investigation, the experimental mice appeared to be more lively and have a generally healthier appearance, even when the tumor mass was as large or larger than in the control animals.

Summary. A cold water extract of leaves of the plant *Symphytum officiale* was given to mice bearing spontaneous or transplanted tumors. The tests involved 73 control and 52 experimental mice bearing spontaneous mammary tumors of various initial sizes, and 71 control and 63 experimental animals bearing transplanted mammary tumors. Survival time of the spontaneous tumor mice that received comfrey extract was increased an average of 59% as compared with the controls. In the transplanted tumor tests, tumor weight at autopsy averaged 24% less in the comfrey extract mice as compared with the controls. These differences between control and experimental groups are statistically significant.

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Potentiating Effect of K-Virus on Mouse Hepatitis Virus (MHV-S) in Weanling Mice.* (28793)

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In a recent review of subacute viral hepatitis in man(1) it was suggested that this serious variant of hepatitis resulted from the interplay of several host and viral factors, one of which might be the potentiating effect of a second viral agent preexisting as a latent infection of the liver. The present studies represent an experimental approach to this hypothesis.

K-virus, a member of the papova group of viruses known to infect weanling or adult mice without producing overt disease(2,3), has been used at a latent agent in the livers of mice later infected with the mouse hepatitis virus (MHV-S)(4). In these short-term experiments the latent infection enhanced the hepatic necrosis induced by the MHV-S agent. Although these results may not be applicable directly to human hepatitis viruses, they do indicate that a naturally-occurring latent viral infection may potentiate the effects of a hepatitis virus.

Materials and methods. The MHV-S agent (obtained from Dr. John D. Nelson, Rockefeller Inst.) was prepared as a 10% liver-saline homogenate which had an LD_{50} of 10^{-7} in suckling ICR mice by the intraperitoneal route. K-virus (obtained from Dr. Lawrence Kilham, Dartmouth Med. School) was prepared as a liver-spleen-kidney homogenate made into a 10% suspension with medium 199, having an LD_{50} of approximately 10^{-5} in suckling mice by the intracerebral route. Homogenates were stored at -30°C.

Female weanling ICR mice (Charles River Mouse Farms, Inc., Boston, Mass.) weighing 9-15 g were used in all experiments. Representative animals were shown to be free of *Eperythrozoon coccoides* by examination of peripheral blood smears following splenectomy.

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TABLE I. Results Showing Potentiating Effect of K-virus on Acute Hepatic Necrosis (4th Day) Induced by MHV-S in Weanling ICR Mice.

Group	No. mice	Viruses inoculated	Results	
			General	Microscopic
I	15	K-virus	No illness	No hepatic necrosis. Kupffer cell prominence and scattered mitoses.
II	25	MHV-S	Inconstant mild illness	Hepatic necrosis in 19/25 with 30.8% lobules involved. 2/25 with confluent lesions.
III	30	K-virus, MHV-S 4 days later	Marked acute weakness, wasting	Hepatic necrosis in 28/30, with 60.0% lobules involved. Confluent and submassive necrosis in 8/30.

The overall experimental design is summarized in Table I. Seventy-five weanling mice were given 0.05 ml of K-virus by the intraperitoneal route on day 1. On day 4, 50 of these animals and 40 untreated mice were inoculated with 0.1 ml of MHV-S homogenate by the intraperitoneal route. Four days later, at the time of maximum acute hepatic necrosis, animals from each experimental group (K-virus only, MHV-S only and K-virus followed by MHV-S) were anesthetized with ether, exsanguinated and their livers removed for microscopic examination. The remaining mice in each group were sacrificed for study on the 14th day after MHV-S inoculation at a time when the acute hepatic lesion had usually subsided.

Results. The pathological effects on the mouse livers of K-virus alone (Group I), MHV-S alone (Group II) and K-virus followed in 4 days by MHV-S (Group III) are shown in Table I. Many mice, especially in the group receiving both K-virus and MHV-S, became acutely ill 2 to 3 days after the inoculation of MHV-S, developing weakness, pallor and weight loss. The mice receiving K-virus only showed no overt signs of acute illness.

