# Effect of Friend Virus in Swiss and DBA/1 Mice on $Fe^{59}$ Uptake.* (26358)

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Friend Virus Disease is typically associated with malignant reticulum cell proliferation and pronounced erythroblastosis in adult mice of Swiss, DBA/2(1-3) and DBA/1 strains. Grossly, the most striking feature is the associated progressive hepatosplenomegaly. Latent period in susceptible mice is relatively short after inoculation of virus.

The erythroblastosis which was regularly observed prompted us to undertake a study of some of the features of erythropoiesis in the disease. The 24 hour uptake of $Fe^{59}$ was one of the parameters selected for study, because of its marked sensitivity as an index of erythropoiesis(4,5). This report describes a marked peripheral erythroblastosis and erythrocytosis which parallels spleen involvement in the disease and the marked selective uptake of $Fe^{59}$ by the spleen, beginning very early in the disease.

*Materials and methods.* Mice employed in this study were HA/ICR Swiss and DBA/1 mice 6-8 weeks old, obtained from our own breeding colonies and maintained on a diet of Derwood-Morris pellets.

Friend Virus was obtained through the courtesy of Dr. Charlotte Friend, Sloan-Kettering Inst., New York City. The disease was maintained by both cellular and cell-free passages of infected spleens. The resultant disease was the same regardless of type of inoculum used. At the beginning of our study, the virus was in its eighth serial passage in this laboratory and percentage of "takes" with a $10^{-1}$ inoculum in our mice at this passage was 85-100%. A pool of virus was obtained by harvesting a large number of infected spleens. A 10% fine mince suspension of the infected spleens in cold Locke-Ringer solution was homogenized in a Ten Broeck grinder and centrifuged for 10 minutes at 5000 G in a refrigerated centrifuge. A broth culture of *E. coli* was added to the supernatant prior to filtration through a HA-grade millipore filter. Filtrate was cultured in broth to check impermeability of the filter to *E. coli*. Pooled filtrate was stored in sealed ampoules in a dry ice chest at −70°C. Dilutions ranging from $10^{-1}$ to $10^{-6}$ were made of the pooled filtrate. Two-tenths of a milliliter of the dilutions from $10^{-1}$ to $10^{-6}$ were injected intraperitoneally into mice. A portion of the mice were sacrificed at regular intervals for studies described below. A minimum of 15 mice were used to establish each point in Fig. 1-4 and for each average figure in Tables I, II, III.

Twenty-four hours prior to sacrifice, one $\mu$c of $Fe^{59}$ in 0.5 ml of unbuffered 0.9% NaCl solution was injected into the jugular vein of each mouse. At time of sacrifice, mice were bled from aorta and various organs removed and weighed. Radioactivity in blood and organs was measured in a Nancy-Wood well-type scintillation counter. Radioactivity in an aliquot of the original $Fe^{59}$ solution used for injections was similarly measured. Uptake of $Fe^{59}$ in the blood and organs was calculated by a previously described method(6).

White cell and rbc counts were performed on blood from the aorta with the use of standard human diluting pipettes and hemocytometers. Blood films were stained with Wright-Giemsa Stain. Hematocrits were done on aorta blood with Drummond micro-hematocrit capillary tubes.

*Results.* Tables I and II show 2 striking features of Friend Virus Disease in Swiss and DBA/1 mice. First, there is a progressive rise in hematocrit throughout the course of the disease. Secondly, there is a marked increase in 24 hour uptake of $Fe^{59}$ by the spleen. This is particularly striking at 7 days post infection when there is greater than a 5-fold increase in spleen uptake as

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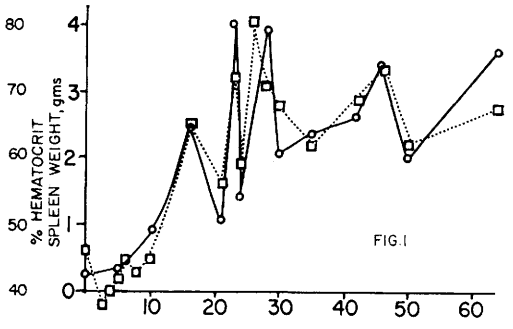


