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DRUG DEVELOPMENT AGAINST VIRAL DISEASES

ANNUAL REPORT

GREGORY H. TIGNOR, SC.D.

1 FEBRUARY 1989

SUPPORTED BY

U.S. ARMY RESEARCH AND DEVELOPMENT COMMAND, FORT DETRICK,
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Two hundred and ninety-four drugs have been tested for efficacy against CCHF (strain 10200). Geometric mean times to death were calculated for control mice (VC) and for drug-treated mice (VR). Tests with 294 drugs resulted in a mean VR score of 1.0, and 75th and 90th percentile scores of 1.1 and 1.2 respectively. Thirty drugs had VR scores greater than 1.2. Ribavirin efficacy is inversely dependent upon virus dose. After multiple doses of drug, efficacy is significantly higher than that observed after single dose treatment.

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CCHF virus antigen occurs very early in Kupffer cells lining the liver sinusoids. Animals treated with a single dose of ribavirin have very little or no demonstrable CCHF antigen in these cells. Infected Kupffer cells have been positively identified by immunoperoxidase staining on serial sections of paraffin embedded liver tissue sections using either anti-MAC-1 antibody or anti-CCHF antibody.

Ribavirin-resistant CCHF virus has been isolated from the livers of ribavirin-treated mice showing no clinical signs of illness and necropsied on the seventh day after infection. Virus was isolated from the liver and was used as source in a ribavirin drug test. The VR score in mice treated with 50 mg/kg was 1.0 and the VR score in mice treated with 100 mg/kg was 0.9.

Three hundred and nine drugs have been tested in the LCM model. Thirty-one drugs have been identified as being of potential interest because they fall into the upper 90th percentile of all drugs tested this year. Of these 31 drugs, five have been re-tested with similar results.

Squirrel monkeys from Charles River Research Primates Corporation were tested with AVS# 1968. Drug treatment beginning before and continuing after exposure to virus resulted in a statistically significant depression in viremia levels on day 2 after exposure. 3 of 4 treated animals were still depressed on day 4 and day 7. Antibody developed in all injected animals with the earliest appearance recorded on day 7 (1/4). Treatment with both AVS 1968 and AVS #1 resulted in an early depression in viremia, although later viremia levels (day 4) were uniformly higher than those seen in group 1, treated with AVS 1968 alone. Drug treatment beginning after virus exposure (Group 2) produced no detectable differences from control animals exposed to virus alone.

AVS 1968 treatment alone did not produce uniform levels of interferon in monkeys until several doses of drug had been given, i.e., on days -1 and +1. Twenty-four hours after the first dose of drug, only one of four animals had detectable interferon. By day 2, all animals were positive. Interferon levels remained high through day 14. However, interferon levels were consistently lower in animals given AVS 1968 and yellow fever virus.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. (NIH) 86-23, Revised 1985).

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INTRODUCTION

This report is divided into four sections. The results of drug testing in two different murine models are shown in the first two sections. The results of drug testing in a primate model are given in the third section. The fourth and final section deals with biological safety considerations addressed during the drug testing in both the murine and primate models.

PRIMARY TESTING WITH CONGO-CRIMEAN HEMORRHAGIC FEVER VIRUS (CCHF)

NUMBER OF DRUGS TESTED

Two hundred and ninety-four drugs have been tested for efficacy against CCHF (strain 10200). The results in virus ratings (VR) are given in Table 1. The method for deriving the virus rating is given in a later section. Detailed data for each drug were submitted at frequent intervals throughout the year.

DESCRIPTION OF THE MODEL AND ANALYSIS OF DATA

DRUG TESTING

Coded drugs were received and submitted to screening. With few exceptions at the time of drug screening, no drug information was available other than some details regarding solubility. Thus, information on drug relationships was not available prior to initiation of drug testing. In some repeat tests, effort has been directed toward the study of efficacy at varying drug or virus doses, but this has not been done on a routine basis.

The CCHF model is as follows. Drugs have been tested for toxicity in infant mice (1-2 days old) at 50 mg/kg. Those which were not toxic were subjected to testing against CCHF, strain 10200, passage 11 in infant mouse brain tissue. Each drug was given to infant mice in a volume of 0.075 mls, i.p. Fresh drug doses are prepared for all tests. Forty-five minutes later, virus at 50 LD50's was inoculated ip in a volume of 0.075 mls. Mock-treated mice were given tissue culture medium DMEM as control. Virus titrations were carried out at the same time. Mice were observed daily.

In multiple dose experiments, the initial procedure was the same. However, 4 additional doses of drug were given at daily intervals for a total of five drug doses. All mice were housed in a modified class 3 facility using isolators. Personnel entering the facility wear positive-pressure respirators and participate in the University's respirator program. Sentinel mice were inspected for intercurrent murine infections throughout the year.

ANALYSIS OF DATA.

Geometric mean times to death were calculated for control mice (VC) and for drug-treated mice (VR). The geometric mean time to death (VC) is equal to the nth root (where n=the total number of animals) of the product of each day with mortality raised to the power of the number of animals dying on that day. In this calculation, survival is defined as 28 days. The geometric mean time to death (VR) for each drug is equal to the ratio of the geometric mean time to death for each drug divided by VC. A single drug, ribavirin (AVS #1), was used in each test as a measure of variation in test sensitivity.

METHOD FOR MEASURING ANTIVIRAL DRUG EFFECT

$$VR = \frac{\text{GEOMETRIC MEAN SURVIVAL TIME (DRUG)}}{\text{GEOMETRIC MEAN SURVIVAL TIME (PLACEBO)}}$$

There was an inverse relationship between the geometric mean survival time (VC) and the CCHF virus dilution inoculated into the mice. At higher virus concentrations, the VC was lower; as the virus dose decreased, the VC increased. These data are shown in Figure 1A.

A similar relationship was observed in multiple dose tests conducted in parallel with the tests described above. However, in the multiple dose (placebo) tests, there was less variation about the VC at any given virus dose. The fit of the regression line (0.9) is better than for single dose tests (0.8). On the other hand, the mean survival time was reduced in the multiple dose tests. The reduction in VC was dependent upon the virus dose with the reduction more marked using moderate doses of virus and less marked with high doses of virus. These data are shown in Figure 1B.

SUMMARY OF RESULTS OF DRUG TESTING.

DISTRIBUTION OF VR SCORES.

Tests with 294 drugs resulted in a mean VR score of 1.0, and 75th and 90th percentile scores of 1.1 and 1.2 respectively. The lowest score was 0.6 and the highest, excluding ribavirin, was 1.8. Ninety percent of the drugs tested had a VR score less than 1.2. Thirty drugs had VR scores greater than 1.2. Thirty drugs fell into the lowest 10th percentile with a VR score equal to or less than 0.9. These data also show that most drugs tested had no effect (VR score of 1.0) on CCHF virus infection.

The VR scores and CCHF virus doses used for all tested drugs are given in Table 1. The distribution of VR scores is shown in a histogram in Figure 2. The percentile ranking for each VR score is given in Figure 3.

CCHF VIRUS DOSE USED IN DETERMINING VR SCORES

Most drugs were tested against 50 LD50's of virus as shown in Figure 4. The actual mean test dose was 49.636 LD50's (1.696 logs) with a standard deviation of

26.019 (0.3 logs). Some tests were done with higher or lower doses of virus to test drug efficacy and/or potency.

TEST SENSITIVITY AND REPRODUCIBILITY

VARIATION IN SENSITIVITY

The sensitivity of the test system was monitored last year (1987) and this current reporting year (1988) by inclusion of ribavirin (AVS #1) in each test.

In 1987, the VR score for ribavirin (VR+) value was compared after 19 trials. The mean VR+ was 2.6 with a minimum of 1.6 and a maximum of 3.5 (range of 1.9) giving a standard deviation of 0.6. Sixty-three percent of the observations were between 1.6 and 2.0.

A similar analysis was done for data from 12 tests in 1988. The mean VR+ was 2.3 with a minimum of 1.4 and a maximum of 3.1 (range of 1.7) resulting in a standard deviation of 0.5. These results are remarkably similar to those obtained in 1987 and suggest that data obtained in these two years can reasonably be considered comparable.

EFFECT OF CCHF VIRUS DOSE ON RIBAVIRIN VR SCORES

The range of VR scores for the positive control included in each test reflects the fact that the VR+ score is inversely dependent upon virus dose. Relatively small variations in virus test dose produce significant changes in the VR+. For example, a change from 50 LD50's (1.7 logs) of virus to 100 LD50's (2.0 logs) results in a decrease of the VR+ from between 2.2-2.5 to 1.7. However, there is also inherent variation in that the VR+ score varies at a single virus dose. For example, the VR+ ranges from 2.2 to 2.5 at fifty LD50'S of virus. These data are shown in Figure 5.

Variation of 0.3 logs in virus dose has been observed consistently in our experimental results as discussed above. Therefore, the variation in VR score for ribavirin may reflect in part error, insensitivity, or other lack of precision in virus dose determinations. While the potency of ribavirin in the CCHF model is inversely related to virus dose between 5 and 200 LD50's, the VR+ doesn't fall below 1.4, thus demonstrating significant efficacy of this drug over the entire virus dose range.

Finally, the regression of VR+ upon virus dose provides a means whereby any given test can be evaluated for goodness of fit with accumulated data. If a future data point lies outside the 95% confidence limits of the regression line, that test result might be considered "suspect" in both sensitivity and reproducibility.

EFFECT OF MULTIPLE DRUG DOSES ON RIBAVIRIN VR SCORES

A series of simultaneous experiments were conducted to determine differences between single drug dose and multiple drug dose experiments using ribavirin. In earlier years, we observed that drugs effective in single dose experiments became more effective in multiple dose experiments. Since mice, albeit in varying numbers, survive CCHF infection in both single and multiple dose experiments, one possible influence which could elevate the VR+ is for the survival time of control mice (VC) to decrease after multiple placebo injections. The VR+ is a ratio of the geometric mean survival time of ribavirin treated animals divided by the control geometric mean survival time. A decrease in the control VC would automatically raise the VR+. A decrease in the VC was found in these experiments.

The VC for single and multiple injection drug tests is shown in Figure 6. The VC is less variable after multiple placebo injection confirming the analysis of data by linear regression presented above. In addition to being less variable, the VC is also reduced after multiple placebo injection.

The VR after ribavirin multiple dose treatment is significantly higher than that observed after single dose treatment as shown in Figure 7. One way to resolve whether the increase in VR was attributable to a decrease in virus replication was to look at the target organ in CCHF infection of infant mice.

PATHOGENESIS OF CCHF VIRUS IN INFANT MICE.

In the 1987 annual report, we presented data suggesting that CCHF virus infection was associated with multiplication in the liver as the primary target organ. Virus titers were higher in the liver than in the blood from day 3 to day 7. Virus appeared very late after infection in other tissues including the brain, heart, and spleen. Ribavirin-treated mice showed a reduced viremia and lower virus titers in liver tissue.

