same. Cases did not break out simultaneously in the same household or in institutions. Males were more frequently affected than females, and only a few cases in children, 10 to 12 years old, were observed. At the time of the epidemics, Arribalzaga looked, without success, for epizootic diseases in the afflicted area and for changes in weather or sanitary conditions that might account for the outbreak. Attempts to isolate viruses, Leptospira, or bacteria from patients were also unsuccessful. He concluded, on the basis of differential diagnosis, that a virus was probably responsible for the disease. It was postulated that the source of contamination was something in the field, since all the cases but two failed to provide evidence of direct human-to-human transmission (*5*).

In 1956, Domingo Duva published a report (*34*) on 25 patients whom he attended personally from February to September 1953 and from April to August 1954 in Mechita, a small town in Bragado Parish. Mechita, with a population of 2,150, is a typical rural area with unpaved streets and idle land even in the center of town. Of the 25 patients, 5 were youngsters between 12 and 16, and the remaining 20 were adults—16 men and 4 women. None of them had been working in the fields. The cases were scattered about the town. Pigs, rabbits, dogs,

FIG. 1. PROVINCE OF BUENOS AIRES, ARGENTINA: NORTHWESTERN PARISHES IN WHICH AHF WAS FIRST RECORDED



(REPRODUCED FROM PIROSKY, et al, 1959)

and an unusual number of rats were found nearby the patients' homes. Remarking on the lack of interhuman contagion, Duva considered the possibility of a leptospiral infection.

Though there are no publications or official records on cases during 1955, 1956, or 1957, physicians in the endemic area claimed that indeed there had been cases every year and that their attempts to report them to public authorities had met with unsuccessful results. The situation came to a head, however, in 1958. It so happened that the Minister of Public Health, a physician with knowledge of the problem, had landholdings in the endemic area. The epidemic that year was more severe than ever, and it received wide publicity through newspapers, radio, and television. All these circumstances combined to press the public health authorities into action.

At last local physicians had support from the public and the government. Work could start on looking for the etiology of the disease and developing preventive measures. Commissions were organized. One, headed by Dr. Pirosky, was appointed by the National Ministry of Public Health; another, under Dr. Martínez Pintos, was set up by the Provincial Ministry of Public Health; a third was organized by the Medical School of Buenos Aires University at the invitation of local medical groups in the endemic area. This last team was made up of personnel from the Department of Infectious Diseases, then headed by Dr. Humberto Ruggiero, and the Department of Microbiology and Parasitology, under Dr. Daniel Greenway. The present author was engaged in a full-time program of teaching and research at the latter department during this period.

Field trips from the city of Buenos Aires, where the laboratories were located, to the endemic area, especially the Junín Regional Hospital, were conducted to collect materials for the isolation of infectious agents. Blood, spinal fluid, urine, feces, and pieces of organs from fatal cases were inoculated in experimental animals and in media for growing bacteria and fungi. The search was centered on pathogenic microorganisms, viruses and Leptospira in particular, that would be most likely to be involved because of the epidemiological pattern.

Guinea pigs proved to be the most susceptible of the animals used. They developed a pathological picture similar to the human disease, with exaggerated hemorrhagic manifestations.

From three patients the Buenos Aires University team isolated three strains of an agent—in one instance from the blood, in another from the urine (a case with hematuria), and in the third from organs of a fatal case (*81*). The agent was named "Junín virus," because the work had been performed with materials collected from the Regional Hospital in the city of Junín. Independently, Dr. Pirosky's team isolated a virus with which it was possible to reproduce the disease in man (*93*). Even though the agent isolated by the Buenos Aires University group was also capable of producing the disease in humans (*108*), it was not until 1961 that the viruses isolated by both teams were compared to determine whether they were one single agent or different viruses producing similar clinical manifestations (*69*).

Studies directed toward finding Junín virus in nature were started, and successful isolations were achieved with wild mice (*79*) and mites (*85*). The agent's presence in wild mice and in an arthropod, plus its sensitivity to sodium desoxycholate and the lack of interhuman infection, gave reason to place it in the arthropod-borne group of viruses.

Junín was tested against all the other viruses in the collection of The Rockefeller Foundation Virus Laboratories and found to bear an antigenic relationship to a strain of Tacaribe virus isolated from bats in Port of Spain, Trinidad, in 1956 (*32*). Since

Tacaribe had primacy, a new complex with this name was born. Further isolations of Junín from patients and wild animals in nature by different laboratories resulted in the collection of some 100 or more strains.

Shortly after the isolation of Junín virus, an epidemic with clinical symptomatology similar to that of AHF appeared in Bolivia. Serological study showed the presence of antibodies against Junín virus in convalescents (*129*). The following year, in a similar outbreak in San Joaquín Valley, Bolivia, another virus was isolated and named Machupo. This agent showed a relationship to Junín in the complement-fixation test but not in the serum neutralization test. (*46*).

Still another agent, designated Portillo virus, was isolated at the Children's Hospital in the city of Buenos Aires in 1963 in the course of an etiological study of a children's disease known as uremic-hemolytic syndrome. This virus, shown to be related to Tacaribe and Junín by complement-fixation (*70*), is currently under study at Yale University to fully determine its relationship to the other members of the Tacaribe group.

Shortly after the designation of Portillo virus, the Belém Laboratories in Brazil announced that their team had isolated a virus related to the Tacaribe group from wild rodents. This agent was recognized as a new member and given the name Amaparí (*87*). In 1966, Pichinde virus, also related to the group, was isolated from wild rodents in Colombia. This agent is currently under study at the World Reference Center for Arboviruses at Yale University (*122*). And finally, not long ago the Communicable Disease Center in Atlanta, Georgia, isolated two strains from wild rodents trapped in Tampa, Florida, and found the virus to be related to Amaparí and Pichinde. It has been named Tamiami (Chamberlain, personal communication).

Thus, the Tacaribe group now has the following seven members: Tacaribe, Junín, Portillo, Machupo, Amaparí, Pichinde, and Tamiami. Further investigations will define the relationships among the various members of this group. In the course of isolating these agents, two important facts have been brought to light. One is that viruses from the Tacaribe group have been isolated from wild rodents in the United States. The other is that the uremic-hemolytic syndrome, a clinical condition of unknown etiology reported in the United States (*60*), has the same clinical manifestations as the disease in Argentina which has been proven in at least 60 per cent of the cases studied to be caused by an agent of the Tacaribe group (*66*). Clearly, there is a need for better dissemination of knowledge about human disease produced by viruses of the Tacaribe group. The present paper is intended to make available in English a summary of the knowledge acquired to date about Argentine hemorrhagic fever—which has been traced in at least 70 per cent of the cases to Junín virus.

2. GENERAL ECOLOGY OF ARGENTINA

Geography

Argentina owes its wide range of climate to sharp variations in altitude and a latitudinal extension of over 33 degrees, from 21°55′S to 55°3′S. The wedge-shaped continental territory, 3,700 km long and 1,700 km across at its widest point, covers approximately 2,800,000 km² (Fig. 2).

The Andean mountain chain, which extends the entire length of South America, forms a natural division between Argentina and Chile. In the north, the Andean ranges extend east through approximately one third of the Argentine territory, but farther south the width of the mountainous border diminishes sharply. All the territory east of this chain, comprising by far the greater part of the country, has the character of a plain, rising from sea level at the Atlantic coast to the Andean foothills. The highest elevation in the Argentine Andes is the Aconcagua, a 7,021-meter peak north of the Río Negro. In the Province of Córdoba there are three short parallel ranges belonging to another formation older than the Andes.

In all parts of the republic, January is the warmest month of the year, and June and July are the coolest.

The main watersheds belong to the La Plata system, which is made up of the Paraná, Uruguay, Pilcomayo, Bermejo, Paraguay, and La Plata rivers. Other important rivers are the Colorado, the Negro, the Chubut, and the Deseado, which form the Patagonian system and flow into the Atlantic. In the interior there is the Andean snow-fed Desaguadero system. And finally, there is the Dulce river, which starts in the highlands, undergoes various name changes, and finally flows into the Mar Chiquita lagoon in the Province of Córdoba.

The railroad system, which has a total of 44,000 km of track, stems outward from the port of Buenos Aires and connects with lines in neighboring Chile, Bolivia, and Paraguay. The main roads also fan outward from the capital. Neither the railroads nor the highways seem to bear any direct relation to the spread of AHF, though there are numerous fences along these routes, and also between fields, that constitute an important shelter for rodents.

There is aviation service to all the major cities of the interior. Exchanges with international service are made at the heavily trafficked Ezeiza Airport, which is situated a few miles from the city of Buenos Aires.

Administratively, Argentina is divided into a federal district, 22 provinces, and a territory. The Federal District (city of Buenos Aires) is the political, economic, and cultural capital of the country. The provinces most affected by AHF are Buenos Aires, Santa Fe, La Pampa, and Córdoba.

The Argentine people, some 23,000,000 strong, are generally first, second, or third generation Europeans, the heaviest migrations having come from Spain and Italy, with smaller groups from Germany, England, France, Belgium, Holland, and Switzerland. Spanish is the national language—and therefore the language in which much of the existing literature on AHF has been published. In general, the country is sparsely populated, with an average density of about seven per-

FIG. 2. ARGENTINA: MAJOR POLITICAL SUBDIVISIONS; PRINCIPAL RIVERS, ROADS, AND RAILROADS

sons per square kilometer, but there is a high concentration in Greater Buenos Aires, where some 7,000,000 people have their homes.

Economy

Argentina is essentially an agricultural country. Around 5,220,000 tons of corn are harvested each year from 3,420,000 hectares, mainly in the AHF endemic area. Wheat is cultivated on about 5,250,000 hectares and yields over 5,000,000 tons a year. Nearly half of this produce—2,570,000 tons of corn and 2,500,000 tons of wheat—is exported annually. Flax is abundant, and Argentina is the first producer of this oleaginous plant in the world. Oats, barley, and rye are also cultivated on a broad scale.

The bulk of the nation's agricultural products, with certain exceptions, come from the AHF endemic area. The exceptions are sugar cane, from Tucumán, Salta, and Jujuy; cotton, from Chaco, Formosa, Corrientes, and Santiago del Estero; grapes, from Mendoza, San Juan, and the Río Negro Valley; and citrus fruits, apples, and pears from Río Negro, Entre Ríos, Corrientes, Misiones, Salta, Tucumán, and Jujuy.

Forests cover more than 100,000 hectares. Quebracho extract and timber are the most important forest products, and they come mainly from the Province of Misiones.

Argentina's fishing industry is greatly underexploited. Though the country has a "marine pampa" extending along the Atlantic coast from the La Plata river to the Beagle canal and covering an area of 1,000,000 km^2, there are only a few fishing centers—among the most important, Mar del Plata, Necochea, and Rawson (Chubut).

The country also has vast mineral wealth potential, but so far the only important efforts at exploitation have been limited to petroleum and iron, mainly in Patagonia and in the northwestern provinces—Salta, Jujuy, and Mendoza.

Meat, grapes, wine, sugar, oil, yerba mate (Paraguayan tea), and textile manufactures are old and characteristic Argentine industries. Heavy and light industries, such as the manufacture of cars, refrigerators, television sets, and petrochemical products—plastics, synthetic detergents, india rubber, insecticides, synthetic fibers, and the like—have been developed since World War II.

Cattle raising, which already thrived during the Spanish colonial period, is the oldest and most important rural activity. Argentina is the world's number one exporter of meat. Of approximately 43,500,000 head of cattle, most are concentrated in the provinces of Buenos Aires, Santa Fe, Corrientes, and Entre Ríos. Sheep raising is extensive mainly in the southern provinces. In all, the country has about 50,000,000 sheep, and its wool exports place it internationally in third place, after Australia and New Zealand. There are about 7,300,000 horses, which are raised mainly in the provinces of Buenos Aires, Santa Fe, and La Pampa.

Endemic Area

General description

The AHF endemic area basically includes the provinces of Buenos Aires and La Pampa, the southern part of Santa Fe, and the eastern part of Córdoba. It is situated in the richest part of the country, commonly referred to as the Pampa region. This district includes the most populous provinces and is scored by many principal and secondary roads and railways. It has a temperate, healthful climate. Though it enjoys a large amount of sunshine, generally there is adequate rainfall as well. The area extends over nearly 10 degrees of latitude, but the climate shows little variation. It never snows. Light frost may sometimes occur during the cold months, but the vegetation is never frozen.

The advent of European civilization in Argentina produced a marked change in the flora of this region. Useful trees and plants from every part of the world—cereals, alfalfa, grasses, and all varieties of fruit—were introduced. The Australian eucalyptus, in particular, thrives in the Pampa. Other varieties that have been planted extensively include the acacia, the sycamore, the paradise tree, and several types of evergreens. The trees are especially seen close to homes in the rural areas.

In winter the land is plowed in preparation for spring planting. In the spring there are fields of flax and wheat, and in the summer, corn. Alfalfa and pasture are seen at all times of the year. The fields are frequently from 50 to 80 hectares in size. During summers with humidity and abundant rain—for example, the summer of 1964—grassland vegetation is luxuriant. This condition is accompanied by an increase in the wild rodent population, because the vegetation provides abundant food as well as protection against predators such as owls and hawks. If the rodent population gets too high, epizootics and intra- and interspecific fighting—similar to the 1958 Microtus crash in Oregon—develop among these animals by the end of the winter (*30*).

Most of the towns in the endemic area are small. Generally the streets are unpaved and there is no running water or sewage disposal system. Distances between large cities with proper sanitary facilities are usually quite great.

The rural dwellings, scattered over the large areas between the towns, are typically made of adobe, rarely of concrete. The floors and patios are made of brick or plain earth; roofs are of wood and zinc; and the insulation usually consists of adobe, straw, or sometimes wattle and daub. A home generally has one or two bedrooms and an adjoining room that serves as kitchen and dining room. Tin sheds, chicken coops, and pigpens are found near the house. The privy installed over a cesspool only a few feet from the house completes this unit. Drinking water is obtained from a pump, well, spring, or cistern. The boundaries between these dwellings and the open fields are wire fences, reed grass, or shrubs and do not form a barrier for wild rodents.

