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Protection Against Junin Virus by Immunization with Live Tacaribe Virus. (30251)

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A new and important group of South American viruses has been recently recognized. It comprises Junin virus, the causative agent of Argentinian hemorrhagic fever(1,2), Tacaribe virus, which was recovered from bats in Trinidad(3) and Machupo virus, the causative agent of Bolivian hemorrhagic fever (4). The 3 viruses are almost indistinguishable by the complement-fixation (CF) test (4,5). However, they can be clearly differentiated by the plaque neutralization test(4,6) with a slight, but consistent, one way reaction between Tacaribe antibody and Junin virus.

Tacaribe virus is not known to be pathogenic for any adult laboratory animal; however, Junin virus is pathogenic for the adult guinea pig. By immunizing adult guinea pigs with Tacaribe virus and then challenging them with Junin virus, the immunological relationship between these two viruses could be further clarified. The results of this experiment and of serological tests with bloods obtained from both immunized and control animals are the subject of this report.

Materials and methods. Tacaribe virus, prototype strain TRVL 11573. Infectious stock virus used for immunization of guinea pigs and in the plaque neutralization test was prepared from suckling mouse brains (SMB) harvested 7 days after intracerebral inoculation, and made up as a 20% suspension in phosphate buffered saline (PBS)(7) with 0.5% bovine plasma albumin (BPA). This virus has had 22 serial passages in suckling mice (NIH-General Purpose); the virus used in the preparation of sucrose-acetone extracted(8) CF antigen had undergone one additional suckling mouse passage.

Junin virus, XJ strain. The infectious stock virus used in the challenge experiments and the plaque neutralization tests was prepared from a suspension of newborn guinea pig (NIH-Hartley) spleens harvested as animals sickened after intraperitoneal inoculation, and made up as a 20% suspension in PBS with 5% BPA. Previously this virus was passaged twice in guinea pigs, 13 times in newborn mice and 23 times more in guinea pigs. The CF antigen was prepared by sucrose-acetone extraction(8) of SMB harvested 7 days after intracerebral inoculation; this virus had undergone 2 passages in guinea pigs and 17 in newborn mice.

Immunization and challenge. Forty adult female guinea pigs (300-400 g) were immunized with a single intramuscular inoculation of 0.1 ml of stock Tacaribe virus diluted 1:20 in medium 199; this contained 3×10^5 plaque forming units (PFU) of virus. A similar number of control animals was inoculated with 0.1 ml of 20% normal SMB which was also diluted 1:20 in medium 199. Complement-fixation and plaque neutralization tests were performed on unpooled samples of sera obtained from individual control and immunized guinea pigs which were bled out by cardiac puncture prior to immunization and on days 14, 21, 32 and 49. Groups of immunized and control animals were challenged intraperitoneally with 0.1 ml of serial 10-fold

dilutions of stock Junin virus on the 14th day. The LD_{50} endpoint was calculated by the Kärber method(9).

Complement-fixation test. We have used the quantitative CF procedure of Kent and Fife(10) modified for use in microtiter equipment(11). Five 50% hemolytic units of complement and serial 2-fold dilutions of antigen were added to serial 2-fold dilutions of heat-inactivated (56°C for 30 minutes) serum; the hemolytic system was added after incubation at 4°C for 18 hours and the mixture was incubated in a 37°C water bath for 1 hour. The test was read as percentage hemolysis by comparison with simultaneous standardized controls on each plate. When 35% (or less) hemolysis occurred the CF reaction was considered positive; the highest dilution of serum which gave a positive reaction with any dilution of the antigen in the block titration was recorded as the serum titer.

Plaque neutralization test. Unheated serum, diluted 1:4 in PBS with 0.5% BPA, was added to serial 10-fold dilutions of virus in the same diluent; after incubation at room temperature for 1 hour, 0.2 ml of the mixture was inoculated into each of two 2-oz bottles of a continuous line of embryonic rhesus monkey kidney cells (MA-104); following adsorption for 2 hours at 35°C the cultures were overlaid once with 5 ml of an agar medium(12), modified by including the neutral red and by decreasing the concentration of bovine serum to 2%. Plaques were read after incubation at 35°C for 8-10 days. The difference in titer between control and experimental sera represents the titer of neutralizing antibody.

