conditions of complete isolation from other cell lines. A lag phase of 7 weeks preceded rapid multiplication. Cultivation of these cells is apparently not dependent on the presence of a herpes-like virus similar to the leukovirus found in Burkitt's lymphoma. The line has been cultured continuously for 1 year.

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Porphobilinogen and Porphyrin Synthesis in Splenomegaly of Some Murine Leukemias* (33558)

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Splenomegaly has been employed to measure the degree of infectivity of leukemia viruses in mice (1). Since the erythropoietic activity in mice increases after infection with murine leukemia viruses (2), the rise in Δ-aminolevulinic acid dehydratase (5-aminolevulinate hydro-lyase, E.C. 4.2.1.24) (DA LAD) and porphyrin producing activities representing heme synthesis was considered to reflect the extent of infection with these viruses in the host systems. Tengerdy reported that DALAD activity increased in tissues of mice infected with Friend virus (FV) (3), and we also observed (4) increased DALAD and porphyrin activities in spleens of mice infected with FV and Rauscher virus (RV). Although splenomegalic response was similar in mice infected with these viruses, the porphyrin activity in spleens of mice infected with FV was twice the activity observed in spleens of mice infected with RV. Experiments were conducted to examine the effects of FV infection on the enzymic activity of various strains of mice and to correlate the sensitivity of the enzymic system with the degree of viral infection.

Materials and Methods. BALB/c, DBA/2, BDF_1 (C57BL/6♀ × DBA/2♂)F_1, and C57BL/6 mice 7–10 weeks of age and weighing 18–23 g, were inoculated intraperitoneally with 0.2 ml of a $10^{-1.3}$ dilution of a plasma virus preparation of FV. The DBA/2 mice were inoculated subcutaneously with 0.2 ml of 1×10^6 leukemic cells in physiologic saline prepared from spleens of DBA/2 stock leukemic (L1210) mice. Five mice from each group of normal, virus infected or L1210 inoculated animals were sacrificed by cervical dislocation at designated intervals. Spleens were removed, weighed, washed three times

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with 5 ml of cold 0.1 *M* phosphate buffer and homogenized in a Teflon glass homogenizer. The homogenate was centrifuged at 20,000*g* for 45 min at 4°. The resulting supernatant fluid was employed as the source of enzyme. The incubation mixture of 3 ml, contained 5 μmoles of Δ-aminolevulinic acid, 5 μmoles of glutathione, supernatant (equivalent to 5 mg of protein), and phosphate buffer. After incubation of the mixture at 37° for 30 min, 1.0 ml of 10% trichloroacetic acid containing 0.1 *M* $HgCl_2$ was added. The precipitate was separated by centrifugation and the clear supernatant fluid filtered through Whatman No. 1 filter paper. Aliquots of the clear filtrate were employed for the assay of porphobilinogen (PBG) and porphyrin formation by the method of Shemin (5).

Results. The results on the development of splenomegaly, and the increase in the PBG and porphyrin producing enzyme activities in each strain of mice after FV infection are shown in Fig. 1. The average spleen weight of the three strains of mice used in this study was approximately 100 mg. After infection with FV, the spleen weight of the BALB/c mice increased to 1200 mg in 12 days, 1600 mg in 14 days and reached a peak value of 1800 mg in 21 days. In the BDF_1 mice splenomegaly was observable on day 7 after infection and the increase thereafter was rapid with an average spleen weight of 2200 mg in 21 days. Significant increase in spleen weight was not observed in C57BL/6 mice after infection.

