Clinical and epidemiological patterns of Argentine haemorrhagic fever

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The epidemiology of Argentine haemorrhagic fever (AHF) is closely related to cricetine rodents acting as natural hosts of Junin virus. The endemo-epidemic area, which has increased 5 times since the disease was first recognized 15–20 years ago, is located in a densely populated region of Argentina. It has been shown that the virus of LCM is active in humans and rodents of the AHF endemic area; this demonstrates the simultaneous presence of two arenaviruses pathogenic for man in a given geographic location.

The disease is characterized by haematological, renal, neurological and cardiovascular changes. Electron microscopy and immunohistochemical studies have shown cytopathic changes, characteristic intracellular virus-like particles, and antigenic determinants of Junin virus in different organs from 9 cases of AHF. No deposits of immunoglobulins or C3 were found in the kidneys; in addition, an absence of fibrinogen and C3 in the hepatocytes and of immunoglobulins in the spleen was observed. These findings suggest a direct viral pathogenic action in the human disease.

Ultrastructural and immunofluorescence studies in tissues of guinea-pigs inoculated with two strains of Junin virus revealed the presence of the same types of virus-like particles and antigenic determinants of Junin virus as were encountered in the human subjects with AHF.

EPIDEMIOLOGICAL CHARACTERISTICS

Epidemics of AHF were recognized more than 20 years ago in the north-west of the Province of Buenos Aires. The endemo-epidemic area is located in the humid pampa, in latitudes 33°–37° south and longitudes 59°–64° west (Fig. 1). This region is the richest farming land of Argentina, with a temperate climate and an average annual rainfall of 1000 mm.

In 1958, the cases were limited to an area of approximately 16 000 km², with a population of 270 000. However, over the years the endemo-epidemic region has extended progressively northwards and westwards. In 1963, cases of AHF were confirmed in the south-east of the Province of Córdoba, and between 1964 and 1967 new areas were detected in the Province of Buenos Aires. Cases began to appear later in the south of the Province of Santa Fé. At present, the endemo-epidemic region covers an area of approximately 100 000 km², with a population of more than 1 million. Thus, in a period of 17 years the area in which AHF is endemic has increased 5 times. Although the region is still relatively small in comparison with the total size of Argentina (almost 3 million square kilometers and 25 million people), the progressive extension of AHF and the fact that 50% of the population live in the endemo-epidemic region or within 50 miles of it, greatly increase the public health importance of the disease.

Between 1958 and 1974, almost 16 000 cases of AHF were notified on clinical grounds. However, until 1965 the virological diagnosis of the disease was only attempted in some of the notified cases. Because of this and for other reasons, an epidemiological unit was established in 1965 in Pergamino (a district of 3000 km² and a population of 75 000, located less than 150 miles west of the city of Buenos Aires). Table 1 shows the results obtained from 1965 to 1974. The diagnosis was confirmed in 64% of the notified cases, most often by means of a serological conversion in complement fixation (CF) tests, but in some patients by the isolation of Junin virus from blood obtained during the acute phase or from autopsy specimens. The cases listed as doubtful are