The livers of the group treated with K-virus only were grossly normal and showed only prominence of Kupffer cells and scattered mitoses in parenchymal cells on microscopic examination (Fig. 1). After 4 days a majority of the mice receiving MHV-S only had slightly pale and mottled livers, with clear-cut hepatitis characterized microscopically by circumscribed foci of liver cell loss, disruption and eosinophilic condensation, reactive neutrophilic and mononuclear inflammation and local vascular congestion (Fig. 2). Hepatic necrosis was typically monolobular in these animals, 30% of the lobules in an average liver showing areas of destruction; confluent or submassive necrosis was rarely seen (2/25). Most of the livers of the group receiving both K-virus and MHV-S (Group III) were small and yellow on the fourth day after MHV-S inoculation. All but 2 of these 30 mice had hepatitis that involved an average of 60% of the liver lobules, and 8 had extensive confluent hepatic necrosis (Fig. 3). The actual cellular pattern of parenchymal destruction resembled that seen in the mice receiving MHV-S alone, but the extent was exaggerated and the zones of cell loss often involved several adjacent lobules. Pneumonitis was not observed in any of the experimental animals.

All animals sacrificed 14 days after MHV-S inoculation had livers which were grossly normal. Microscopic examination revealed only small isolated foci of subsiding hepatitis in 7/15 of the MHV-S mice and in 11/20 of the group receiving both K-virus and MHV-S.

Discussion. These studies with 2 naturally-occurring viral infections of mice suggest that a latent systemic infection with one viral agent may alter the host response to a second virus. Specifically, prior inoculation of K-virus, a DNA intranuclear virus of the papova group that induces only reticuloendothelial cell swelling in the infected livers of adult and weanling mice(5), enhanced the acute liver cell necrosis and inflammatory response produced in weanling mice by MHV-S, a cytoplasmic RNA virus(6).

A similar enhancing or potentiating effect

has been described with other dual infections. Coxsackie A-14 infection has been shown to increase the pathogenic action of type I poliomyelitis virus in monkeys(7), and inapparent *Trichinella spiralis* infection of rats led to increased susceptibility to disease caused by encephalomyocarditis virus (EMC)(8). The pathogenicity of mouse hepatitis virus (MHV-1) in weanling mice was enhanced by prior infection with *E. coccoides*(9) and by latent infections with the Friend and the Moloney leukemia agents (10). Thus, there is substantial experimental evidence that simultaneous infection with two agents may lead to enhanced clinical and pathological disease.

Comparison of the histologic appearances of mouse livers infected with MHV-S only (Group II) with those harboring both MHV-S and K-virus (Group III) suggests that the latent agent promotes the destructive action of MHV-S on the liver cells, but it sheds little light on the possible mechanism of enhancement. Since K-virus seems to affect hepatic reticuloendothelial cells (Table I) (5), it may facilitate the entrance of MHV-S into the mouse liver. Alternatively, K-virus may depress the resistance of the liver cells proper in some manner that permits survival and unusually rapid replication of MHV-S, a mechanism demonstrated in double infections with *T. spiralis* and EMC(8) and *E. coccoides* and MHV-1(9). Since infection with K-virus involves many body tissues(2), its enhancing action on MHV-S may be mediated through inapparent systemic effects. Further studies are being carried out to explain the potentiation observed in the current experiments.

Acute MHV-S hepatitis was clearly enhanced by prior infection with K-virus but subacute progressive hepatitis was not produced. Nonetheless, these preliminary observations lend support to the theory that severe atypical or progressive human hepatitis may result from coincident inapparent infections with other viral agents, and they may indicate a worthwhile approach to the further study of human hepatitis.

Summary. Prior infection of weanling ICR mice with K-virus, an agent that alone causes no obvious liver cell damage, enhanced the

