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TECHNICAL REPORT

SOME EFFECTS OF 7,12-DIMETHYLBENZ(A)ANTHRACENE AND BENZO(A)PYRENE ON FRIEND VIRUS LEUKEMOGENESIS

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May 1978

Prepared for

THE U.S. DEPARTMENT OF ENERGY
UNDER CONTRACT

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ABSTRACT

The effects of the chemical carcinogens, 7,12-dimethylbenz(a)-anthracene (DMBA) and benzo(a)pyrene (BP), on leukemia induction by Friend leukemia virus (FLV) have been evaluated in SJL/J mice. In the viral carcinogen controls, FLV doses of 0.1 SED injected IP caused early leukemia in up to 50% of the recipient animals, which died with characteristic leukemia induced splenomegaly around day 40 after virus injection. The remainder died between 120 and 260 days after FLV, with some splenomegaly but also notably with other symptoms, such as enlarged lymph node and thymus glands. In the chemical carcinogen controls, a single dose of DMBA or BP (up to 50 µg DMBA and 500 µg BP were tested) was not found to have any significant tumorigenic effect by itself in this study, except in one experiment where DMBA was dissolved in olive oil instead of the usual synthetic oil, trioctanoin. However, similar doses of DMBA, given in conjunction with FLV, were found to be unexpectedly protective with regard to the leukemogenic effect of FLV, particularly when given 5 hrs after the virus. On the other hand, pretreatment with BP had an opposite effect, resulting in increased leukemogenesis in SJL/J mice and also in normally virus resistant B10SJF1 mice.

INTRODUCTION

A number of synergistic interactions between viral and physical (1,2) and also between viral and chemical carcinogens (3-6) have been reported. Most of these studies, however, utilize radiation or chemicals which mimic radiation effects on DNA and/or in vitro systems. In the present studies we have been investigating in vivo effects of the polycyclic hydrocarbons, 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BP), on leukemogenesis induced by injection of low doses of Friend leukemia virus (FLV). DMBA (8,9) and BP (10,11) are known to produce sarcomas and carcinomas in adult mice, mostly at the site of application or injection. DMBA also causes an increased incidence of breast tumors in certain rat strains (12). Although a single injection of these hydrocarbons is effective when given to neonatal animals (13), in adults repeated administration is normally required to produce an effect (7,10,11), or they must be given in conjunction with promotors of carcinogenic action (9). The purpose of the present study was to determine if these agents also showed a synergistic effect when given in single dose to normal adult mice in conjunction with a known viral carcinogen.

MATERIALS AND METHODS

Animals - Four to six week old female SJL/J and C57Bl/10 mice were purchased from Jackson Laboratories and held in our facilities until use at 10-12 weeks. The B10SJF1 hybrids were produced in our facilities by crossing C57Bl/10 females and SJL/J males. Female

hybrid mice were used for experiments when they were 10 to 14 weeks old. All animals were maintained in plastic cages with filter tops and were given acidified water and sterilized food ad libitum. The animal room was temperature and humidity controlled and maintained at a 12 hr light cycle.

Virus - FLV obtained from NCI was maintained by passaging in SJL/J mice. FLV rich plasma of leukemic SJL/J mice was titered for virus by a spleen enlargement assay. One spleen enlargement dose (SED) is defined as that amount of virus that causes splenomegaly in 50% of SJL/J mice by day 14. The plasma was diluted with phosphate buffered saline to give the appropriate SED. The virus was injected intraperitoneally.

Carcinogens - 7,12-DMBA or BP (Sigma) was dissolved in trioctanoin, a pure synthetic oil (Eastman Kodak), and injected intraperitoneally as indicated. In the experiment of figure 1, olive oil was used as the vehicle instead of trioctanoin.

Parameters monitored - Mice were kept for 250 to 300 days after treatment. They were checked for deaths daily and their condition at autopsy was noted. Leukemia was monitored with periodic white blood cell counts, hematocrit determination, and microscopic examination of blood smears.

RESULTS

In these studies the effects of DMBA or BP given 5 hours before or 5 hours after FLV were contrasted with the effects of the virus alone or the chemical alone. For control purposes an additional set of mice not exposed to either carcinogen was also maintained

and regularly checked for evidence of spontaneous cancer development.