The corn and sunflower harvest coincides with the autumnal peak of the AHF epidemic cycles (April and June). The manual harvesting of the crops employs a great number of laborers from the town or from other provinces, and usually an entire family, including women and children, participates. During this time the laborers, most often coming from other places, build huts of maize stems and straw on the ground over the field where the crop is being harvested. Thus, both their working and leisure time is spent in intimate proximity with the wild rodents.

Fauna: wild and domestic

The Pampa has a great variety of bird life, including hawks, falcons, owls, herons, storks, swans, partridge, plovers, ducks, chajas, and many other species known by native names. Frogs, toads, and ants are ubiquitous.

The search for the etiological agent of AHF in the animal reservoir was concentrated mainly on wild rodents because of their high population density in the corn fields.

Field rats and mice (suborder Myomorpha, family Cricetidae) are present not only in the cornfields but also in the untilled lands surrounding the towns in the endemic area. There are many South American species in the Myomorpha suborder, among them *Akodon arenicola hunteri, Akodon arenicola beatus, Hesperomys murillus murillus, Hesperomys laucha,* and *Oryzomys flavescens* (*54*). *Akodon arenicola hunteri* stays away from the human habitat; even when all kinds of food are available it prefers to eat dead congener, regardless of whether it is

in captivity or in a wild environment. *Hesperomys laucha* seems to be present only in the eastern Pampa in the Province of Buenos Aires (A. Cabrera and J. Yepes). *Hesperomys bimaculatus bonaerensis* is considered a geographic form of *H. bimaculatus bimaculatus* (A. Cabrera *et al.*). It is found almost exclusively in the northeastern part of the Province of Buenos Aires. *Hesperomys murillus murillus* is a species of the Argentine Pampa that inhabits the Province of Buenos Aires and eastern Córdoba. In the central provinces and toward the southwest, another subspecies, *H. murillus cordobensis,* is recognized (A. Cabrera and J. Yepes). *Oryzomys flavences* is present in a large area of Argentina and the southern part of Uruguay, except at high altitudes.

In addition to Cricetidae and native South American mice, there are also rats and mice imported from Europe. These latter rodents reproduce in such numbers in the endemic area that they are considered real pests.

The Rattus genus is represented by two species, the black rat (*Rattus rattus rattus*) and the Norwegian rat (*Rattus norvegicus*). These species are enemies and never live together. They migrate en masse during the summertime and are not even stopped by rivers.

With respect to naturalized exotic species of Muridae, *Mus musculus musculus* has been reported to be a plague in the Province of Buenos Aires (R. Ringuelet and R. H. Aramburu, 1957). It lives close to man, invading human habitations and nesting in the furniture or in quiet places of the house. It eats food found in people's homes—fruit, cheese, candy, and the like, and it also chews on materials such as wood, paper, and clothing to make a powder for its nest. It is also found away from houses in big nests in the stubble. Two other species of the genus Mus were described by José Yepes in 1935: *M. musculus* Linn. and *M. musculus brevirostis* Waterh. The first is found throughout the entire country, and the second in the eastern part, close to La Plata river.

Other rodents abundant in the area include those of the Caviidae family, Cabiinae subfamily. *Cavia pamparum* is a plague in the Pampa. It is similar to the common guinea pig, but lives in the wild state. Other members of this family are *Microcavia australis* and *Galea musteloides littoralis;* both shun man.

As a consequence of the discovery of sylvatic plague in Argentina by Dr. de la Barrera, many studies dealing with the distribution of wild animals in the country were conducted. These studies are very useful, especially in showing the distribution of the rodents from which Junín virus has been isolated (*119*).

Other wild animals very common in the endemic area are *Ctenomydae porteonsi porteonsi* and the skunk, *Conepatus suffocans gibsoni,* usually seen along the roadsides. From the order Marsupialia there are opossums, of which the *Didelphis paraguayensis paraguayensis* and the red opossum, or *Lutreolina crassicaudata bonari,* are real pests. Among the bats, *Myotis albescens, Histiotus montanus, Lasiurus borealis blosseoillii, Lasiurus cinereus villosissius,* and *Tadarida brasiliensis* have been recognized in Argentina. The hare, order *Lagomorfos,* family Leporidae, *Lepus europaeus europaeus* is extremely abundant, and so is the partridge. Both species are avidly hunted as food delicacies. In the process, there is ample opportunity for the hunters to come in contact with reservoirs of AHF.

Among the less common species are the viscacha (*Lagostomus maximus maximus*), the quirquincho (family Dasypodidae, species *Chaetaphractus villosus,* and the pampean mulita (*Masypus septemcinetus*), which are seen less frequently every day. The same is true of the coypu (*Myocastor coypus bonariensis*), the common fox

(*Pseudalopex gymnocercus*), and the minor ferret (*Galictis cuja huronax*). The "cat of the straw" (*Lynchailurus pajeros pajeros*) and the *Oncifelis geoftroyi* have almost disappeared (*54*).

Dogs and cats, the good friends of man, are always present in the home.

As stated before, cattle, horses, sheep, and pigs exist in large numbers and constitute one of the area's major sources of wealth. The poultry usually found close to homes are chickens, hens, ducks, turkeys, and geese.

As a rule, both wild and domestic animals have a great number of ectoparasites—mites, fleas, and the like. In the Province of Buenos Aires at least 6 families, 8 subfamilies, 12 genera, and 19 species of fleas have been identified (*20*).

Although the area has an abundance of mosquitoes, no attempts at virus isolation have been made.

3. EPIDEMIOLOGICAL OBSERVATIONS

The high incidence of AHF among corn harvesters is so well recognized that the affliction is called "stubble disease." Though the present trend in the area is toward mechanized picking, there are times when it is impossible to harvest corn with machines. This is the case, for example, when the plants have fallen over because of strong winds.

Although AHF bears an apparent relationship to the rural environment, frequently there are patients who have not left the towns or cities in the epidemic area. These urban areas, however, are not free of rodents.

It is noteworthy that the annual period of the epidemics coincides with the rainy season and that, in a given outbreak, weekly and monthly fluctuations in the number of patients interned at the public medical centers appear to be related to climatic factors, especially temperature and rainfall (*54*).

The morbidity data since 1958 show considerable fluctuation in the annual incidence of the disease:

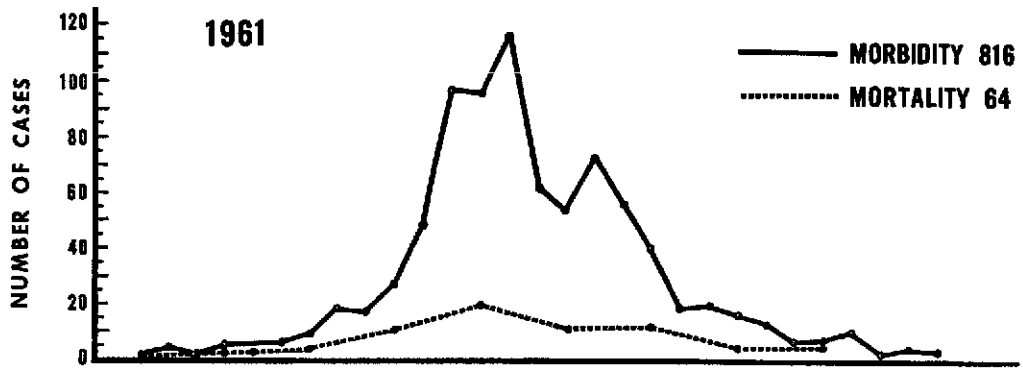
Year	Number of cases	Mortality (% of cases)
1958	283	19
1959	1,027	6.31
1960	335	6.27
1961	816	7.84
1962	362	?
1963	653	4.42
1964	3,026	?
1965	148	?
1966	643	?
1967 until Sept. 6	1,059	8.69

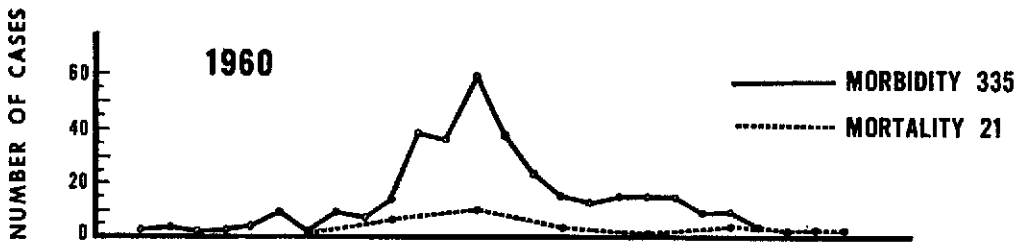
Mortality, however, has shown a tendency to decline, thanks to improved diagnostic and therapeutic measures that permit early recognition of the disease and timely treatment of its severe and typical forms. Delayed diagnosis of severe cases accounts for the high mortality reported during the first large epidemic in 1958. Also, in these earlier years, cases of medium and mild severity may have escaped detection. Thus, in more recent years, as the total number of reported cases increased, the proportion of deaths became lower.

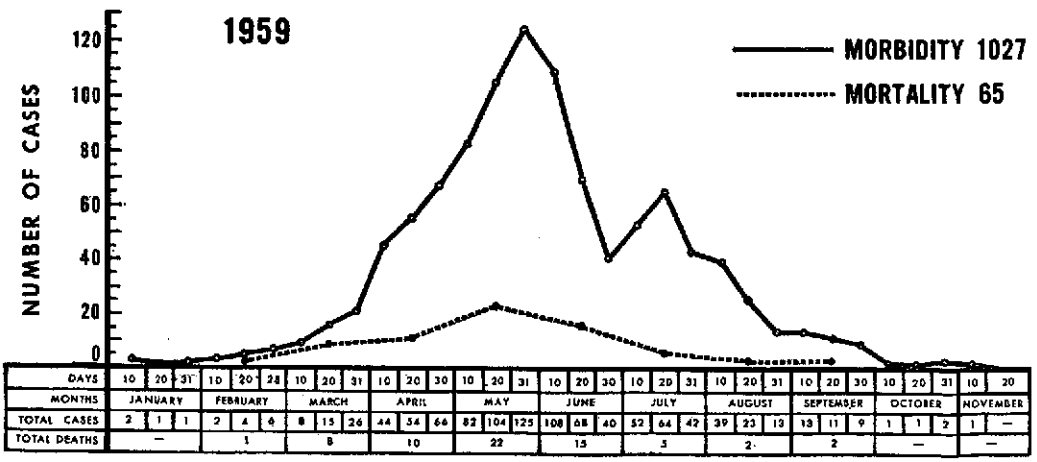
In general, the first cases occur late in March, the epidemic reaches its peak in May and June, and after that the cases decrease steadily during July and remain very low in August and September, when the morbidity curve drops to nearly zero (Fig. 3). It should be kept in mind that this distribution corresponds to the fall and winter months in the Southern Hemisphere. Only sporadic cases have been observed during the rest of the year.

Simultaneous outbreaks within a group of people working in the same field is a frequent observation and suggests the likelihood of infection from a common source. Human-to-human transmission has been almost entirely ruled out. Epidemiological observations have shown that human subjects are not sources of infection for the healthy population around them. Even with patients in the same room or ward in hospitals, only rarely has the disease developed among the medical staff or other persons in close contact with the patients (*111*). Nor could any data be found to support the pos-

FIG. 3. ARGENTINE HEMORRHAGIC FEVER: MORBIDITY AND MORTALITY CURVES, 1959, 1960, AND 1961







(Reproduced from Martínez Pintos, 1962)

sible role of food or drinking water in the transmission of AHF. When the disease appears in an area, it becomes an endemic focus for several years thereafter, suggesting that the source of infection is something that remains in the environment once it is established.

The endemic nature of AHF in certain localities in the Province of Buenos Aires is well explained by the presence of rodents infected with the virus (*79, 84, 112*). The same is true for the Province of Córdoba (*26, 124*).

There is a definite parallel between the density of rodents and the incidence of AHF. Seasonally, the rodent population is highest in autumn and winter, which is when the AHF epidemics are at their peak, and lowest in summer, when only sporadic cases are observed (*30*). Also, the lands directly associated with AHF infection are those devoted to field crops, where rodents are most populous, rather than cattle-raising lands. The great majority of cases occur among people living in primitive conditions out in the open, where contact with rodents is facilitated. Disease-afflicted homes are frequently located on the border of uninhabited grasslands or open fields. The few cases observed among people living in cities may be accounted for by brief trips to the countryside.

Repeated isolations of Junín virus from wild rodents (*26, 79, 84, 91, 112*), as well as several isolations from their ectoparasites (*4, 85, 89*), point convincingly to a connection between the appearance of AHF in humans and the presence of rodents infected with a virus from the zoonoses group, the disease probably being transmitted through the animals' excreta or their blood-sucking ectoparasites.

In 1959, 449 wild rodents were trapped in the endemic area. Of these, 52.8 per cent were *Mus musculus;* 24.4 per cent, *Hesperomys laucha* (Dumarest); 11.1 per cent, *Akodon arenicola;* 6.2 per cent, *Oryzomys flavences* (Waterhouse); and 5.3 per cent, *Rattus sp.* Ten strains of Junín virus were isolated from the *Mus musculus,* one from the *Hesperomys laucha,* and one from the *Akodon arenicola* (*79*). In the Province of Córdoba, 46 strains were isolated from *Calomys laucha* alone, and additional strains were taken from *Akodon azarae, Akodon obscurus, Calomys musculinus,* and *Oryzomys flavences* (*26*). Other workers have isolated Junín virus from wild guinea pigs, or *Cavia pamparum* (cuis), and from hares (*Lepus europaeus*) as well (*4, 22*). There may be an even wider distribution in nature, but not many attempts to isolate virus from other sources have been made to date.

