A Dose-Response Curve Employing Thrombocytopenia Induced by the Friend Leukemia Virus.* (30624)

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The interaction of viruses with platelets has been much investigated in recent years. Thrombocytopenia is present in human viral infections(1-6). Recently, significant thrombocytopenia has been demonstrated with the Friend Leukemia Virus within 48 hours after infection in several different strains of mice, and is a major factor in the hemorrhagic diathesis associated with this entity(7). In the murine leukemias, characteristic virus particles have been demonstrated in the platelets, and in the cytoplasmic vacuoles and subcellular components of the megakaryocytes indicating their importance as sites of production and activity of the virus(8,9).

In studying viral infectivity, procedures for quantitation of infectivity are of great importance. In certain instances, a predictable quantitative relationship exists between the dilution of virus inoculated, and a second parameter, readily accessible to precise measurement. Rowe and Brodsky demonstrated such a relationship with respect to splenomegaly in Friend Virus Leukemia(10). In this report, a linear relationship will be demonstrated between dilution, or dose of virus and the fall in the platelet count, or degree of thrombocytopenia.

Materials and methods. Mice. Weanling female (16 to 18 g) mice of the "General Purpose" non-inbred Swiss Webster mouse strain from the Huntingdon Farms production colony were maintained in plastic cages 8 to 10 per cage and fed Purina laboratory chow, small, with water *ad libitum*.

Virus. The Friend Leukemia Virus used was obtained through the courtesy of Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md. This virus was maintained by serial passage in mice at weekly intervals of both clarified spleen suspension and infected whole blood respectively, both inoculated intravenously. The spleens were homogenized in a mortar and pestle at 4°C and suspended to 20% by weight in Hanks' Solution containing 125 units of penicillin and 125 μg of streptomycin per ml. This suspension was clarified by centrifugation at 2,500 r.p.m. for 20 minutes, and the supernatant centrifuged again at 5,000 r.p.m. for 10 minutes.

Blood was obtained from infected mice by decapitation and maintained at 4°C. This blood was collected undiluted, in heparinized centrifuge tubes. Penicillin and streptomycin were added as above. The whole blood was inoculated intravenously immediately after collection.

The virus titer was determined by the graded assay technique of Rowe and Brodsky (10). Using this method the expression of potency of virus preparations as determined by the dose response assay titer was arbitrarily defined as the dilution of the virus preparation, which corresponded to a mean spleen weight of $10^{2.7}$ mg (500 mg) on the regression line, *i.e.*, the dilution of the preparation calculated to produce spleen enlargement to 500 mg in 2 weeks when inoculated intravenously in 0.2 ml volume. This titer is referred to as the 500 mg spleen enlarging dose (SED 500)(10). In these experiments the Friend virus titer was SED 500 2.1.

Platelet counts. Blood for platelet counts was obtained in individual mice by puncture of the retroorbital venous plexus of the eye using heparinized capillary tubes. The platelets were counted directly using phase microscopy by the method of Brecher and Cronkite (11). All platelet counts were performed by the same technician.

Plan of experiment. Serial 10-fold dilutions of infected spleen homogenate and infected whole blood were prepared in chilled physiologic saline solution, and the dilution tubes maintained in an ice bath. The vari-

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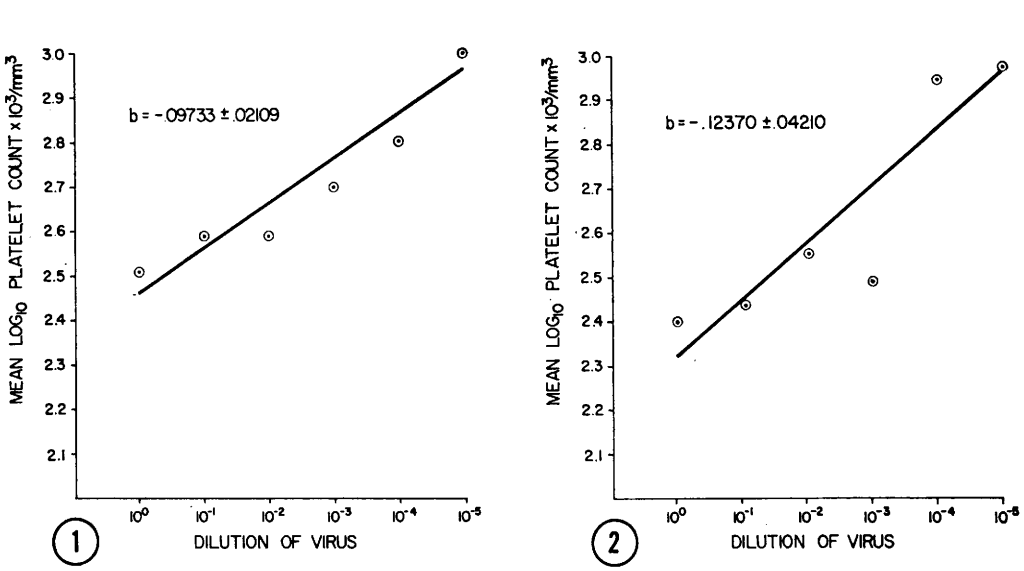

