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PROGRAM FOR PREPARATION OF IMMUNE  
GLOBULIN AGAINST BOLIVIAN HEMORRHAGIC  
FEVER

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<p>The project provided 145 ten ml ampoules of gammaglobulin prepared from 222 units of plasma donated by persons known to be immune to Bolivian Hemorrhagic Fever. Donors were recruited from the rural area of San Joaquin, Bolivia, where a temporary plasmapheresis unit was established, using project funds. The collected plasma was fractionated in the United States where one half was retained for prophylactic or therapeutic use following potential laboratory accidents. The remainder was returned to Bolivia to be used in treatment or prophylaxis of contacts to known cases. Dosage levels were determined by experiments performed in rhesus monkeys by the United States Army Research, Infectious Disease Laboratories, Fort Detrick, Maryland.</p>			

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### Background information

Bolivian hemorrhagic fever was first recognized in 1959 as an important disease in Bolivia, with fatality rates ranging from 30 to 100%. It was primarily a rural disease found in Orabayaya and San Joaquín in the Provinces of Itenez and Mamoré, in the Amazon Basin of Bolivia. The disease was characterized by fever and hemorrhages in the buccal and gastrointestinal mucosa.

By the end of 1962, it was established that epidemics were caused by Machupo virus which was related to, but different from, the Junin virus, causative agent of Argentine hemorrhagic fever which had been identified some years before.

In 1963 the virus was isolated from the rodent Calomys callosus and was named Carvalho strain. In 1964, as a result of the systematic extermination of rodents in San Joaquín, the number of human cases showed a downward trend finally reaching zero.

In 1968 new cases again appeared in San Joaquín, Río Negro, and other localities, showing a tendency for the disease to attack other geographical areas. This hypothesis was confirmed when there were six cases in that year in rapid succession, all of them fatal, in the locality of Cayoba in the Province of Itenez, near Magdalena. In 1969 a total of nine cases occurred in Magdalena. In the first half of 1971, there were six cases in the town of Cochabamba, all of them fatal, except one who carried the disease to the town of Tarija. In the second half of the same year a small epidemic outbreak occurred for the first time in the Province of Yacuma, with a total of four cases, all fatal.

Transmission of the virus following accidental laboratory exposure or person to person transmission within the hospital has been demonstrated. It was also known that infection with the virus and recovery could occur since serologic surveys of Bolivians demonstrated a significant number with high titers of antibody against the Machupo virus and no history of antecedent clinical illness.

This project was therefore undertaken to obtain sufficient quantities of plasma from immune donors to prepare a high titered gamma globulin, both for prophylaxis and possible therapy in cases with known laboratory or hospital exposure.

### Accomplishments

Using the list of persons who had been tested for Machupo virus neutralizing antibody by the Gorgas Memorial Institute/MARU laboratories,

## Foreword

Bolivian hemorrhagic fever, caused by the Machupo virus, is an important, highly lethal disease, affecting primarily the rural populations of the Bolivian Amazon Basin. The reservoir of the virus, Calomys callosus, is a small rodent and transmission of the disease to man is primarily through rodent urine, feces or saliva. The absence of any effective vaccine or therapeutic procedure forces reliance upon rodent control as the only preventive measure. Such programs in rural areas are only partially effective and highly costly due to complex administrative problems.

Studies of the basic pathogenesis of the virus have been under way for almost ten years. Investigations of virus attenuation looking toward possible vaccines have been undertaken more recently. Laboratory accidents with the virus are a special hazard requiring prompt administration of immune serum or plasma. Direct person to person exposure has also occurred, especially in hospital environments, with fatal termination of some cases.

For these reasons, the development of an high titered immune globulin against Bolivian hemorrhagic fever had high priority both for the laboratory and field. Accordingly, the present program was undertaken to fill this need. The project was a joint administrative responsibility of the Bolivian Ministry of Social Welfare and Public Health, United States Army Medical Research, Infectious Disease Laboratory, and the Pan American Health Organization.

The objectives of the project were as follows:

1. To obtain at least 200 units of plasma from Bolivian hemorrhagic fever immune donors, using the plasmapheresis method.
2. To prepare an immune globulin in laboratories in the United States.
3. To use the gamma globulin prepared for emergency prophylaxis in selected contacts of cases occurring in Bolivia.
4. To use a portion of the immune globulin for emergency prophylaxis in laboratory workers in the United States exposed to the Machupo virus.
5. To provide resources for future collection of plasma, as required.
6. To evaluate in the laboratory and in the field the degree of protection obtained by BHF immune globulin.

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### Accomplishments

Using the list of persons who had been tested for Machupo virus neutralizing antibody by the Gorgas Memorial Institute/HARU laboratories,

individuals were selected who had titers of 1:64 or greater against BHF virus. These individuals were then screened to meet the criteria for plasmapheresis donors including age, sex, level of plasma proteins, level of hemoglobin and negative tests for Chagas disease and hepatitis B antigen. From the final list, 15 individuals were selected to be used as donors by the plasmapheresis technique. All were residents of Province of Beni in or near the small village of San Joaquin, Bolivia.