With mouse ectoparasites, it was demonstrated by Pirosky and co-workers in 1959 that mites (*Mesostigmata* without further identification) were able to transmit the Junín virus infection experimentally (*89*). Further investigations in mites yielded Junín virus from *Echinolaelaps echidninus* (Berlese) taken from nests of naturally infected *Mus musculus* (*85*). Another successful isolation from mites was made in 1965, this time from *Eubrachilaelaps rotundus* taken from *Akodon azarae* (*4*).

Research has not yet shown exactly how the virus is transmitted from infected rodents to healthy people. It is not clear whether they become infected by contact with the rodents' excreted urine, or by their ectoparasites (Gamesoid mites), from which the virus has been isolated. Among the rodents themselves, it is most probable that circulation of the virus is maintained by the ectoparasites of the animals serving as vectors. Man can be infected without the intervention of arthropods; this has been proven by numerous Junín virus infections among laboratory workers. But whether or not this is true in the natural environment no one knows, even though a number of hypotheses may be formulated. If the human infections acquired in the field come

from an environment contaminated with the urine of infected rodents, without any intervention of arthropods, the lack of infection in summertime could be explained by the natural sterilization of the field by ultraviolet radiation from the sun.

Although there are few well-documented instances of human-to-human transmission, there is a theoretical possibility that with increased adaptation to man and the development of persistent chronic infection the virus may propagate by human contact in the absence of an arthropod vector.

From the public health standpoint, AHF is not the most serious problem in Argentina. In addition to the summer diarrhea and other diseases of children that constitute a major problem throughout all of South America, Chagas' disease and tuberculosis involve a great number of adults, and the sequelae usually cause permanent handicaps. However, the fact that AHF afflicts workers in the farthest reaches of the country at harvest time gives it great economic, as well as social and political, importance.

4. THE DISEASE

Clinical Description

AHF is an acute infectious disease of 7 to 15 days' duration followed by a long convalescent period of approximately one month; with few exceptions, there are no sequelae.

For the most part, the persons who contract the disease are newcomers in the endemic area who have contact with the field. The patients are usually males—corn harvesters or people involved in other rural activities. The disease is unkown or unrecognized in infants, and only few cases in children have been recorded. Morbidity is higher in males than in females, the ratio being approximately 5 to 1.

The clinical symptoms are not always uniform, but the typical case is diagnosed readily; the local physicians were so impressed by the signs and symptoms that it was named for its hallmark (*enfermedad del sello*). In a fully developed, "classical" set of symptoms and laboratory findings, the complex includes involvement of the renal, cardiovascular, hematic, and nervous systems.

This essay synthesizes the observations on the clinical characteristics of the disease made by different teams of investigators from 1955 up to the present (*3, 5, 35, 40, 57, 62, 74, 90, 91, 105, 106, 109, 110, 113, 114*), plus the personal observations of the author as part of a team and/or as an individual physician.

In general, the disease is considered to have mild, moderate, and severe forms, though sometimes the characteristics overlap. After an asymptomatic incubation period of 7 to 16 days, three stages can be differentiated: the initial period, the acute phase, and convalescence. In 3 to 30 per cent of the patients, depending on the severity of the epidemic, death occurs before the convalescent period. Although these three states are not found in all cases of AHF, the phasic course of the disease is beyond doubt, and the suggested division in distinct periods should lead to a better understanding of the essence of the pathological changes and their sequential appearance.

In the first stage—the initial or invasion period—the usual onset is vague with grippe-like symptoms, such as malaise, fatigue, chills, and lumbar backache. In around 5 per cent of the cases, however, the first symptoms appear suddenly with severe prostration and lumbar and limb pains. Fever and accentuated asthenia are always present regardless of the type of onset (*113*). The bulbar and palpebral conjunctivas are injected without any secretion or tearing. The face becomes edematous and flushed, as in measles. Headache, postorbital pain on moving the eyeballs, and subpalpebral edema are almost constant. Stiffness is present in the neck and the muscles of the costovertebral canals. The patient sometimes complains of feeling as if he were strapped down to a pale.

The patient is dull, drowsy, confused, and often apathetic. He does not respond immediately to questions. Tremor of the tongue during its protrusion is accentuated, as in general paresis of neurosyphilis. The

patient's gait is unsteady, and active movement is slow. Frequently there is tremor of the upper extremities when the arms are extended forward at shoulder level, as in hyperthyroidism. Another common symptom is noninflammatory painless generalized lymphadenopathy.

Enanthemas are present on the buccal mucosa. At the juncture of the soft and hard palate one can usually see a number of small hemorrhages, petechiae, and/or some twenty to thirty microvesicles of clear content, without color change in the surrounding area. They are not painful; they are present from four to six days, increasing in number during the acute phase (*114*).

Halitosis, anorexia, thirst, nausea, vomiting, and epigastralgia are sometimes present. Constipation is frequent, and only in a few patients does one observe a brief period of diarrhea. The feces in these cases are fluid–homogeneous, without mucus, but frequently with an admixture of fresh blood and sometimes melena. The diarrhea lasts two or three days and usually ceases without any therapeutic measures. Meteorism and cecal gurgling have been noted. In women, there may be uterine hemorrhages. Bradycardia with arterial hypotension is present in 90 per cent of the cases (*113*), and dizziness is observed in a large proportion as well. As a rule, the spleen and liver are not enlarged; palpation in the kidney area, however, is very painful.

The initial or invasion period lasts two to four days, and passage to the fully developed, or acute, stage is accompanied by an accentuation of the same symptomatology.

The fully developed disease usually presents hemorrhagic injury characterized by bleeding of the gums, epistaxis, and in some few severe cases, hematemesis, melena, and enterorrhagia. A rash first appears on the trunk, primarily in the axillae and on the chest, and spreads to the extremities. It is usually petechial, and in some cases confluent, so that large areas of the skin assume a purpuric appearance.

The fever is of a remittent type; the pulse is slow compared with the temperature rise; and arterial hypotension, usually moderate, is observed in all patients. If the blood pressure drops precipitously, this is a sign of grave prognostic significance, particularly if associated with oliguria. Marked decrease in diuresis, sometimes resulting in complete anuria in the second period, as often observed in 1958, may be found (*109*). In these cases, the oliguria or anuria is followed by prolonged polyuria; in others, the development of acute renal failure leads to uremic coma, followed by death. Signs indicating progressive central nervous system involvement, ranging from disorientation and delirium through convulsions to stupor and finally coma, as terminal manifestations, are frequently seen in severe cases. A characteristic odor, different from that of any other ward in the same hospital, is present wherever AHF patients are confined.

Although the patient is extremely thirsty, he refuses to drink, in order to avoid spasms of the laryngeal and pharyngeal muscles, as observed in rabies.

When the disease is at its height, the patient's temperature may reach 39° to 40°C. In this stage, cutaneous hyperesthesia and contraction of the muscles under the skin are observed upon stimulation. Photophobia, strabismus, and nystagmus may also be seen.

In some patients, gastrointestinal disorders are conspicuous. The tongue is dry, and coated with a thick brown layer. Halitosis, with an odor of decay, especially in cases with bleeding of the gums, oral mucosa, and nasopharynx, is usually present.

Changes in the respiratory organs are usually slight and infrequent; the most common finding is a dry cough.

The final, or convalescent, period begins with a drop in temperature and usually

lasts two to four weeks, or even longer in severe cases. During the recovery period, the fever diminishes by lysis. Hyperemia of the face and mucous membranes disappears and the hemorrhages on the skin are reabsorbed. The blood pressure and pulse rate become normal, and only changes detectable in the clinical laboratory may remain. Physical examination may only reveal a general weakness and a tendency to fatigue, which can persist for some weeks.

During this period some patients have a special gait similar to that presented by dengue. Usually in convalescence there is a partial and gradual loss of hair, followed eventually by the growth of new hair to supplant what was lost. This is especially noted among women. Even if all the hair is changed, there is never a time of complete alopecia.

The recovery is usually without sequelae, though there have been a few cases in which parkinsonism was present for one or two years and others in which albuminuria persisted for a long time. Uncomplicated cases are seldom fatal; the prognosis is negative only in cases with serious nervous disturbances or severe hemorrhagic manifestations or both—especially when there are hematemesis, melena, and enterorrhagias—and also in persons with alcoholic habits, and in the aged. During the recovery period latent chronic infections such as amebiasis may exacerbate.

In 1.2 to 1.8 per cent of the cases, relapses were observed 15 days or a month after the original onset, with repetition of the previous symptomatology (*106*).

Diagnosis

Differential diagnosis

AHF must be differentiated from other acute infectious diseases that share some of the same symptomatology, such as influenza, rickettsiosis, leptospirosis, toxoplasmosis, and typhus.

Laboratory investigation is necessary in order to make a differential diagnosis. Sometimes it is impossible to attempt to differentiate this disease from others on clinical grounds alone. Etiological investigation through serology and isolation of the infectious agent will confirm or reject any of the diagnoses mentioned above.

Clinical laboratory diagnosis

The routine laboratory procedures, such as complete blood count and urinalysis, are of great value in diagnosis. Generally, a clinical picture such as that described in the previous section, associated with thrombocytopenia, leukopenia, and special cells in the urine sediment, will be labeled as AHF until such time as a contrary diagnosis can be demonstrated.

Blood

Regardless of the severity of the disease, the laboratory findings will generally reveal a normal or lowered erythrocyte sedimentation rate during the first week (1 to 7 mm in the first hour, using Westergren and Wintrobe). This value rises during the second and third week, particularly if bacterial complications develop. In mild cases it remains normal. The hematocrit shows normal value rises, except in cases of severe dehydration, where the increase in cells is proportionate to the degree of hemoconcentration.

The number of red cells is usually normal at all stages of the disease, except when the hemorrhagic manifestations are exaggerated and there is consequent anemia in proportion to the blood loss.

Leukopenia due to granulopenia is invariably found. The total white blood cell count begins to fall by the second day of fever and drops to a low of 1,900 to 3,000 by the fourth or fifth day after onset. Cases with values as low as 400 leukocytes per

mm^3 have been observed. The leukopenia is accompanied by aneosinophilia and neutropenia (*5, 91, 109, 126*).

The neutrophils suffer severe alterations, with marked anisocytosis and toxic granulations. There are a great number of neutrophils with crooked-shaped nuclei. The nuclei of the neutrophilic granulocytes are marginated.

There is nuclear chromodiffusion, sometimes accompanied by pycnotic condensations of the chromatin, with partial breakage of the nuclear membrane, which give the appearance of flaked nuclei.

At the peak of infection, atypical mononuclears, some of them showing vacuolation of nuclei and cytoplasm similar to hystiocytes, are characteristic findings (*126*).

Recovery starts at the same time that the oliguria disappears, the number of white blood cells begins to increase either slowly or suddenly, and leukocytosis appears. The corresponding normal white cell differential count signifies the reappearance of eosinophils and monocytes with normal nuclei and the disappearance of any toxic granulations in neutrophils and plasmatic cells that may have been present. Any earlier increase in white cells than what would be expected from the normal course of disease suggests a focus of bacterial infection somewhere in the organism.

The number of platelets decreases somewhat during the initial stage, and even more sharply at the height of the disease. Values as low as 6,000 per mm^3 have been encountered. The average in most cases is between 30,000 and 40,000 per mm^3. Giant and morphologically abnormal platelets may be seen on smears. During convalescence the number returns to normal.

The coagulation time is longer than normal. In severe cases, periods of as much as an hour, with the blood kept in a tube at 37°C, have been observed. Clot retraction is incomplete (*110, 127*). Using more sophisticated techniques such as the thromboelastographic method, it was demonstrated that 65 per cent of the patients present hypocoagulability during the acute stage, while in convalescence the tracings are normal in 77 per cent of the cases, and sometimes even a reactional hypercoagulability is present. Only in a very few cases does the hypocoagulability persist in convalescence. The hypocoagulability is translated by the lengthening of "r" and "k" and the narrowing of "am" (*100*). Fibrinogen is found to be lowered in cases of marked hypocoagulability, and occasionally fibrinolysis is observed.

Bleeding time is increased. The plasma prothrombin time and concentration are normal.

The ionic calcium is normal or lowered during the initial and acute periods, and always normal during convalescence.

The total proteins in blood plasma are in the lower normal range. The zonal electrophoresis of the proteins generally shows hypoalbuminemia with a moderate increase in alpha 1 and 2 globulins, a normobeta globulin level, and a normogamma globulin level. During convalescence the hypoalbuminemia still remains, but the gamma globulin increases, and thus the inversion of the proteic rate does so too (*37, 107*).

The total lipemia and cholesterolemia may reach very low values; cholesterol ranges of from 58 to 138 mg/100 ml have been observed. The direct bilirubin is negative and the indirect is in the normal range, only having shown to be increased in a few fatal cases. Liver function tests, thymol turbidity, and Hanger's test also give normal results.

The electrolytes are without severe alterations except in grave cases where hyponatremia and hypochloremia, accompanied by a slight decrease in the CO_2 combining power, have been observed. Potassium is normal or increased. Blood sugar is increased to values of from 120 to 150 mg/100 ml, but it gradually returns to

normal in days or weeks. In some patients, values as high as 200 mg/100 ml late in the acute period, followed by a sudden normalization of the sugar level in about two days, have been reported.

The urea is normal or increased; cases of 40 mg/100 ml, with oliguria or anuria present, have been recorded. When the anuria or oliguria last, the values drop sharply to normal in one or two days.

The white cell count, the differential white cell count, the number of platelets, and bleeding and coagulation times normalize their values independently of the evolution of the disease, whether toward cure or death.

In bone marrow there are macrophages with leukocytes phagocyted—a fact that may account for the central destruction of white cells reported by Vucetich (*126*). During convalescence the red cells of the bone marrow reveal hypofunction.

CSF spinal fluid findings have been normal even in the presence of spinal muscular contraction.