In the current reporting period, we have looked at the target organ by both immunofluorescence and immunoperoxidase techniques using both frozen and paraffin-embedded tissue. Three days after inoculation of virus, CCHF virus antigen occurs in what appear to be Kupffer cells lining the liver sinusoids. (Figure 8, top). Animals treated with a single dose of ribavirin have very little or no demonstrable CCHF antigen in these cells. (Figure 8, bottom)

Later in the infection process in untreated animals, CCHF virus antigen is present in numerous clusters of hepatocytes widely distributed throughout liver tissue and in occasional tissue macrophages. Infection of Kupffer cells is not prominent at this time. (Figure 9, top). CCHF antigen is only occasionally seen in liver tissue of ribavirin-treated animals, sometimes in a putative Kupffer cell or sometimes in an hepatocyte. (Figure 9, bottom)

Putative infected Kupffer cells have been positively identified by immunoperoxidase staining on serial sections of paraffin embedded liver tissue sections using either anti-MAC-1 antibody or anti-CCHF antibody. In consecutive tissue slices, Mac-1 antibody stained cells, i.e., Kupffer cells, were found on day 3 to be also stained with anti-CCHF antibody as detected by the immunoperoxidase technique. (Figure 10 top & bottom)

There was very little CCHF virus antigen in the liver tissue of animals treated with multiple doses of ribavirin. Only isolated infected cells were found during the time

frames examined by immunofluorescence. Seven days after infection, infectious virus was isolated from the liver of mice treated with multiple doses of ribavirin, but only at 1:1000 dilutions of the homogenized tissue. These data suggest, but do not prove, that ribavirin treatment may result in the production of defective virus in the liver tissue of treated-mice.

RIBAVIRIN RESISTANCE IN THE CCHF MODEL

Ribavirin-treated mice showing no clinical signs of illness were necropsied on the seventh day after infection. Virus was isolated from the liver and the blood. The virus titer in the liver (2.3 log LD50'S) was higher than the titer in the blood (2.0 log LD50'S). Virus from liver tissue was used as source in a ribavirin drug test . Mice were infected with a low dose of the liver virus (2.5 LD50'S) and treated with ribavirin at either 50 mg/kg or 100 mg/kg. The VR score in mice treated with 50 mg/kg was 1.0 and the VR score in mice treated with 100 mg/kg was 0.9. The ribavirin VR scores in mice infected with 2.5 LD50's of CCHF liver virus which had not been passaged through ribavirin-treated animals were 2.7 and 3.0 for 50 mg/kg and 100 mg/kg respectively.

DRUGS OF POTENTIAL INTEREST IN THE CCHF MODEL

Thirty-one drugs after, at least one test, have given a VR score in the upper 90th percentile. Of these 31, only 5 have yet been confirmed in re-testing. There are 6 drugs which have VR scores in the 90th percentile for both CCHF and LCMV. Of these six, three had not been tested prior to this year They are AVS#'s 0002, 4071 and 4217. Many of the drugs with VR scores in the 90th percentile have consecutive VR numbers (e.g., 3606, 3607, 3608.) The meaning of this is not known to us since we have no data regarding the drugs other than some solubility information. (Table 3)

PRIMARY TESTING IN THE LCM MODEL

NUMBER OF DRUGS TESTED

Three hundred and nine drugs have been tested in the LCM model. VR scores have been sent to the contract officer as they became available to us. The detailed data are not, therefore, included in this report. However, a summary of our results is presented in Table 2.

DESCRIPTION OF THE LCMV MODEL

Adult mice are inoculated with 50 mg/kg of drug i.p. in a volume of 0.4 mls to 0.7 mls depending upon the weight of the individual mice. Forty-five minutes later, mice are inoculated with 50 LD50's of LCM virus (LCMV) i.p. The virus strain is propagated by intracerebral passage in inbred C3H mice. Random bred CF-1 mice from Charles River are used for drug tests. The identity of the virus strain has been monitored by examination of infected mouse tissue by immunofluorescence. The virus stock titers 5.0 to 5.4 log LD50's by intraperitoneal inoculation of random bred mice.

HISTOPATHOLOGY OF LCMV INFECTION

Little additional work has been done on the pathogenesis of LCMV in mice. A summary of our past findings is a follows. Mice suffer from severe, multisystemic disease, with necrotizing inflammation of lymphoid tissues, parotid salivary glands, pancreas, splenic red pulp, liver, intestine and mesentery. They also have a mild focal choriomeningitis. The majority of leukocytes, regardless of type, in all tissues examined were undergoing necrosis. No lesions were found in submaxillary or sublingual salivary gland, kidney, heart, eye, lacrimal gland, thyroid, trachea, or lung.

CORRELATION BETWEEN THE GEOMETRIC MEAN SURVIVAL TIME OF LCMV INFECTED CONTROL MICE (VC) AND VIRUS DOSE.

There is a poor relationship between the VC and the virus dose in the LCMV model. Ten-fold dilutions of virus make little or no difference in the geometric mean survival time of control mice. (Figure 11) These data suggest that it may be difficult to associate drug potency with changes in survival time in a linear basis. Further work will be done during the coming year.

DISTRIBUTION OF LCMV VR SCORES

A histogram giving the frequency distribution of VR scores is shown in Figure 12. The mean VR score is 1.0 with a range extending from 0.7 to 1.8. The relationship between VR score and percentile ranking is given in Figure 13. VR scores of 1.3 or higher are in the 90% of all drugs tested.

VIRUS DOSE USED IN DETERMINING VR SCORES

Most of the drug testing was done using a virus dose of 50 LD50's as shown in the histogram (Figure 14) relating virus doses and the number of drugs tested.

DRUGS OF POTENTIAL INTEREST IN THE LCMV MODEL

Thirty-one drugs have been identified as being of potential interest because they fall into the upper 90th percentile of all drugs tested this year. Of these 31 drugs, five have been re-tested with similar results.

The most promising, in terms of VR scores, is AVS# 4070 which has VR's of 1.7 and 1.8 against test doses of 100 and 63 LD50's respectively. This drug has a higher VR score in the LCM model than we have observed with any other drug including ribavirin (AVS# 1) or any of its analogues which are known to us.

There are 6 drugs which are in the 90th percentile of all drugs tested in both the LCM and the CCHF model. In addition to ribavirin, (AVS#1, 206), there are 2 other drugs which seem of interest. They are AVS #4071 and 4217 which have given high VR's in both LCM and CCHF. However, additional re-tests are required to confirm these screening data. These data are presented in detail in Table 3.

RESULTS OF TESTING IN THE YELLOW FEVER PRIMATE MODEL

EXPERIMENTAL PROTOCOL

Squirrel monkeys from Charles River Research Primates Corporation were assigned to groups and tested as follows. The drug tested was AVS# 1968.

Groups 1, 2, and 3 as shown in Table 4 were bled for virus titers on days -1,+2,+4,+7, +14 and +21 days. Tests for neutralizing antibodies were done using serum taken on on days -1, +7, +14, and +21.

Group 4 was bled for interferon titers on day -1, and 16 hours after each treatment with the compound and on day 14.

Group 5 was treated with AVS# 1968 and AVS# 1 simultaneously. The dose of AVS #1 used was 50 mg/kg administered by the oral route. These animals were bled following the schedule for Groups 1, 2, and 3.

Groups 1,2,3, and 5 were given yellow fever virus on day 0.

RESULTS OF PRIMATE TESTING WITH YELLOW FEVER VIRUS AND AVS# 1968.

MORTALITY

Although the virus dose (100,000 PFU in 0.2 mls subcutaneously) was the same as in previously described experiments, there was no mortality in injected animals. In previous experiments, mortality ranged from 40% to 83%. There is no known reason for the fact that these animals did not die, although we do know, from experience, that squirrel monkeys, in general, are less susceptible to lethal yellow fever virus infection. In other experiments in this laboratory, squirrel monkeys have not died from yellow fever virus exposure.

WEIGHT.

Female monkeys were randomly assigned to groups. A statistical analysis of the groups by weight did not reveal significant differences among the groups. (See Tables 5 and 6)

VIREMIA AND ANTIBODY.

Drug treatment beginning before and continuing after exposure to virus resulted in a statistically significant depression in viremia levels on day 2 after exposure. 3 of 4 treated animals were still depressed on day 4 and day 7. Differences between group 1 and group 3 were not significant by T test, but were significant by chi-square test. Antibody developed in all injected animals with the earliest appearance recorded on day 7 (1/4).

COMBINED THERAPY.

Addition of AVS #1 to the experimental protocol (Group 5) resulted in an early depression in viremia, although viremia levels on day 4 were uniformly higher than those seen in group 1.

Drug treatment beginning after virus exposure (Group 2) produced no detectable differences from control animals exposed to virus alone. Viremia levels were similar in the two groups. Animals uniformly made HAI antibody somewhat earlier than did animals in group 1. These results are in Table 5. The statistical analysis is presented in Table 6.

INTERFERON.

Serum from animals in Group 4 was assayed for alpha/beta interferon by the following procedure. Test sera were diluted ten-fold in DMEM and adjusted to pH 2 by addition of HCl, held for 24 hours, and readjusted to pH 7.3. Half-log dilutions of each serum were incubated with Vero cells for 18 hours prior to infection of cells with VSV-Indiana using a dose which was 100 PFU's. The results, expressed as $\log_{10}$ titers, are given in Table 7. The drug did not produce uniform levels of interferon in monkeys until several doses of drug had been given, i.e., on days -1 and +1. Twenty-four hours after the first dose of drug, only one of four animals had detectable interferon. By day 2, all animals were positive. Interferon levels remained high through day 14.

ADDITIONAL YELLOW FEVER VIRUS TESTS WITH DRUG AVS# 1968 IN THE PRIMATE MODEL

EXPERIMENTAL PROTOCOL

Four drug-treated monkeys from the experiment described above were used in an additional test with a different virus strain. Eight weeks after exposure to drug, animals were divided into two groups. Half (2) were inoculated with yellow fever virus only (Dakar, passage 9 in infant mouse brain tissue, 400,000 LD50's in 0.2 mls, subcutaneously) and two were pre-treated with AVS# 1968 on day-1 before inoculation of virus. Drug-treated animals were given 25 mg/kg of drug on days 1,3,5, and 7 by oral intubation.

RESULTS

There was no mortality. Monkeys were bled as in the original experiment. The viremia, antibody, and interferon studies have not been completed.

BIOLOGICAL SAFETY CONSIDERATIONS

During the past year, serious questions regarding our biological safety practices were raised by the staff and chairperson of a senatorial subcommittee on oversight of governmental management. The principal investigator appeared before that subcommittee to respond to some of the many issues which were raised. In addition, other questions were raised by Mr. Ralph W. Kuehne, Safety Officer, USAMRIID. After an inspection by Mr. Kuehne, we were somewhat pleased that the overall impression of the physical facility and the procedures, policies and equipment being used was generally favorable and considered adequate to minimize the risk of infection to laboratory workers and animal caretakers. However, Mr. Kuehne raised other issues to which we have responded.

Outlined below are the steps which we have undertaken to implement suggestions received from Mr. Kuehne. Many of the measures which we have taken are far in excess or any requirements or suggestions presently incorporated in any of the several biological safety guidelines for BL-3 level work.

An RC-2 centrifuge in room 600 of LEPH has been modified with a disinfectant trap connected to tygon tubing and a vacuum pump as suggested.

Powered air, positive pressure, HEPA-filtered, face-shield respirators (NIOSH/MSHA approved) have been provided for use in rodent and primate rooms.

A respirator program has been established by the Occupational Health Officer.

The pressurization relationships in the 8th floor facility are being improved in a 3-part program. The immediate response has been to rebalance the existing system in order to increase the differential pressure to 0.04" wg as recommended. Within the next 2-4 months, the exhaust system will be modified to provide a quantum improvement in performance. Within 4-6 months, a computerized pressure monitoring system that will alarm adverse conditions as well as provide trend reports on pressure relationships will be provided.

While emergency power systems are not present, several conditions exist to provide a margin of safety. If power fails, positive sealing pneuma-valves actuate which seal the supply and exhaust ducts at each room. These utilize compressed air for actuation and are backed up by compressed nitrogen cylinders. The long-term goal is to supply emergency power to the building systems. The infrastructure for emergency power distribution within the LEPH building is currently being designed and is scheduled to be installed within the year.