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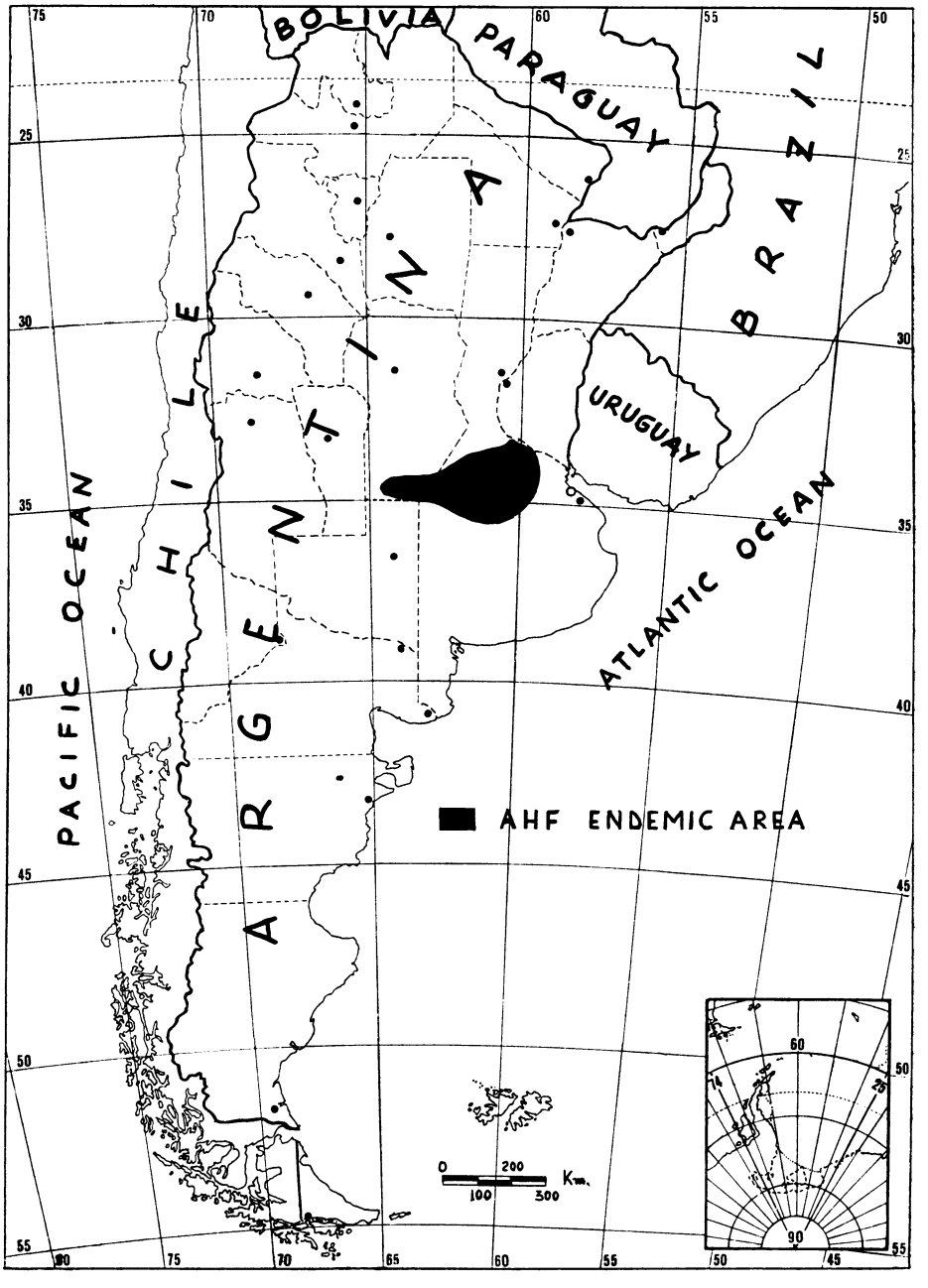

Fig. 1. Map of Argentina showing the area in which AHF is endemic.

Table 1. Cases of AHF studied in Pergamino from 1965 to 1974

Year	Clinical diagnosis (notified cases)	Virological diagnosis		
		confirmed [a]	negative	doubtful
1965	63	17	40	6
1966	175	111	44	20
1967	341	214	73	54
1968	87	46	36	5
1969	549	333	151	65
1970	456	265	152	39
1971	310	208	57	45
1972	268	196	54	18
1973	435	273	87	75
1974	391	296	85	10
Totals	3075	1959	779	337

[a] CF conversion of paired sera and/or isolation of Junin virus from acute blood or autopsy specimens.