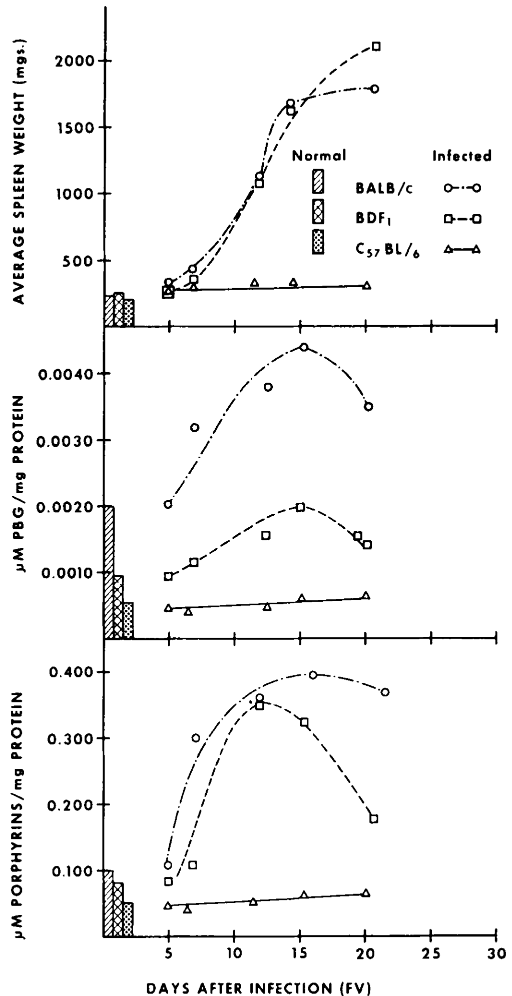

FIG. 1. Effect of Friend virus inoculation on spleen weight, PBG, and porphyrin production by the spleens of mice.

The initial PBG levels in the BALB/c, BDF_1, and C57BL/6 strains of mice were 0.002, 0.0009, and 0.0005 μmole/mg of protein, respectively. In the BALB/c mice infected with FV the PBG level doubled in 15 days and then dropped to 1.5 times the initial value 21 days after infection. The PBG values at 15 days after infection were elevated to twice the control values in BDF_1 mice but at 21 days these values dropped to approximately normal levels. There was no definite increase in PBG values in C57BL/6 mice at any time during the experiment.

The initial porphyrin levels (Fig. 1) were 0.1, 0.08, and 0.05 μmole/mg of protein in the spleens of noninfected BALB/c, BDF_1, and C57BL/6 mice, respectively, the porphyrin producing activity in spleens of BALB/c and BDF_1 mice remained unchanged from the controls when measured on day 5 after FV infection. The porphyrin level in the spleens of BALB/c mice rose to 0.3 μmole in 7 days and 0.4 μmole in 15 days after infection; the latter value was almost 4 times that of the controls. Thereafter, the activity remained essentially unchanged in these mice. In the BDF_1 mice, the porphyrin level rose to 0.34 μmole in 12 days and dropped to a level of 0.15 μmole in 21 days after infection. No

major changes in the porphyrin level were observed in the spleens of C57BL/6 mice after infection with FV. The response of the spleens of DBA/2 mice to FV infection was approximately the same as that observed with the spleens of BALB/c mice.

The results of the experiment on the relationship of splenomegalic response and elevation of the enzymic activity to the quantity of virus injected are shown in Fig. 2. Significant increases in the spleen weights, PBG levels and porphyrin activities in the spleens were observed in mice inoculated with 10^{-1} to 10^{-5} dilutions of the virus. The average spleen weights of mice 14 days after inoculation with 10^{-1} to 10^{-6} dilutions of FV were 1200, 450, 200, 140, 140, and 120 mg, respectively. The average spleen weight of control mice was 100 mg. Thus, the increase in the spleen weight of mice inoculated with 10^{-1} dilution was 12 times that of the control value. Seven days after infection the PBG level in the spleens of mice inoculated with 10^{-1} dilution of the virus was 3 times and the porphyrin producing activity was 5 times more than the control values.

These effects of infection with FV were compared with that produced in the spleens of mice inoculated with leukemia L1210. The results of this experiment are summarized in Table I. Splenomegaly was not discernible in mice 4 days after inoculation of leukemia L1210 or FV. The PBG value in the spleens of leukemic (L1210) mice remained normal; there was a slight increase in the PBG value of mice infected with FV. The porphyrin production in spleens of leukemic (L1210) mice or mice infected with FV was not affected at the time. After 7 days, the average spleen weight of the mice inoculated with leukemia L1210 was almost twice that of the FV infected mice and over four times that of the normal mice. However, only a slight increase in the PBG value and porphyrin production was observed in leukemic (L1210) spleens, while these activities had increased significantly in the spleens of FV infected mice.

