produced inhibitor may well have diffused through the agar medium and thus had a negligible effect. Hence, in the immediate area of infection the ratio of infectious virus to virus inhibitor remained high and plaque formation could therefore continue. If, on the other hand, such an infected area was covered with fluid medium, then the inhibitor substance accumulated, and the ratio between infectious virus and inhibitor substance was lowered, probably reaching an equilibrium. In this instance, the cells were protected, at least partially, from destruction during viral multiplication, and therefore no CPE appeared.

Summary. Auto-inhibition of DA virus plaque formation was observed in tissue cultures during serial passages of this virus. An inhibitor (interferon-like) substance was found in the virus-free culture fluids. The presence of such an inhibitor substance seemed to be the determining factor for the 2 unusual phenomena observed with the DA virus: 1) plaque formation under agar overlay without CPE in parallel fluid cultures, and 2) "prozone" reaction in plaque formation.

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Effect of Chemotherapeutic Agents on Friend Virus-Induced Leukemia in Mice.* (26938)

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This study was undertaken to (a) establish methods for testing of chemotherapeutic agents against the Friend virus-induced leukemia (FVL) of mice(1) and (b) investigate the effect of a number of "established" and newer drugs.

Sugiura and Stock(2,3) have studied the effect of a variety of compounds on FVL using the spleen weight 3 weeks after virus inoculation as the criterion of evaluation. Effective compounds also were tested for their ability to increase survival time of infected animals. Mirand, Prentice, Hoffman and Grace(4) demonstrated that soon after inoculation, Friend virus produces a marked increase in uptake of Fe^{59} by the spleen. Moreover, this disease in HA/ICR Swiss mice is associated with malignant proliferation of the splenic reticulum, stimulation of the erythroid and myeloid elements of the spleen and liver, incerased hematocrit, presence of immature and mature erythrocytes in the blood, and leukocytosis(5,6).

Materials and methods. Adult mice (25-30 g), HA/ICR Swiss strain, were inoculated with the Friend virus† from a virus pool, originating from the ninth bi-weekly passage into Swiss mice in our laboratory, which was

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prepared from 10% spleen filtrates. The spleens from infected animals were harvested freshly, homogenized in a Ten Broeck grinder, and suspended to 10% by weight in cold Locke-Ringer solution. The suspension was clarified in a refrigerated centrifuge at 5000 g for 10 minutes, and the supernate filtered through a HA millipore filter. The filter was checked for ability to retain bacteria by adding *E. coli* to the suspension prior to filtration. Serial dilutions of infected and normal spleen filtrates prepared in a similar fashion were made with chilled physiologic saline. Mice were inoculated intraperitoneally with 0.2 ml of these filtrates. Daily drug administration was started 24 hours after virus inoculation and continued for 11 days. On the 12th day, part of the animals were injected intravenously with 1 μc of Fe^{59}. Iron uptake in red blood cells, spleen and liver was measured 24 hours later in a Nancy Wood well-type scintillation counter as previously described(4). Hematocrit and spleen weight measurements were made on all mice. A group of animals was followed for survival and median survival times (MST) estimated. Tests for *in vitro* antiviral effect were performed by incubating for 2 hours at 4°C dilutions of the virus filtrate with equal volumes of various concentrations of the drug under study. The drug-virus mixtures were then injected intraperitoneally into mice. Effect was evaluated as described above.

The following drugs were used: "Established" cancer chemotherapeutic agents; 5-fluoro-2′-deoxyuridine (5-FUDR), 6-mercaptopurine (6-MP), and amethopterin (methotrexate, MX). The following newer mercaptouracil derivatives, synthesized by Bardos (8,7,11) were studied: 5-mercaptouracil (AB-050)(8-10), 5, 5′ Diuracilyl disulfide (AB-051), 5-(Acetylmercapto)uracil (AB-053), 5-(Methylmercapto)uracil (AB-054). These drugs were shown to be thymine antagonists in microbiologic studies. A new antagonist of both folinic acid (but not folic acid) and riboflavin was included; 2,4-diamino-7,8-dimethyl-2,4-deoxyalloxazine (AB-200)(9-10). A series of ethylenimidophosphorocarbamates were also studied: Benzyl N-(bis-(ethylenimido)phosphoro) carbamate (AB-103), Ethyl N-(bis-(2,2-dimethylethylenimido)phosphoro)carbamate (AB-132)(12-15), and Dimethyl N-(N′, N′-bis (2-chloroethyl(amidophosphoro) carbamate (AB-155)(11). Solvents used are indicated in Table II. All drug solutions were prepared freshly before injection.