Urine

Albuminuria begins to show in the initial period and increases to 0.10 to 0.50 g/1000 ml during the acute stage. Those patients whose initial albuminuria disappears have a second rise to 3 or 4 g/1000 ml lasting one or two days, with normalization immediately thereafter.

Cylindruria is present early in the disease, sometimes preceding albuminuria. The amount of cylinders is unrelated to the degree of albuminuria. In general, the cylinders are hyaline, hyaline-granulose, and epithelial; some are erythrocytic.

Vacuolated epithelial cells are a characteristic finding in the urine and are used as a criterion for diagnosis. First described by Milani, these cells were studied in detail by Palatnik (*75, 76*). They are 18 to 45 μ in size—about twice the size of a lymphocyte. They have hyaline, intensely eosinophilic inclusions, which stand out sharply in the cytoplasm. The inclusions—round, half-moon, or ring shaped—are 10 to 26 μ in size. In the later stages of the disease large inclusion bodies, along with granular inclusion forms, are observed in the cytoplasm. The inclusion maintains a stable configuration during the initial and acute stages. At the beginning of convalescence the forms decrease in size and number. Soon thereafter, they disappear, and they are not present in the late stage of convalescence, after the disease is over, or in the case of relapse. The inclusions are Fuelgen negative, but they have Fuelgen positive corpuscles; with PAS they present a typical and stable morphology. Palatnik suggests that the granules represent viral material and that the inclusions are related to the intracellular cycle of the virus.

Pyocytes and erythrocytes are often seen throughout the entire course of the disease, and for this reason a daily check of the urine is advisable. Macrohematuria was observed in only a few cases. The 17-ketosteroids are lowered, especially in severe cases.

ECG

Modifications of the ECG were found in 71 per cent of the patients. The most characteristic alterations consisted of an elevated ST interval, with upper concavity, followed by an exaggerated T wave. In 20 per cent of the patients a flat or inverted T wave was found. Sometimes the P wave had low voltage and the PR interval was extended. The QT interval was found to extend moderately in 14 per cent of the patients. The U wave was found in 9 per cent of the cases (*101*).

Low voltage was an infrequent manifestation, as were nodal rhythm and ventricular extrasystoles. No bundle branch heart blocks were observed. An electroballistocardiographic study of eleven convalescents showed normal variants of the

cardiogram in nine cases and probable myocardial injury in two of the cases (*86*).

Pathology

Pathological changes at various stages of the disease have been described by Rivero *et al.* (*97*) and Polak and Jufe (*94*). The lesions or changes were not specific for AHF and had features similar to those of other diseases and infections accompanied by hemorrhagic diatheses, particularly other hemorrhagic fevers (*48, 118*).

Postmortem examination reveals alterations corresponding to the lesions produced by the virus itself or to intercurrent infections associated with the cause of death. The alterations produced by the virus itself correspond to perturbations of the blood capillary system, reactivation of the reticuloendothelial system, and areas of tissue degeneration, as described below.

Gross lesions

The gross anatomy shows general congestion and various degrees of hemorrhages on the skin and in some of the organs, especially the lung and spleen. Generalized lymphadenopathy appears in almost every case. Infiltration resembling that of bronchopneumonia, necrotic areas in the parenchyma of the lung, and even pleural effusion have been seen. Renal damage, as in nephrosis with acute glomerulitis, is usually observed.

Microscopic lesions

The dominant changes are vascular; vasodilation, with congestion, edema, and hemorrhages, is the main presenting symptom. The chief abnormality occurs in and about the small blood vessels and consists of endothelial swelling, perivascular edema, and diapedetic hemorrhages. There is a cellular reaction characterized by hyperplasia in the reticulohistiocytary system of the lymph nodes, tonsils, spleen, and bone marrow; in the bone marrow there are no mature neutrophil or eosinophil leukocytes.

Dystrophic changes are present in the parenchyma of the organs and in the blood vessels. In the liver, hepatic cells show granular degeneration of the cytoplasm. The central vein is dilated, and the sinusoids contain a deposit of bile pigment. Edema is observed in the connective tissue surrounding the dilated central vein, and lymphocytic infiltration is seen in some of the Kiernan's spaces.

The kidney alterations are mainly in the proximal tubules, where the epithelial cells show alteration of the nucleus, granular cytoplasm, and an indefinite border. The lumina are obstructed by hyaline and cellular casts. Acute glomerulitis is present, with general congestion, microscopic hemorrhages, and edema, as in the other organs. The microdissection of the tubules done by Aoki *et al.* (*2*) showed renal alterations similar to those observed with Korean hemorrhagic fever.

In the spleen, hyaline degeneration of the corpuscular arteries, with focal modifications in the sinuses, is observed.

In the lungs, inspecific bronchopneumonic foci and interalveolar hemorrhages are present.

There is congestion with marked meningeal edema in the brain. Rovere *et al.* claimed the presence of Lafora's amyloid bodies in the cerebral tissue (*98, 99*).

When intercurrent infections are the cause of death, the pathology corresponds to alterations caused by the virus itself at the stage of the disease when death occurred, plus the changes corresponding to the intercurrent infections.

Treatment

Symptomatic treatment and good nursing care are of the utmost importance. There is

no specific treatment except for serum transfusions (*vide infra*). It is imperative to put the patient in bed as soon as possible. The most severe cases appear in persons who continue to work in spite of general malaise and muscular pain. Early rest helps to prevent the disease from becoming severe.

Even though the dry tongue and skin may justify the ample use of fluids in treatment, it is necessary to bear in mind the renal involvement and observe caution in administering liquids. Only in severe cases where vomiting and absolute anorexia are present are parenteral fluids advisable, and then acid-base determinations should be used for control. Otherwise, parenteral injections must be avoided. They can provide means of entry for secondary bacterial infections, and, in addition, because of the hemorrhagic diathesis present in these patients, they can cause hematic collections at the place where the needle is introduced.

The administration of convalescent serum from other patients, though not necessary with mild attacks, is advisable in severe cases. Treatment with convalescent serum is routine in the endemic area, and sometimes its administration can have spectacular results. Within a matter of hours, the condition of a severely ill, semicomatose patient can be reversed. The experience of local physicians has shown that this treatment works better when instituted very early in the disease—no more than five days after onset. From 250 to 500 cc of serum is used depending on the individual patient and the severity of the case. Interestingly enough, even though convalescent serum is administered routinely in Argentina for treatment of AHF, to date no cases of hepatitis have been observed among the patients. As a rule, a patient's friends who have already had AHF donate their blood on such occasions.

The administration of oral antibiotics is considered advisable, particularly after observing the lesions produced by Junín virus in the intestinal walls of experimental guinea pigs. These lesions facilitate the passage of bacteria from the intestine to the blood stream. Medication with antibiotics should help to prevent such complications.

Fresh blood transfusion is advisable in severe hemorrhagic cases. If the blood comes from a person with antibodies against Junín virus there will be the added advantage of neutralizing the virus infectivity, but if antibodies are not present, at least the restitution of volemia and the administration of platelets, white and red cells, and all the other factors needed for coagulation will help return the organism to its equilibrium. In general, no more than 500 cc of whole blood is given at one time; transfusions are repeated if necessary.

More detailed information about treatment and the effects of specific drugs can be found in the literature (*18, 56*). Basically, the criteria outlined above are applied.

5. JUNÍN VIRUS

An infectious agent of AHF was first isolated from victims of the 1958 outbreak. Three different strains were obtained: one from the blood of a patient in the acute stage; a second from another patient's urine, which contained a large amount of blood; and a third from a suspension of viscera (brain, liver, spleen, and kidney) taken at the necropsy of a fatal case. The strains were isolated by inoculation of guinea pigs. Even though many hosts were used in the laboratory in an effort to isolate the causative agent, the guinea pigs were the first to show a response characterized by death with hemorrhagic manifestations resembling those presented in human beings (*62*). Indeed, the hemorrhagic characteristics were exaggerated. All three strains behaved similarly in this host.

Intracerebral inoculation of a pool of infected guinea pig organs in suckling mice produced ataxia, spasms, and occasionally paralysis of the hind legs. The infected mouse brain fixed complement with human convalescent serum but not with serum taken from a patient in the acute stage of the disease.

Since the agent passed through a Chamberlain L5 candle, failed to multiply in cell-free media, and could not be seen under the visible light microscope, it was considered to be a virus. It was given the name Junín, after the city where the materials had been collected. The etiological role of the virus was subsequently demonstrated by inoculation of infectious material in a volunteer and by multiple accidental infections in laboratory workers, some of them fatal. In the same epidemic, Pirosky and his group (*91*), working independently, also isolated several strains of a virus. One of the members of this team reproduced the disease in himself by autoinoculation. Further studies showed that the viruses isolated by the two teams were indistinguishable by complement-fixation (*69*).

Physical and Chemical Properties

The virus is very sensitive to acidification. When kept on dry ice, unless it is perfectly sealed, the diffusion of CO_2 into the ampoule will cause it to become inactivated.

Infected organs, or suspensions of infected organs in a pH 7.2 buffer with 0.75 per cent bovine albumin, can be stored for years at –70°C in an electrically operated freezer without loss of infectivity. If the material is frozen and thawed, the infective titer of the virus drops with each step. Lyophilization of infected suspensions has proved to be a good method of stock conservation, since the decrease of virus titer is minimal.

The resistance to this virus at different temperatures has been studied, using M-199 free of any serum as diluent and checking the virus titer after exposure to 56° and 37°C. Ten minutes' exposure at 56°C decreased the titer in the logarithmic expression by one; thirty minutes killed the virus totally. After one hour at 37°C the titer decreased by 0.09 logs, after six hours by 0.37 logs, and after twenty-three hours by 0.56 logs.

The virus was shown to be very sensitive to sodium desoxycholate, as is generally the

case with arboviruses. It is inactivated by trypsin, chloroform, and ether. The optimum pH for virus viability is on the alkaline side, close to neutrality (pH 7.1 to 7.4). It has more resistance to alkaline than to acid pH; in a diluent free of proteins, very light acidity kills it. When protective substances like bovine albumin or rabbit serum are added to the diluent, the lability of the agent is decreased, as is the case with many other viruses. A phosphate buffer of pH 7.1 to 7.4 with 0.75 per cent bovine albumin is routinely used as diluent. A tenfold concentration of albumin in the same pH 7.2 buffer did not prove to be better; on the contrary, the infectivity tended to decrease.

The effect of pH, temperature, and ultraviolet light on infected guinea pig plasma has been studied. No significant decrease in infectivity was observed between pH 5.5 and 9.5 after six hours at 4°C or between pH 6.5 and 9 after eighteen hours at this same temperature. However, a significant decrease was found after eight days at 4°C, three days at 25°C, twenty-six hours at 37°C, and ten minutes at 56°C. With the ultraviolet light at a distance of 67 cm, a minimum exposure of thirty seconds was necessary to achieve significant inactivation; after forty seconds, 99.9 per cent of the virus was inactivated (*12*).

Lajmanovich *et al.* (*49*) determined the size of Junín virus, strain XJ, in the plasma of infected guinea pigs by means of ultracentrifugation with a Spinco preparation. Assuming spherical shape and a density of 1.2 g per cc, they calculated that the diameter was between 18 and 25 mμ. The same source of virus was used to obtain a pellet, which, resuspended in 1/50 of the primitive volume in a phosphate buffer of pH 7.4, was chemically analyzed for nucleic acid determination. The authors concluded that Junín is an RNA virus (*78*).

Electron microscopic study of Junín virus was attempted by Dr. Zaharzevski in infected lymph nodes of guinea pigs, and images compatible with the characteristics described for Junín virus were found (*132*).

Behavior in Animals and Tissue Cultures

Experimental animals

Guinea pigs

As stated before, the guinea pig was the first laboratory animal to show characteristic responses to the virus. After inoculation of virus materials by any of several routes (*vide infra*), it may become sick and die, usually within 11 to 20 days and sometimes later, depending on the amount of virus present in the inoculum. As a rule, animals that do not appear to have been given sufficient virus to produce disease are held for 40 days before they are considered free from infection. Even then, when no apparent disease ensues, additional information can be obtained by testing the sera of the survivors for complement-fixing (CF) antibodies. A single injection of mouse-adapted XJ strain, even in amounts of 100 mouse LD_{50} or less, produces a high titer of CF antibodies in the guinea pigs. According to Casals (*21*), it is possible, but unlikely, that this amount of inert virus could stimulate the production of CF antibodies; it seems reasonable to think that the guinea pigs can suffer a nonfatal, inapparent infection. The XJ strain, originally highly pathogenic for guinea pigs when inoculated intraperitoneally, appears to lose this pathogenicity after about 40 passages in newborn mice (*69*). The reason for this loss of pathogenicity is as yet undetermined. It may be due to selection in the mouse of a less virulent virus population, to environmental conditions such as diet and handling of the guinea pigs, or to higher resistance of North American guinea pigs as compared with the Argentine animals.

Intraperitoneal, intramuscular, and subcutaneous routes of inoculation are routinely

used, although other routes can be chosen. Intracerebral inoculation of guinea pigs usually gives a picture of encephalitis instead of the hemorrhagic manifestations. Tests using nebulization; corneal scarification; and intravenous, skin, and oral administration have also produced infection, even though mortality is lower. The virus usually tends to be localized in the lymph nodes and the spleen. Fever begins on the seventh or eighth day after inoculation and remains high until the last day of life, when a marked hypothermia suddenly occurs. Loss of weight is present from the beginning and increases after the fever appears. Viremia is constant, and the titer continues to rise until death (*13*). The study of blood and bone marrow shows absolute leukopenia, relative and absolute lymphocytosis, decreased eosinophils, slight anemia (normochromic and normocytic), increased reticulocytes, increased alkaline phosphatases in the leukocytes, inhibition of mature myeloblasts in the bone marrow, and an increased red blood cell count. From the first day of inoculation until the animal's death there is a decreased number of platelets (*39*).