Discussion. Splenomegaly as a parameter of FV infection in mice had been reported

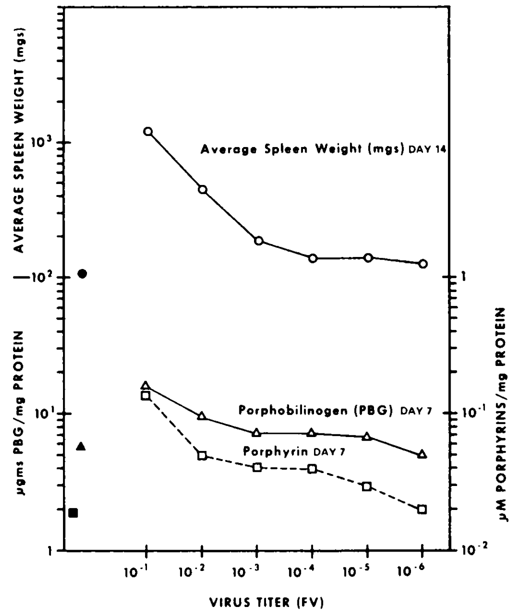

FIG. 2. Relationship of spleen weight, PBG, and porphyrin production by the spleens of mice infected with gradual amounts of Friend virus. (Friend virus was titrated and inoculated i.p. into BALB/c mice. One group of mice was sacrificed on day 7 corresponding to the time of the earliest high level of activity after virus inoculation, to estimate PBG and porphyrin producing activities in the spleens. Another group was sacrificed on day 14, the time of maximal splenomegalic response, and the spleen weights were recorded. The solid symbols represent control values.)

previously (1). The data presented here show that mice susceptible to infection with FV developed splenomegaly shortly after viral infection. Mice (C57BL/6) resistant to FV infection failed to exhibit an increase in spleen weight. Sassa *et al.* (6) recently reported significant increases in heme synthesis in FV ascites tumor lines. In the present study a correspondence was observed between the increase in the spleen weights after infection with FV, and the extent of elevation of PBG and porphyrin producing activities of these spleens. The mice that failed to manifest splenomegaly also failed to exhibit changes in the enzymic activity to produce PBG and porphyrin in these spleens. In these studies the changes in the enzymic activity could be detected earlier than the splenomegalic response and the former parameter was almost

TABLE I. Comparison of Effect of Leukemia (L1210) and Friend Virus on Spleen Weight and DALAD Activity.[a]

Inoculated	Spleen wt. (mg)	PBG (μmoles/mg of protein)	Porphyrins (μmoles/mg of protein)
Sacrifice (day 4)			
Normal (control)	120	0.0013	0.063
Leukemia[b] (L1210)	130	0.0014	0.051
Friend virus[c]	130	0.0022	0.064
Sacrifice (day 7)			
Normal (control)	140	0.0015	0.065
Leukemia[b] (L1210)	600	0.0022	0.072
Friend virus[c]	340	0.0035	0.256

[a] Results represent an average of five mice per group. In each instance the enzyme assay was performed on the equivalent amount of soluble protein.

[b] Leukemia (L1210) Z line inoculated s.c. (1×10^6 cells/0.2 ml).

[c] Friend virus (plasma) inoculated i.p. (10^4/0.2 ml).

as sensitive a measure of FV infection as was splenomegaly.

The current observations suggest that susceptibility to FV infection and PBG plus porphyrin production which are intimately involved in heme synthesis are genetically controlled and the enzyme activity leading to heme synthesis is influenced by the genetic background of the animals. The precise nature of these reactions in determining the response to viral infection does however remain to be elucidated. That the production of splenomegaly per se even when accompanied by an increase in the activity of heme synthesizing enzymes does not necessarily result in increased virus replication was determined in a separate study in which phenylhydrazine was injected into C57BL/6 mice prior to inoculation of FV. Injections of phenylhydrazine resulted in considerable increases in PBG, porphyrin, and spleen weight, however, these increases failed to influence the susceptibility of these mice to infection with FV (Table II).