Results. Adult guinea pigs were protected against challenge with Junin virus 14 days after immunization with live Tacaribe virus administered intramuscularly. Titration of stock Junin virus was performed simultaneously in control and immunized animals covering a series of 10-fold dilutions from 10^{-1} to 10^{-8}. The LD_{50} was 6.5 dex* in the control group and less than 1.0 dex in the immunized group. Thus, the protection index was greater than 5.5 dex (Table I).

* Decimal exponent(13).

TABLE I. Protection of Guinea Pigs Against Junin Virus Challenge 14 Days After Immunization with Tacaribe Virus.

	LD_{50}	Protection index
Control	6.5*	>5.5
Immunized	<1.0	

* Dex value(13) as calculated by the Kärber method(9).

Results of the CF tests with sera from both groups of animals are shown in Fig. 1. Sera from the Tacaribe virus-immunized guinea pigs had significant CF antibody titers on the 14th day in tests with both Junin and Tacaribe antigens. However, the sera reacted with the Junin antigen to a lower degree than with the homologous antigen. The titers continued to rise until termination of the experiment on the 49th day.

Results of the plaque neutralization tests with sera from the same animals are shown in Fig. 2. Significant homologous neutralizing antibody titers developed by the 14th day; a progressive increase in titer was demonstrated on days 21 and 32, at which time the titer was higher than the plaque neutralization test could detect with sera diluted 1:4. Sera taken on the 49th day were the fist to show minor cross-reactions with Junin virus.

Discussion. While our studies were in progress, Parodi and Coto reported a similar experiment in a Buenos Aires journal(14). They immunized adult guinea pigs intramuscularly with 0.2 ml of a 10% suspension of Tacaribe virus-infected suckling mouse brain. Some animals were given a single immunizing dose while others received 2 or 3 at 15-day intervals between inoculations. All animals were challenged with Junin virus, also administered intramuscularly, 15 days after the last inoculation of Tacaribe virus. Guinea pigs which had received a single inoculation of Tacaribe virus resisted the challenge with 3 dex LD_{50} of Junin virus, but those which had received 3 inoculations withstood the challenging dose of 5 dex LD_{50}. Thus, the findings in both laboratories indicate that adult guinea pigs can be protected against the Junin virus by immunization with live Tacaribe virus.