Studies of coagulation factors with the XJ strain show increased deficiencies up until just before death. Coagulation time rises from the normal 3′ 20″ to 60′ with lack of clot retraction (*19*).

Histaminemia was determined one day before inoculation and again on the third, seventh, and tenth days thereafter. Uninoculated controls were tested simultaneously. It was found that a significant decrease in histaminemia is apparent in animals after the seventh day of infection; there is increased histaminemia, reaching normal values, in sick animals just before death; and there is no difference in histamine release between normal and infected lung tissue (*131*).

A study of inter-guinea pig contamination rate, using 16 strains of Junín virus, was performed by placing normal guinea pigs in the same cage with the inoculated animals and in neighboring cages. Although 100 per cent of the inoculated guinea pigs died with the characteristic picture, only 11.3 per cent of the other guinea pigs in the same cage died, and none of the normal animals in neighboring cages died (*42*). Since the elimination of Junín virus through the urine of infected guinea pigs starts to appear on the seventh day after inoculation, contamination of the uninoculated animals placed in the same cage probably occurred through contact with the infected urine. Despite numerous attempts, Junín virus has never been isolated from the stools of infected animals.

Infected guinea pigs show alterations in their immune response to other antigens, such as red cell preparations from different species (*83*). They also manifest an increase in susceptibility to *escherichia coli* endotoxin administered by the parenteral route (*41*).

A detailed study of the seric proteins in infected guinea pigs was performed using electrophoresis on paper and a gel of polyacrylamide (*17*).

The fairly precise relation between the amount of virus inoculated and the incubation time between inoculation and appearance of a given symptom has long been recognized. In 1939, for example, with rabbit papilloma, the time interval between inoculation in the rabbit skin and appearance of papillomas is related to the concentration of the inoculum by a mathematical formula (*15*). In the present case, a linear relationship between intramuscularly inoculated doses of Junín virus and the time of the guinea pigs' death can be determined. However, the formula cannot be applied with a high concentration of virus; it is useful only with dilutions starting at 10^{-4} (*27*).

The gross anatomy of guinea pigs infected with Junín virus shows only conspicuous hemorrhagic manifestations, consisting of petechiae, ecchymosis, or hematic suffusions

on the skin, suprarenal glands, kidneys, and small and large intestines, and usually areas of lung hepatization. The liver and spleen are generally normal in size, except when a long incubation period leads to fatty degeneration of the liver and consequent enlargement. Hypertrophy of Peyer's patches is a constant finding. Bacteriological studies in this animal, as in human beings, show negative results (*38*).

The pathological picture of Junín virus in guinea pigs closely resembles that presented by epizootic hemorrhagic disease in deer. This fact led to the development of a complement-fixing system with the New Jersey deer strain for purposes of comparison with Junín virus. The two agents were found to be completely different antigenically, however (*71*).

Histological examination of different organs consistently shows general congestion, with microhemorrhages scattered throughout, as in human beings (*9, 10*). In sections of the CNS and ganglion stained with Seller's technique it is possible to observe intracytoplasmic inclusion bodies similar to those described in the urine of humans infected with this same virus (*76*) or with Portillo virus (*70*) and in mouse brain infected with Tacaribe virus (*32*).

Mice

Mice, the animals most widely used for research in virology, have been very helpful in the study of Junín virus. Indeed, importance should be given not only to white mice for research but also to wild mice present in the endemic area. When studies on this virus were first started, even though the disease had been reproduced in guinea pigs, there was no way of ascertaining the rise of antibodies in human convalescents until newborn mice were available as a host for the neutralization test and as a source of complement-fixing antigen from their infected brains (*82*). Intracerebral inoculation of a pool from infected guinea pig organs in newborn mice produced a disease nine days later, with signs of encephalitis, ataxia, tremors, and convulsions. In those mice that survived one or two days longer, paralysis of the hind legs appeared as well. Newborn mice are also susceptible to the virus inoculated intraperitoneally, although not to the same extent as with the intracerebral route.

Adult mice are susceptible, too, but to a lesser degree, and only by intracerebral inoculation, not intraperitoneal. The titer is lower, infection is irregular and spotty, and some mice survive without showing any symptomatology.

The incubation period of the virus in newborn mice inoculated intracerebrally depends on the particular virus strain and the amount of virus present in the inoculum. With the prototype strain, the disease usually starts around the eighth or ninth day and the mice are held for 21 days to determine the endpoint in terms of LD_{50}. With other strains, the incubation period is shorter (five days), but it is always necessary to keep the animals 21 days to reach the endpoint, and in some cases 28 days, as observed by Sabattini (*26*).

Since newborn mice were found to be susceptible to inoculation by more than one route, this host was used for measuring the neutralization of infectivity of human convalescent sera. Infected mouse brain was taken as the source of Junín virus. Later, the growth curve of the virus in the infected mouse brain was studied by measuring the infectivity in HeLa cells, and the optimum time to harvest the brain for use as complement-fixing antigen was determined. The prototype XJ strain was utilized in the assay (*69*).

A comparative study of Junín virus multiplication in the brain of 24- and 72-hour-old suckling mice was carried out by inoculation of 10^{-2}, 10^{-4}, and 7×10^{-2} particles from another strain of this virus. Both groups of mice were found equally sus-

ceptible, and there was a plateau at the maximum titer between the third and the eighth day after inoculation. At the highest level of inoculum, interference was also observed (*47*). The production of interference in HeLa cells, using infected mouse brain as inoculum, especially in recently harvested material, was a very common finding when this type of work was done routinely.

Newborn mice are susceptible to natural infection as well. When exposed for 16 days to 2,000 acarids collected from the nest of a field mouse, newborn mice showed typical signs of infection. The virus responsible for this infection was propagated in newborn mice and was shown to cross-react with human strains in complement-fixation tests (*89*).

Mice infected with strains of Junín virus show signs of encephalitis regardless of the route of inoculation; histologically, the brain is infiltrated with lymphocytes and perivascular cuffing (*82*). The alterations in the kidney, bone marrow, and blood vessels are very slight compared with those found in man and guinea pigs (*10*).

Newborn mice have proved to be a good host for the primary isolation of Junín virus. Of 34 strains directly isolated in newborn mice, 22 were recovered in the first passage, 1 after a blind passage, and 11 after two blind passages (*26*). Investigators at the Córdoba Institute of Virology found newborn mice to be more effective than guinea pigs for virus isolation.

The brain of newborn mice has been used in an attempt to localize the viral antigen. The Weller and Coons method, using fluorescent antibodies, was applied to brain sections taken from mice 11 days after inoculation. The results showed a large amount of fluorescent material, stained greenish-yellow, which corresponded to specific viral antigen particles (*88*).

A more exhaustive study of this problem is under way. In particular, efforts are focused on determining the relationship between maximum fluorescence and the length of time after virus inoculation is completed, and on the use of this information as a tool for earlier diagnosis of AHF (*58*).

Embryonated hens' eggs

The prototype XJ strain of Junín virus was adapted to the chick embryo (*61*), and a study of the biological properties of the virus adapted to this host was carried out (*62*). The adapted strain produced pocks on the chorioallantoic membrane similar to those presented by herpes simplex or smallpox. The specificity of the lesions was demonstrated by a neutralization test; sera taken from patients during the acute period of the disease failed to neutralize the pocks, but convalescent sera did. Furthermore, human gamma globulin taken from convalescent sera and labeled with lissamine-rhodamine B200 reacted specifically with infected membranes but failed to react with normal or with Junín-virus-infected membranes previously blocked with the same unlabeled gamma globulin.

Following inoculation of the chorioallantoic membrane, the virus reached its maximum titer in the embryo in five days. The highest infective titer was found in the amniotic fluid, followed in decreasing order by the allantoic fluid, the brain, the chorioallantoic membranes, the yolk sac, the liver, and the heart. In the last-mentioned organ there were only traces of virus. The adapted strain was inoculated endovenously into the allantoic sac, the amniotic sac, and the yolk sac to determine the best route for obtaining the highest concentration of virus in the allantoic fluid harvested seven days later. The chorioallantoic membrane route gave the highest concentration of virus in the fluid.

The virus adapted to eggs was unable to agglutinate red blood cells from one-day-old or adult chickens, humans, mice, rats, guinea pigs, hamsters, or toads (*Bufjo arenarum*), whether in saline or exposed to pHs ranging from 5.8 to 7.6. Human sera

from cases of Korean hemorrhagic fever and Colorado tick fever were unable to neutralize the virus.

A description of the pathology of the virus in the different organs of the chick embryo has been reported (*62*). After 14 passages in chick embryos, the virus showed no change in pathogenicity for guinea pigs or newborn mice. For that reason, no further studies were performed.

Other laboratory animals

In rats, as in mice, Junín virus is pathogenic for the newborn. The localization of the virus in different regions of the brain has been studied (*29*).

The newborn chick is also susceptible to intracerebral inoculation of the virus, showing paralysis followed by death (Villa Lucio, personal communication).

Junín virus is pathogenic for infant laboratory hamsters, but the infected hamster's brain is not as suitable as mouse brain for production of complement-fixing antigen, since the antigen appears in the brain only after the onset of symptomatology (*128*). The distribution of the virus in hamsters was studied by Bruno Lobo *et al.* (*14*), using fluorescent antibody techniques.

One primate, the marmoset, or titi, of Panama (*Saguinus geoffroyi*), was found to have great susceptibility to inoculation by the peripheral route, not only to Junín but also to the related Machupo, Tacaribe, and Amaparí viruses (*77*).

Wild animals

The isolation of virus from field mice (*Hesperomys laucha laucha*) was reported by Pirosky *et al.* (*92*). Two out of 20 mice captured in a maize plantation situated within the epidemic area showed signs of infection after a period of seven days in isolation from other mice. A virus was isolated from brain tissue suspensions of the diseased mice by intracerebral inoculation into newborn white mice. Antigens prepared from infected mouse brain gave positive complement-fixation reactions with human convalescent sera from the epidemic under study. No virus was found in the brains of healthy field mice, though their sera did show neutralizing antibodies that suggested recovery from natural infection.

On the basis of observations of wild mice trapped in the endemic area in 1959, Pirosky *et al.* claimed that these animals are infected in nature in the same manner as human beings, showing illness followed by either death or recovery. In the latter case the sera were found to have neutralizing antibodies for the virus. As a consequence, the team concluded that wild mice cannot be the reservoir of the virus in nature and that it is more likely that mites, from which the virus has been isolated (*89, 92*), are the reservoir.

In 1965 a new epidemic broke out in the Province of Buenos Aires. It followed directly after an epizootic in a particularly high population of wild rodents (*125*). Even though the virus apparently killed a good number of the rodents, the survivors seemed to carry this virus, or at least a related one, chronically and asymptomatically (*50*). Hence, they cannot be definitely rejected as reservoirs.

Attempts on the part of the present author's team to isolate viruses from wild rodents resulted in the recovery of ten strains from *Mus musculus,* one from *Hesperomys,* and one from *Akodon* (*79, 84, 112*). The trapped animals seemed to be healthy. The strains were recovered from pools of organs (liver, kidney, brain, and spleen) and inoculated in guinea pigs. From guinea pigs, in turn, they were inoculated intracerebrally into newborn mice. The infected mouse brain was used as a source of complement-fixing antigen for virus identification.

In the Province of Córdoba, which was first recognized as an endemic area in 1963 (*123*), an attempt to isolate viruses from

wild rodents yielded 46 strains of Junín from *Calomys laucha.* In addition, four strains of Junín and five strains of other viruses were obtained from *Akodon* (*26*). The same investigators tested the brain and viscera of the wild mice as complement-fixing antigen in the presence of a known hyperimmune serum; 2 out of 35 *Calomys* were found to fix complement. The starting antigen was a 20 per cent suspension of organs taken separately. In one of the *Calomys,* the CF titers were as follows: brain, 1:128; spleen, 1:4; kidney, 1:8; and liver, 1:32. Strains of Junín virus were also isolated from these same two *Calomys.* On the other hand, no complement-fixing antigen could be obtained in two other *Calomys* from which virus had been isolated. Thus, while the use of organs from wild rodents for the preparation of CF antigens is easier and more direct for epidemiological purposes, it is less sensitive than virus isolation for studying the infective rate in wild animals (*26*).

Isolation of Junín virus from other wild animals has been reported in *Cavia pamparum* (cuis) and their flea ectoparasites (*24*), *Mesostigmata* without further identification (*89*), *Echinolaelaps echidninus* (*85*), and *Eubrachilaelaps rotundus* (*4*). The hare (*Lepus europeaus*) has been found infected in nature; two attempts at virus isolation from this animal yielded two strains of Junín virus (*4*).

Tissue cultures

The virus proved capable of being propagated serially in HeLa cells. Appropriate cell viability during the long experimental period was obtained by the use of a suitable combination of growth-promoting and maintenance media, as well as by frequent fluid changes during the period of viral synthesis. A characteristic cytopathogenic effect was observed during the first passage and did not change during ten consecutive transfers of the agent. Evidence that the cytopathogenic agent was Junín virus was derived from fluorescent antibody studies and from neutralization tests with sera from animals immunized with virus that had been propagated in newborn mice (*67*). Infected HeLa cells were examined at intervals, stained with Giemsa, and treated with fluorescent antibody. Foci of 1 to 30 cells (usually 6 to 10) appeared with basophilic cytoplasmic inclusions; these inclusions varied in size and shape, were often near the nucleus, and were frequently surrounded by a clear halo. After three days, cytopathic changes become visible without staining. These consisted of foci of shrunken granular cells that fell off the glass. At this stage the inclusions were larger. During the next 24 hours the foci enlarged and became confluent. By the seventh day destruction of the cell sheet was marked, although islands of unaffected cells persisted and giant cells could be seen. Fluorescent antibody studies two days after inoculation showed antigen in small round cytoplasmic inclusions in individual cells. Groups of cells containing antigen were also seen, and the infection appeared to be spreading from one infected cell to surrounding cells. Antigen was not observed in the nucleus. The subsequent stages resembled those shown by Giemsa staining; the inclusion visible by both methods appeared to correspond (*95*).