Though infection with leukemia virus (FV) or inoculation of leukemia L1210 results in the occurrence of splenomegaly, the biochemical (7, 8) and morphological (9, 10)

TABLE II. Effect of Phenylhydrazine Hydrochloride ($\Phi NHNH_2 \cdot HCl$) on Normal and Friend Virus Infected Mice.[a]

Treatment	Av spleen wt. (mg)	PBG (μmoles/mg of protein)	Porphyrins (μmoles/mg of protein)	FV[e] titer
Normal (control)	80	0.0004	0.003	—
$\Phi NHNH_2 \cdot HCl$[b]	280	0.0005	0.03	—
FV[c]	120	0.0005	0.05	$<10^2$
$\Phi NHNH_2 \cdot HCl$ and FV[d]	600	0.0007	0.09	$<10^2$

[a] The values given are for five mice per group.

[b] Mice (C57Bl/6) were given two injections of neutralized $\Phi NHNH_2 \cdot HCl$ (40 mg/kg) on the first day and one injection on the second day. The values are given for mice sacrificed on day 13 after treatment.

[c] Inoculated with approximately 10^5/0.2 ml of FV. The values are given for mice sacrificed on day 12 after FV infection.

[d] Mice were inoculated with FV 1 day after completion of treatment with phenylhydrazine hydrochloride and sacrificed on day 12 after FV infection.

[e] Friend virus titer was determined by inoculating the donor plasma into recipient mice.

factors involved in the development of splenomegaly are different with these two murine leukemias.

Summary. The production of porphobilinogen and porphyrins by spleen extracts of Friend virus (FV) infected mice depends on the initial enzymic activity in the organ and varies with the amount of virus injected and the strain of mice employed. The increase in the enzymic activity appears to be as sensitive a measure of FV infection as was the splenomegalic response. Only minor changes in the enzymic activity were observed during the development of splenomegaly induced by leukemia L1210.

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Viral Inhibition in Adenovirus 5 Treated Hamster Cells (33559)

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It has been shown by Pereira *et al.* (1) that human adenovirus type 5 causes a fatal infection of newborn hamsters with liver cell necrosis and characteristic nuclear changes of adenovirus infection. However, these authors could not make a categorical statement regarding virus multiplication in this host. This is the only human adenovirus thus far reported to be lethal for the newborn hamster. This effect is in contrast to that caused by other human adenoviruses [types 3 (2), 7 (3), 12 (4), 18 (5), and 31 (6)], namely, malignant cellular transformation. A study was undertaken to investigate the interactions between adenovirus 5 and hamster embryo fibroblasts (HEF) in culture and to determine whether this virus would behave *in vitro* as it did *in vivo*.

Materials and Methods. Cells. Pregnant golden Syrian hamsters (*Mesocricetus auratus*) were obtained from the Animal Production Section, Laboratory Aids Branch, NIH. Embryos were harvested on days 14–16 of gestation and cell cultures were prepared by the Tissue Culture Section, Laboratory of Virology and Rickettsiology, DBS, by seeding cells into 2-oz bottles. Chick embryo fibroblast (CEF) monolayers were prepared from 10–12-day-old embryos and seeded in a similar manner. Cell sheets were used for testing only when they had become confluent monolayers. The human diploid cell strain, WI-38, was supplied as confluent monolayers of cells by the Tissue Culture Section. Human embryonic kidney (HEK) cell cultures were obtained as complete monolayers from Flow Laboratories, (Rockville, Md.). A human epithelial cell line (KB strain) and human amnion cell culturees (HA strain) were obtained from Dr. K. O. Smith, NIH.

Media. Eagle's basal medium with 2% fetal calf serum containing 400 units of penicil-