ing development of inflammation seems likely to contribute to an increase in the released amounts of such biologically active substances on subsequent exposure to endotoxin, leading to more extensive hepatocyte damage. The notion that hepatocellular injury may be mediated by recruited and activated macrophages has been presented by several authors[14,15]. However, another possibility, that the hepatocytes from rats with inflammation, which are already in a metabolically subnormal state[13], may be readily affected by the direct action of endotoxin, cannot be excluded.

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Effect of propane sultone pretreatment on Friend virus leukemogenesis in mice

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Summary. Propane sultone (PS) injected i.p. 24 or more hours before Friend leukemia virus increased the incidence of lymphoma in SJL/J mice and at a higher dose increased the incidence of erythroleukemia in B10SJF$_1$ mice. PS at the same time also decreased hematopoietic stem cell clonogenicity.
Key words. Mouse erythroleukemia; propane sultane; Friend leukemia virus; carcinogenesis; clonogenicity.

Various chemical carcinogens can affect cultured cells[1,2] or whole organisms[3-7] to make them more susceptible to viral oncogenesis. This report describes effects of propane sultone (PS) on Friend leukemia virus (FLV)-dependent oncogenesis in virus sensitive SJL/J mice and in relatively virus resistant B10SJF$_1$ mice.

PS, a sulfonated-hydrocarbon, monofunctional alkylating agent[8,9] is known to be carcinogenic in rats when ingested[10,11] or introduced parenterally[12,13]. In vitro PS transformed human epithelial cells with greater efficiency than human fibroblasts[14,15], suggesting similarity with natural situations where the frequency of carcinomas is greater than that of sarcomas. PS is used in certain industrial chemical processes[16,17] and is related to ethyl and methyl sulfonate, which are used in genetics to produce point mutations[18]. Like methyl methane sulfonate (MMS), PS attaches to DNA[17-19]. MMS is known to produce single strand breaks in DNA that resemble X-ray caused lesions and are quickly repairable[1], and the PS-caused damage to DNA, although not as well characterized as that of MMS, may be similar[2,8,9,20].

In vitro treatment of human fibroblasts with various carcinogens, which caused DNA damage that led to DNA-repair synthesis, increased the frequency of transformation produced by subsequent SV40 infection[2]. Our results show that PS and FLV, given in vivo at doses that cause little or no malignancy when either is given alone, can produce the malignancy characteristic of the virus in 80% of mice injected with both the chemical and viral carcinogen.

Materials and methods. Female, six-week-old SJL/J mice were purchased from Jackson Laboratories and held for 2 weeks before being entered into experiments. B10SJF$_1$ mice were bred by us from Jackson stock (C57BL/10J males × SJL/J females). Both male and female B10SJF$_1$ mice were used when they were 8–12 weeks old. There was no significant effect of sex or age on the response measured. Animal housing and maintenance were standard as described previously.

PS (Sigma) was diluted with physiological saline and injected i.p. at 100 or 175 mg per kg of b.wt. Groups that received only FLV were injected with saline instead of PS.

Our FLV stock and its preparation was described previously[3,21]. Approximately one tenth SED (spleen enlargement dose) was injected into SJL/J mice and 100 SED was injected into B10SJF$_1$ mice. The day of virus injection was taken to be day 0 in each experiment. Groups that received PS only were injected with saline on day 0.

The measurement of the induction of antibody against antigens on sheep red blood cells by the plaque forming (PFC) technique was previously described[22]. The enumeration of

Numbers of PFC and CFU-S in SJL/J mouse spleen cells after exposure of the animals to PS (100 mg/kg). Mean ± 1 SE of 3 or more experiments

Time after PS	Plaques per 10^6 spleen cells	% of control	CFU-S per 10^5 spleen cells	% of control
5 h	1318 ± 221	130	6.12 ± 0.34	66
1 day	1158 ± 148	115	5.20 ± 0.29*	51*
2 days	993 ± 76	98	3.91 ± 0.26	38*
5 days	1592 ± 264	158	9.56 ± 0.48	93
7 days	732 ± 121	72	10.60 ± 0.60	104
10 days	937 ± 73	93	–	–
20 days	1335 ± 198	132	–	–
Control	1010 ± 63	–	10.19 ± 0.28	–

*Indicates means that significantly differed from the control.

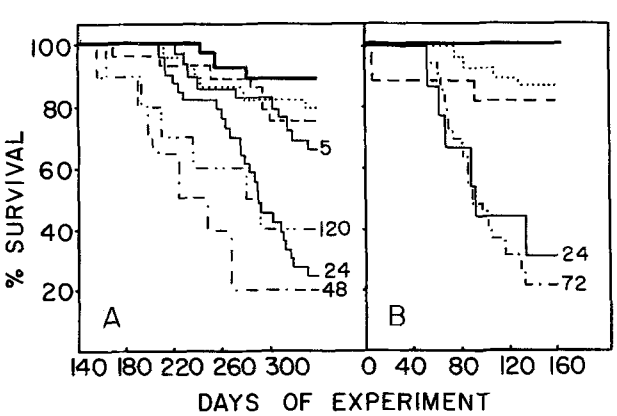
hematopoietic stem cells by the spleen colony forming technique (CFU-S) followed the method of Till and McCulloch[23] as used by us previously[22]. Significance of the difference between survival curves was assessed by Colton life table statistics[24] and significance of differences between mean values of CFU-S and PFC readings was assessed by Student's t-test.