Partial regeneration of the cells occurred 21 days after inoculation. The cultures remained chronically infected, and up to 3 log TC_{50} per ml could be found after 60 days. Serial passages of three strains of Junín virus were accomplished without difficulty. Usually the passages were carried out at seven-day intervals, and from 69 to 79 passages were undergone (*16*). Attempts with other tissue cultures resulted in reproduction of Junín virus in the kidney cells of guinea pigs, rabbits, and hamsters, and in the fibroblastic growth of chicken and mouse embryo. Under direct microscopic observation, however, no cytopathogenic effect was present (*33*).

Plaque production of this virus in Rhesus monkey kidney cell monolayers was reported by Henderson and Downs (*44*). Plaque assays were performed using the Hsiung-Melnick overlay supplemented with lactoalbumin hydrolysate and yeast extract, as recommended by Coleman. Plaques started to develop by the sixth to eighth day, but at this early stage they appeared as small, irregularly shaped clusters rather than as discrete, clear areas.

Clear plaque areas that could be counted directly or as clusters developed progressively by the ninth or eleventh day. Tacaribe virus behaved similarly in this tissue culture, and a study of the antigenic relationship between these two agents using the plaque neutralization test was carried out. The endpoints were clear-cut, and there appeared to be a one-way cross.

With the MA-111 cell line in cell monolayers under a fluid medium of M-199 with 2 per cent fetal calf serum added, maximum virus titers of 6 to 7 $\log_{10}$ plaque-forming units (PFU) were obtained between two and five days after inoculation, depending on the amount of virus in the dose. With a single agar overlay, as described by Henderson and Downs, Junín, like Tacaribe and Machupo, produced clear plaques between six to ten days after inoculation (*128*).

The reproduction of Junín virus in a continuous Vero cell line of kidney tissue cultures from the African green monkey (*Cercopithecus aethiops*) was reported by Rhim *et al.* (*96*) and is now used routinely by Dr. Buckley at the YARU unit at Yale for all the viruses of the Tacaribe group. With the same maintenance medium as reported for the HeLa cell line (*67*), the cytopathogenic effect is evident at three to five days after inoculation.

Personal experience has shown that a complement-fixing antigen can be obtained from infected Vero cells. The infected tissue is frozen and thawed twice and then centrifuged for one hour at 40,000 rpm. After this, the sediment is resuspended in a saline or Veronal buffer of 1:50 or 1:100 the volume of the initial infected material. Homogenization of the sediment is done by slight sonication. At the same time, uninoculated Vero cells are treated in the same way as a normal control. Titers of 1:64 or more, depending on the amount of virus present in the infected tissue and on the concentration of the resuspension fluid, are obtained, and no anticomplementarity has been found.

With the Dulbeco method, Vero cells behaved similarly to the Rhesus monkey kidney cell monolayers in the studies by Henderson and Downs (*44*). Observations were also made of the PR5 strain from infected mouse brain; when diluted $10^{-0.5}$ it yielded from 50 to 100 plaques. The cell monolayer was stained with neutral red five days after inoculation, and two days later the final reading was taken.

With Junín virus, the plaque method, using Vero cells and agar overlay, gives more clear-cut results than fluid cultures in tubes; it also avoids the cell regeneration seen with the latter.

6. IMMUNOLOGICAL INVESTIGATION

Characterization: Tacaribe Group

The method introduced by Clarke and Casals for the study of arboviruses, especially the sucrose-acetone extraction of infected mouse brain as complement-fixing antigen, proved to be the most effective technique for the identification and immunological characterization of Junín virus (*25*). While the antigen prepared by this method never showed hemagglutinating properties with any of the strains of Junín virus tested, it gave high titers in the complement-fixation test, especially when the infected mouse brain was harvested at the peak of virus reproduction (*69*). This material permitted a comparative study with all the other viruses available at The Rockefeller Foundation Virus Laboratory, currently the YARU unit at Yale University.

Hyperimmune serum for the homologous system is obtained by immunization of four- to eight-week-old mice, as is done routinely in arbovirus research. It is now possible to obtain large volumes of ascitic fluid, which is as good as serum for serological work, by virus immunization followed by the use of the relatively nonvirulent ascites cell neoplasm Sarcoma 180/TG. The titers of the ascitic fluid antibodies compare favorably with those of the sera, and more than 30 times as much antibody can be obtained per mouse (*116*).

In exhaustive complement-fixation tests, Junín antigens were tried against hyperimmune sera for practically all known arboviruses, as well as a few other agents that were included for purposes of a differential diagnosis—herpes, Q fever, rickettsialpox, murine typhus, Newcastle disease, mouse encephalomyelitis, and encephalomyocarditis. The only serum that cross-reacted with the Junín CF antigen was that of Tacaribe, a virus repeatedly isolated from bats and once from mosquitoes by Downs *et al.* (*32*) in Trindidad. This serum, with a homologous titer of 1:256, reacted against Junín antigen with a titer of 1:32. Not only did the mouse immune serum cross-react with the antigen, but human AHF convalescent sera reacted with the antigen from Trinidad as well.

To determine the relationship more precisely, two neutralization tests were performed, one with Junín virus and the other with Tacaribe virus, using the same specimens of immune and control sera. A Junín guinea pig serum with a titer of 1:32 against 10,000 TCD_{50} of homologous virus failed to protect against Tacaribe virus, and a Tacaribe serum, which at constant dilution 1:2 protected against 100 TC_{50} of homologous virus, failed to show any protection against Junín virus. Thus it was demonstrated that Junín and Tacaribe are two distinct agents (*69*). Other investigators using the same CF technique to compare Tacaribe virus with strains of Junín virus from different epidemics arrived at the same results—that is to say, they found the same type of differences in serum titer between the homologous and heterologous Junín and Tacaribe systems (*1*).

Although some clinical resemblance may exist between the Korean and Argentine hemorrhagic fevers, sera from six patients convalescing from the former failed to react

to a Junín complement-fixing antigen (*69*).

In the absence of a hemagglutinating antigen for Junín, sera known to have CF or neutralizing antibodies for this virus were tested against hemagglutinating antigens prepared from the following arboviruses: group A: Mayaro, Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), Venezuelan equine encephalitis (VEE), Aura, and Una; group B: St. Louis encephalitis (SLE), Ilheus, Powassan, Bussuquara, yellow fever, dengue Type II, Japanese B encephalitis (JBE), Central European tick-borne encephalitis, louping-ill, Omsk hemorrhagic fever, and Russian spring–summer encephalitis (RSSE); group C: Marituba, Oriboca, and Caraparú; and Bunyamwera, Germiston, Cache Valley, Guaroa, California encephalitis (CE), Tahyna; Guama, Koongol, Witwatersrand, Ketapang, Bakau, Neapolitan sandfly fever, Icoaraci, Akabane, Ingwavuma (SA AN 4165), Sathuperi, Manzanilla, Tacaiuma, and Bwamba. No cross–reactions were observed.

Further studies to discover possible antigenic relationships with viruses other than those from the arthropod-borne group were performed with measles virus and mouse adenovirus (*43*). With the former the complement-fixation and measles hemagglutination-inhibition tests were used. No cross–reaction between Junín and measles could be found by complement-fixation, using either of the heterologous systems for comparison, or by hemagglutination-inhibition using measles virus as antigen. With mouse adenovirus, no cross–reaction with either of the antigens against either of the heterologous systems could be detected. The reason for studying these two antigenic relationships was that both measles virus (*45*) and mouse adenovirus (*43*) had been reported to be capable of propagating in newborn mice when inoculated intracerebrally, with behavior similar to that observed for Junín. Moreover, the inclusion bodies found in the cells of the urine sediment in both Junín and measles infections are similar. And finally, Bergold has observed that the image of Tacaribe group viruses resembles that of the adenoviruses under the electron microscope; they have the same number of capsomers (*7*).

Extending the study of antigenic relationships between Junín virus and other pathogenic organisms, sera from AHF convalescents with known titers against Junín and experimental sera obtained in mice using the XJ and RP strains of Junín were checked by microagglutination against Leptospira from the following groups: icterohaemorrhagiae, javanica, canicola, ballum, pyrogenes, cynopteri, autumnalis, pomona, australis, grippotyphosa, hebdomadis, bataviae, hyos, biflexa, and semaranga. No ability to agglutinate Leptospira was shown by any of the sera tested except the XJ mouse immune serum, which reacted to Leptospira ballum, strain Mus 127. The same reaction, in a lesser degree however, was shown by the sera of normal mice from the same colony used as controls (*68*).

In short, up to now the only agents that have reacted to Junín virus are the other members of the Tacaribe group, with which it shares a common complement–fixing antigen. Junín can be differentiated by the neutralization test and, according to Cuadrado and Casals (*31*), by immunoelectrophoresis on agar. This latter method seems to provide a means for detecting specific differences among Amaparí, Junín, and Tacaribe viruses. With this technique, Cuadrado found Portillo virus to be different from Junín (personal communication).

Experimental Vaccination

Owing to the seriousness of the disease caused by Junín virus and the consequent public pressure for action, attempts have been made to develop a vaccine to help pro-

tect the exposed population in the endemic area.

A formalin–inactivated vaccine was prepared from mouse brain tissue infected with a pool of several strains of Junín virus. The vaccine, produced and distributed by the National Institute of Microbiology, Buenos Aires, was given to more than 15,000 persons beginning in May 1959. The regular course of vaccination consisted of three injections of 1 cc each, followed by a booster each subsequent year. Some vaccinees received only one injection, others as many as seven. The vaccination program was eventually discontinued for administrative reasons, and no evaluation of the efficacy of the vaccine was ever carried out.

Since Junín and Tacaribe viruses were found to be antigenically related, while the latter lacked pathogenicity for guinea pigs, several investigators started looking into the possibility of using Tacaribe virus as a vaccine against infection from Junín (*80, 120*).

Adult guinea pigs immunized with one inoculation of 300,000 PFU of Tacaribe virus were protected against challenge with Junín virus 14 days later (*120*). The guinea pigs immunized with Tacaribe virus had complement–fixing antibodies against Tacaribe and Junín virus at 14 days, the level against Junín virus being lower. Neutralizing antibodies against Tacaribe virus were detectable at 14 days, but neutralizing antibodies against Junín virus were negligible even after 49 days.

In another experiment (*28*), guinea pigs were inoculated with live Tacaribe virus by the intramuscular route. It was possible to isolate infectious virus from the lymph nodes for a period of 48 days after inoculation, although not consistently. Virus was present in the serum on the seventh day and in the liver on the thirteenth day. There was no immunity against challenge with Junín virus in the first three days, partial resistance appeared after seven days, and complete immunity was established thereafter. Complement-fixing antibodies against Tacaribe and Junín viruses were detected on the thirteenth day, the titer against the homologous antigen being higher.

The development of immunity against Junín virus infection after inoculation with Tacaribe virus has been explained by interference rather than other mechanisms.

Thus, in another experiment (*128*), passive immunization against Tacaribe virus in suckling hamsters was obtained through immunization of the mothers, but the passive antibodies against Tacaribe virus did not afford protection when the hamsters were challenged with Junín virus. In still a another study, guinea pigs infected with Tacaribe virus developed CF antibodies, but not neutralizing antibodies, against Junín (*120*).

Interference between Junín and other antigenically unrelated viruses, such as VEE, has been demonstrated using the white mouse as host (*59*).

7. ISOLATION AND IDENTIFICATION OF JUNÍN VIRUS

Special Precautions

As a rule, no special precautions beyond those routinely followed in the handling of arboviruses are available. Infections in laboratory workers and animal keepers are very common in places where Junín virus is present, and unfortunately they can be quite severe. It is thought that these laboratory infections are contracted either from aerosol in the environment, as is the case with many other arthropod-borne viruses, or through cuts or minor abrasions in the skin (*102, 111*). It is advisable, therefore, to send materials for etiologic diagnosis to laboratories specialized in this kind of research, at least until vaccination for laboratory workers against this virus can be performed. In Argentina, where the disease is endemic, there are at least three centers—two of them in the city of Buenos Aires and one in the city of Córdoba—where this kind of investigation can be done.

In the United States, where the disease could well appear in a person coming from the endemic area—whether an American citizen returning from a business or pleasure trip, or an Argentine entering as an immigrant or a visitor—it is necessary to know what institution should take care of this kind of research. When there is a suspicion of AHF, even if the patient has not been out of the country, an explanation of the circumstances should be sent to the National Communicable Disease Center in Atlanta, Georgia, or to the WHO Reference Center of the Yale Arbovirus Research Unit (YARU) at Yale University, New Haven, Connecticut, for clarification of the etiology.

Clinical Sources of Materials for Testing

AHF is a systemic disease in which the infectious agent is present in the blood almost from the beginning, and usually the viremia persists throughout the febrile period (*11*). It is diagnosed etiologically by recovery and identification of the virus from the patient's blood, urine, and organs, and/or by demonstration of a rise in antibody titer in serum during convalescence.

Blood and pieces of organs from fatal cases are most suitable for virus isolation. Successful results have been achieved with serum stored at −70°C for periods of a year or longer. The virus has also been isolated from urine, which seems to be a good source as well. It has never been isolated from feces.

On primary isolation, certain strains show a selective pathogenicity for particular hosts. This is an important diagnostic consideration, since a strain can be lethal for a guinea pig, for instance, and show no lethal infection in suckling mice.