Battery powered emergency lights have been installed to supplement the existing emergency lighting. In addition, battery powered emergency lights have been installed in room 600 of LEPH. A battery powered emergency power source has been installed for the biological safety cabinet located in room 600. The emergency operating source is a Powermaker Uniinterruptable Power System by Topaz.

Infusion pumps have been connected to timers so that disinfectant can be added to autoclave drains on both the 6th and 8th floors at 30 minute (or greater, as needed) intervals during the working day. The pump used is a liquid metronics metering pump (model A-151-92) with associated 35 gallon tank and variable timing device.

To provide the suggested uniform coverage in infected animal areas, the following disposable clothing items have been provided: (1) TYVEK maximum coverage coveralls with zipper fronts, elastic closures at wrists and ankles; (2) Hi-tech TYVEK hoods with over the shoulder coverage; (3) Non-conductive, water repellant TYVEK foot covers.

An emergency alarm has been installed in the four involved animal areas. By pushing an emergency button in any of these four areas, an audible alarm and visual signal is activated in an office which is always manned during the working day. The alarm and visual signal indicate the specific room from which the emergency signal arises.

We have arranged, with assistance from the University Biologic Safety Officer, for each bank of HEPA filters to be DOP tested at the time of installation. This will be done via contract with an independent firm, ENV Services, Inc. Of course, the individual doing the DOP testing will have to undergo vacccination as required for those working in the 8th floor facility.

The suggestion that the manual be updated yearly has been passed along to the University's administration.

Additional magnehelic gauges will be installed where readable from the clean side of the animal containment area. One magnehelic was deemed inaccurate and was repaired. All instruments within the facility have been calibrated. A mangehelic guage has been installed in a visible position in the corridor outside room 600 of LEPH and indicates an air pressure differential of 0.05" wg.

The pressure differential alarm on the eight floor is tied into the computerized building automation system (CBAF). At present, the alarm system detects changes only between the dirty and the clean corridors within the animal facility. Thus, the alarm detects only mean pressure differentials. In the future, sensors will be added to individual animal holding rooms. Within the next 4-6 months, a computerized monitoring and the control system will be added. This will far exceed the requirements suggested.

Following implementation of the above procedures, the contractor was re-inspected by Mr. Kuehne. We have not yet received communication regarding the outcome of that site-visit.

There are a host of other voluntary measures which we have taken to safeguard the public even further For example, our entire inventory of virus materials is now computerized. Amounts of virus containing material and the disposition of such material are tracked from storage to disposal.

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
1	1.4	200 LD50'S
1	1.7	100 LD50'S
1	2.1	100 LD50'S
1	2.1	100 LD50'S
1	2.2	50 LD50'S
1	2.3	50 LD50'S
1	2.3	63 LD50'S
1	2.4	31 LD50'S
1	2.5	4 LD50'S
1	2.6	20 LD50'S
1	2.9	13 LD50'S
1	3.1	6 LD50'S
2	2.2	12 LD50'S
2	2.2	13 LD50'S
52	1.1	12 LD50'S
52	1.1	13 LD50'S
71	1.0	12 LD50'S
71	1.0	13 LD50'S
78	1.2	8 LD50'S
181	1.1	8 LD50'S
181	1.1	13 LD50'S
231	1.2	12 LD50'S
2582	0.9	50 LD50'S
2770	1.1	50 LD50'S
2870	1.1	12 LD50'S
2870	1.1	13 LD50'S
2873	1.0	12 LD50'S
2873	1.0	13 LD50'S
2874	0.9	12 LD50'S
2874	0.9	13 LD50'S
2874	0.8	50 LD50'S
2874	1.3	50 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
2876	1.0	12 LD50'S
2876	1.0	13 LD50'S
2876	1.1	50 LD50'S
2877	1.0	50 LD50'S
2878	0.9	50 LD50'S
2883	1.4	50 LD50'S
2884	1.4	50 LD50'S
2885	1.4	50 LD50'S
2886	1.0	50 LD50'S
2888	1.6	50 LD50'S
2889	1.0	12 LD50'S
2889	1.0	13 LD50'S
2890	1.1	12 LD50'S
2890	1.1	13 LD50'S
2890	0.9	50 LD50'S
2891	0.9	12 LD50'S
2891	0.9	13 LD50'S
2891	0.9	50 LD50'S
2916	1.2	12 LD50'S
2916	1.2	13 LD50'S
2926	0.9	50 LD50'S
2927	1.0	50 LD50'S
2928	0.9	50 LD50'S
2929	1.0	50 LD50'S
2959	1.0	12 LD50'S
2959	1.0	13 LD50'S
3505	1.6	50 LD50'S
3530	1.1	50 LD50'S
3531	1.0	50 LD50'S
3532	0.9	50 LD50'S
3533	0.9	50 LD50'S
3534	1.0	50 LD50'S
3535	1.1	50 LD50'S
3536	1.0	50 LD50'S
3537	1.0	50 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
3538	1.0	40 LD50'S
3539	TOXIC	
3540	0.9	50 LD50'S
3541	1.0	50 LD50'S
3542	1.0	100 LD50'S
3543	TOXIC	
3544	1.0	100 LD50'S
3545	1.1	50 LD50'S
3546	1.3	50 LD50'S
3547	1.3	50 LD50'S
3548	1.3	50 LD50'S
3549	1.1	50 LD50'S
3550	1.0	100 LD50'S
3551	1.0	40 LD50'S
3552	0.8	50 LD50'S
3553	0.6	50 LD50'S
3554	1.0	100 LD50'S
3556	1.0	100 LD50'S
3557	1.0	100 LD50'S
3558	1.0	100 LD50'S
3559	1.1	100 LD50'S
3560	1.0	100 LD50'S
3561	1.0	100 LD50'S
3562	1.0	100 LD50'S
3563	0.6	50 LD50'S
3573	1.2	50 LD50'S
3574	1.1	50 LD50'S
3575	0.9	50 LD50'S
3576	0.7	50 LD50'S
3577	1.0	50 LD50'S
3578	0.9	50 LD50'S
3579	1.1	50 LD50'S
3580	0.8	50 LD50'S
3581	1.1	50 LD50'S
3583	0.7	50 LD50'S
3584	0.9	50 LD50'S
3602	0.9	50 LD50'S
3603	1.0	50 LD50'S
3605	0.9	50 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
3606	2.2	50 LD50'S
3607	1.4	50 LD50'S
3608	1.2	50 LD50'S
3609	0.9	50 LD50'S
3610	0 7	50 LD50'S
3611	0.7	8 LD50'S
3612	1.0	50 LD50'S
3614	1.0	40 LD50'S
3615	0.9	40 LD50'S
3625	0.7	50 LD50'S
3677	0.9	40 LD50'S
3678		TOXIC
3679	0.8	50 LD50'S
3680	1.1	50 LD50'S
3703	1.1	50 LD50'S
3704	0.7	50 LD50'S
3705	1.6	8 LD50'S
3706	1.2	50 LD50'S
3707	0.7	50 LD50'S
3708	0.8	50 LD50'S
3709	0.8	50 LD50'S
3710	0.9	50 LD50'S
3711	0.8	150 LD50'S
3712	1.0	150 LD50'S
3713	1 3	150 LD50'S
3714	1.0	150 LD50'S
3715	1.0	150 LD50'S
3716	1.1	150 LD50'S
3717	1.0	150 LD50'S
3720	1.1	150 LD50'S
3721	1.1	150 LD50'S
3722	1.1	150 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
3723	1.1	150 LD50'S
3724	1.1	150 LD50'S
3725	1.0	40 LD50'S
3726	1.1	40 LD50'S
3906	1.0	100 LD50'S
3906	1.0	50 LD50'S
3907	0.9	40 LD50'S
3908	0.9	40 LD50'S
3910	0.8	40 LD50'S
3911	1.0	100 LD50'S
3911	1.1	50 LD50'S
3912	1.0	50 LD50'S
3913	1.0	50 LD50'S
3914	1.0	50 LD50'S
3915	1.0	50 LD50'S
3916	1.1	50 LD50'S
3917	1.0	40 LD50'S
3918	1.2	50 LD50'S
3919	1.1	40 LD50'S
3920	1.0	40 LD50'S
3923	1.0	40 LD50'S
3924	1.0	40 LD50'S
3935	0.9	50 LD50'S
3936	0.8	50 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
3937	0.9	50 LD50'S
3938	0.9	50 LD50'S
3939	0.9	50 LD50'S
3940	0.9	50 LD50'S
3941	0.9	50 LD50'S
3942	0.9	50 LD50'S
3943	1.0	50 LD50'S
3944	0.9	50 LD50'S
3945	0.9	50 LD50'S
3946	0.9	50 LD50'S
3947	1.0	50 LD50'S
3966	TOXIC	
3985	0.9	6 LD50'S
3986	0.9	6 LD50'S
3987	1.0	6 LD50'S
3988	0.9	6 LD50'S
3989	0.9	6 LD50'S
3990	1.0	6 LD50'S
3991	1.1	6 LD50'S
3992	0.9	6 LD50'S
3993	0.9	6 LD50'S
3994	1.0	6 LD50'S
3995	0.9	6 LD50'S
3996	1.0	6 LD50'S
3997	1.0	6 LD50'S
3998	1.0	6 LD50'S
3999	0.8	6 LD50'S
4000	0.9	6 LD50'S
4001	0.9	6 LD50'S
4002	0.9	6 LD50'S
4003	1.1	6 LD50'S
4004	0.9	6 LD50'S
4006	1.1	6 LD50'S
4042	1.0	40 LD50'S
4043	0.9	40 LD50'S
4044	0.9	40 LD50'S
4045	1.2	31 LD50'S
4046	0.9	40 LD50'S
4047	0.9	31 LD50'S
4048	1.0	31 LD50'S
4049	1.0	3 LD50'S
4050	0.9	31 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

SINGLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
4051	1.0	31 LD50'S
4052	1.1	31 LD50'S
4053	1.0	31 LD50'S
4065	1.0	6 LD50'S
4068	1.0	6 LD50'S
4070	TOXIC	
4071	2.3	6 LD50'S
4073	1.1	6 LD50'S
4094	1.1	31 LD50'S
4095	1.0	31 LD50'S
4103	0.9	6 LD50'S
4104	1.0	79 LD50'S
4108	0.9	79 LD50'S
4109	1.1	31 LD50'S
4110	1.0	79 LD50'S
4111	0.9	79 LD50'S
4112	0.9	79 LD50'S
4113	1.0	79 LD50'S
4114	0.9	79 LD50'S
4115	1.0	79 LD50'S
4116	1.0	79 LD50'S
4117	1.0	79 LD50'S
4118	0.9	79 LD50'S
4119	1.0	31 LD50'S
4120	1.0	31 LD50'S
4121	0.9	79 LD50'S
4122	1.0	31 LD50'S
4123	1.0	31 LD50'S
4124	1.0	31 LD50'S
4125	1.0	31 LD50'S
4126	1.1	31 LD50'S
4127	1.0	31 LD50'S
4128	1.0	31 LD50'S
4129	0.9	31 LD50'S
4130	TOXIC	
4131	0.9	31 LD50'S
4135	1.0	31 LD50'S
4136	1.0	31 LD50'S
4137	0.9	31 LD50'S
4138	0.9	31 LD50'S
4139	1.2	31 LD50'S
4140	1.0	31 LD50'S
4149	1.0	31 LD50'S
4205	1.0	31 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