DMBA - Figure 1 shows the effect of 10 µg of DMBA given by intraperitoneal injection 5 hours before or 5 hours after 0.1 SED FLV. When given 5 hours after the virus, DMBA had a significant protective effect when the survival of these mice is compared to that of mice given 0.1 SED of virus alone from the same preparation. This protective effect is illustrated by a generally longer survival time than that seen for FLV given alone, and a smaller number of animals dying with the normal symptoms of Friend leukemia. When given 5 hours before FLV, this protective effect is still suggested but the difference is not significant. All of the mice that died after day 120, and none of the mice that died before this time, had enlarged lymph nodes and thymus glands, as well as enlarged spleens. The incidence of the later deaths with enlarged lymphoid organs appears to be uneffected by DMBA. Of the 4 mice in the DMBA only group that died, 3 had large tumors at the injection sites, while 1 appeared to die of infection. It should be noted that the experiment of figure 1 was the only instance where olive oil was used as the carcinogen vehicle, and it was also the only instance where solid tumor development at the site of carcinogen injection in the group receiving carcinogen only was observed.

Figure 2 shows the results of an experiment where a lower dose of FLV (0.015 SED) was used in conjunction with three doses of 50 µg DMBA in trioctanoin. The lower dose of FLV used in this experiment had only minimal early leukemogenic effect, and only those mice that died before day 100 were free of lymphoid organ enlargement. The fact that DMBA had no effect on survival in this