As a matter of routine, guinea pigs and newborn mice are simultaneously inoculated for virus isolation. Two or four guinea pigs and at least two litters of mice are used for each attempt. The guinea pigs, preferably

between 250 and 350 g, are inoculated by the intraperitoneal route (0.15 to 1.0 cc) or intramuscularly or subcutaneously (0.3 to 0.5 cc) with original material from a patient. The usual material is whole blood, serum, or clots macerated in saline or some other diluent. If the disease had reached a sufficiently advanced stage in the patient, neutralizing antibodies or other inhibitors may be present in the serum. For this reason, it is advisable to use not only undiluted serum or whole blood, but tenfold dilutions of the same materials as well, so as to dissociate the virus-antibody complex. In many instances, guinea pigs show no signs of illness after inoculation with undiluted blood, whereas disease becomes definitely apparent after a 10^{-2} or greater dilution. The same precautions should be taken with mice, and intracerebral and subcutaneous injection should be done simultaneously in any one animal.

When urine is used, the guinea pigs should be inoculated by the intramuscular or subcutaneous routes.

It is usually at least a month, sometimes longer, before a viral diagnosis can be pinned down, especially when it is necessary to wait for the convalescent serum sample to demonstrate a rise in antibodies against the virus.

Procedures such as the fluorescent antibody technique, while available as an investigator's tool, have not yet been applied in the routine diagnosis of Junín infections. The presence in the patient's urine of giant cells, presumably containing viral antigen, could provide a likely means for detecting Junín virus during the acute stage of the disease.

With routine serology techniques, the specific diagnosis can be speeded up by sacrificing some of the inoculated suckling mice on the seventh or eighth day and using their infected brains as crude antigen in the form of a 10 or 20 per cent suspension in saline or Veronal buffer. After light centrifugation, the supernatant acts as a good complement-fixing antigen, with a titer only one or two dilutions lower than that obtained with the sucrose-acetone treatment. If the antigen from the suspect material (and not the controls) reacts against a known serum with a good titer for Junín, this means that the agent belongs to the Tacaribe group; if it does not react, a blind passage in other mice can then be performed. Serology techniques are essential for the diagnosis of Junín virus, because many agents can kill mice or guinea pigs after similar incubation periods. Since guinea pigs are not a good source for antigen, infection with Junín virus in this animal can be shown in two ways: (1) if it becomes sick or dies, blood or pools of organs may be transferred to newborn mice by the intracerebral route to obtain an antigen for the CF test; or (2) serum from a surviving animal may be tested for CF antibodies against Junín. If the complement-fixation test is positive, then the virus is a member of the Tacaribe group (Junín, Machupo, Portillo, Amaparí, Pichinde, Tamiami, or Tacaribe), and a neutralization test will be necessary to determine its precise identity.

Neutralization and Complement-Fixation Tests

For final identification, neutralization of the suspected isolate by specific Junín immune serum is required.

The isolated strain is diluted serially in tenfold steps to an appropriate endpoint (usually 10^{-10}). A prototype strain of Junín virus is treated in the same way. Each dilution of each virus strain is distributed into three rows of test tubes in 0.2 cc amounts. To the first row an equal amount of Junín immune serum, undiluted and unheated, is added; to the second, normal serum from the same host as the Junín immune serum (usually mice or guinea pig

serum), also undiluted and unheated, is added. To the third row, diluent is added. Incubation is carried out at 37°C for one hour, after which the chosen hosts are inoculated with the contents of each row separately.

The difference between the virus titers in the immune serum and the normal serum will reveal the extent of specific neutralization; the difference between the titers in the serum and the diluent will show the extent of nonspecific inhibition. The set with the prototype strain of Junín virus will indicate homologous protection of the serum used for virus identification.

The endpoint in each group is considered to be the dose sufficient to infect or kill 50 per cent of the host (mice, guinea pigs, tissue culture). It may also be taken as the average plaque-forming unit per ml in each group. A difference of 2.5 $\log_{10}$ between the two groups is usually considered significant, but identification can be suggested by a smaller difference.

A number of facts must be taken into consideration in performing the neutralization test. In the first place, newborn mice are more suitable than guinea pigs for reasons of economy and space. If newborn mice are going to be used, it is best to keep only eight mice in each litter to avoid overcrowding, since they must be observed for 21 days to reach the endpoint in the case of determining LD_{50} for titration. Newborn mice can also be used to determine the ID_{50}. For this purpose, all the animals are sacrificed on the seventh or eighth day after inoculation, and a crude antigen is prepared by diluting macerated mouse brain in 10 or 20 per cent saline or Veronal buffer. A complement-fixation test is then performed against a Junín immune serum with a high titer and another negative control (Shope, 1967). The mouse brain having a positive reaction to the immune serum will be considered to be infected with Junín virus, and the negative mice to be uninfected. The titration is done in the same way as before, and in this case the units will be ID_{50}. The same procedure cannot be used with guinea pigs because this animal is not a good producer of Junín CF antigen. The measurement of antibodies around the thirtieth day after inoculation of the virus serum mixtures is not reliable because there is great variation in individual response to the same inoculum, and even though the virus in the mixture may have been neutralized or killed, it may still be a good antigen capable of inducing an immune response in the guinea pig.

To diagnose a Junín virus infection in Argentina, it is only necessary for the isolated virus to react by CF with Junín antisera, or else for paired sera to show serological conversion with Junín antigen. For this reason, it is not known for sure whether the 100 or more strains of Junín virus mentioned in Chapter 1 are really one single agent, or whether they are different viruses with a common complement-fixing antigen.

Human convalescent sera from Junín infection have antibodies against Tacaribe virus as well, but to a lesser degree (*69*); human convalescent sera from cases of uremic-hemolytic syndrome have antibodies against Junín virus; and human convalescent sera from Bolivian hemorrhagic fever have antibodies against Machupo and Junín viruses and to a lesser extent against Tacaribe virus (*51*). It would not be surprising, therefore, if a careful study of the many virus strains currently labeled Junín were to show that some of them are Machupo, Amaparí, or new entities in the group.

The isolation of Junín virus from human beings directly in tissue cultures has not been reported, even though theoretically there is no reason why this could not be done.

To develop the system—antigen, serum, or ascitic fluid—in mice for comparison between viruses in the Tacaribe group or between strains of the same virus, the current methodology for arbovirus research is followed (*130*).

8. SPECIAL STUDY OF THE 1963 OUTBREAK

From the time that Junín virus was originally isolated up to 1963, no comprehensive serological study of an entire epidemic had been carried out to discover the exact role of Junín virus. Only scattered data on the rise of antibodies against this agent were available.

At the beginning of 1963, a well-planned serological study was started to determine the relationship of Junín virus to all the clinically diagnosed cases of AHF. For this purpose, two or three serum samples were taken from each patient for antibody investigation—one at the time of the initial medical examination, a second thirty days later, and in many instances a third two months after the onset (*63*). In addition to the serum samples, epidemiological data were collected on each patient at the first medical examination. This information included the patient's full name, sex, age, places of residence and work, and previous history of vaccination. With respect to the current illness, the date of onset, the degree of severity, and the presence or absence of similar cases within the same household were recorded. Finally, the number of the patient's record in the hospital was noted. These data, together with detailed findings from all the tests performed, were assembled in a professorship thesis for the Medical School of the University of Buenos Aires (*64*).

Four public medical centers located in Junín, Rojas, Chacabuco, and Salto took part in the program. From the 653 clinically diagnosed cases reported during that year (Table 1), it was possible to obtain 430 paired sera. Curves showing morbidity, number of cases studied through serology, and number of cases with serological conversion for Junín during the year are presented in Figure 4. In the complement-fixation test, 69.55 per cent of the paired sera studied showed serological conversion for Junín virus (*72*). The geographical distribution of these positive cases is shown in Table 2.

The paired sera without serological conversion for Junín, plus 40 other paired sera with conversion for Junín virus used as a control, were tested by hemagglutination-in-

FIG. 4. CURVES COMPARING REGISTERED CASES OF AHF WITH SEROLOGICAL RESULTS, 1963

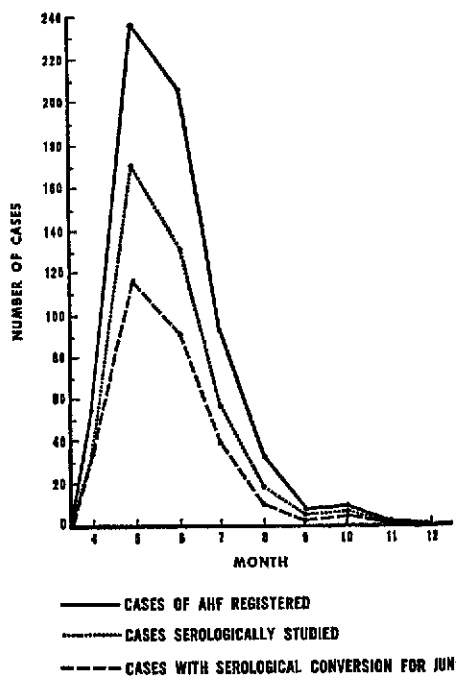


TABLE 1. CLINICALLY DIAGNOSED CASES OF AHF: GEOGRAPHICAL AND CHRONOLOGICAL DISTRIBUTION, 1963

Places	14-15*	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42-43	44-45	46-47	48-49	50-51	Date unknown	Totals
Totals	8	36	84	114	123	94	66	42	25	19	7	4	3	7	2	2	—	—	1	13	650†
Salto	5	10	27	35	33	25	16	14	3	3	—	1	—	1	—	—	—	—	—	1	174
Rojas	2	10	15	23	23	15	8	8	1	2	—	—	—	—	—	—	—	—	—	1	108
Junín	—	1	10	20	27	13	10	4	3	6	4	1	1	2	—	—	—	—	1	2	105
Chacabuco	—	9	20	21	22	23	17	8	5	6	—	—	—	2	—	—	—	—	—	—	133
Gral. Viamonte	—	2	—	3	4	2	3	2	—	1	—	—	—	—	—	1	—	—	—	—	18
Bragado	1	—	3	3	—	4	1	2	1	—	—	—	—	—	—	—	—	—	—	—	15
Alem	—	—	1	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Chivilcoy	—	1	—	3	—	1	—	—	—	—	1	1	1	—	—	—	—	—	—	—	8
Lincoln	—	—	1	1	5	3	4	2	4	—	—	—	—	—	—	—	—	—	—	—	20
Gral. Sarmiento	—	—	2	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	4
Gral. Arenales	—	—	—	—	—	—	1	1	—	—	1	—	—	—	—	—	—	—	—	—	3
Pergamino	—	—	—	—	1	2	—	—	2	—	—	—	—	—	—	—	—	—	—	—	5
Pehuajo	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
Córdoba	—	—	—	1	4	—	4	—	1	—	—	—	1	1	2	—	—	—	—	—	14
Others	—	—	1	1	1	1	—	1	3	1	—	1	—	—	—	—	—	—	—	—	10
Unknown	—	3	4	2	2	2	2	—	1	—	—	—	—	1	—	1	—	—	—	9	27

* Week of the year; for example, 14 corresponds to the week of March 31 to April 6, 1963.
† Plus 3 cases from Carmen de Areco.

TABLE 2. CASES OF AHF WITH SEROLOGICAL CONVERSION FOR JUNÍN VIRUS AS AGAINST ALL AHF CASES SEROLOGICALLY STUDIED, 1963

Places	14-15*	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42-43	44-45	46-47	48-49	50-51	Undeter.	Totals
Totals	5/6	22/22	38/53	58/84	48/82	50/64	28/36	19/29	10/15	3/7	4/6	0/2	2/3	2/6	1/2	1/1	—	—	1/1	5/8	297/427†
Salto	3/3	6/6	12/18	13/29	9/21	11/16	2/5	5/7	1/1	—	—	—	—	—	—	—	—	—	—	—	62/106
Rojas	2/2	9/9	8/10	17/22	11/18	9/10	7/7	6/8	1/1	0/1	—	—	—	—	—	—	—	—	—	0/1	70/89
Junín	—	1/1	9/9	14/15	11/19	11/12	6/8	2/2	2/2	2/3	2/4	0/1	1/1	1/2	—	—	—	—	1/1	0/2	63/82
Chacabuco	—	4/4	3/8	5/7	7/11	11/13	5/5	2/6	1/2	0/2	—	—	—	1/2	—	—	—	—	—	—	39/60
Gral. Viamonte	—	2/2	—	1/2	1/1	2/2	1/1	1/2	—	1/1	—	—	—	—	—	1/1	—	—	—	—	10/12
Bragado	0/1	—	3/3	3/3	—	1/3	1/1	2/2	1/1	—	—	—	—	—	—	—	—	—	—	—	11/14
Alem	—	—	1/1	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/2
Chivilcoy	—	—	—	1/1	—	1/1	—	—	—	—	1/1	0/1	0/1	0/1	—	—	—	—	—	—	3/6
Lincoln	—	—	1/1	—	4/5	2/2	2/4	1/2	2/4	—	—	—	—	—	—	—	—	—	—	—	12/18
Gral. Sarmiento	—	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/1
Gral. Arenales	—	—	—	—	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	1/1
Pergamino	—	—	—	—	—	1/2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/2
Pehuajo	—	—	—	—	—	—	—	—	—	—	1/1	—	—	—	—	—	—	—	—	—	1/1
Córdoba	—	—	—	1/1	4/4	—	2/3	—	0/1	—	—	—	1/1	0/1	1/2	—	—	—	—	—	9/13
Others	—	—	1/1	—	1/1	0/1	—	—	2/2	—	—	—	—	—	—	—	—	—	—	—	4/5
Unknown	—	—	0/2	2/3	0/2	0/1	1/1	—	0/1	—	—	—	—	—	—	—	—	—	—	5/5	8/15

* Week of the year; for example, 14 corresponds to the week of March 31 to April 6, 1963.
† Plus 3 positive cases from Carmen de Areco.

hibition, using the techniques of Clarke and Casals (*25*), against the following arboviruses: from group A: EEE, WEE, Mucambo, and Mayaro; and from group B: SLE, yellow fever, dengue Type II, Omsk hemorrhagic fever, Ilheus, and Kyasanur Forest disease (*65*). In the 40 paired sera with conversion for Junín virus, no conversions for arboviruses from groups A or B were found. In those without conversion for Junín, no antibodies against group A were found, but group B told a different story: some true conversions for SLE virus antigen and some activity against other members of group B were found; however, the responsible virus was not determined. In some instances it was noticed that a high antibody level against SLE was present in the first sample but that the titer against the same virus in the second sample, taken usually a month later, showed a decrease of twofold dilutions or greater. This decline in the hemagglutination-inhibition (HI) antibody level was good confirmatory evidence of a recent infection with one of the group B arthropod-borne viruses. Indeed, parallel findings were made during an outbreak of SLE in Tampa, Florida (*36*).