AVS NUMBER	VR+	VR	VIRUS DOSE
MULTIPLE DRUG DOSES			
1		2.9	12 LD50'S
1		3.7	50 LD50'S
1		3.8	50 LD50'S
3980	3.7	1.1	50 LD50'S
3982	3.7	1.2	50 LD50'S
4201	3.7	1.2	50 LD50'S
4204	3.7	1.1	50 LD50'S
4206	3.7	1.0	50 LD50'S
4207	3.7	1.0	50 LD50'S
4208	3.7	1.0	50 LD50'S
4213	3.7	1.0	50 LD50'S
4214	3.7	1.0	50 LD50'S
4215	3.7	0.9	50 LD50'S
4215	3.7	0.9	50 LD50'S
4216	3.7	1.0	50 LD50'S
4217	3.7	1.2	50 LD50'S
4218	3.7	1.0	50 LD50'S
4219	3.7	1.0	50 LD50'S
4220	3.7	1.0	50 LD50'S
4221	3.7	1.2	50 LD50'S
4224	3.7	1.0	50 LD50'S
4225	3.7	1.4	50 LD50'S
4226	3.7	1.0	50 LD50'S
4227	3.7	1.1	50 LD50'S
4231	3.8	1.2	50 LD50'S
4232	3.8	1.0	50 LD50'S
4233	3.8	1.1	50 LD50'S
4235	3.8	1.1	50 LD50'S
4239		TOXIC	
4240		TOXIC	
4241	3.8	1.1	50 LD50'S
4242	3.8	1.1	50 LD50'S
4243	3.8	1.1	50 LD50'S
4244	3.8	0.9	50 LD50'S
4245	3.8	1.1	50 LD50'S

TABLE 1. RESULTS OF TESTING IN THE CCHF MODEL (CONTINUED)

AVS NUMBER	VR+	VR	VIRUS DOSE
MULTIPLE DRUG DOSES			
4246	3.8	1.0	50 LD50'S
4247	3.8	1.1	50 LD50'S
4249	3.8	1.0	50 LD50'S
4251	3.8	1.1	50 LD50'S
4254	3.8	1.1	50 LD50'S
4255	3.8	1.0	50 LD50'S
4256	3.8	1.0	50 LD50'S
4257	3.8	1.1	50 LD50'S
4258	3.8	1.0	50 LD50'S
4262	3.8	1.0	50 LD50'S
4261		TOXIC	

FIGURE 1A. CORRELATION BETWEEN THE GEOMETRIC MEAN SURVIVAL TIME (VC) OF CCHF VIRUS INFECTED MICE AND THE CCHF VIRUS DOSE IN SINGLE DOSE TESTS

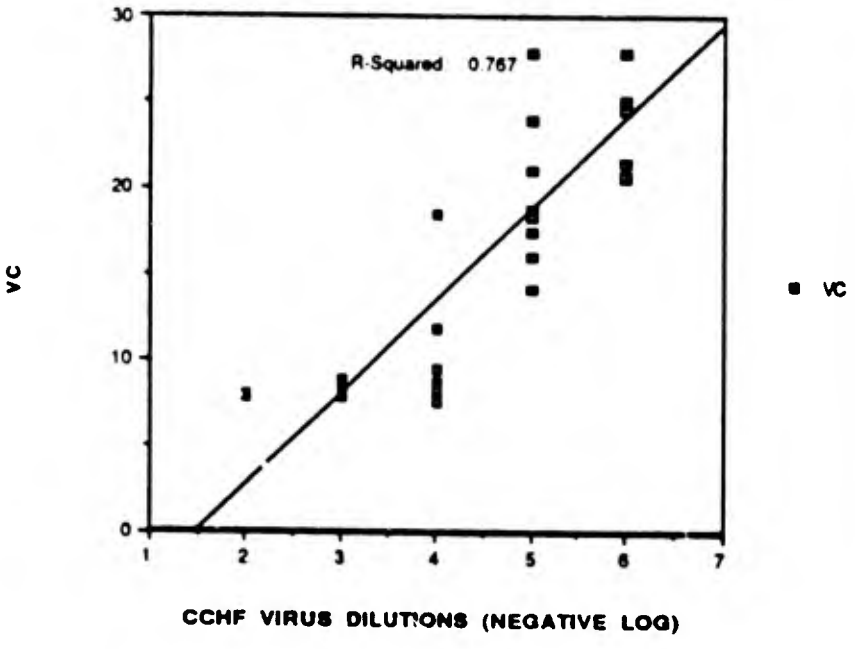


FIGURE 1B. CORRELATION BETWEEN THE GEOMETRIC MEAN SURVIVAL TIME (VC) OF CCHF VIRUS INFECTED MICE AND THE CCHF VIRUS DOSE IN MULTIPLE DOSE TESTS