Table 2. Age and sex distribution of virologically confirmed cases of AHF studied in Pergamino from 1965 to 1974

Age group (years)	No. of patients		
	male	female	totals
0-14	89	65	154
15-19	176	40	216
20-29	364	87	451
30-39	362	71	433
40-49	277	61	338
50-59	176	47	223
60-69	96	22	118
> 70	21	5	26
Totals	1561	398	1959

those in which the virological studies could not be completed, generally because an insufficient number of serum samples were available. It is worth while mentioning that in a recent study, neutralizing antibodies were detected in about 10% of CF-negative convalescent serum samples from a random group of patients (Barrera Oro et al., unpublished observations). It is therefore reasonable to assume that the proportion of virologically confirmed cases is higher than that shown in Table 1.

The risk is not uniform throughout the endemic region. Between 1965 and 1974 a total of 8728 cases were notified; the 3075 notifications from Pergamino therefore represent 35% of all cases for that period. Considering that Pergamino occupies a very small area of the AHF region, and that the majority of its 3075 cases became infected within the district, it is apparent that there are marked differences in risk within the endemo-epidemic area and that the risk of becoming ill is greater in newly affected regions.

The epidemics have a seasonal distribution, with a peak incidence in the month of May. The disease is 4 times as prevalent in males as in females and is more prevalent among rural workers than in the urban population. Table 2 shows the age group and sex distribution of patients in whom a virological diagnosis of AHF had been established among those studied in Pergamino from 1965 to 1974.

Wild rodents are the reservoir of Junin virus. Although several species of Cricetidae and Muridae coexist in the endemic region, field and experimental studies have shown that only members of the Cricetidae appear to be effective reservoirs of the virus (Sabattini et al., unpublished observations). *Calomys musculinus* and *C. laucha* develop a persistent, inapparent infection with Junin virus; there is prolonged viraemia and the virus is regularly recovered from oral swabs. These properties ensure the maintenance of the virus in nature. In experimentally infected *C. musculinus*, evidence of horizontal transmission has recently been obtained (Sabattini et al., unpublished observations).

The seasonal variations in the size of rodent populations—with an increase during the epidemic months—explain the seasonal prevalence of AHF. At least two factors account for the predominance of the disease in rural workers; Cricetidae are found almost exclusively in the fields, and the annual increase in their numbers coincides with the corn harvesting season and therefore with an increase in the male population at risk.

During 1969 and 1970, the activity of the virus of lymphocytic choriomeningitis (LCM) was observed in humans and rodents in the AHF endemic area (1). CF antibodies against LCM virus antigens were found in serum samples from more than 20 *Mus musculus* that had been captured in the city of Pergamino (2). At the same time, an agent isolated from a *Mus musculus* trapped in the AHF endemo-epidemic area of the Province of Córdoba, was

identified as a strain of LCM virus (3). It is worth while noting that strains of Junin virus were isolated from 2 *C. musculinus* captured in the same place and at the same time as the *M. musculus* from which the strain of LCM had been recovered. This was the first demonstration of the coexistence of two arenaviruses pathogenic for man in a given geographical area.

A strain of LCM was also isolated from the blood of a patient with a clinical diagnosis of AHF during the acute phase of the disease (Maiztegui et al., unpublished results). In addition, a serological conversion for LCM in the CF and neutralization tests was found in several patients from the Provinces of Buenos Aires, Córdoba, and Santa Fé in whom a presumptive clinical diagnosis of AHF had been made (4).

A retrospective study of paired serum samples from about 3000 notified cases of AHF revealed the simultaneous presence of CF antibodies to both Junin and LCM viruses in 80 of these patients; in most of them, the antibodies were detected in the convalescent serum sample and the titres were similar (4). There are several possible explanations of this finding. One is that the patients may have acquired simultaneous infections with both viruses; this hypothesis is supported by the fact that both viruses are present in rodents from the same geographic regions where the patients acquired the infection. Another possibility is that the patients were infected with a different agent, antigenically related to both Junin and LCM viruses. A third possibility is that the patients may have been infected at some time in the past with one of these viruses and more recently with the other; the second infection would then have acted as a booster, giving a secondary heterologous reaction.