In summary, of all the cases studied, 69.55 per cent showed conversion for Junín virus by CF; 20.32 per cent showed activity for group B arboviruses, and 10.13 per cent had no antibodies against any of the viruses tested.

Lack of serological conversion by the CF test in 30 per cent of the patients could at first be attributed to poor sensitivity of the reaction; however, in view of the HI test results with group B antigens, this assumption appears unlikely. It would seem that some other viruses causing the same clinical picture as AHF are involved.

The presence of SLE virus, or at least a very closely related agent, has already been demonstrated in serological surveys (*73, 115*). However, the clinical effects of this virus in man have never been described in Argentina; even though cases of encephalitis occur, virological diagnosis is rarely available.

It was unexpected to find SLE or a closely related agent producing a hemorrhagic disease. Since no neutralization tests were performed, and only a few members of the group B arboviruses were included in the serological study, some doubt arose as to the identity of the virus, but it is known from the same study that the agent is neither Omsk hemorrhagic fever nor Kyasanur Forest disease.

Attempts to isolate viruses from acute-period sera (kept at −70°C) from AHF patients with conversion for group B arboviruses but not for Junín yielded three infectious agents. The investigators at YARU demonstrated that two of the strains were SLE. The third agent is still under study. It is different from SLE, but still closely related; it may be Ilheus.

Cases clinically diagnosed as AHF with serological conversion for SLE or some other group B arbovirus occurred at random over the entire epidemic area and throughout the full course of the outbreak (Fig. 5 and Table 3). These cases were treated at several separate medical centers, and apparently none of the attending physicians saw any difference from patients with a clinical diagnosis of AHF who showed conversion for Junín. The physician in charge of the Rojas Center stated that in his judgment there was no distinction in the severity of the illness between the two groups of patients. He observed severe, mild, and doubtful cases in both of them (personal communication).

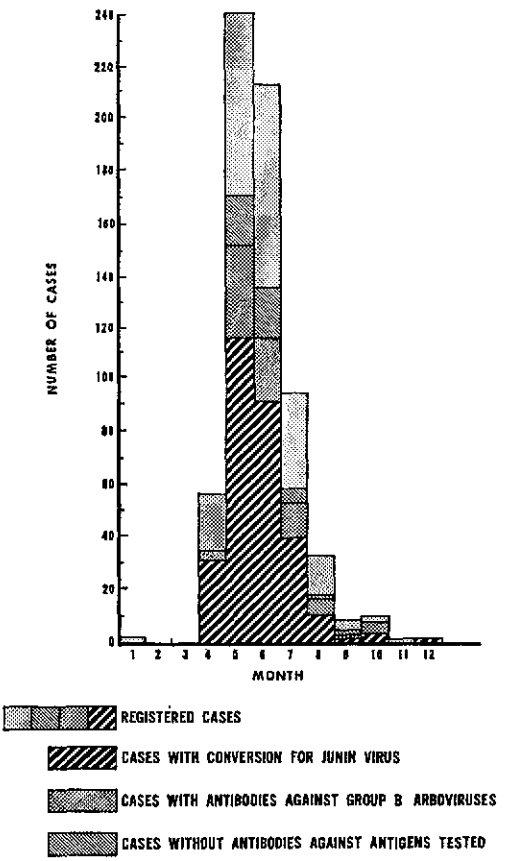
Information was exchanged with the author on the various clinical manifestations of AHF—the hemorrhagic, nervous, and

TABLE 3. AHF CASES SEROLOGICALLY NEGATIVE WITH RESPECT TO JUNÍN VIRUS: CASES WITH ANTIBODIES FOR GROUP B ARBOVIRUSES AS AGAINST TOTAL JUNIN-NEGATIVE CASES TESTED FOR GROUP B, 1963

Places	14-15*	16-17	18-19	20-21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42-43	44-45	46-47	48-49	50-51	Undeter.	Total
Totals	0/1†	—	7/11	7/9	14/23	4/5	1/6	8/9	3/4	2/3	2/2	1/1	0/1	3/3	—	—	—	—	—	1/1	53/79
Salto	—	—	4/5	2/2	3/4	1/1	0/1	2/2	—	—	—	—	—	—	—	—	—	—	—	—	12/15
Rojas	—	—	0/2	3/4	5/7	1/1	—	2/2	—	—	—	—	—	—	—	—	—	—	—	—	11/16
Junín	—	—	—	0/1	0/5	0/1	0/2	—	—	0/1	2/2	1/1	—	1/1	—	—	—	—	—	1/1	5/15
Chacabuco	—	—	3/4	2/2	4/4	—	—	3/4	1/1	2/2	—	—	—	1/1	—	—	—	—	—	—	16/18
Gral. Viamonte	—	—	—	—	0/1	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	1/2
Bragado	0/1	—	—	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/2
Alem	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chivilcoy	—	—	—	—	—	—	—	—	—	—	—	—	0/1	—	—	—	—	—	—	—	0/1
Lincoln	—	—	—	—	1/1	—	1/2	—	2/2	—	—	—	—	—	—	—	—	—	—	—	4/5
Gral. Sarmiento	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gral. Arenales	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pergamino	—	—	—	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/1
Pehuajo	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Córdoba	—	—	—	—	—	—	0/1	—	0/1	—	—	—	—	1/1	—	—	—	—	—	—	1/3
Others	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unknown	—	—	—	—	1/1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/1

* Week of the year; for example, 14 corresponds to the week of March 31 to April 6, 1963.
† Numerator: Number of cases with antibodies for group B.
Denominator: Number of cases checked against group B after knowing they were negative against Junín.

FIG. 5. HISTOGRAM OF REGISTERED CASES OF AHF AND SEROLOGICAL RESULTS, 1963



mixed forms. There are no data as yet to show whether the cases produced by SLE virus remain in the nervous form (*103, 104*) or in the hemorrhagic, or whether they can occur with the signs of both.

Further studies on this matter will be carried out.

Cases from the same cornfield, breaking out during the same week and even on the same day, and treated at the same public medical center, are produced sometimes by Junín and other times by a group B arbovirus.

With this new knowledge about the etiology of AHF, it will be necessary to look for viruses in mosquitoes from the endemic area and to explore other ways in which SLE virus can be transmitted to human beings.

Later, in 1964, at least one case of AHF without conversion for Junín or a group B arbovirus showed conversion for Maguari (BEAR 7272) from the Bunyamwera group (*66*). Since this antigen was not used in the 1963 tests, it could not be correlated with the 10.13 per cent of cases that had no antibodies for Junín or group B.

9. RECENT EXTENSION OF AHF TO NEW AREAS

Between 1955 and 1963, AHF was considered to be endemic only in the Province of Buenos Aires in an area approximately 20,000 km² (*53, 55*) with a population of about 250,000. In 1963, a new endemic area was recognized in the Province of Córdoba when 14 cases were registered. The complement-fixation test showed that 9 out of 13 were infected with Junín virus.

Vanella studied 76 cases in the Province of Córdoba over the period 1963-1964, and his clinical and epidemiological findings have been presented in a report (*123*). The latest data available from the Córdoba endemic area, where the cases are increasing each year, showed a morbidity index of 60: 100,000 and a mortality rate of 6 per cent.

In 1963, the main focus of the epidemic was in Salto, a parish in the Province of Buenos Aires where the disease had not been recognized before. In 1967, however, the epidemic was centered in the parish of Pergamino (also in the Province of Buenos Aires), where scattered cases had been observed in previous years. Only five cases from this area were clinically recognized in 1963; two of these were studied serologically, and one of them proved to be a Junín infection. The most recent news on morbidity and distribution of the disease in the Province of Buenos Aires appeared in *La Nación* on September 6, 1967. This report states officially that in 1967 Pergamino had 331 cases and 32 deaths; Rojas, 195 and 30 deaths; Salto, 167 and 12; Junín, 123 and 3; Nueve de Julio, 8 and 1; Chacabuco, 100 and 3; Carlos Casares, 3 and 1; Bragado, 9 and 3; Bartolomé Mitre, 87 and 7; Alberti, 7 and no deaths; Capitán Sarmiento, 3 and no deaths; and Colón, 26 and no deaths.

Since 1964, the Provinces of La Pampa and Santa Fe have had scattered cases in zones nearby the Province of Buenos Aires.

From all this data, it is clear that the endemic area is expanding every year and at the same time coming increasingly closer to the populous cities of Buenos Aires and Córdoba.

10. FINAL CONSIDERATIONS

AHF and its associated Tacaribe-group viruses have become a new public health problem in the Americas during the last decade (*117*). Numerous efforts have been made to clarify the epidemic-epizootic aspects of the disease and to explore possible approaches to immunoprophylaxis.

Among the many Argentine institutions that are devoting concentrated efforts to the study of the disease are the National Ministry of Public Health, through the Malbran Institute (*4, 8, 91, 121*); the National Institute of Agricultural Technology (INTA), through the Zoonoses Institute (*22, 23*); the Buenos Aires Provincial Ministry of Public Health (*54, 57*); the Córdoba Provincial Ministry of Public Health, through the Córdoba Institute of Virology (*26, 124*); and the University of Buenos Aires, through the School of Medicine (*40, 72*). In addition, individual physicians in the endemic area are doing everything they can to help combat the disease.

In response to a request for sera from persons with previous history of AHF for CF testing purposes, a local physician from Junín drew blood from all the patients he had attended from 1953 to 1962. In this way, 147 sera were obtained, and the results of this study are shown in Table 4. Factors taken into account were the number of years since the disease occurred and the patient's place of residence.

Although the study of the 1963 epidemic did show that 70 per cent of the AHF cases had CF antibodies against Junín virus during the convalescent period, this retrospective survey could not provide very much further concrete data on the subject because of insufficient knowledge about CF reaction against Junín when patients were in convalescence.

TABLE 4. PRESENCE OF JUNÍN VIRUS CF ANTIBODIES IN PERSONS WHO HAD AHF FROM ONE TO TEN YEARS BEFORE SEROLOGICAL TEST *

No. of Years after AHF	Number of Cases Positive by CF/Number of Cases Studied in Each Parish							
	Junín	Rojas	Chacabuco	Bragado	Vlamonte	Chivilcoy	Lincoln	Total
Totals	25/54	1/4	15/33	9/28	13/24	3/3	0/1	66/147
1 year	2/8	0/1	4/8	1/4	4/7	—	—	11/28
2 years	6/12	1/2	2/5	3/4	3/4	2/2	—	17/29
3 years	8/17	—	3/5	1/8	1/3	—	—	13/33
4 years	8/11	0/1	3/8	3/6	4/5	1/1	0/1	19/33
5 years	1/5	—	3/6	0/2	1/3	—	—	5/16
From 6 to 10 years	0/1	—	0/1	1/4	0/2	—	—	1/8

* Based on blood samples provided by C. Magnonf.

The information in Table 4 does not include the former patients' sex, because only 9 of the 147 subjects were females—5 positive and 4 negative. Of the remaining 138 males, 61 showed antibodies in the complement-fixation test and 77 did not. Since no serological tests were performed with these patients' sera at the time of the illness, the present results cannot be interpreted as necessarily proving the persistence of antibodies to Junín virus. This is especially true since it is now known that some of the cases clinically labeled as AHF show conversion to viruses other than Junín. It has also been shown that with laboratory-acquired infections the CF antibodies disappear within a year (*6*). Furthermore, it is possible that continuing residence of former patients in the endemic area may expose them to new contacts with the virus, thus creating a booster effect.

Although no neutralization tests were performed with these 147 sera, neutralizing antibodies have been detected as long as 14 years after an illness (*6*). However, since no neutralization-test surveys are available to show the rate of antibody prevalence among persons living in the endemic area who have never had the clinical syndrome, it is hard to interpret such long-lasting antibodies.

There are instances of persons who have had AHF more than once. A patient from Junín was hospitalized three times, in three different years, with a diagnosis of AHF. If neutralizing antibodies persist for life, then there must be more than one virus involved in the etiology, as the serological findings from the epidemic seen to indicate. Each subsequent epidemic brings additional information about serological conversion for viruses other than Junín.

Prevention of human infection can be accomplished in several ways. One method is to reduce the population of infected animals in the endemic area. The spreading of rodenticides and acaricides from helicopters has not been well received. And with reason: the first big AHF epidemic, in 1958, appeared shortly after fumigation of the area with dieldrin and, since the etiological agent of the disease was unknown, many people felt that this toxic substance was to blame for the disease, or at least for creating a predisposition thereto (*52*). This may be a coincidence, but it is true that fumigation with dieldrin could trigger a biological imbalance, as has been claimed by many Argentine investigators, and it is hard to prove that this is not so.

Another approach to reducing human infection would be to introduce mechanized equipment for picking corn. Corn harvesters, however, are not the only people who contract the disease, and even if mechanical corn pickers were available, there are times when harvesting has to be done by hand.

Still another avenue, and one worthy of serious consideration, is to immunize the population at risk. However, a vaccine has yet to be developed. A problem is to determine what will go into it. If the serological findings and the results of the virus isolation attempts from the 1963 epidemic are carried through, then it would appear as though the vaccine would have to contain agents of SLE and some other group B arboviruses, in addition to Junín. Moreover, many Junín virus strains would have to be studied comparatively by neutralization tests to see if they are alike.

Once all this information is in hand, attention will still have to be given to the development of a faster and more economical method than the neutralization test for evaluating the vaccine's effectiveness in man.

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