Results. All $B10SJF_1$ mice that received neither PS nor FLV survived for the entire 300-day observation period. Less than 15% SJL/J mice that received no treatment died between days 240 and 300 of the experimental observation period of a spontaneous lymphoma characteristic of this mouse strain (fig.).

We observed significantly increased incidence of malignancy, without any effect on the latency or course of the disease, after PS injection in two types of experiments. Firstly, when relatively low doses of FLV were injected 24 or more hours after PS into virus-sensitive SJL/J mice (fig. A) and secondly when higher FLV doses were injected into virus-resistant $B10SJF_1$ mice 24 h after injection of PS at maximum, well tolerated doses (fig. 1B). The malignancy found in the SJL/J mice was a lymphoma associated with little or no spenomegaly and no increases in peripheral white blood cell numbers; while the malignancy in $B10SJF_1$ mice was a typical Friend erythroleukemia[25]. When PS was injected 24 or more hours before FLV there was a significant synergism between the chemical and viral carcinogens. Thus the survival curve of mice so treated were significantly lower than an experimental survival curve calculated by assuming that the PS and FLV effects are additive. This point is also seen directly by comparing the survival of mice given PS and FLV only 5 h apart.

The above described incidences of malignancy were found only with the specified virus and PS doses. When higher FLV doses were used in SJL/J mice erythroleukemia was produced and PS, at the same dose that caused an increase in lymphoma incidence (i.e. 100 mg/kg) in the previous experiment had no effect. Moreover, when 100 mg/kg doses of PS were used in $B10SJF_1$ mice no effect on the incidence of FLV erythroleukemia was observed. These negative data are not shown in the figures.

Induction of humoral immunity against sheep red blood cells (PFC assay) was not affected by PS, but viability of spleen-colony forming hematopoietic stem cells in the bone marrow (CFU-S assay) was decreased by PS at days 1 and 2 (table). Recovery from this decrease was complete by day 5 and was maintained at day 7. CFU-S assays were not run on days 10 and 20 after PS treatment.



A Survival of SJL/J mice given a very low FLV dose (approximately 0.1 SED) and/or PS (100 mg/kg). PS was injected 5 h, 24 h, 2 days and 5 days before FLV as indicated. The day of virus injection was taken as day 0. Only one of 192 mice in the experiments summarized in (A) died before day 140. This was a mouse in the PS-5 h group that died on day 40 with an extremely large spleen (10-fold normal). Because this was an exceptional case it was not included in the plot. *B* Survival of $B10SJF_1$ mice given FLV (100 SED) and/or PS (175 mg/kg of b.wt). All of the mice that died after receiving FLV either alone or in combination with PS died with signs of erythroleukemia (i.e., splenomegaly and elevated peripheral with blood cell counts). No lymph nodes or thymus gland enlargement was observed. The day of virus injection was taken as day 0. (Heavy solid line: untreated control groups; dotted line: virus only groups; dashed line: PS only groups; other lines show survival of mice given PS at the indicated number of h before FLV.)

Discussion. Demonstration of interaction between two subthreshold carcinogenic stimuli, such as we report here, underscores the probable complexity of natural cancer induction that may involve chemical and physical carcinogens as well as exogenous and endogenous viruses. Thus descriptions of the conditions necessary for such interactions to occur may help in understanding the mechanisms involved. Our data show that at least one day is necessary after i.p. injection of PS to produce a state of enhanced sensitivity to viral oncogenesis and that different doses of PS can cause increased incidence of different FLV-dependent malignancies.

We used the highest, well tolerated doses of PS in order to maximize the probability of seeing an effect. It would be interesting in future experiments to test whether lower PS doses have similar effect in SJL/J mice. However, we have already observed that 100 mg/kg doses of PS do not affect FLV erythroleukemia in $B10SJF_1$ mice, whereas 175 mg/kg doses of PS do.

PS had no demonstrable effect on the induction of humoral immunity and although other cell-mediated immune functions should be measured our data indicate that a direct PS effect on the virus target cells (hemotopoietic stem cells) is an important aspect of the mechanisms by which PS acts since virus susceptibility was greatest during periods when the hematopoietic stem cells were unable to form spleen colonies.

The FLV virus we used consists of at least two components: a replication defective SFFV component, which causes an early appearing erythroleukemia[25] and an LLV component, which enables the SFFV to replicate and also causes a later-appearing lymphoma when low doses of the virus are inoculated[26]. SJL/J mice present a further complication in that they develop a spontaneous lymphoma after 10 months of age[27]. However, the lymphoma we observed appeared earlier than the spontaneous disease in FLV-free, SJL/J littermates. We cannot distinguish whether the FLV inoculation accelerated the appearance of the spontaneous lymphoma or caused the observed lymphoma directly. Whatever the etiology of the late appearing lymphoma that we observed, PS at 100 mg/kg of b.wt accelerated its development while PS at this dose had no effect on FLV-caused erythroleukemia.