Table 3. Neutralization tests in patients with CF antibodies against Junin and LCM viruses

Patient number	Serum sample	Neutralizing index	
		Junin (XJ Cl3)	LCM (1371)
059/86	Acute	0.0	0.0
	Convalescent	2.8	0.0
P 1988	Acute	1.4	1.0
	Convalescent	3.1	0.7
P 2012	Acute	0.9	2.1
	Convalescent	3.5	2.8
P 2068	Acute	0.0	0.7
	Convalescent	4.2	0.7
P 2085	Acute	0.0	3.0
	Convalescent	3.2	3.0
P 2136	Acute	0.0	1.0
	Convalescent	3.2	1.0
P 2147	Acute	0.2	0.2
	Convalescent	3.3	1.2

Although these possibilities are still under investigation, some observations seem to support the last explanation. The results of neutralization tests with acute and convalescent serum samples from some of the patients indicate a true conversion for Junin virus (Table 3). Also, the agents recovered from the blood of 5 of these cases during the acute infection have been classified preliminarily as Junin virus. In any case, the simultaneous activity of Junin and LCM viruses creates new and challenging diagnostic, therapeutic, and preventive problems in Argentina.

THE DISEASE IN MAN

AHF is characterized by renal, haematological, neurological, and cardiovascular changes. The mechanism of natural infection has not been elucidated; although mites have been implicated, there is no conclusive evidence that mites or any other arthropod vectors play a role in the transmission of AHF. The incubation period appears to be between 8 and 12 days. The clinical disease has been described in detail (5, 6, 7). Although the mortality is between 10% and 20%, in general the outcome is favourable: an acute febrile period of 8–10 days' duration is followed by a prolonged convalescence with loss of hair, but usually without permanent sequelae. In the severe cases, instead of the gradual defervescence the neurological and/or haemorrhagic manifestations increase; convulsions, profuse bleeding, coma, shock, or a combination of these signs results in death of the patient within 48–72 hours.

Although the manifestations of the disease are characteristic only in the moderate and severe clinical forms, the recognition and diagnosis of mild cases appears to be improving. Some observations suggest that the proportion of less severe forms of AHF is higher in areas where the disease has been

present for years. Whether this difference is a real one or results from better diagnosis remains to be determined. Nevertheless, this is one of the factors that should be taken into account when interpreting statistics that indicate a decreasing mortality in certain parts of the endemic area.

There is general agreement that in the great majority of cases Junin virus infections cause clinical disease. However, subclinical forms have been observed (Maiztegui et al., unpublished observations, Weissenbacher et al., unpublished observations). AHF is not usually contagious, but transmission from man to man can occur (8). Junin virus has occasionally been isolated from oral swabs and from the urine of patients (7) and viraemia is present throughout the acute febrile period, which lasts from 7 to 12 days (9). It is noteworthy that CF or neutralizing antibodies are not detected earlier than 3–4 weeks after the onset of symptoms, and in some patients even later. In this respect, preliminary results of a study in progress indicate that the immune response is impaired in patients infected with Junin virus (Arana et al., unpublished results).

During the acute phase of AHF there is progressive leukopenia and thrombocytopenia, with counts falling to 1000–2000 white cells and 50 000–100 000 platelets per mm^3. Suarez et al. have shown that there is bone marrow inhibition (10), that the platelet half life is reduced in some cases (11), and that there are no platelet antibodies in such patients (11).

A study by Schwarz et al. (12) revealed changes in several clotting factors; the bleeding time and the clot retraction were prolonged, in agreement with the reduction in the number of platelets. The prothrombin time (Quick) was abnormal in half the patients and the recalcified plasma time (Howell) was abnormal in more than half. Similarly, reductions were observed in Factors II, VII, and X. Fibrinogen levels were low and, in some of the very ill patients, the presence of fibrinogen degradation products—in the absence of fibrinolysis—indicated a process of intravascular coagulation; in these cases, a moderate reduction in Factors V and VIII was also observed. Independently, Agrest et al. (13) have also reported a consumption coagulopathy in a case of AHF with acute renal failure.

