SIXTEENTH MEETING OF THE
ADVISORY COMMITTEE ON MEDICAL RESEARCH

Washington, D.C.
11-15 July 1977

VIRAL HEMORRHAGIC FEVERS - RESEARCH NEEDS

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VIRAL HEMORRHAGIC FEVERS - RESEARCH NEEDS*

Dr. Bond has reviewed the current epidemiological situation with respect to Argentine (Junin) and Bolivian (Machupo) hemorrhagic fever. An assessment of contemporary research needs requires a brief summary of current knowledge of the biology of these arenaviruses.

The Role of Cricetid Rodents

Although Junin virus has been recovered from other species, it is almost certainly a parasite of *Calomys musculinus* and *Calomys laucha*, two closely related sympatric field mice found in southern South America. These mice reach highest population densities in the fertile cultivated fields of the Argentine pampa. Machupo virus has been isolated only from a slightly larger mouse *Calomys callosus* inhabiting the grassland-forest edges of eastern Bolivia and adjacent Paraguay and Brazil. Virus distribution covers only a relatively small portion of Beni Province in Bolivia.

Each virus in its respective rodent hosts produces clinically silent chronic infection. Such rodents continuously excrete large amounts of virus in the urine and this is thought to represent the source of human infection since no arthropod has been incriminated in biological transmission of either virus (as for that matter any other arenavirus). Human disease, therefore, is influenced by a number of factors:

1. Rodent population density and proximity to human dominated habitats.
2. Prevalence of chronic virus infection in rodent populations.
3. Density and activity of human populations.
4. Levels of basic human sanitation with respect to rodent exposure.

In a given endemic region, virus transmission to man appears to be a function largely of the first two factors. *Calomys* rodent populations are cyclic and there is both experimental and field evidence in the case of Machupo virus to suggest that high population density also is associated with high prevalence of chronic virus infection. The cyclic 3-5 year peaks in Argentine hemorrhagic fever strongly imply a similar phenomenon in the case of Junin virus.

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Disease Control through Rodent Control

The Beni Province of Bolivia is principally cattle country, sparsely inhabited by man. There hemorrhagic fever is mainly a disease acquired by contact with peridomestic Calomys. Rodent control programs of limited geographic extent have proven highly successful in halting and preventing epidemics in the larger towns of the endemic region, none of which has more than 10,000 people. The adjacent province of Santa Cruz, with more population, more agriculture and probably more Calomys callosus has not experienced hemorrhagic fever. This is due to the fact that the rodent in this region is infected with Latino virus, another arenavirus, but one which is unlikely to be acutely pathogenic for man. Some years ago, work was undertaken with Machupo and Latino viruses in Calomys from each province to explore the feasibility of biological control of Machupo by introduction of either Latino virus or Santa Cruz Calomys, or both into Beni Province. Although small but definite differences were found in chronicity of infection and quantitative virus shedding in these reciprocal studies, the conclusion was that this elegant approach would not work. Indeed it appeared likely that introduction of Machupo-infected Calomys into Santa Cruz Province would be followed by outbreaks of hemorrhagic fever.

The situation in Argentina is quite different. The endemic area and the human population subscribed by it is much larger. Disease is apparently acquired in the fields rather than in or near the home. Direct rodent control does not seem a feasible approach. Nor has any natural avirulent arenavirus been found in Argentine Calomys.

Disease Control through Vaccination

Biohazards in Arenavirus Research

Although there are few documented instances of direct person to person transmission of Machupo and Junin viruses, severe and even fatal disease has resulted from exposure of laboratory workers to virus-containing droplets. Safety during serious work toward vaccines for such agents depends either on availability of personnel who are immune through prior infection or of special laboratories engineered to protect both the external environment and the experimenter.

Junin Virus Vaccines

About 700 persons have been successfully immunized (neutralizing antibodies) with a strain of Junin virus attenuated for guinea pigs by routine passage through suckling mice. Further human trials were suspended because of questions relating to possible adventitious agents in the vaccine seed and in the suckling mice used to grow the
vaccine virus. No data are available on experimental neurovirulence of Junin virus or the vaccine strain in non-human primates nor on possible teratogenic effects of the agent. Because of the basic rodent biology of arenaviruses in which chronic infection and circulation of antigen-antibodies are thought to lead to "late" disease such as glomerulonephritis, some workers have questioned whether it is wise to intentionally infect the several millions of persons it would require to prevent a few hundred cases and the usually less than 100 fatalities caused by Junin virus annually.