A refrigerated centrifuge and portable generator had previously been installed in San Joaquin by the MARU staff. This was supplemented with additional equipment and supplies needed for a complete plasmapheresis unit. A local Bolivian physician was trained in the plasmapheresis technique in the Roswell Park Memorial Institute, Buffalo, New York. A second Bolivian physician was assigned by the Ministry of Social Welfare and Public Health to the Hospital in San Joaquin where the plasmapheresis unit was established. Two medical consultants from the Pan American Health Organization were employed consecutively during the ten weeks the unit was in operation. During this time 222 units of plasma were obtained from the 15 donors. The plasma was transported directly to the National Institute of Health Laboratories in La Paz, Bolivia, for storage, prior to transport to the United States.

The collected plasma was then transferred to the Department of Public Health Biologic Laboratories, Boston, Massachusetts, where it was fractionated for its globulin and albumin components. Tests for sterility and safety were performed by the same laboratories prior to final packaging of the biologicals. The gross yield of globulin was 145 -10 ml vials of which 133 were considered satisfactory for human use. The globulin was then transferred to the United States Army Research, Infectious Disease Laboratories, Fort Detrick, Maryland for final testing in monkeys.

The results of the preliminary dose response study of the prophylactic efficacy of BHF immunoglobulin in rhesus monkeys is shown in the attached table. On the basis of these results a dosage of 0.2 ml-1.0 ml per kg was suggested as a range for effectivity in preventing severe disease in humans following actual inoculation with Machupo virus. A protocol was then prepared for the utilization of BHF gamma-globulin, copy of which is given in appendix n° 2. One half of the total amount of gammaglobulin with the protocols, was then returned to Bolivia for further use and field evaluation in humans.

TABLE 1. A PRELIMINARY DOSE RESPONSE STUDY OF THE PROPHYLACTIC EFFICACY OF BOLIVIAN HEMORRHAGIC FEVER IMMUNE GAMMA GLOBULIN (HUMAN ORIGIN) IN RHESUS MONKEYS

Dose <sup>a/</sup> ml/Kg	Clinical Signs			Deaths	Viremia
	Positive/ Total	Severity (number)	Duration (days)		
1.5	0/3	- (3)	-	0/3 <sup>c/</sup>	0/3
0.5	3/3 <sup>b/</sup>	± (3)	7	0/3	0/3
0.15	3/3	++ (2) +++ (1)	14	1/3	1/3
none	3/3	+++ (3)	all died	3/3	3/3

- a. Three monkeys per dosage group were inoculated subcutaneously with 1000 plaque forming units of Machupo virus. Four hours later they were inoculated intramuscularly with the RHF immune human gamma globulin dosage indicated in the column.
- b. The clinical signs in the three monkeys in this dosage group only were limited to a pallid complexion that was suggestive of a transient anemia. No other clinical signs were apparent.
- c. Previous, similar studies indicated that all surviving monkeys are immune to challenge at 60 days postinoculation. This suggests that infection occurred despite the absence of clinical signs.

A PROTOCOL FOR THE UTILIZATION OF BOLIVIAN HEMORRHAGIC FEVER GAMMA GLOBULIN

The following protocol is intended as an aid in establishing the efficacy of Bolivian hemorrhagic fever gamma globulin (BHFGG) in the prophylaxis of the disease in man. The purpose is to determine the levels of passive antibody that occur as a result of a given dosage of BHFGG and to assess the efficacy of passive antibody in preventing illness or infection. It is assumed that the BHFGG usage will be limited primarily to persons working under a high risk of exposure.

A series of serum samples should be collected from all persons who receive the gamma globulin. The samples should be collected immediately prior to and on days 4, 14, 28, 42, and 84 following administration of the BHFGG. Sera should be collected aseptically from clotted blood samples and stored in a frozen state until they can be shipped for assay. Five ml serum samples are desirable. In the event of multiple dosages, the sera should be taken at two week intervals between doses and at the intervals shown above following the last dosage. Each serum sample should be labeled with the name of the donor, cedula number if available and the date collected. A clinical data sheet should be prepared for each person showing the date(s) of gamma globulin administration, date(s) of possible exposure and all clinical data including laboratory tests.

Exposed persons should be observed for clinical signs and symptoms of HHF, and these observations should be carefully recorded. Clinical laboratory tests, particularly leucocyte counts, differentials and hematocrit values, would be most useful. They should be done prior to exposure, at 30 day intervals during the period of exposure and during any febrile or clinical episode. The sera plus copies of all clinical and laboratory data should be sent to Chief, CD, Pan American Health Organization, 525 23rd St. N.W. Washington, D.C., 20037, U.S.A.

The dose of gamma globulin to be given will probably be individually determined in each instance of exposure or potential exposures. For example, the degree of exposure which would occur if a pathologist were to lacerate his skin during an autopsy might warrant a different dosage than that of medical personnel engaged in routine patient care. Our data suggest that a dosage of 0.2 ml - 1.0 ml/Kg would be effective in preventing severe disease in the face of an actual inoculation with virulent virus. These figures are based entirely on preliminary rhesus monkey studies however, and they may not be wholly applicable to prophylaxis in man.