FIG. 1 and 2

ous dilutions were inoculated within one hour after preparation. Each mouse was inoculated with 0.2 ml, and 8 to 10 mice were inoculated per dilution of virus. Two weeks after inoculation the mice were bled from the retroorbital venous plexus of the eye. Platelet counts were obtained. The mice were then killed by cervical fracture and the spleens weighed on a Shadowgraph tumor weighing scale to the nearest 5 mg.

Experiments. Regression analysis of 2-week dose-response functions. Fig. 1 shows the dose response curve for the mean platelet count at each dilution of virus, the calculated regression line, and slope of the regression line for infected spleen homogenate. Fig. 2 shows the dose response curve for the mean platelet count at each dilution of virus, the calculated regression line and the slope of the calculated regression line for infected whole blood inoculum. Regression lines were fitted by the method of least squares(12). With this bioassay the infectivity titer was arbitrarily defined as that dilution of virus which would cause the platelet count to fall to 500×10^3 per mm^3 (mean platelet count $10^{2.7} \times 10^3$ per mm^3). This titer is referred to as the Platelet Depressant Dose 500,000 (PDD 500,000). The point 2.7 was selected since it was approximately at the midpoint of the portion of the curves and consequently least likely to be unduly influenced by variations in slope of the regression line.

The titer or PDD 500,000 for the infected spleen homogenate was determined to be $10^{-2.3}$, and that for infected whole blood was $10^{-3.0}$. These titers corresponded well with the 50% infectious dose (ID 50), calculated from the same criteria, by the method of Reed and Muench(13). The ID 50 for infected spleen homogenate was $10^{-2.0}$, and that for infected whole blood was $10^{-2.8}$.

Discussion. As indicators of infectivity, splenomegaly and thrombocytopenia obviate the necessity for standard extinction dilution titration. A death dependent assay with Friend Leukemia Virus requires several months to obtain results and is too erratic for short term observation. Fifty per cent infectious end points, based on thrombocytopenia of 500,000 per mm or less at 2 weeks, as the criterion of infection, provide an acceptable method for calculating virus titer. This has been shown to be true for spleen weights of 500 mg or more(10). However, as stressed by Rowe and Brodsky(10), the dose response assay has several distinct advantages over the Reed and Muench method: (1) Dose response assay permits determination of titer, when the end point is not

reached, by extrapolation of the regression line. (2) The dose response assay provides an indicator of titer at a single virus dilution. (3) The character of the dose response curve provides a check on the normality of mouse response.

As an indicator of infectivity for Friend Leukemia Virus infection, thrombocytopenia offers several definite advantages over splenomegaly. Spleen weight can vary widely with changes in the physiologic status of the animal. Starvation, infection and stress may produce significant changes in spleen weight.

It has been recently demonstrated that many strains of mice relatively resistant to the Friend virus on the basis of splenomegaly and the subsequent development of a leukemic state will nevertheless show significant thrombocytopenia after infection with the Friend virus(3). Therefore, the thrombocytopenia is probably a more sensitive indicator of infection than splenomegaly. Finally, using thrombocytopenia the virus titer can be obtained without killing the mouse.

Summary. The Friend Leukemia Virus causes significant thrombocytopenia within 48 hours after infection in Swiss Webster female mice. This thrombocytopenia has been demonstrated to be dependent upon the dilution of virus inoculated. A linear relationship exists between dose of virus and degree of thrombocytopenia. A dose-response curve using thrombocytopenia can be used to assay the Friend Leukemia Virus.

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Immunology and Serology of *Anaplasma marginale*. II. Nature of the CF Antigen.* (30625)

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A previous report(1) described the purification of *Anaplasma* complement-fixing (CF) antigen. The antigen was produced from the sonicate of *Anaplasma*-infected bovine red blood cells by a process of differential centrifugation followed by treatment with an ion exchange resin. The antigen possessed high CF titer, low anticomplementary activity and did not contain the color due to hemoglobin which interferes with interpretation of hemolysis in the CF test. Preliminary chemical studies indicated that the antigen was lipoprotein in nature. Since the CF antigen is employed routinely for diagnosis of anaplasmosis(2), studies were conducted to determine its nature.

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