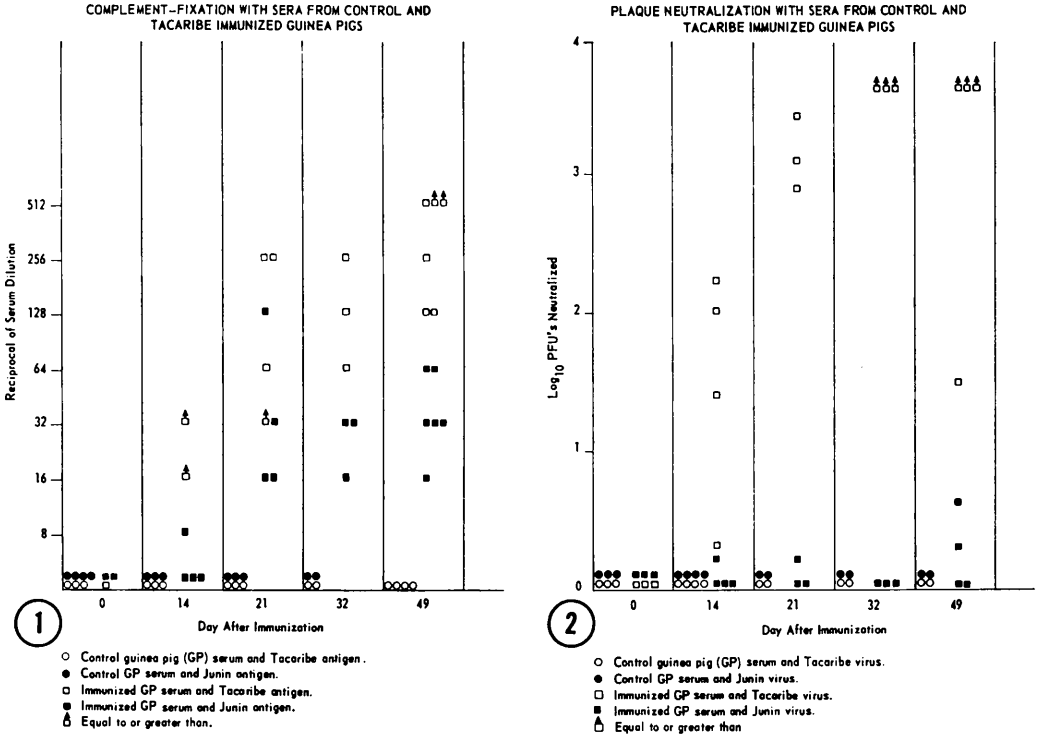

FIG. 1 and 2

Parodi and Coto did not perform serological tests on their guinea pig sera. We were able to show that the sera from our animals immunized with Tacaribe virus contained large amounts of homologous antibody as measured by CF and plaque neutralization tests. The occurrence of reactions by CF test between Junin antigen and sera from Tacaribe virus-immunized animals had been established before(4,5). The CF antibody titers we recorded were consistently lower in the heterologous than in the homologous system. It was also noted previously(4,6,15) that hyperimmune sera from animals immunized with Tacaribe virus reacted with the Junin virus in the plaque neutralization test. Although our guinea pigs were not hyperimmunized such a reaction may have occurred with 2 of the sera obtained from our guinea pigs 49 days after immunization (Fig. 2).

Tacaribe virus is not known to be pathogenic for any adult laboratory animals. Although Downs and co-workers(3) initially isolated the virus from sick Artibeus bats, they were unable to produce experimental Tacaribe infection in several species of these bats. This does not preclude the possibility that Tacaribe virus can multiply in the adult guinea pig. In fact, the antibody responses observed in our Tacaribe virus-immunized animals support this possibility. The titers would seem to be greater than might be expected from a single injection of a small antigenic mass of the virus. If Tacaribe virus multiplies in the adult guinea pig, then the possibility must be considered that the observed protective effect against Junin virus challenge might be due to interference between the two viruses. This problem can be settled only by further experimental work.

Summary. Adult guinea pigs immunized with the live Tacaribe virus are able to resist challenge with a large dose of Junin virus to which they are normally susceptible. Sera from the Tacaribe virus-immunized guinea pigs contain abundant homologous antibody demonstrable by both complement-fixation and plaque neutralization tests. The complement-fixing antibody titers of these sera with

the heterologous Junin antigen are moderately high. However, the amount of antibody determined by the plaque neutralization test with Junin virus is negligible.

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Effects of Hydrogen Ion Concentration on Transport Characteristics of p-Aminohippurate by Rabbit Kidney Slices.* (30252)

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Earlier studies concerned with the mechanism by which p-aminohippuric acid (PAH) is actively transported by rabbit kidney slices have demonstrated that certain chemicals, when added to the medium, exert specific effects on the accumulation of PAH or on the displacement of intracellularly accumulated PAH(1-4). Although many parameters of this transport system have been studied, almost all of the data reported have been obtained from experiments conducted at pH 7.4.

In this paper are reported observations on the effects of certain hydrogen ion concentrations with or without various test agents on (a) the accumulation of PAH by slices and (b) displacement of preaccumulated PAH.

Materials and methods. Young adult rabbits weighing approximately 2 kg were sacrificed by a blow on the head, exsanguinated and the kidneys removed immediately. The kidneys were promptly placed in an ice cold saline bath of 0.13 *M* NaCl-0.02 *M* KCl. Thin slices were prepared by the method of Forster and Copenhaver(2). After brief blotting on filter paper, 280 to 320 mg of the slices were weighed and added to a 25 ml flask containing 2.7 ml of ice cold Cross-Taggart medium(1). It was necessary to replace 0.1 ml of the 0.1 *M* sodium phosphate with 0.1 ml 0.2 *M* 2-amino-2-methyl-1,3 propanediol (propanediol) in order to cope with the wide pH range (pH 5.0 to 12.0)

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