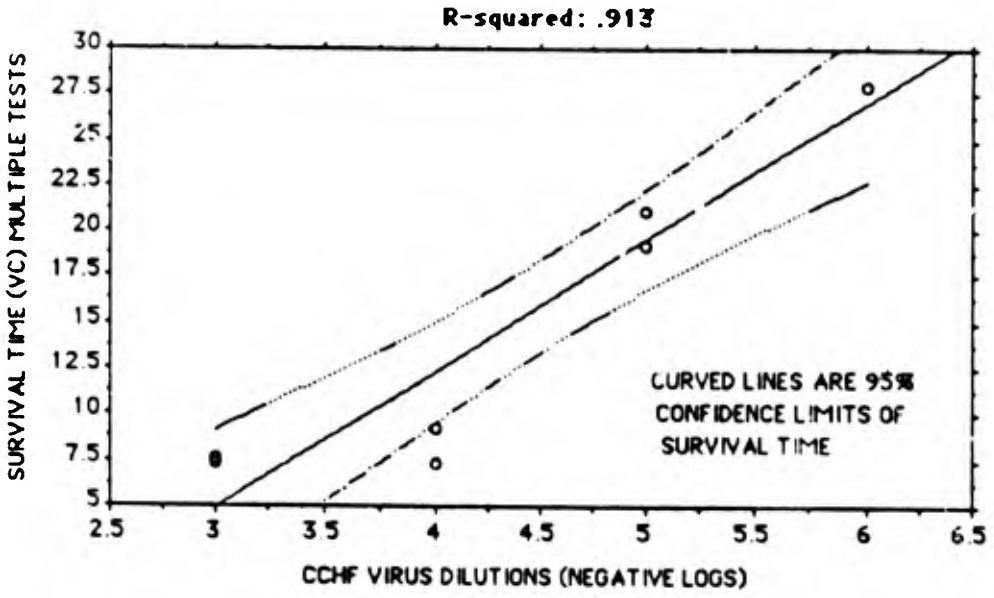


FIGURE 2. FREQUENCY DISTRIBUTION OF VR SCORES IN CCHF DRUG TESTS

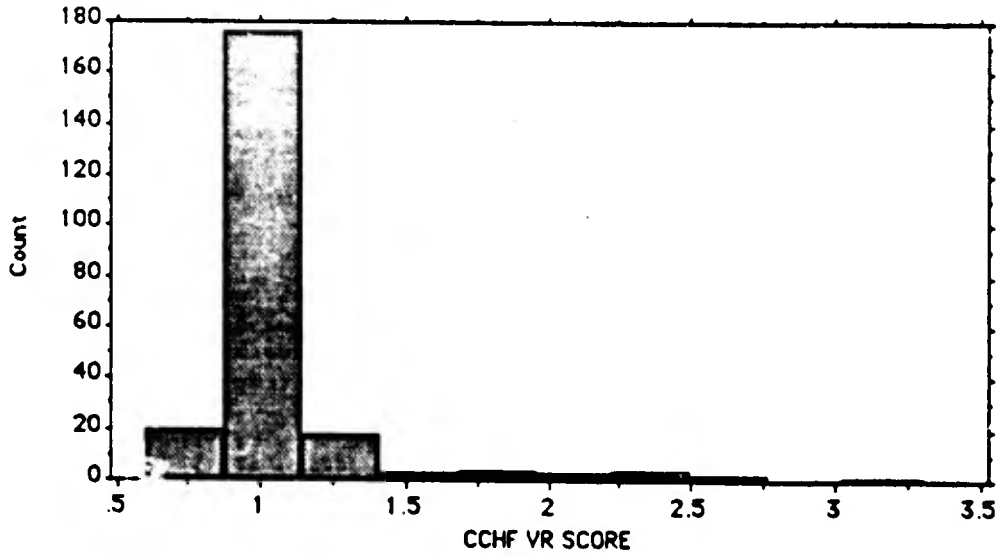


FIGURE 3. PERCENTILES FOR VR SCORES IN CCHF TESTS

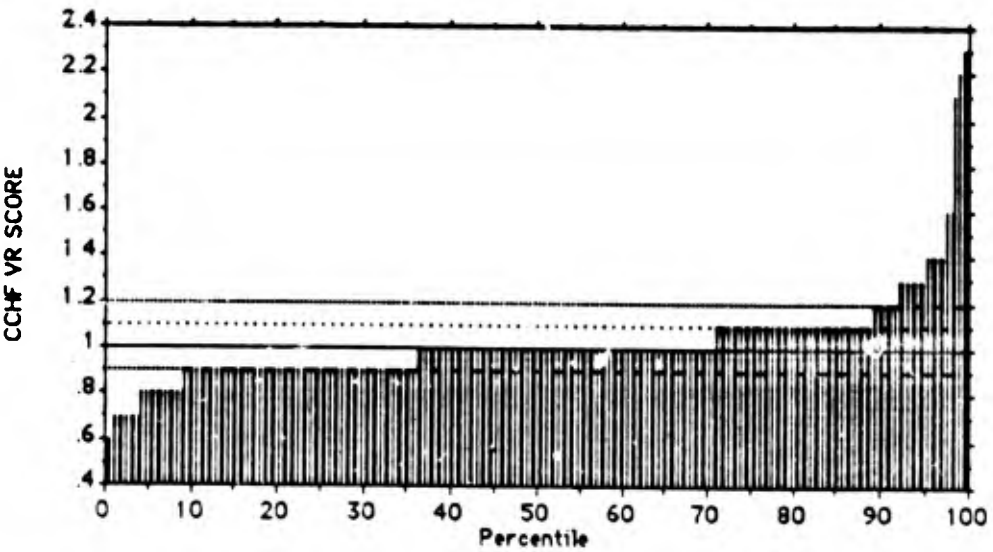


FIGURE 4. FREQUENCY DISTRIBUTION OF VIRUS DOSES (LD50'S) IN CCHF DRUG TESTS

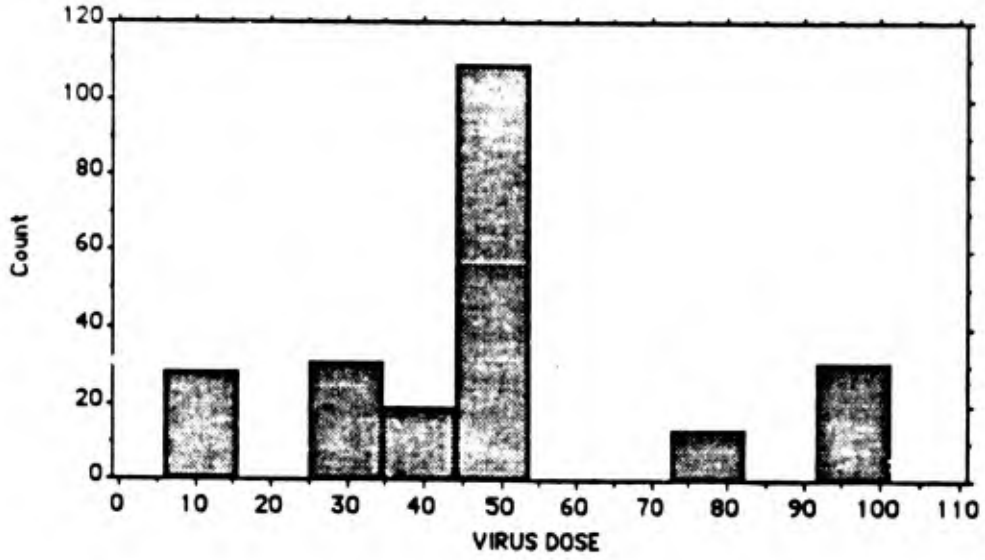


FIGURE 5. CORRELATION BETWEEN RIBAVIRIN VR SCORES AND CCHF VIRUS DOSE

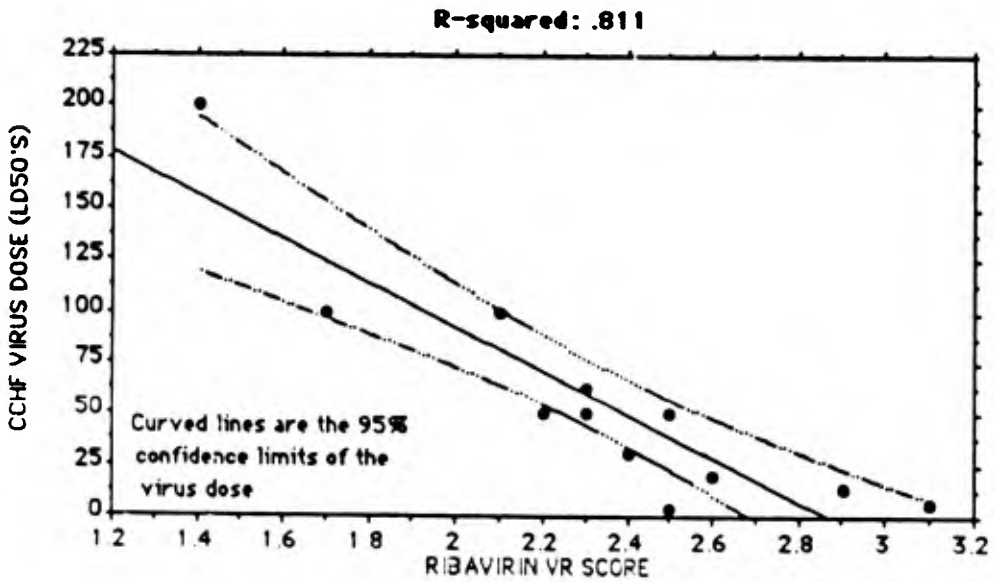
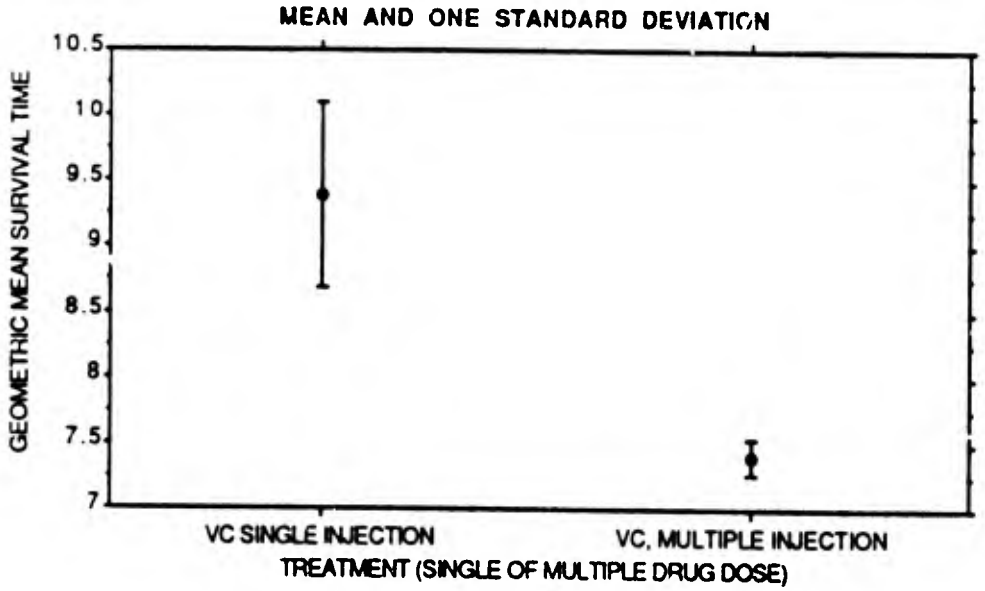
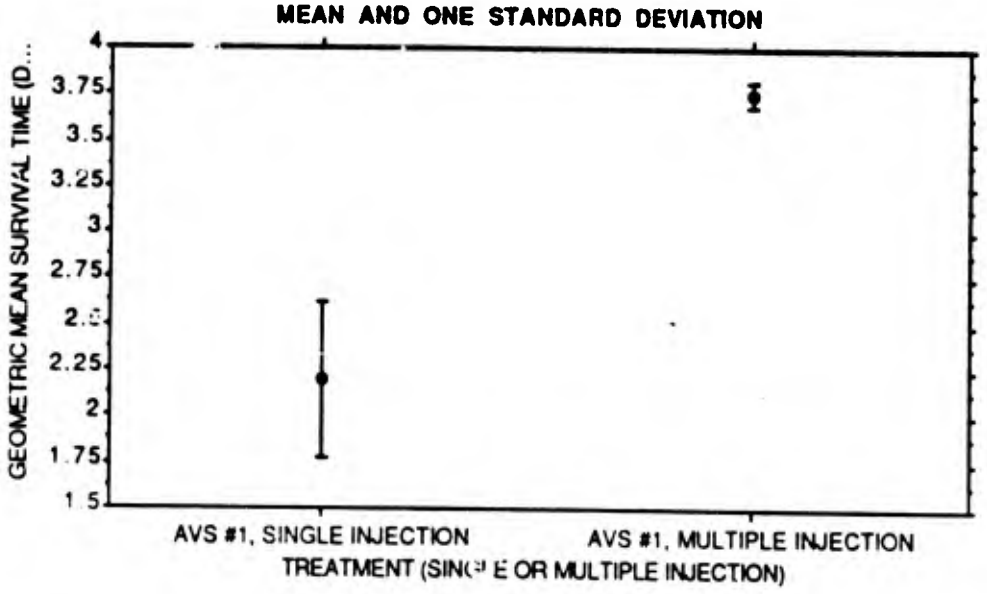


FIGURE 6. COMPARISON OF SINGLE AND MULTIPLE INJECTIONS ON GEOMETRIC MEAN SURVIVAL TIME OF CONTROL MICE



CCHF VIRUS STRAIN DOSE IS 50 LD50'S, I.P.

FIGURE 7. COMPARISON OF RIBAVIRIN VR SCORES AFTER SINGLE AND MULTIPLE DRUG DOSES



CCHF STRAIN 10200 VIRUS DOSE IS 50 LD50'S, I.P.

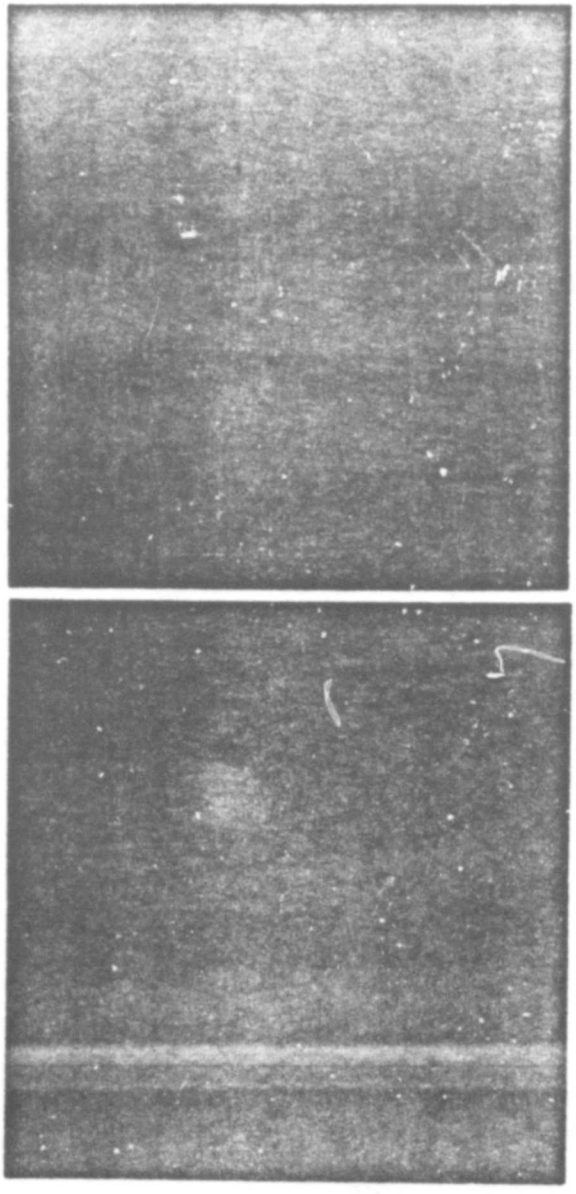


FIGURE 8. EARLY APPEARANCE OF CCHF VIRUS ANTIGEN IN INFECTED AND R BAVIRIN-TREATED INFANT MICE

The effect of a single dose of ribavirin on the distribution of CCHF virus antigen in infant mouse liver tissue 3 days after inoculation of virus.

TOP. CCHF antigen occurs predominantly in Kupffer cells lining the liver sinusoids of a placebo-treated animal.

BOTTOM. CCHF virus antigen does not appear in either cells lining the sinusoids or the hepatocytes.

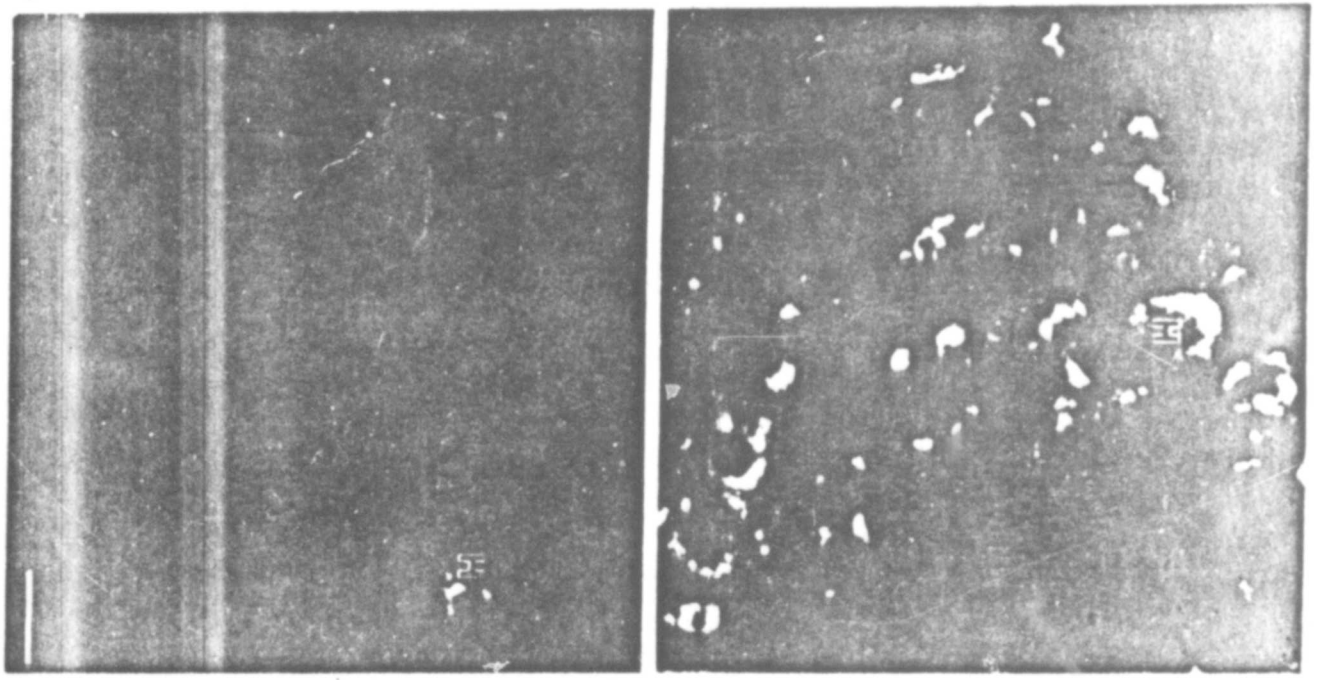


FIGURE 9. LATE APPEARANCE OF CCHF VIRUS ANTIGEN IN INFECTED AND RIBAVIRIN-TREATED INFANT MICE

The effect of a single dose of ribavirin on the distribution of CCHF virus antigen in infant mouse liver tissue 7 days after inoculation of virus.