Almost invariably, there is an impairment of renal function, with oliguria and albuminuria. Anuria is infrequent, except in terminal cases; in the urinary sediment, there are hyalin-granular casts, round cells with cytoplasmic inclusions (14) and, less frequently, haematuria. Dávalos et al. (15) reported that glomerular filtration, renal plasma flow, and creatinine clearance were within normal limits, indicating that the proximal segment of the nephron is not affected. The results of this functional study are in agreement with recent electron microscopy and immunofluorescent observations (16), which revealed severe damage in the distal and collecting tubular cells of the kidney without significant alterations in the glomeruli or proximal tubules.

Neurological signs and symptoms are very common, indicating diffuse changes in the central nervous system. Usually, there are no meningeal signs, and the cerebrospinal fluid is within normal limits (17).

Electrocardiographic changes indicative of myocardial involvement are present during the acute febrile period (18, 19); there is moderate bradycardia and hypotension, and shock is observed in the severe clinical forms of AHF.

Enzymatic changes, particularly in glutamic-oxalacetic transaminase, lactic dehydrogenase, and creatine phosphokinase were observed by Mandó et al. (20). In some patients, there is hyperglycaemia and glucosuria.

The treatment of AHF is mainly symptomatic, aiming at the maintenance of a correct fluid and electrolyte balance. Convalescent plasma is given on the assumption that it may exert a specific effect, and because beneficial results have been reported (21, 22). A controlled therapeutic trial to determine its effectiveness is in progress.

Examination of the gross pathology and light microscopy studies have shown nonspecific alterations similar to those described in other haemorrhagic fevers (23). A recent study using electron microscopy and immunohistochemical techniques revealed the presence of specific lesions in tissues from 5 human cases of AHF (24); the same lesions were found in 4 further cases studied during the 1975 epidemic of AHF. In these studies, two types of characteristic intracytoplasmic virus-like particles were seen in the cells of all the organs examined (liver, kidney, spleen, lymph nodes, salivary glands, pancreas, heart, intestine, adrenals, hypophysis, ovary, testis, and brain). These particles differ in morphology, morphogenesis, and location from the viral particles described in cell cultures and tissues of rodents experimentally infected with arenaviruses (Fig. 2). They were located in the lumen of the endoplasmic reticulum cisternae, and apparently originated by a process of budding from the endoplasmic reticulum wall.