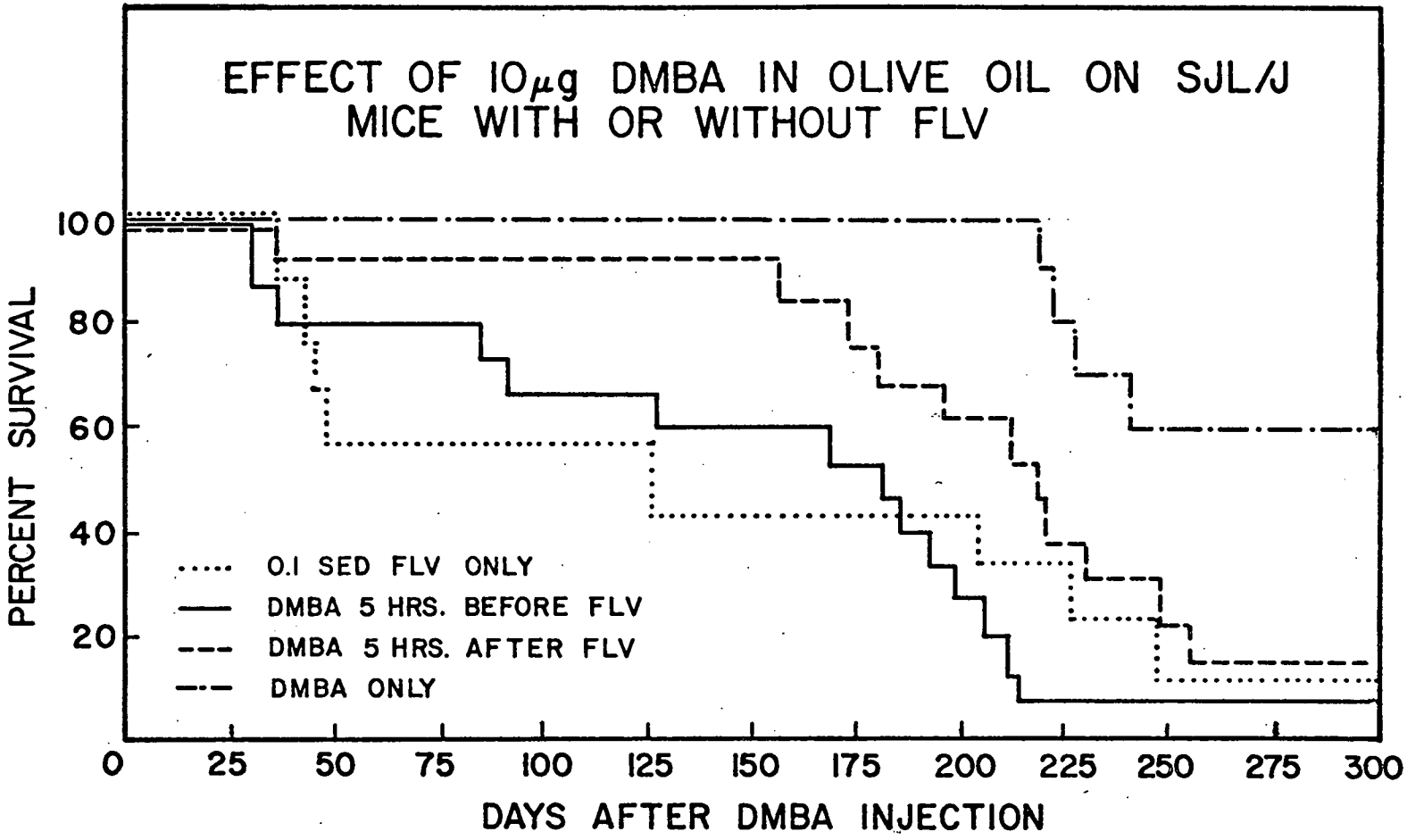

Fig. 1. 10 μg DMBA dissolved in olive oil was injected 5 hrs before or 5 hrs after 0.1 SED FLV into SJL/J mice. The groups that received both viral and chemical carcinogens consisted of 15 mice each. All of the other groups consisted of 10 mice. A control group that received the carcinogen vehicles only had 100% survival by day 300 (not shown in graph).

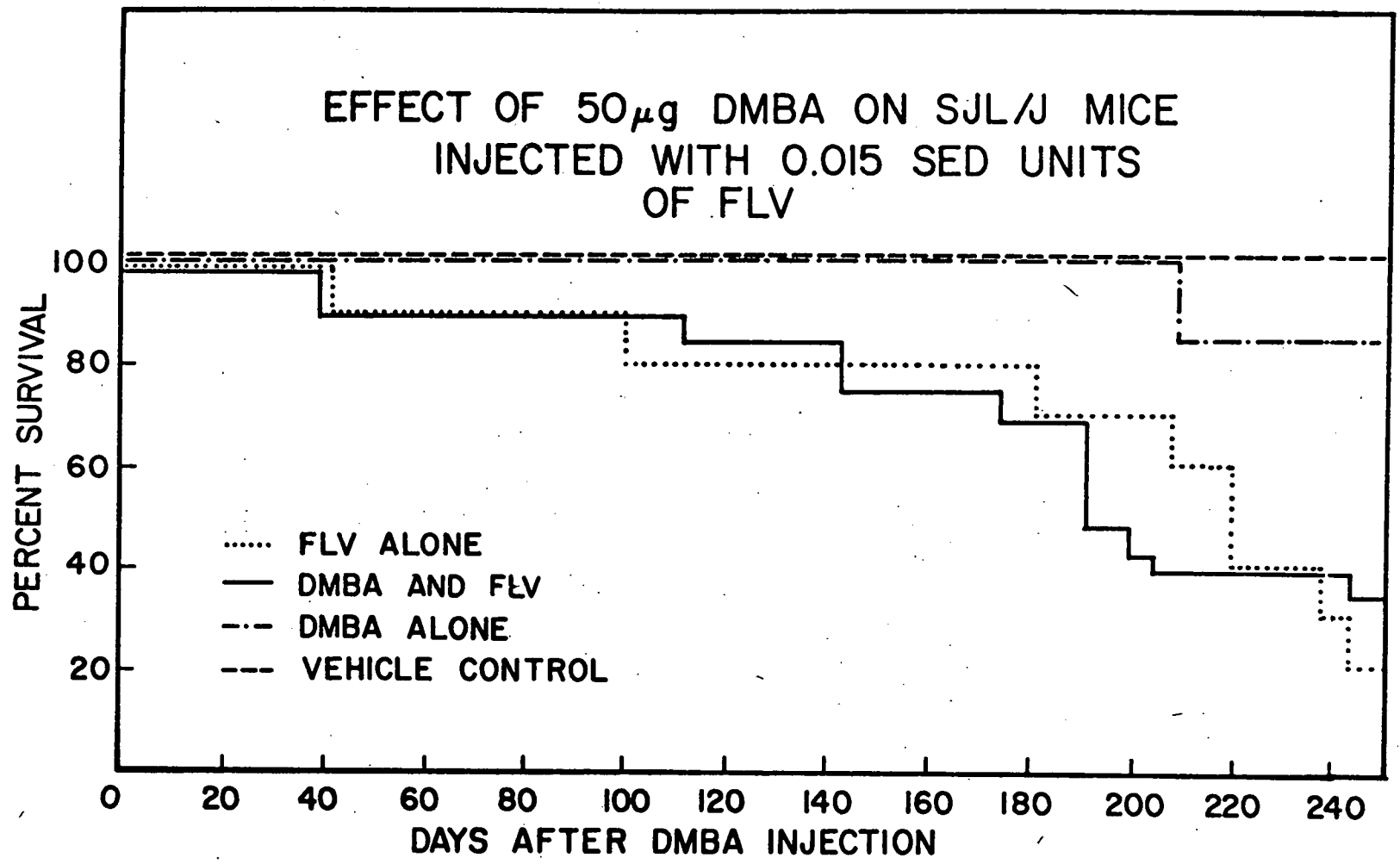

Fig. 2. 50 μg DMBA dissolved in trioctanoin was injected IP into SJL/J mice. 5 hrs later 0.015 FLV was injected. The DMBA injection was repeated again 7 days and 14 days after FLV injection. There were 5 mice in the control group that received vehicles only; 10 mice in each of the groups that received either carcinogen alone and 20 mice in the group that received both chemical and viral carcinogens.