TOP. CCHF virus antigen is present in many clusters of hepatocytes (H) in the liver of an animal given placebo.

BOTTOM. CCHF virus antigen is generally absent from liver tissue of animals treated with ribavirin. A single infected hepatocyte (H) is shown

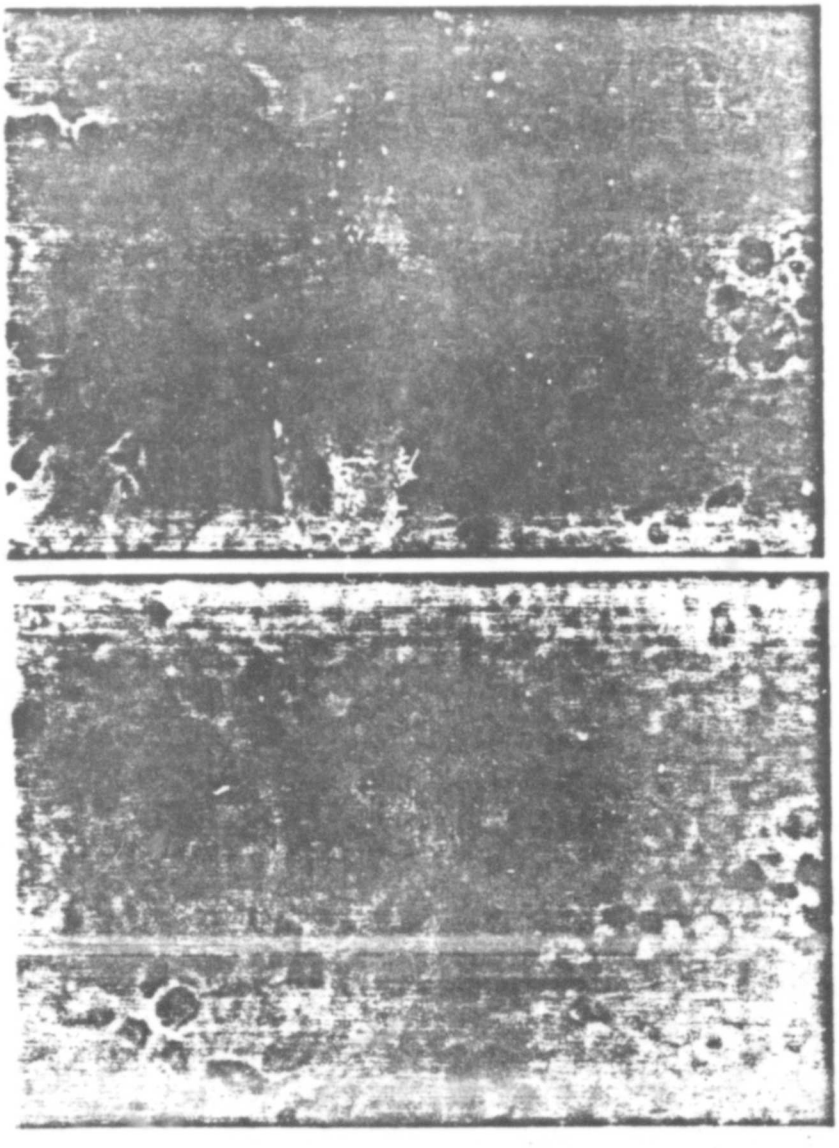


FIGURE 10. IDENTIFICATION OF CCHF VIRUS INFECTED CELLS AS KUPFFER CELLS

The distribution of MAC-1 antibody and CCHF virus antibody in Kupffer cells.

TOP. MAC-1 antibody reactivity present in a Kupffer cell lining a liver sinusoid.

BOTTOM. In a consecutive section, CCHF virus antibody reactivity occurs in the same Kupffer cell.

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL
MULTIPLE DOSE DRUG TESTS

AVS NUMBER	VR	VIRUS DOSE
1	1.6	63 LD50'S
2	1.3	63 LD50'S
52	1.2	250 LD50'S
71	1.2	63 LD50'S
78	1.0	63 LD50'S
181	1.0	63 LD50'S
206	1.0	50 LD50'S
206	1.3	79 LD50'S
206	1.4	50 LD50'S
206	1.6	250 LD50'S
231	1.1	63 LD50'S
253	2.2	79 LD50'S
1915	1.2	79 LD50'S
2787	1.0	50 LD50'S
2873	1.3	63 LD50'S
2874	1.3	63 LD50'S
2876	1.0	63 LD50'S
2879	1.1	79 LD50'S
2889	1.0	63 LD50'S
2890	1.1	63 LD50'S
2891	1.1	63 LD50'S
2911	1.0	250 LD50'S
2912	1.1	63 LD50'S
2914	1.0	63 LD50'S
2918	1.0	63 LD50'S
2919	1.1	63 LD50'S
2958	1.1	63 LD50'S
2996	0.8	63 LD50'S
3530	1.3	50 LD50'S
3531	0.9	50 LD50'S
3532	1.3	50 LD50'S
3533	1.0	50 LD50'S
3534	1.0	50 LD50'S
3535	1.1	50 LD50'S
3536	1.0	50 LD50'S
3537	1.1	50 LD50'S
3538	1.1	40 LD50'S
3539	0.9	50 LD50'S
3540	1.0	50 LD50'S
3541	0.9	50 LD50'S
3542	1.0	50 LD50'S
3543	TOXIC	

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL (CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
3544	0.9	50 LD50'S
3545	1.2	50 LD50'S
3546	1.0	50 LD50'S
3547	1.1	63 LD50'S
3548	1.0	50 LD50'S
3549	0.9	50 LD50'S
3549	1.0	63 LD50'S
3550	1.0	50 LD50'S
3551	1.0	50 LD50'S
3552	0.7	40 LD50'S
3553	1.0	50 LD50'S
3554	1.0	50 LD50'S
3556	1.0	50 LD50'S
3557	1.0	50 LD50'S
3558	1.2	50 LD50'S
3559	0.9	50 LD50'S
3560	0.9	50 LD50'S
3561	1.0	50 LD50'S
3562	1.1	50 LD50'S
3573	0.9	100 LD50'S
3574	0.9	50 LD50'S
3575	1.0	100 LD50'S
3576	1.0	50 LD50'S
3578	1.0	50 LD50'S
3579	1.0	50 LD50'S
3580	1.5	50 LD50'S
3581	1.1	50 LD50'S
3582	0.9	100 LD50'S
3583	1.0	50 LD50'S
3584	1.0	50 LD50'S
3602	1.0	50 LD50'S
3603	0.8	50 LD50'S
3605	0.9	50 LD50'S
3606	0.9	50 LD50'S
3606	1.1	250 LD50'S
3607	1.0	50 LD50'S
3608	0.9	50 LD50'S
3609	1.0	50 LD50'S
3610	1.0	50 LD50'S
3611	0.9	50 LD50'S
3612	0.9	50 LD50'S
3613	1.7	50 LD50'S
3614	1.2	63 LD50'S
3625	1.2	50 LD50'S

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL (CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
3647	1.0	50 LD50'S
3672	0.9	40 LD50'S
3677	1.1	100 LD50'S
3678		TOXIC
3679	1.3	50 LD50'S
3680	1.0	50 LD50'S
3703	0.9	50 LD50'S
3704	0.9	50 LD50'S
3706	1.0	50 LD50'S
3707	0.9	50 LD50'S
3708	1.0	50 LD50'S
3709	1.0	50 LD50'S
3710	0.9	50 LD50'S
3711	1.0	50 LD50'S
3712	1.0	50 LD50'S
3713	0.9	50 LD50'S
3714	0.9	50 LD50'S
3715	0.9	50 LD50'S
3716	0.9	50 LD50'S
3717	1.0	50 LD50'S
3720	0.9	50 LD50'S
3721	0.9	50 LD50'S
3722	0.9	50 LD50'S
3723	0.9	50 LD50'S
3724	1.1	50 LD50'S
3725	0.9	50 LD50'S
3726	1.1	50 LD50'S
3893	1.4	150 LD50'S
3906	0.9	50 LD50'S
3907	1.1	50 LD50'S
3908	1.4	50 LD50'S
3910	1.2	50 LD50'S
3911	1.2	150 LD50'S
3912	1.5	150 LD50'S
3913	1.1	150 LD50'S
3914	1.0	150 LD50'S
3915	1.0	150 LD50'S
3916	1.0	150 LD50'S
3917	1.6	50 LD50'S

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL
(CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
3918	1.0	150 LD50'S
3919	1.0	150 LD50'S
3920	1.7	50 LD50'S
3923	1.0	150 LD50'S
3924	1.1	150 LD50'S
3935	1.2	150 LD50'S
3936	1.0	150 LD50'S
3937	0.9	150 LD50'S
3938	1.0	150 LD50'S
3939	1.2	150 LD50'S
3940	1.2	150 LD50'S
3941	1.0	150 LD50'S
3942	1.2	150 LD50'S
3943	1.3	150 LD50'S
3944	1.0	150 LD50'S
3945	1.8	50 LD50'S
3946	1.0	150 LD50'S
3947	1.1	150 LD50'S
3966	1.2	150 LD50'S
3980	1.0	150 LD50'S
3981	TOXIC	
3982	1.1	150 LD50'S
3984	1.0	150 LD50'S
3985	1.1	50 LD50'S
3986	1.2	50 LD50'S
3987	1.2	50 LD50'S
3988	0.9	150 LD50'S
3989	1.2	50 LD50'S
3990	1.2	50 LD50'S
3991	1.1	50 LD50'S
3992	1.3	50 LD50'S
3993	1.2	50 LD50'S
3994	1.2	50 LD50'S
3995	1.1	50 LD50'S
3996	1.1	50 LD50'S
3997	1.1	50 LD50'S
3998	1.0	50 LD50'S
3999	1.0	50 LD50'S
4000	1.1	50 LD50'S
4001	1.3	50 LD50'S
4002	1.3	50 LD50'S
4003	1.3	50 LD50'S
4004	1.1	50 LD50'S
4005	1.1	50 LD50'S
4006	1.2	50 LD50'S
4042	0.9	100 LD50'S
4043	0.9	100 LD50'S