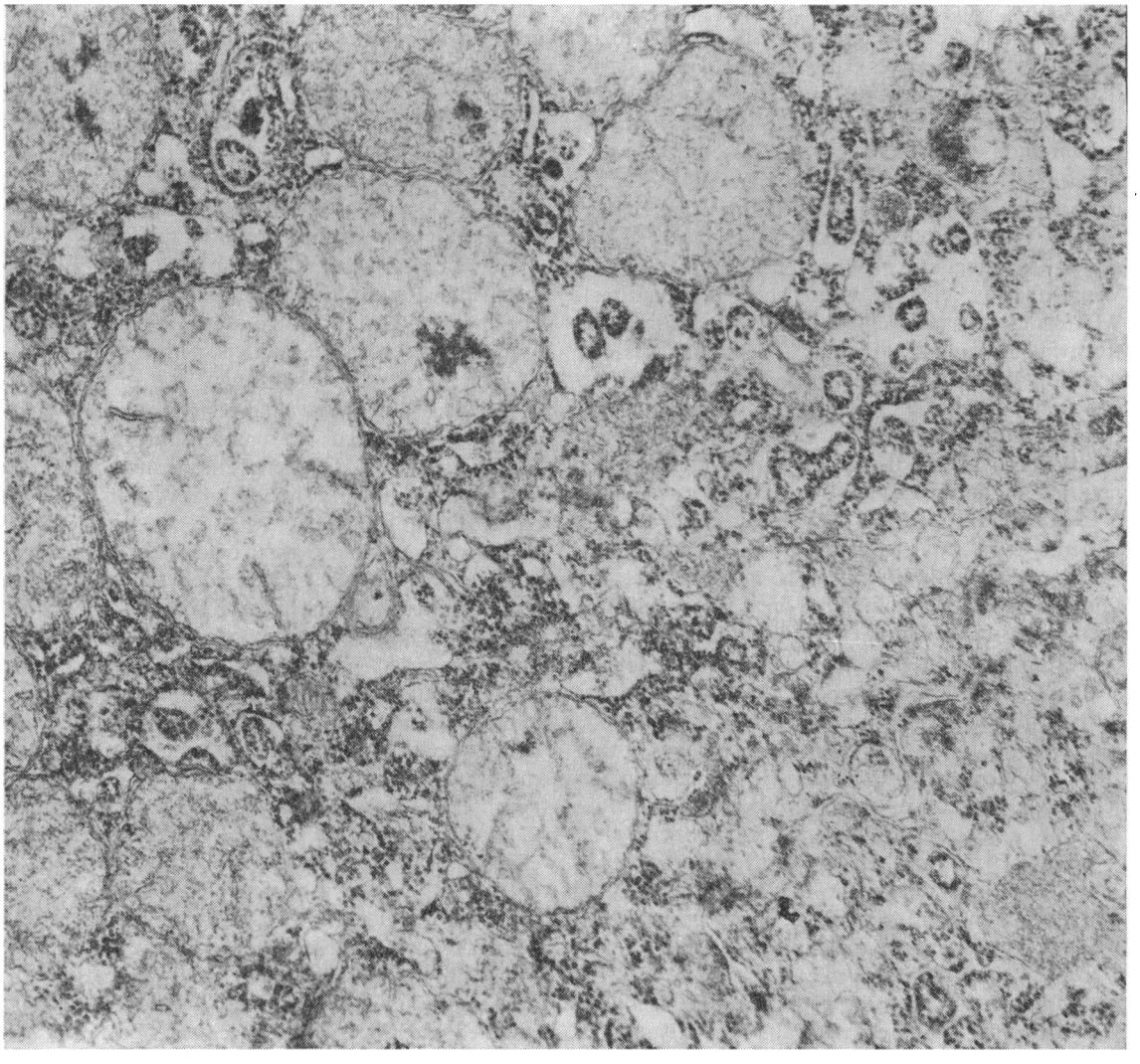

Fig. 2. Part of the cytoplasm of a hepatocyte. Virus-like particles can be seen in the lumen of the endoplasmic reticulum cisternae (× 45 000).

One of the two particle types was heterogeneous in size and shape, measuring 80–300 nm, and was invested by a single envelope, similar to the endoplasmic reticulum wall; within the particle, an electron-lucid matrix contained a variable number of ribosome-like granules. The second type of particle was smaller, uniform in size and of spherical shape, measuring 60 nm in mean diameter; within the particle there was an electron-dense matrix with a finely granular aspect in the centre, and it was also invested by a single membrane similar to the endoplasmic reticulum wall. The number and distribution of both types of particle was variable for each organ, as well as for the different cases examined. In no case were any alterations observed in the plasma membrane or extracellular virus-like particles. Although it is not possible to determine on morphological grounds the significance of these virus-like particles, it appears that the larger, electron-lucid and pleomorphic type of particle results from the

convoluted appearance of the rough endoplasmic reticulum, which gives rise to pseudo-viral images. It remains to be demonstrated if the smaller, electron-dense particles found in the tissues of humans with AHF are true virions, or if they represent a specific cytopathic effect produced by Junin virus.

In addition to the virus-like particles, and coincident with them, marked nonspecific cellular damage and constant cytopathic changes were observed in all the organs examined. Intranuclear bodies and 3 types of cytoplasmic change were seen: (*a*) the presence of an electron-dense reticular network in which small interconnected tubular images could be distinguished; (*b*) an accumulation of ribosomes; and (*c*) a remarkable irregular shape of the endoplasmic reticulum, characterized by circumvolutions and pseudo-viral images.