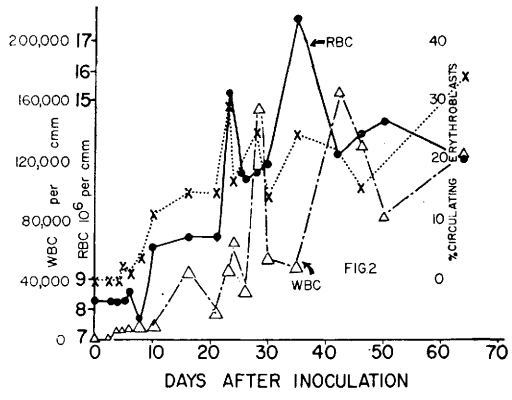


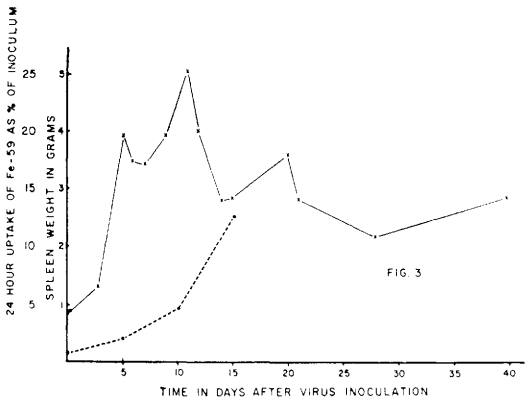


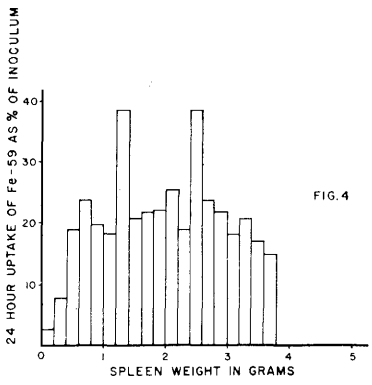


FIG. 1. Increase of spleen wt ○, and % hematocrit □ in Swiss mice infected with Friend Virus ($10^{-1}$).

FIG. 2. Changes in circulating blood cells in Swiss mice with time after infection with Friend Virus. ●, RBC; △, WBC count; and ×, % circulating erythroblasts, same mice as in Fig. 1.

FIG. 3. Mass of spleen, ⊙, and 24 hr uptake of $Fe^{59}$, ×, in Swiss mice infected with Friend Virus ($10^{-1}$).

FIG. 4. 24 hr uptake of $Fe^{59}$ in spleens of Swiss mice infected with Friend Virus ($10^{-1}$) as a function of the spleen wt in g.

compared to uninfected controls. This is especially interesting because the increase in $Fe^{59}$ uptake by the spleen precedes the onset of signs of the characteristic disease syndrome. In contrast to selective uptake of $Fe^{59}$ by the spleen, other organs show no significant increase in uptake as compared to the controls.

Figures shown in Table I and II under controls represent values obtained at 7 days. Control measurements were also done at 14, 21, 28, 35 and 40 days. However, since values obtained on those days were essentially the same as those obtained at 7 days they were not included in this table.

The hematocrit was slightly below normal at 7 days (Tables I and II). This led to a more detailed study of post-infection hematocrit in Swiss and DBA/1 mice. Since Swiss and DBA/1 mice responded similarly to Friend Virus, subsequent data will pertain only to Swiss mice. Fig. 1 shows the hematocrit pattern following infection. Hematocrit levels dropped immediately post-infection, reached minimal level at about 3 days, then rose sharply. There was a close parallel between the rise in hematocrit and splenic weight. At 16 days average spleen weight was about 20 times that of uninoculated control animals.