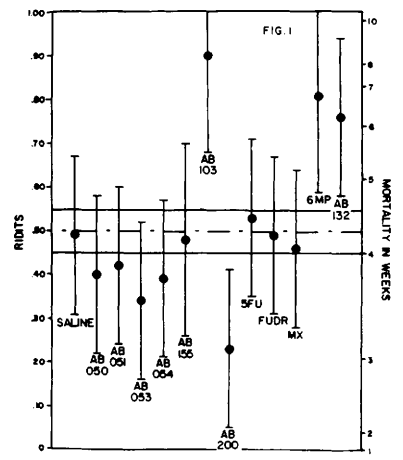
TABLE I. Relation of Spleen Weight and 24 Hour Fe^{59} Uptake to Dilution of Friend Virus Determined 14 Days after Virus Inoculation in Swiss Mice.

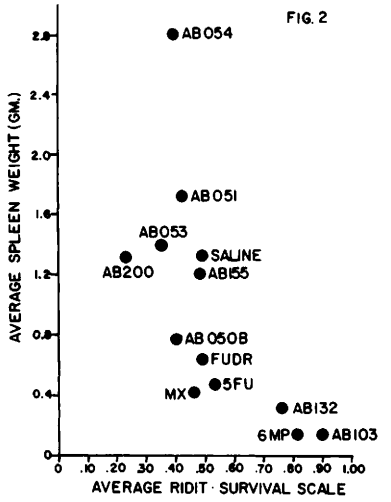
Inoculation*	Dilution of virus	Spleen wt (g)†	% of inoc. Fe^{59} taken up by spleen in 24 hr†	Hematocrit
	10^{-1}	1.83 ±.9	16.0 ± 3.6	47
Friend virus infected spleen filtrate	10^{-2}	.35 ±.02	7.6 ± 2.6	44
	10^{-3}	.27 ±.04	5.9 ± 2.3	42
	10^{-4}	.13 ±.03	3.9 ± .3	43
	10^{-5}	.16 ±.03	2.7 ± 1.0	42
	10^{-6}	.14 ±.02	2.6 ± .6	41
Normal spleen filtrate	10^{-1}	.14 ±.03	2.7 ± .04	42
None	—	.17 ±.04	2.3 ± .3	41

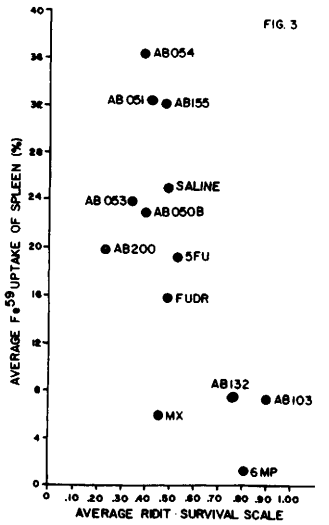
* An avg of 10 mice/dilution.
† ± S.D.

Results and discussion. Table I shows the relationship between spleen weight and 24 hour Fe^{59} uptake in the spleens of mice injected with various dilutions of FV filtrate. The splenic response closely follows the virus dilutions. Normal spleen filtrate resulted in a response similar to that of the non-injected control. Dilutions above 10^{-4} did not appear to be infectious.

Table II summarizes the effect of the various chemotherapeutic agents on spleen size, splenic uptake of Fe^{59}, and mortality. Fifteen mice were used in each group, 5 for Fe^{59} uptake and ten observed for mortality. AB-103, AB-132, 6-MP, and MX were the only drugs that caused significant decreases in spleen size and Fe^{59} uptake, compared to the control values. Only the former 3 prolonged survival time significantly as seen by analysis of the mortality data by the ridit method (Fig. 1)(16). The average ridit and 95% confidence interval is plotted for each treatment. The relationship between Fe^{59} uptake (or spleen weight) and survival on the ridit scale can be seen in the scatter diagrams of Fig. 2 and 3. It appears that when drug







treatment retains Fe^{59} uptake (or spleen weight) in the normal range (AB-103, AB-132, and 6-MP), there is an increase in survival time. MX appears to be an exception by significantly decreasing Fe^{59} uptake and spleen weight without increasing survival. On the other hand when Fe^{59} uptake (or spleen weight) becomes abnormal, there is little or no relationship to survival. There appears to be good correlation between Fe^{59} uptake and spleen weight. It is possible that these parameters are more sensitive indicators of drug effect than mortality, indicating differences between various treatments which cause no change in survival.