Antigenic determinants of Junin virus were detected in the cytoplasm and nuclei of the cells of the organs examined by immunofluorescence techniques in all cases (Fig. 3). The nuclear fluorescence was granular and similar in size to the nuclear bodies observed with the electron microscope. The presence of specific nuclear fluorescence has been reported by Garay in neurons of mice inoculated with Junin virus (25), and by Mims in different organs of LCM carrier mice (26).

In other immunofluorescence experiments, no deposits of immunoglobulins or C3 were found in the glomeruli of patients with AHF. It is therefore unlikely that immune complexes are implicated in the pathogenesis of AHF. In addition, there were no deposits of immunoglobulins in the liver. The search for fibrinogen and C3 in the cytoplasm of hepatocytes gave consistently negative results. In other observations, neither C3 nor immunoglobulin was

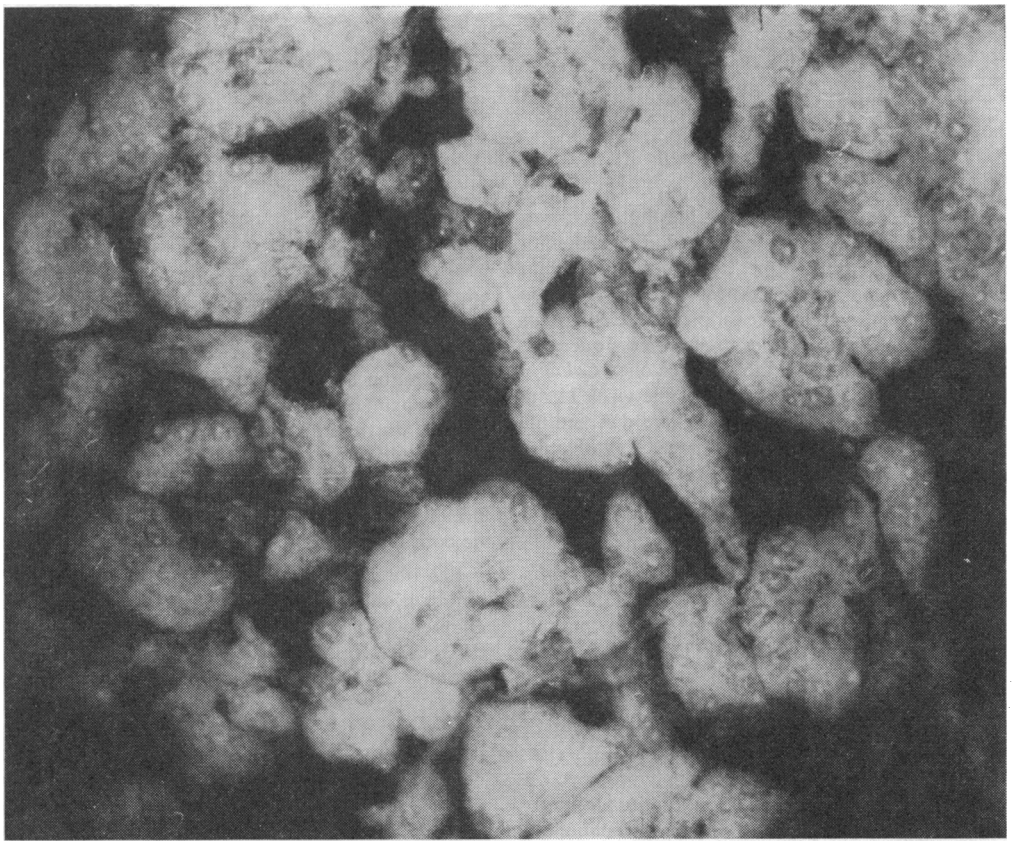

Fig. 3. Direct immunofluorescence: pancreas cryostat section, treated with fluorescein-labelled anti-Junin virus mouse ascitic fluid. Positive staining in the cytoplasm and in the nuclei is observed (original magnification × 250).

found in the spleen of the cases examined. These findings indicate profound alterations in the cellular mechanisms of protein synthesis.