Accompanying the rise in hematocrit and increase in splenic weight were changes in circulating blood elements. Fig. 2 demonstrates the progressive increase in numbers

TABLE I. 24-Hour Uptake* of $Fe^{59}$ and Hematocrit ICR/HA Swiss Mice at Weekly Intervals Following Inoculation with Friend Virus ($10^{-1}$).

| | 7 days | 14 days | 21 days | 28 days | Controls† |
|---|---|---|---|---|---|
| Blood | 21.1 ± 5.4 | 28.1 ± 3.4 | 25.3 ± 6.3 | 25.9 ± 4.5 | 26.9 ± 3.7 |
| Liver | 18.6 ± 3.9 | 13.0 ± 6.4 | 8.1 ± 1.8 | 10.8 ± 5.0 | 11.3 ± 2.9 |
| Spleen | 17.6 ± 2.8 | 12.3 ± 4.4 | 13.8 ± 5.8 | 11.1 ± 1.5 | 3.0 ± .67 |
| Kidney | 1.0 ± .9 | 1.1 ± .3 | .9 ± .22 | 1.1 ± .2 | .8 ± .2 |
| Femur | .11± .4 | .11± .1 | .15± .13 | .2 ± .1 | .3 ± .16 |
| % hematocrit | 39.5 ± 3.3 | 57.4 ±11.3 | 63.6 ±11.8 | 71.1 ± 8.1 | 43.5 ± 1.9 |

* Uptake in 24 hr expressed as % of inoculum.
† Controls at 7 days are shown. These are essentially identical with those at subsequent times.

TABLE II. 24-Hour Uptake* of $Fe^{59}$ and Hematocrit in DBA/1 Mice at Weekly Intervals Following Inoculation with Friend Virus ($10^{-1}$).

| | 7 days | 14 days | 21 days | 28 days | Controls† |
|---|---|---|---|---|---|
| Blood | 24 ± 6.1 | 26.1 ± 2.1 | 25.9 ± 1.2 | 25.1 ± 6.2 | 27.2 ± 1.4 |
| Liver | 14.5 ± 2.1 | 15.1 ± 1.8 | 12.1 ± 2.4 | 15 ± 1.3 | 13.4 ± 1.2 |
| Spleen | 18.5 ± 3.4 | 14.2 ± 1.2 | 14.8 ± 1.8 | 12.8 ± 4.3 | 2.8 ± .54 |
| Kidney | 1.2 ± .8 | 1.1 ± .5 | 1.1 ± .13 | .9 ± .23 | 1.2 ± .8 |
| Femur | .11± .05 | .13± .08 | .14± .09 | .18± .09 | .3 ± .09 |
| % hematocrit | 37.8 ± 1.2 | 62.3 ± 8.5 | 60.3 ± 9.5 | 74.8 ± 7.2 | 41.5 ± 1.2 |

* Uptake in 24 hr expressed as % of inoculation.
† Controls at 7 days are shown. These are essentially identical with those at subsequent time.

TABLE III. Spleen Weight and $Fe^{59}$ Uptake of Spleen and Hematocrit at 14 Days after Friend Virus Inoculation in Swiss Mice at Various Dilutions of Virus.

| | Inoculum, dilution of virus filtrate | | | | Normal spleen filtrate† | No filtrate inoculated |
|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-6}$§ | $10^{-1}$ | — |
| Spleen wt (g) | 1.83 ± .9 | .35 ± .02 | .27 ± .04 | .14 ± .02 | .14 ± .03 | .17 ± .04 |
| $Fe^{59}$ uptake,* % | 16.0 ±3.6 | 7.6 ±2.6 | 5.9 ±2.3 | 2.6 ± .6 | 2.7 ± .04 | 2.3 ± .3 |
| Hematocrit, % | 51 | 44 | 42 | 41 | 42 | 41 |
| $Fe^{59}$ uptake/g‡ | 9.9 ±3.0 | 24.1 ±7.9 | 23.4 ±5.5 | 18.7 ±4.3 | 20 ±3.5 | 14.9 ±4.3 |