Recent animal work also has shown that infection with Tacaribe virus, another arenavirus thought to be avirulent for man, produces Junin neutralizing antibodies and protection against virulent virus challenge.

Machupo Virus Vaccines

Serial passage of two Machupo virus strains led to progressive attenuation in virulence for guinea pigs and Rhesus monkeys in one but not the other. A major antigenic change was observed as well but monkeys successfully infected with the high passage (Sm80) strain were resistant to challenge with a large dose of wild Machupo virus. Viremia in such animals was minimal or absent. Interestingly, this "vaccine" virus was found to kill suckling Calomys callosus and to produce a silent, non-chronic infection in adult rodents. Further work on this strain has been dropped for the present because of the general concern about long-term consequences of arenavirus infection in man. Sm80 virus failed to grow to sufficient titer in cells suitable for human vaccines to serve as source of a potent inactivated vaccine.

Efforts continue at the U.S. Army Medical Research Institute for Infectious Diseases to make a killed Machupo virus vaccine. A strain highly virulent for monkeys (and probably man) does produce sufficient antigen to protect monkeys against challenge. The concern here, of course, is with inactivation and safety testing.

Specific Research Needs

I. Argentine Hemorrhagic Fever

A. Epidemiology and Ecology

1. Detailed retrospective study of a large group of AHF patients infected at least 10 years previously. If subsequent mortality data can be included with adequate reexamination of survivors, some major questions regarding late effects, if any, of AHF could be answered.
2. Rodent-based viral mapping of geographic extent of Junin virus distribution. Is the virus truly spreading or are conditions favorable for rodent and viral infection prevalence increasing over a wider area?

3. Detailed experimental documentation of chronicity, vertical vs. horizontal transmission and urinary virus shedding in both Calomys musculinus and Calomys laucha. It is important to decide which of these rodents is the basic virus reservoir/vector.

4. An experimental study of the biology of the attenuated Junin virus in Calomys rodents. If this agent is efficiently transmitted from rodent to rodent, but urinary excretion is less than with field strains, it might be possible to "vaccinate" the wild rodent population. A better in vitro marker than those now available is needed to discriminate wild and vaccine strains for such work.

5. A controlled study to determine whether convalescent plasma (passive antibody) is really of value in treatment of the disease.

B. Vaccine Development

1. Live Attenuated versus Inactivated

Only the competent authority in Argentina can make this basic decision. In the former case, critical needs appear to be:

a. virus cloning and preparation of stocks in cells certified for human vaccine production.

b. serial passages in guinea pigs and/or monkeys to test for reversion to virulence.

c. examination of neurovirulence and teratogenic potential in monkeys.

d. the retrospective AHF study cited under IA 1.

e. redocumentation of efficacy and safety in man by phased clinical studies with emphasis on careful documentation of patterns of virus shedding in initial trials.
If an inactivated vaccine is desired, first priority should go to selection of a virus strain which grows well enough in vaccine-quality cell cultures to be immunogenic. This may mean use of human-virulent Junin virus which implies a special laboratory designed to control the biohazard which accompanies manipulation of large quantities of such an agent.

Much good work has been done on Argentine hemorrhagic fever in that country. PAHO should carefully consider the merits of increased support of a program which holds high promise of achievement of the goal of effective control of a significant disease in Latin America.

2. **Bolivian Hemorrhagic Fever**

   a. **Epidemiology and Ecology**

   Although there is less disease than in Argentina, two major problems require attention.

   1. Basic diagnostic capability in Bolivia still does not exist. At least one laboratory should be capable of measuring antibodies in man and mouse. This is most conveniently done by fluorescent microscopy. Inactivated antigens can be prepared at CDC, Atlanta and supplied. Even better techniques are currently under development.

   2. The geographic extension of Machupo virus has not been systematically surveyed in more than 10 years. Human disease is a much less reliable index than in Argentina because of the sparse population and limited facilities for medical care and communication.

   3. Bolivian hemorrhagic fever may be a disease of the future in Santa Cruz Province. Road and rail links into Beni are under construction. For that reason, it is important to determine present geographic boundaries between Machupo and Latino viruses.

   b. **Vaccine Development**

   1. It seems probable that an inactivated Machupo vaccine will soon be available for clinical testing. Initial studies should be done in persons already immune to the disease. PAHO should follow developments closely and assist in the execution of these clinical trials.