The results of the electron microscopy studies, the immunohistochemical observations, and the functional abnormalities detected in several previous studies suggest that in humans with AHF Junin virus produces a direct pathogenic action.

Since guinea-pigs are known to provide a good experimental model of the disease, and in order to find out if the ultrastructural and immunofluorescence findings reported in humans could be reproduced experimentally, one group of guinea-pigs was inoculated with a strain of Junin virus that had been isolated from one of the human cases, while another group was inoculated with a prototype strain of Junin virus (XJ strain). Virus-like particles, cytopathic changes, and antigenic determinants of Junin virus similar to those found in humans were also encountered in different tissues of the guinea-pigs experimentally infected with the two strains of the virus (27).

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RÉSUMÉ

ASPECTS CLINIQUES ET ÉPIDÉMIOLOGIQUES DE LA FIÈVRE HÉMORRAGIQUE D'ARGENTINE

Les rongeurs de la famille des Cricétidés hôtes naturels du virus Junin jouent un rôle important dans l'épidémiologie de la fièvre hémorragique d'Argentine. *Calomys musculinus* et *Calomys laucha* contractent une infection chronique inapparente; la virémie est prolongée et le virus est régulièrement observé dans les prélèvements buccaux. La transmission horizontale a été prouvée chez *Calomys musculinus* infecté expérimentalement par le virus Junin. Quelque 16 000 cas de fièvre hémorragique d'Argentine ont été notifiés depuis que la maladie a été identifiée il y a environ 17-20 ans, et depuis lors l'étendue de la zone endémo-épidémique, inscrite dans une région où vit plus de la moitié de la population argentine, a augmenté de cinq fois.

On a mis en évidence l'activité du virus de la chorio-méningite lymphocytaire chez l'homme et les rongeurs dans les parties affectées par l'endémie des provinces de Buenos Aires, Cordoba et Santa Fé. D'autre part, des études sérologiques effectuées sur des malades ayant fait l'objet d'un diagnostic clinique de fièvre hémorragique d'Argentine ont permis de déceler, par la réaction de fixation du complément, la présence simultanée du virus de Junin et de celui de la chorio-méningite lymphocytaire.

La maladie est caractérisée par des altérations hématologiques, rénales, neurologiques et cardio-vasculaires. Il y a une leucopénie et une thrombocytopénie marquées dues à l'inhibition de la moelle osseuse ainsi que diverses altérations des facteurs de coagulation, avec les signes d'une coagulation intravasculaire dans certaines formes cliniques graves.

On observe presque toujours de l'oligurie, de l'albuminurie, ainsi que la présence de cylindres granuleux-hyalins et de cellules rondes à inclusions cytoplasmiques. Une étude fonctionnelle et des observations par microscopie électronique et immunofluorescence ont montré que le tube proximal du néphron n'est pas altéré, alors que les cellules du tube distal et du canal collecteur sont gravement détériorées.

Le traitement de la fièvre hémorragique argentine est principalement symptomatique; on administre du plasma de convalescent dont on escompte un effet spécifique.

Les observations tant macroscopiques que microscopiques n'ont fait apparaître que des altérations non spécifiques. Toutefois, dans neuf cas de fièvre hémorragique d'Argentine, des observations ultrastructurelles et immuno-histo-chimiques ont révélé des lésions spécifiques: deux types de particules intracytoplasmiques de caractère viral, des modifications cytopathiques et des déterminants antigéniques du virus Junin ont été trouvés dans les organes de tous les sujets examinés. D'autres observations ont permis de déceler des altérations graves des mécanismes cellulaires de la synthèse des protéines. Ces observations font penser que dans le cas de la fièvre hémorragique d'Argentine le virus aurait une action pathogène directe.

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