* 24-hr uptake of $Fe^{59}$ of whole spleen as % of inoculum.
† Normal spleen filtrate without virus.
‡ 24-hr uptake of $Fe^{59}$ in spleen expressed as % of inoculum/g spleen.
§ $10^{-4}$ and $10^{-5}$ not included; data similar to that seen for $10^{-6}$.

of circulating leucocytes, primarily granulocytes, erythrocytes and erythroblasts. The rise in erythroblasts to 34% on the 64th day led to additional studies designed to assess the role of the spleen in the increased erythropoiesis. Fig. 3 shows 24 hour $Fe^{59}$ uptake in the spleen as related to spleen size. This demonstrates that the increase in $Fe^{59}$ uptake begins very early and precedes splenic enlargement. $Fe^{59}$ uptake levels off at about the time the spleen reaches its maximum size (about 2.5 g). Table III demonstrates in more detail the correlation between splenic weight, $Fe^{59}$ uptake and hematocrit. While total uptake of $Fe^{59}$ by the spleen is greater with the more advanced disease and larger splenic mass, uptake on a per gram basis decreases from earlier levels and falls to a point well below control levels.

*Discussion.* These studies demonstrate that Friend Virus produces, soon after infection, a remarkable increase in selective uptake of $Fe^{59}$ by the spleen, accompanied by an increased splenic mass, an increase in circulating leucocytes and an increase in hematocrit characterized by a marked increase in circulating erythroblasts and erythrocytes. While there is an increase in number of white and red cells, a rise in platelets was not evident in peripheral blood.

Repeated histologic examination of bone marrow of infected animals at various stages

of the disease consistently revealed no hyperplasia. This observation, coupled with the findings described above, points strongly to the spleen as the main source of the erythroblastosis, increased red cell count, and hematocrit in peripheral blood. The very early rise of $Fe^{59}$ uptake by the spleen suggests that the virus may exert a direct stimulating effect on erythroid elements in the spleen. Continued stimulation results eventually in a markedly increased hematocrit with many immature erythrocytes in peripheral blood. Increase in leucocytes, without evidence of increased medullary activity, suggests that the myeloid elements of the spleen may also be stimulated.

As the disease progresses there is decreased uptake of $Fe^{59}$ by the spleen on a weight basis. This may be due to malignant proliferation of reticulum cells in the spleen so that the ratio of blood forming elements to reticulum cells diminishes progressively with advancing disease.

Unlike Polyoma Virus(8,9,10), the Friend Virus is apparently strain specific(1,2,3,7). Adult mice of Swiss and DBA/2 strains are highly susceptible whereas mice of PRI, $C_{57}$ Bl/6, A, $C_3H$ and ($C_{58}$ $\times$ BALB/c) $F_1$ strains are nonreactive. Our data indicate that DBA/1 strain is susceptible to the Friend Virus, to the same magnitude as HA/ICR Swiss mice.

*Summary.* 1. These studies demonstrate that Friend Virus produces in HA/ICR Swiss and DBA/1 mice very soon after infection, a marked increase in selective uptake of $Fe^{59}$ by the spleen. This selective uptake is not found in other tissues in Friend Virus Disease. 2. Friend Virus Disease characteristically produces a malignant proliferation of splenic reticulum cells, but is further characterized by a progressive elevation of hematocrit and rbc count which is related to splenic involvement in the disease. 3. The DBA/1 strain is shown to be susceptible to Friend Virus to the same magnitude as HA/ICR Swiss mice.

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## Thymectomy and Thymic Grafts in Mouse Viral Leukemia.*† (26359)

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Thymectomy has been found to decrease greatly the incidence of spontaneous leukemia in AK(1) and C58 mice(2) and that induced by whole body irradiation in C57B1 mice(3), by administration of methylcholanthrene to DBA mice(4), or by neonatal injection of leukemia virus in C3H or AK mice(5,6,7).

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