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL (CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
4044	1.0	100 LD50'S
4045	0.7	40 LD50'S
4046	1.0	100 LD50'S
4047	0.7	40 LD50'S
4048	0.8	40 LD50'S
4049	0.8	40 LD50'S
4050	0.7	100 LD50'S
4051	0.8	40 LD50'S
4052	0.9	100 LD50'S
4053	1.1	40 LD50'S
4064	1.0	63 LD50'S
4065	1.0	63 LD50'S
4068	0.9	63 LD50'S
4070	1.7	100 LD50'S
4070	1.8	63 LD50'S
4071	1.0	100 LD50'S
4071	1.6	63 LD50'S
4073	1.0	63 LD50'S
4094	0.9	100 LD50'S
4095	0.9	100 LD50'S
4103	1.2	63 LD50'S
4104	1.0	63 LD50'S
4108	1.0	63 LD50'S
4109	0.9	100 LD50'S
4110	0.9	63 LD50'S
4111	1.1	63 LD50'S
4112	1.0	63 LD50'S
4113	0.9	100 LD50'S
4113	1.5	63 LD50'S
4114	1.2	63 LD50'S
4115	1.0	63 LD50'S
4116	1.3	63 LD50'S
4117	1.0	63 LD50'S
4118	1.1	63 LD50'S
4119	1.3	63 LD50'S
4120	1.0	63 LD50'S
4121	1.0	100 LD50'S
4122	0.9	100 LD50'S
4123	0.9	100 LD50'S
4124	0.9	40 LD50'S
4125	0.9	40 LD50'S
4127	0.9	40 LD50'S
4128	1.0	40 LD50'S
4129	0.7	40 LD50'S

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL (CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
4130	0.8	40 LD50'S
4131	0.8	40 LD50'S
4135	0.9	100 LD50'S
4136	0.9	40 LD50'S
4137	1.4	40 LD50'S
4138	0.7	40 LD50'S
4139	0.9	40 LD50'S
4140	0.9	40 LD50'S
4149	1.1	40 LD50'S
4201	1.1	40 LD50'S
4203	1.1	40 LD50'S
4204	1.1	40 LD50'S
4205	1.0	40 LD50'S
4206	1.1	40 LD50'S
4207	0.9	40 LD50'S
4208	1.1	40 LD50'S
4209	1.0	10 LD50'S
4211	1.0	10 LD50'S
4213	1.0	40 LD50'S
4214	1.0	40 LD50'S
4215	0.9	40 LD50'S
4216	1.0	40 LD50'S
4217	1.3	40 LD50'S
4218	1.0	40 LD50'S
4219	1.5	40 LD50'S
4220	1.0	40 LD50'S
4221	1.0	40 LD50'S
4224	TOXIC	
4225	0.9	40 LD50'S
4226	1.2	40 LD50'S
4227	1.0	40 LD50'S
4228	1.0	40 LD50'S
4229	1.0	63 LD50'S
4231	1.1	10 LD50'S
4232	1.0	10 LD50'S
4233	0.8	10 LD50'S
4235	1.2	10 LD50'S
4236	1.0	10 LD50'S
4239	TOXIC	
4240	TOXIC	
4241	0.7	10 LD50'S
4242	0.7	10 LD50'S
4243	0.8	10 LD50'S
4244	0.8	10 LD50'S
4245	1.0	10 LD50'S
4246	0.8	10 LD50'S
4247	0.8	10 LD50'S

TABLE 2. RESULTS OF TESTING IN THE LCM MODEL (CONTINUED)

AVS NUMBER	VR	VIRUS DOSE
4248	0.9	79 LD50'S
4249	1.0	10 LD50'S
4250	1.0	79 LD50'S
4252	1.1	10 LD50'S
4253	0.9	79 LD50'S
4254	0.8	10 LD50'S
4255	0.7	10 LD50'S
4256	1.0	10 LD50'S
4258	0.8	10 LD50'S
4259	TOXIC	
4261	TOXIC	
4263	TOXIC	
4264	0.9	79 LD50'S
4265	0.9	79 LD50'S
4266	0.9	79 LD50'S
4267	1.0	79 LD50'S
4268	1.1	79 LD50'S
4269	1.0	10 LD50'S
4270	0.9	79 LD50'S
4271	0.8	79 LD50'S
4272	TOXIC	
4273	0.7	10 LD50'S
4274	1.2	79 LD50'S
4276	1.0	79 LD50'S
4277	0.8	10 LD50'S
4278	1.1	79 LD50'S
4279	1.5	10 LD50'S
4280	1.0	79 LD50'S
4281	0.9	79 LD50'S
4526	1.2	250 LD50'S
4528	1.3	250 LD50'S
4529	1.0	250 LD50'S
4530	1.0	250 LD50'S
4531	1.1	250 LD50'S
4532	0.9	250 LD50'S
4533	1.1	250 LD50'S
4587	1.0	250 LD50'S
4588	1.0	250 LD50'S
4589	0.9	250 LD50'S
4598	TOXIC	
4600	1.1	250 LD50'S
4601	1.3	250 LD50'S
4602	1.2	250 LD50'S
4603	1.1	250 LD50'S
4604	1.1	250 LD50'S
4606	1.1	250 LD50'S