experiment agrees with the results of figure 1, since nearly all of the deaths in this experiment were of the type which is unaffected by DMBA.

In the experiment of figure 3 the dose of FLV used was higher than the usual 0.1 SED, as is apparent from the increased number of leukemic deaths in the group that received virus only. The results of DMBA treatment in conjunction with this virus dose confirm the conclusion drawn from figure 1 above that DMBA is protective against the early leukemogenic action of FLV. In this case a single exposure to DMBA was as effective as 3 exposures given a week apart.

BP - In the experiment illustrated by figure 4, 500 µg BP was given to SJL/J mice 5 hours before or after 0.1 SED FLV. The results indicate that pre-treatment with BP potentiated FLV carcinogenesis. This conclusion is confirmed by the experiments reported in Table 1, where various doses and schedules of BP increased leukemogenic death in normally resistant B10SJF1 mice. With B10SJF1 mice all of the deaths were due to erythroleukemia, characterized by hepato- and splenomegaly with no instances of lymphoid organ involvement.

DISCUSSION

Evidence was obtained that hydrocarbon carcinogens affect Friend viral leukemogenesis. BP increased this effect of FLV, while DMBA apparently decreased it. These effects were observed when FLV produced symptoms of early erythroleukemia as originally described for this virus (15), i.e. gross hepato- and splenomegaly, increased white blood cell counts and hematocrit. However, in the group of