Experiments were undertaken to determine the effect of drugs on FV *in vitro*. Drug-virus mixtures were inoculated into mice and evaluated as in the previous treatment. At the maximal drug levels tolerated by the recipient mice, AB-103, AB-132, 6-MP and 5-FU decreased splenic Fe^{59} uptake and spleen weight, but only AB-103 and 6-MP increased survival time.

Of the drugs tested both by us and by Sugiura and Stock(3), the latter authors reported the following results: 5-FUDR (40 mg/kg/day)–negative; MX (1.5 mg/kg/day)–"slightly inhibitory"; 5-FU (35 mg/kg/day)–"moderately inhibitory", and 6-MP (30 mg/kg/day) – "markedly inhibitory". They treated the animals for only 7 days and sacrificed 3 weeks after inoculation.

Summary. 1. A variety of newer and "established" chemotherapeutic agents were tested against FVL in mice. Criteria for effectivity included spleen uptake of Fe^{59}, spleen weight, and mortality. 2. Agents demonstrating both *in vivo* and *in vitro* antiviral activity included AB-103, AB-132, and 6-MP. 3. Good correlation appears to exist between Fe^{59} uptake of the spleen and spleen weights. These 2 parameters correlate with

FIG. 1. Mortality data (analyzed by the ridit method) in HA/ICR Swiss mice infected with Friend Virus (10^{-1}).

FIG. 2. Relationship between spleen wt in g and survival (analyzed by the ridit method) in HA/ICR Swiss mice inj. with Friend Virus (10^{-1}).

FIG. 3. Relationship between 24 hr Fe^{59} uptake and survival (analyzed by the ridit method) in HA/ICR Swiss mice inj. with Friend Virus (10^{-1}).

TABLE II. *In Vivo* Effect of Drugs on Survival Time, Spleen Weights and Splenic Uptake of Fe^{59} in Friend Virus-Infected Mice (10^{-1}).

Drug	Solvent or suspending medium	Dose (mg/kg)	Spleen			HCT	Mean survival time (days)
			Route	Mean wt (g)	Mean Fe^{59} uptake (%)		
5-FU	H_2O	25	S.C.	.47 ± .24†	19.1 ± 6.74†	39.2	27.0
FUDR	H_2O	50	I.P.	.64 ± .51	15.7 ±12.04	36.0	26.0
6-MP	Saline	50	I.P.	.14 ± .02	.90± .41	30.5	40.5
Methotrexate	H_2O	1.0	I.P.	.42 ± .12	3.9 ± 2.76	31.0	25.0
AB-050B	H_2O	50	I.P.	.77 ± .23	22.8 ± 7.0	38.5	23.0
AB-051	CMC*	50	I.P.	1.72 ± .65	32.3 ± 8.1	41.2	22.5
AB-053	"	50	I.P.	1.39 ± .73	25.5 ± 9.63	46.2	23.0
AB-054	"	50	I.P.	2.01 ± .94	36.2 ±14.4	45.0	23.0
AB-200	"	25	I.P.	1.31 ± .55	19.7 ± 8.2	47.3	17.0
AB-103	H_2O	30	S.C.	.18 ± .09	7.3 ± 1.4	38.3	65.0
AB-132	Saline	100	I.P.	.31 ± .25	7.4 ± 2.3	43.5	42.0
AB-155	Warm saline	50	S.C.	1.21 ± .34	32.0 ± 8.4	54.4	19.0
Saline	—		I.P.	1.32 ± .47	24.9 ±11.2	44	26.0

* Carboxymethylcellulose 0.5%. † ± S.D.

survival in cases where they remain in the normal range. Once they become abnormal, no quantitative relationship exists between survival on one hand, and spleen weights and Fe^{59} uptake on the other.

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