FIGURE 11. CORRELATION BETWEEN THE GEOMETRIC MEAN SURVIVAL TIME (VC) OF LCM VIRUS INFECTED MICE AND THE LCM VIRUS DOSE

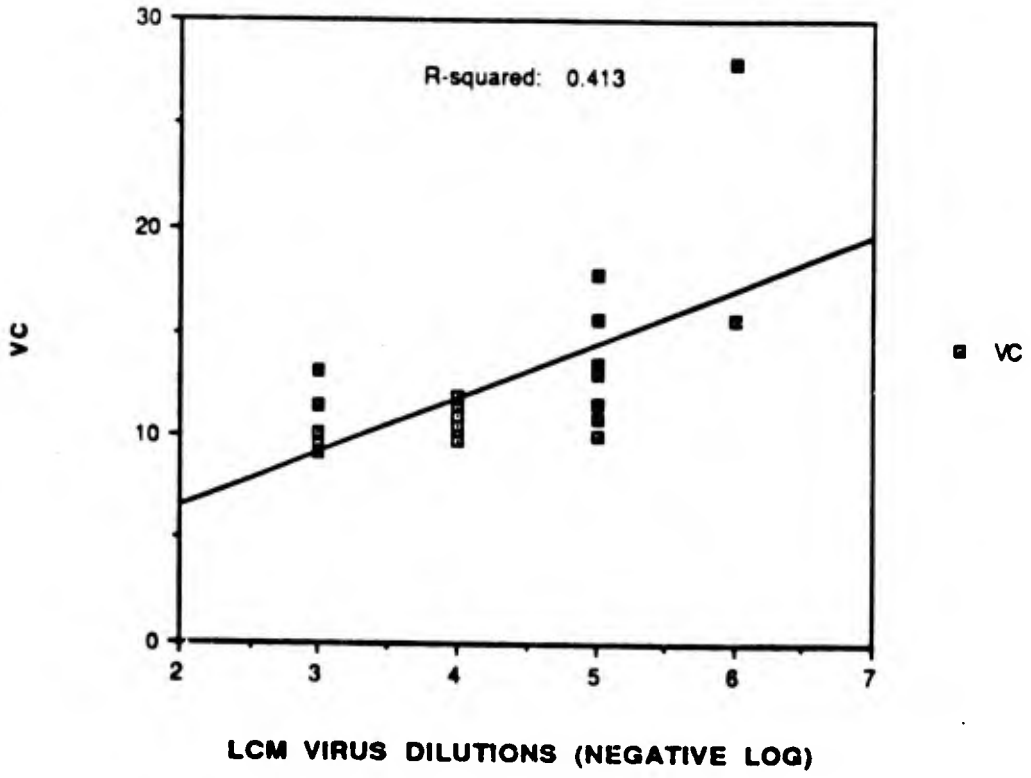


FIGURE 12. DISTRIBUTION OF VR SCORES FOR DRUGS TESTED IN THE LCM MODEL

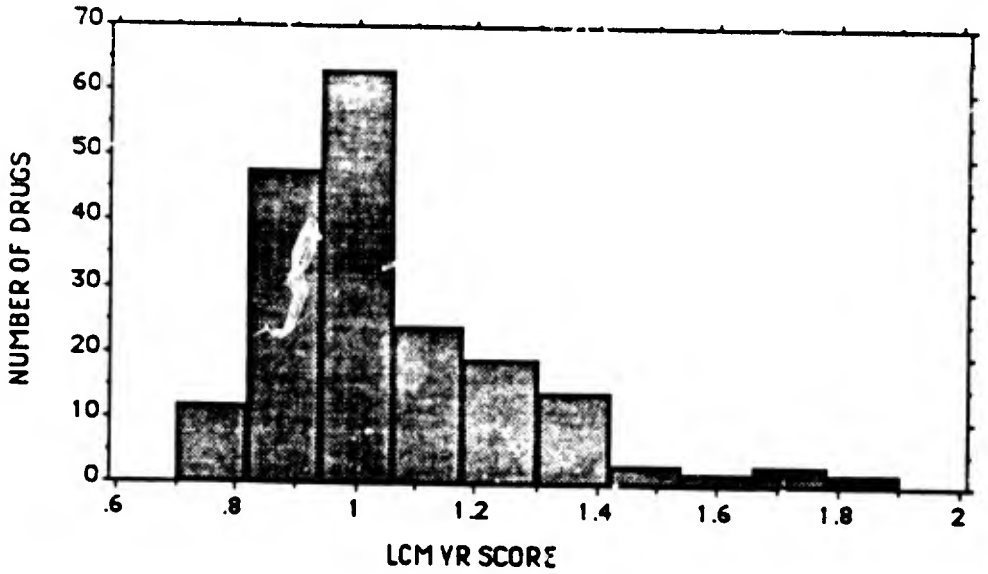


FIGURE 13. PERCENTILES FOR VR SCORES IN LCM TESTS

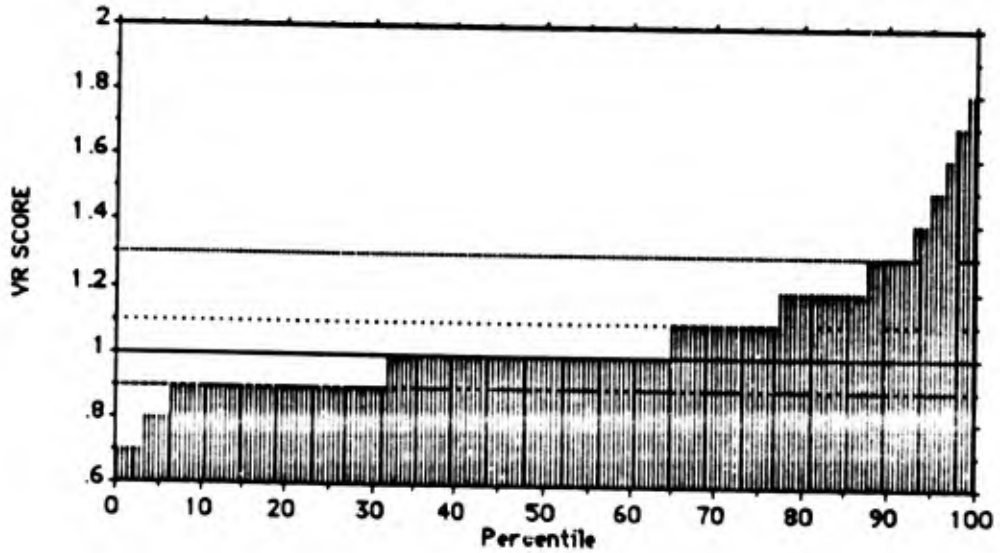


FIGURE 14. FREQUENCY DISTRIBUTION OF VIRUS DOSES (LD50'S) IN LCMV DRUG TESTS

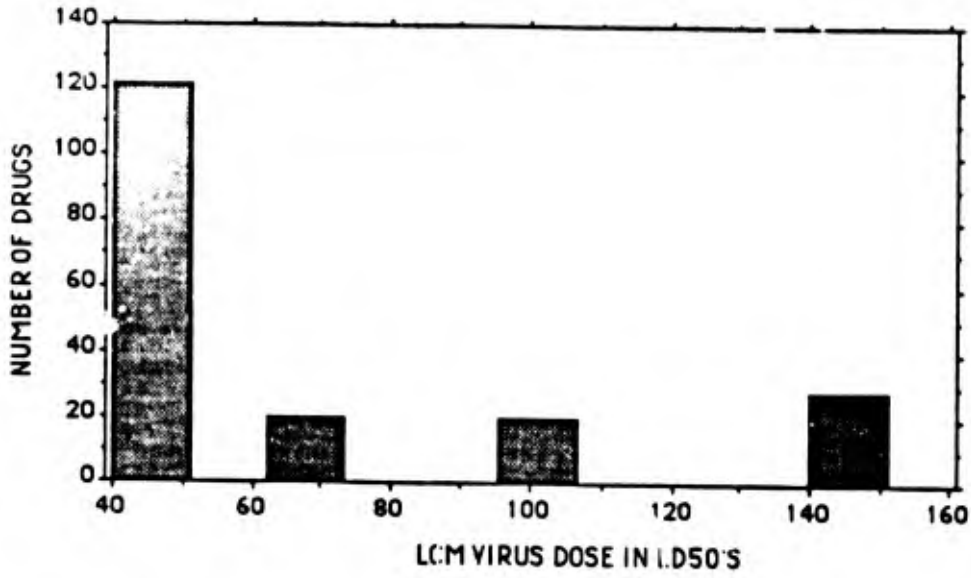


TABLE 3. POTENTIALLY ACTIVE DRUGS
VR SCORES AT OR ABOVE THE 90TH PERCENTILE IN
CCHF (VR > 1.2) AND LCM (VR >1.3) DRUG TESTS
(1988 DATA)

CCHF			LCM		
AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S	AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S
0001	§2.4	§79	0001	1.4*	50*
			0001	1.6	100
0002	2.2	13	0002	1.3	63
0078	1.2	10	0078	1.0	100
0206	§2.6*		0206	1.3	79
			0206	1.4	50
			0206	1.6	100
0231	§1.2	§13	0231	1.1	63
0253	§1.4*	50	0253	2.0*	50
			0253	2.2	79
2872	1.0	8	2872	0.9	100
2872	1.0	50			
2872	2.0*	4*			
2873	1.0	8	2873	1.0	
2873	1.3*	4	2873	1.3	100
2874	0.9	8	2874	1.3	100
2874	1.4*	13	2874	0.9	
2874	0.8*	13			
2883	1.2	13	2883	0.9*	100
2884	1.4	13	2884	0.9*	100
2885	1.4	13	2885	1.0*	100
2888	1.6	13	2888	1.1*	100
2889	1.0	13	2889	1.5*	100
2916	1.2	12	2916	0.9*	100
3530	1.1	50	3530	1.3	50
3532	0.9	50	3532	1.3	50

* DATA FROM 1987
§ MEAN VALUES FROM TWO OR MORE TESTS.

TABLE 3. POTENTIALLY ACTIVE DRUGS:
VR SCORES AT OR ABOVE THE 90TH PERCENTILE IN
CCHF (VR > 1.2) AND LCM (VR >1.3) DRUG TESTS
(CONTINUED)

CCHF			LCM		
AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S	AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S
3546	1.3	50	3546	1.0	50
3547	1.3	50	3547	1.1	100
3548	1.3	50	3548	1.0	50
3573	1.2	50	3573	0.9	100
3580	0.8	50	3580	1.5	50
3606	2.2	10	3606	0.9	50
3607	1.4	10	3607	1.0	50
3608	1.2	10	3608	0.9	50
3613	1.4	10	3613	1.7	50
			3613	1.0	100
3679	0.8	50	3679	1.3	50
3705	1.6	10	3705	NOT TESTED	
3706	1.2	10	3706	1.0	50
3713	1.3	150	3713	0.9	50
3908	0 9	40	3908	1.4	50
3912	1.0	50	3912	1.5	150
3917	1.0	40	3917	1.6	50
			3917	1.1 •	100
3918	1.2	50	3918	1.0	150
3920	1.0	40	3920	1.7	50
			3920	0.9	100

* DATA FROM 1987
§ MEAN VALUES FROM TWO OR MORE TESTS.

TABLE 3. POTENTIALLY ACTIVE DRUGS:
VR SCORES AT OR ABOVE THE 90TH PERCENTILE IN
CCHF (VR > 1.2) AND LCM (VR >1.3) DRUG TESTS
(CONTINUED)

CCHF			LCM		
AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S	AVS NUMBER	VR SCORE	VIRUS DOSE LD50'S
3943	1.0	50	3943	1.3	150
3945	0.9	50	3945	1.8	50
			3945	1.0	100
3982	1.2	50	3982	NOT TESTED	
3992	0.9	6	3992	1.3	50
4001	0.9	6	4001	1.3	50
4002	0.9	6	4002	1.3	50
4003	1.1	6	4003	1.3	50
4045	1.2	31	4045	0.7	40
4070	TOXIC IN INFANT MICE		4070	1.7	100
			4070	1.8	63
4071	2.3	6	4071	1.6	63
			4071	1.0	100
4113	1.0	79	4113	1.5	63
			4113	0.9	100
4137	0.9	31	4137	1.4	40
4139	1.2	31	4139	0.9	40
4201	1.2	50	4201		
4217	1.2	50	4217	1.3	40
4219	1.0	50	4219	1.5	40
4221	1.2	50	4221	NOT TESTED	
4225	1.4	50	4225	0.9	40
4231	1.2	50	4231	1.1	10
4279	NOT TESTED		4279	1.5	10
4528	NOT TESTED		4528	1.5	250
4601	NOT TESTED		4601	1.3	250

* DATA FROM 1987
§ MEAN VALUES FROM TWO OR MORE TESTS.

TABLE 4. EXPERIMENTAL PROTOCOL FOR ANTIVIRAL DRUG TESTING IN PRIMATES USING YELLOW FEVER VIRUS

Group	Dose mg/kg	Route	Schedule (Days)	Yellow fever virus Challenge
1	25	Oral	-1,+1,+3,+5,+7	Day 0
2	25	Oral	+1,+3,+5,+7	Day 0
3	Placebo	Oral	-1,+1,+3,+5,+7	Day 0
4	25	Oral	-1,+1,+3,+5,+7	Diluent
5	25	Oral	-1,+1,+3,+5,+7	Day 0

n=4

TABLE 5. RESULTS OF TESTING IN THE YELLOW FEVER PRIMATE MODEL

		DAY 2		DAY 4		DAY 7		DAY 14
NUMBER	WEIGHT	VIREMIA	AB	VIREMIA	AB	VIREMIA	AB	HAI
GROUP 1								
0141	317	<0.7	0	<0.7	0	4.9	0	640
0981	330	<0.7	0	<0.7	0	<0.7	0	80
0982	373	<0.7	0	<0.7	0	<0.7	80	640
0984	345	<0.7	0	4.9	0	<0.7	0	80
GROUP 2								
0986	342	3.5	0	>4.9	0	<0.7	20	160
0992	424	4.2	0	>4.9	0	<0.7	0	320
0993	292	4.9	0	4.2	0	<0.7	40	160
0994	358	3.5	0	1.4	0	<0.7	20	160
GROUP 3								
0996	334	3.5	0	4.9	0	<0.7	10	160
0997	434	4.9	0	4.9	0	<0.7	20	320
0998	388	4.9	0	4.2	0	<0.7	10	80
0987	439	3.5	0	2.8	0	<0.7	0	40
GROUP 4								
0990	356							0
0999	395							0
0995	385							0
0983	419							0
GROUP 5								
0991	392	<0.7	0	<0.7	0	<0.7	80	640
0988	338	<0.7	0	4.9	0	<0.7	0	160
0989	432	<0.7	0	2.1	0	<0.7	0	40
0985	356	<0.7	0	1.4	0	<0.7	0	160

EXPERIMENTAL PROCEDURE: Male squirrel monkeys were pre-screened for HAI antibodies to yellow fever, SLE, and Ilheus viruses. All were negative at 1:10 dilutions of acetone-treated serum. Monkeys were randomly assigned to test groups and infected as previously described with 100,000 PFU's of yellow fever virus in a volume of 0.2 mls subcutaneously except for Group 4 which was given a placebo in place of infectious virus. Groups were treated as follows: Group 1, CI-246-738 (by oral intubation, 25 mg/kg) one day before and after (See attached protocol); Group 2, drug after virus; Group 3, virus only, placebo before and after; Group 5, drug before and after, no virus, placebo only; Group 5, virus, CI-246-738 and AVS #1 before and after in doses previously described. Bleeding of animals followed exactly the attached protocol directions. Virus infectivity was confirmed by inoculation of infant mice by the intracerebral route.

TABLE 6 STATISTICAL ANALYSIS OF YELLOW FEVER-PRIMATE DATA WITH ANTIVIRAL DRUG AVS# 1968

A. WEIGHT

T TEST (PAIRED, 2 GROUPS, 2-TAIL)

CONTROL	VERSUS	PROBABILITY
GROUP 3		
	GROUP 1	0.0967
	GROUP 2	0.1801
	GROUP 4	0.4964
	GROUP 5	0.6689

B. VIREMIA

T TEST (PAIRED, 2 GROUPS, 2-TAIL)

CONTROL	VERSUS	T TEST PROBABILITY	CHI-SQUARE PROBABILITY
(DAY 2) GROUP 3			
	GROUP 1	0.0032	0.0001
	GROUP 2	0.391	
0.9898			
	GROUP 5	0.0061	0.0001
(DAY 4) GROUP 3			
	GROUP 1	0.2496	0.0001
	GROUP 2	0.7888	0.5934
	GROUP 5	0.1612	0.002

C. GEOMETRIC MEAN HAI TITERS (DAY 14)

A.) T TEST (PAIRED, 2-TAIL)

GROUP 3	GROUP 1	0.425
	GROUP 2	0.2146
	GROUP 5	0.6025

TABLE 7A. INTERFERON IN UNINFECTED DRUG-TREATED (AVS# 1968) MONKEYS (GROUP 4)

Monkey	Day 0	Day 2	Day 4	Day 6	Day 8	Day 14
990	0	2.5	3.5	nt	1.5	2.5
999	0	2.5	3.5	nt	2.0	2.5
995	2.5	3.5	3.0	3.0	2.5	2.5
983	0	3.5	3.5	2.5	2.5	2.5

Drug was given on days -1, +1,+3,+5, and +7. Nt is not tested. Blood drawn on day -1 before drug administration was less than 1:10.

TABLE 7B. INTERFERON IN YELLOW FEVER VIRUS INFECTED DRUG-TREATED (AVS# 1968) MONKEYS (GROUP 1)

Monkey	Day 0	Day 4	Day 7	Day 14
141	0	0	2.5	0
981	0	0	0	0
982	0	1.5	2.0	1.5
984	0	0	1.5	1.5

(DRUG ON DAYS -1, 1, 3, 5, 7) VIRUS ON DAY 0.
MONKEY 981 DID NOT FORM DETECTABLE INTERFERON ON THE DAYS TESTED. NEITHER DID THIS MONKEY DISPLAY A DETECTABLE VIREMIA ON THE DAYS TESTED. HOWEVER, THE ANIMAL SEROCONVERTED (HAI 1:80).

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