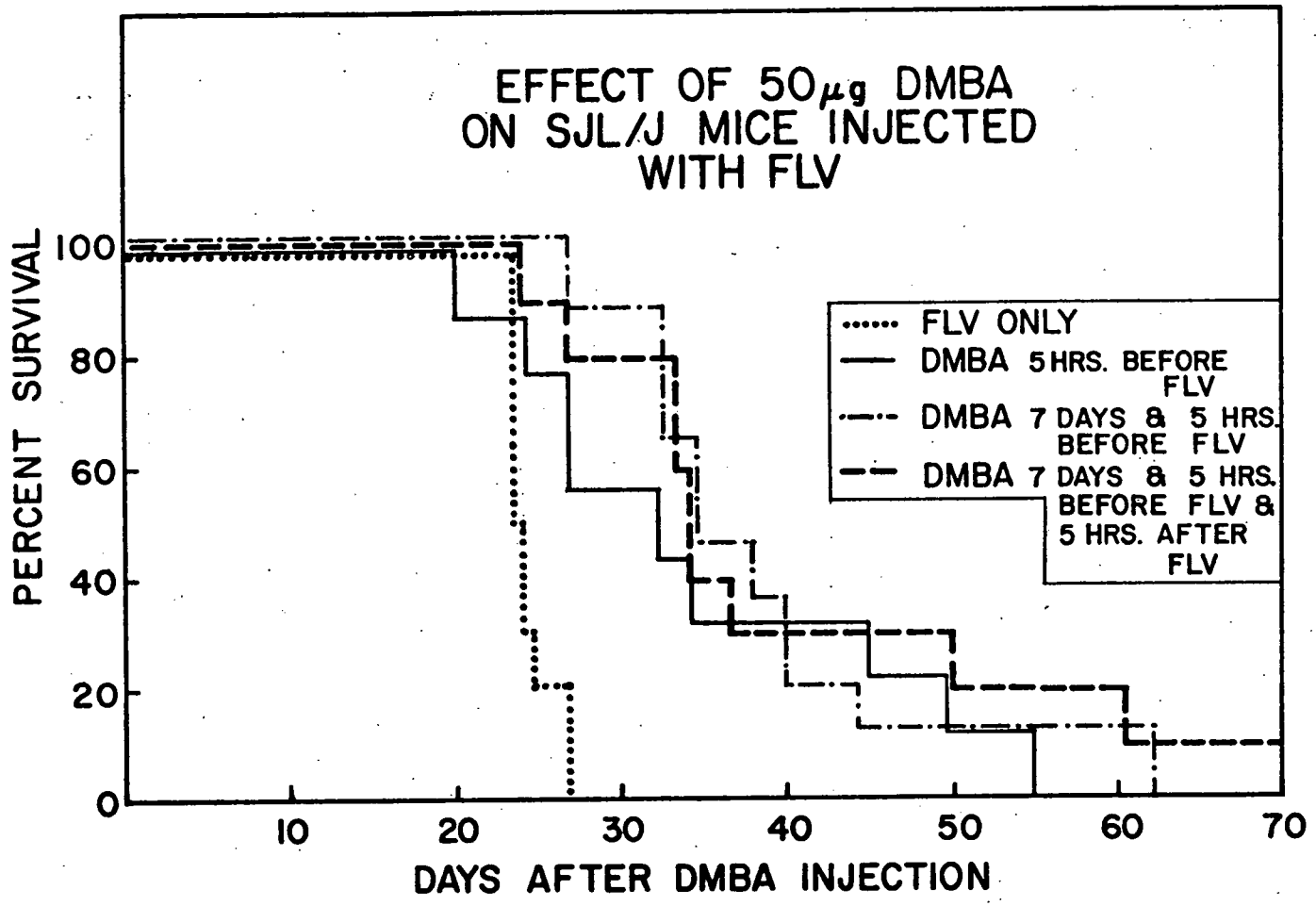

Fig. 3. 50 μg DMBA in trioctanoin was injected IP to SJL/J mice seven days before, 5 hrs before or 7 days and 5 hrs before. All the deaths observed involved erythroleukemia as judged by spleno- and hepatomegaly. There were ten mice per group.