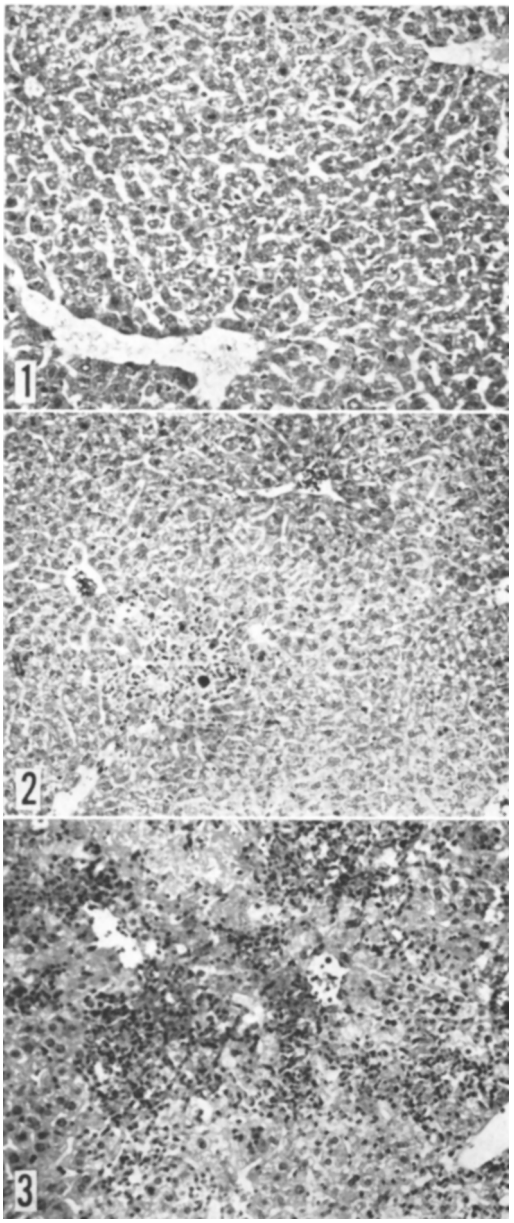

FIG. 1. Kupffer cell prominence and intact hepatic cells in weanling mouse liver, 8 days after infection with K-virus. Hematoxylin and eosin. × 96.

FIG. 2. Focal hepatic necrosis in weanling mouse liver, 4 days after infection with MHV-S. Hematoxylin and eosin. × 96.

FIG. 3. Extensive confluent hepatic necrosis and inflammation in liver of weanling mouse infected with K-virus, 4 days after infection with MHV-S. Hematoxylin and eosin. × 96.

acute (4th day) hepatic necrosis and inflammation produced by subsequent infection with mouse hepatitis virus (MHV-S).

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Effects of Epsilon Amino Caproic Acid (EACA) on Survival of Fibrinogen I^{131} and on Fibrinolytic and Coagulation Factors in Dogs.* (28794)

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Epsilon amino caproic acid (EACA) acts as an inhibitor of the activation of profibrinolysin, and, in higher concentrations, of fibrinolysin itself(1,2). These experiments were undertaken to determine whether or not large oral doses of EACA given to dogs would affect the coagulation mechanism, the fibrinolytic mechanism or the turnover rate of fibrinogen I^{131}.

Methods and materials. Methods for the assay of coagulation factors(3,4), fibrinolytic factors(5) and for turnover of fibrinogen I^{131} (6) have been described. Eighteen mongrel dogs were used in the study. Twenty-four fibrinogen I^{131} survival studies were carried out; 12 on dogs receiving EACA and 12 on dogs which had never received EACA or had received none for 4 weeks or longer. On 6 dogs 2 studies were done, one with EACA and one without. EACA was started 4 days before injection of fibrinogen I^{131} and continued for 8 days thereafter. The dogs ingested 200 mg EACA per kg 3 times a day in meat balls. Coagulation studies were carried out before and after 4 and 12 days of EACA feeding in experimental dogs and, at the same periods, in control dogs. Fibrinolytic assays were carried out before EACA was started and just before fibrinogen I^{131} was injected in the experimental dogs and at similar time intervals in the control group. The presence of I^{131} in recipient plasma interfered with the fibrinolytic assays which, therefore, were not done at the 8-day post-fibrinogen I^{131} period.

Results. Coagulation factors. No significant changes were observed in whole blood clotting time, serum prothrombin time, plasma prothrombin time, plasma thrombin time, platelet count or plasma levels of factors I, II, V, VII, VIII and IX.

Fibrinolytic factors. Fig. 1 illustrates the spontaneous fibrinolytic activity of plasmas and their euglobulin fractions from control and experimental dogs and from a group of 30 normal human subjects. An occasional dog plasma showed fibrinolytic activity (*i.e.,* ability to dissolve added iodinated purified bovine fibrin) which was greater than human. This was suppressed following EACA. Because dog euglobulin activates spontaneously, these fractions were prepared and tested as rapidly as possible. Significant fibrinolytic activity was found in 12 of 36

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