PS at this lower dose was also ineffective in its influence on FLV-caused erythroleukemia in $B10SJF_1$ mice injected with 100 SED of the virus. However, an increased PS dose (75% higher) did significantly increase the incidence of this erythroleukemia in $B10SJF_1$ mice.

Our correlation between PS-caused decrease in CFU-S clonogenicity and increased susceptibility to cell transformation indicates that PS effects target cells directly after i.p. injection since CFU's are major targets of mouse leukemia viruses[28]. By analogy with in vitro studies where the damage of target cell DNA was a requirement for the enhancement of viral transformation[1,2], it is likely that the co-carcinogenic lesions are probably in the target cells' DNA. Analysis of the type of DNA damage produced by PS at low and high doses may determine whether damage that effects the incidence of the lymphoma is qualitatively or only quantitatively different from damage that effects the erythroleukemia incidence in our system.

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The effect of cyclic AMP on Na^+ and K^+ transport systems in mouse macrophages

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Summary. Exogenous cyclic AMP (cAMP) inhibits the Na^+, K^+-cotransport system and stimulates the Na^+, K^+-pump and Na^+, Ca^{2+} exchange in mouse macrophages. These effects are enhanced by inhibition of phosphodiesterase with methylisobutylxanthine (MIX). MIX alone showed little or no effect. A similar response was observed after stimulation of endogenous production of cAMP by isoproterenol.
Key words. Na^+; K^+ transport; macrophages; cyclic AMP; mouse.

It is a classical view that any disturbance of ion metabolism may be corrected by hormonal modulation of gastrointestinal ion absorption and/or renal ion excretion. However, a more complex mechanism was recently suggested by the observation that ion transport may also be submitted to hormonal homeostasis in non-epithelial cells. For instance, in avians, catecholamines may correct the hypokalemia resulting from flight by the stimulation of K^+ entry into red cells. This particular mechanism involves the adenylate cyclase-dependent stimulation of a bumetanide-sensitive Na^+, K^+-cotransport system[1,2]. In mammals, catecholamines may correct the hyperkalemia resulting from muscular exercise by enhancing K^+ gain into striated muscle cells. This mechanism involves the stimulation of a ouabain-sensitive Na^+, K^+-pump by cyclic AMP (cAMP)[3]. Thus, ion transport in non-epithelial cells may be modulated by hormones through second messengers such as cAMP.

Ion transport systems are well characterized at the molecular level in human red cell membranes[4]. On the other hand human erythrocytes, like many other cells, have a cAMP translocating system[5,6]. This regulatory mechanism maintains a cAMP erythrocyte content about 100 times lower than the extracellular cAMP concentration[5]. Furthermore, it catalyzes the rapid incorporation of a small but non-negligible amount of exogenous cAMP[5]. This allowed us to study the effect of internal cAMP on ion transport in intact erythrocytes. We thus observed that exogenous cAMP is able to inhibit the Na^+, K^+-cotransport system in these cells[5]. Unfortunately, the study of cAMP-ion transport interactions is hampered by the fact that human erythrocytes do not have a beta-adrenergic stimulated adenylate cyclase. We thus decided to investigate these interactions further in other non-epithelial cells.

A transport study at the a molecular level requires a large amount of cells, and they must be in suspension under natural conditions. It appeared to us that both conditions were fulfilled by mouse macrophages elicited in the peritoneal cavity by injection of thioglycollate.

2.5–3 ml of sterile thioglycollate medium were injected into the peritoneal cavity of female mice 5–8 weeks old, of the 57 BL/5 ($H\text{-}2^b$) and DBA/2 ($H\text{-}2^d$) inbred strains. 3 to 5 days later, the cells were collected by washing the peritoneal cavity with Hank's balanced salt solution. An average of 10^7 cells were obtained per mouse. More than 80% of these cells presented the morphological aspect of macrophages. A pool of peritoneal cells from 5–15 mice was used in each experiment.

Na^+ and K^+ contents, measured in cells washed once with $MgCl_2$ 110 mM, varied between 15 and 24 mmoles/l·cells and between 50 and 80 mmoles/l·cells, respectively.

The effect of cyclic AMP on the Na^+ and K^+ transport systems of mouse macrophages

Transport system	Control	Isoproterenol 10^{-5} M	cAMP 2 mM	cAMP+MIX 2 mM 0.5 mM
Na^+,K^+-pump				
Na^+ efflux	6.5 ± 4.6	12.1 ± 4.2	17.2 ± 5.8	24.6 ± 6.9
Na^+,K^+-co-transport				
Na^+ efflux	5.1 ± 2.3	0	1.3 ± 0.7	2.7 ± 1.8
K^+ efflux	11.2 ± 3.6	0	9.8 ± 4.6	7.1 ± 0.7
Na^+,Ca^{2+} exchange				
Na^+ efflux	3.7 ± 2.1	10.8 ± 2.9	5.2 ± 3.5	9.5 ± 2.4

Values are given as mean ± SD of 4–7 experiments. Fluxes are expressed as mmol/(l·cells·h).