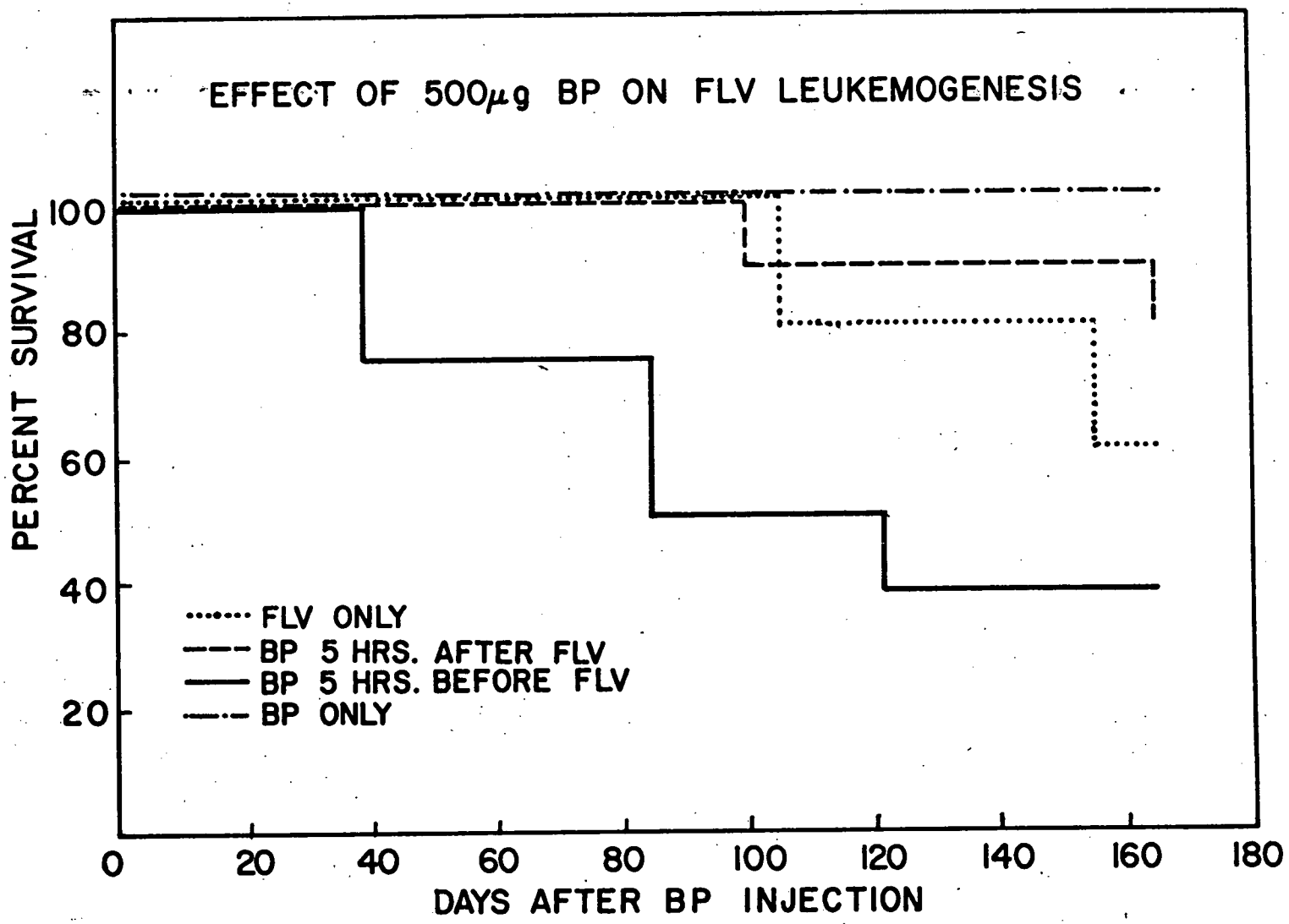

Fig. 4. 500 μg BP was injected IP to SJL/J mice 5 hrs before or 5 hrs after 0.1 SED FLV. There were 10 mice in each of the groups that received both viral and chemical carcinogen; and there were 5 mice in the group that received virus only as well as in the control group that received saline injections only. There was 100% survival in the control group (not shown on graph).

Table I

B10SJF1 Mice Were Treated With BP Dissolved in Trioctanoin and FLV as Indicated. Control Mice Received FLV Only.

	Number of Leukemic Deaths	
	Control	Experimental
Exp. 1 50 µg BP 5 hrs before FLV	0/6	1/6
Exp. 2 50 µg BP 5 hrs before FLV plus 500 µg BP 7 days after FLV	1/6	6/6

SJL/J mice which survived the initial wave of deaths, the survivors often died several months later with different symptoms, more resembling a lymphoma. Neither BP or DMBA given alone seemed to have any effect on this later type of mortality. The lower incidence of late deaths with lymphoid involvement in the group receiving FLV alone, as opposed to both viral and chemical carcinogen (Fig. 1), probably reflects the fact that most of the recipients of FLV alone died before such symptoms usually develop.

We have observed the relatively late development of lymphoid involvement only in these present experiments with hydrocarbon carcinogens and not with others involving the water soluble carcinogen, methyl methane sulfonate (15,16). However, since lymph node enlargement was seen in groups that were also treated with virus only, it is possible that the oil vehicle given these mice for control purposes was instrumental in its induction. The synthetic oil, trioctanoin, was used in all experiments except our first one reported in figure 1. In one set of studies the development of local solid tumors at the site of injection was also observed. However, this occurred only when olive oil was used as the solvent for DMBA. This suggests that the olive oil may have acted as a promotor (9). Histological examination of the solid tumors as well as of enlarged lymphoid organs are now in progress.

Development of a thymic lymphoma has been previously observed to occur in the late course of Friend disease (17), following an early splenic response. A similar situation was also described for the pathogenesis of the closely related Rauscher murine leukemia virus (18). Our data suggest that the early leukemic and late lymphoma effects are differentially sensitive to carcinogen

interaction. Interestingly, the enlarged lymph nodes and thymus glands that are characteristic of late deaths in SJL/J mice infected with FLV were never seen in B10SJF1 mice, despite the fact that many of the deaths occurred around day 150 after FLV injection. It was also the case in this hybrid strain that even the late deaths were influenced by BP injection. Since B10SJF1 mice are fairly resistant to FLV action alone, this observation indicates that the development of lymphoid involvement requires a high degree of virus sensitivity.

The basis for the protective effect of DMBA is not clear and we are presently monitoring plasma titers of virus in mice treated with FLV and DMBA in order to determine whether it may be the result of virus inactivation. Further experiments utilizing various doses of both DMBA and BP are necessary to determine whether the apparent fundamental difference between the effect of DMBA and BP is real.

Another interesting observation involving B10SJF1 mice is that they develop temporarily elevated white blood cell counts in about 30% of animals given 100 SED FLV alone (unpublished). Thus it is possible that some immunological control of the virus occurs in B10SJF1 mice. Experiments to determine whether BP effects the immune system are in progress. Also in progress are other experiments utilizing different timing schedules of hydrocarbon and virus exposure as well as the use of various inhibitors of DNA repair. The latter may determine whether effects on the hosts' DNA are involved.

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