Protective Effect of *Symphytum officiale* on Mice Bearing Spontaneous and Transplant Tumors. (28792)

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For more than 10 years, a project has been under way at the University of Texas with the objective of testing various plant fractions for possible value in cancer therapy (1-10). Most of the work has proceeded by the usual method of injecting test materials into mice bearing transplants of tumor tissue. Also, mouse tumor tissue cultivated in embryonated eggs has been used extensively in this work. The results obtained with these procedures have demonstrated that plants are a promising source of anticancer compounds. It has been noted, however, that extracts effective against transplanted tumors rarely affect the growth of spontaneous tumors.

The present investigation is concerned with a plant fraction which has a protective effect on mice bearing spontaneous tumors. The term protective is used since the increase in survival of tumor-bearing mice receiving this extract appears to be greater than can be accounted for by the retardation of tumor growth. A fairly extensive series of tests with this plant material has now been completed, including mice bearing transplanted as well as spontaneous mammary tumors.

Materials and methods. A cold water extract of dried leaves of the plant *Symphytum officiale,* known as comfrey, was used in these tests. The comfrey extract was prepared by immersing powdered dry leaves in cold distilled water, 20 g per liter, and allowing the mixture to stand 24 hours at ordinary refrigerator temperature. The preparation was then strained through 4 layers of gauze and given to the mice in place of drinking water. The mice were given fresh allotments of the extract once a day, for 5 consecutive days of each week. For the other 2 days of the week, the usual drinking water was utilized.

Tests were made with C3H mice bearing spontaneous mammary tumors, the initial size and growth of which was estimated by squaring the average of 2 measurements across the widest and narrowest tumor dimensions. Mice bearing spontaneous tumors of comparable size and receiving identical treatment, except that they received ordinary drinking water, were used as controls. In the work with mice bearing spontaneous tumors, each animal was essentially a separate experiment with individual records of measurements, weights and observations to the terminal date.

In addition to mice with spontaneous tumors, C3H and DBA mice bearing transplants of mammary tumor tissue were utilized. Mammary tumors, which had been checked through numerous transplant-generations for a period of many years, were used in this work. These transplant tumors were characterized by fairly uniform growth and 100% takes. Animals bearing transplant tumors were placed on the plant extract on the day they received the implants of tumor tissue.

In the tests with spontaneous tumors, the mice were examined daily except Sunday. Each individual experiment was continued until the mouse began to manifest symptoms of distress due to tumor size or to the development of hemorrhagic lesions. This means of determining the end point appeared to be preferable to continuing the tests to the natural death of the animals. A tumor-bearing mouse remains in good health, if given proper care, up to a certain point after which the behavior of the animals changes and a rapid deterioration in health begins.

Evaluation of the procedure was based on the days to termination of the experimental in comparison with the control animals. In the tests with transplant tumors, the results were assessed in relation to tumor weight in the experimental mice as compared with that of the controls at autopsy. In the experiments with both spontaneous and transplant tumors, a record was kept of body weight changes.

Results. Tables I and II summarize the results obtained. Mice bearing spontaneous tumors and receiving the plant extract sur-

TABLE I. Protective Effect of an Extract of *Symphytum officiale* in Drinking Water of Mice Bearing Spontaneous Mammary Tumors.

No. of mice		Initial tumor size, cm^2		Terminal tumor size, cm^2		Duration, days ± S.D.*		Terminal body wt, % of initial	
Control	Exp	Control	Exp	Control	Exp	Control	Exp	Control	Exp
24	23	.91 ±.09	.89 ±.10	3.90 ± .86	4.50 ± 1.14	33 ± 16	50 ± 26	103	106
30	11	1.29 ±.11	1.25 ±.08	4.55 ± 1.11	5.20 ± 1.90	26 ± 11	48 ± 26	105	112
11	9	1.67 ±.08	1.75 ±.16	5.25 ± 1.15	4.38 ± 1.50	24 ± 7	31 ± 25	104	106
8	9	2.34 ±.20	2.43 ±.26	5.56 ± 1.11	7.70 ± 1.20	21 ± 5	32 ± 13	98	107
Totals & averages:									
73	52	1.34	1.37	4.55	5.20	27 ±11.1	43 ±23.6†		

* S.D. = Standard deviation.
† Total avg difference in days to terminal stage is significant (P = <.001).

vived an average of 59% longer than the controls. Of the mice surviving 40 days or more, 11% were controls and 44% were in the experimental group. The average increase in tumor size per day was 25% less in experimental mice as compared with controls.

Spontaneous tumors in 2 mice receiving the plant extract regressed completely within 2 weeks of the treatment. One of these mice bore a single mammary tumor, 0.6 cm in size, and the other mouse had 2 tumors, 0.5 and 1.1 cm in average diameter at the time the extract was added to the drinking water. Such regressions have not been observed in the large number of spontaneous tumors investigated in this laboratory for many years.

Table II reveals that transplant tumors were inhibited in growth 24% in association with the plant extract. This degree of inhibition is equivalent to that which occurred in mice bearing spontaneous tumors. The extract was not associated with any evidence of toxic effects.

Discussion. The results obtained with the *Symphytum* extract indicate that this material had a protective effect on mice bearing spontaneous tumors. The longer survival of the experimental mice, in comparison with the control animals, however, appears greater than would be expected on the basis of the inhibitory effect on tumor growth. It has been reported(6) that *Chelidonium majus* L. given as a supplement to the diet delayed the appearance of mammary tumors in mice and increased the life span about 30%.

Most of the work on the problem of cancer therapy has been carried out from the standpoint of finding drugs which directly interfere with the growth of tumor tissue. But there is the possibility that host resistance to the disease can be so increased that the tumor is secondarily affected. In the present

TABLE II. Effect of an Extract of *Symphytum officiale* in Drinking Water on Growth of Mammary Tumor Transplants in Mice.

Exp No.	No. of mice		Days to autopsy	Tumor size, g ± S.D.*		Tumor size (Control = 100)	Body wt, % of initial
	Control	Exp		Control	Exp		
1	10	10	14	1.07 ± .54	.79 ± .43	74	96
2	5	8	13	1.03 ± .35	.76 ± .50	74	100
3	6	4	15	1.84 ± .74	1.20 ± .39	65	97
4	13	9	15	.87 ± .35	.99 ± .85	104	98
5	13	10	11	.53 ± .25	.51 ± .29	96	98
6	12	9	14	.71 ± .44	.49 ± .30	69	95
7	6	5	33	1.31 ± .77	.82 ± .54	63	96
8	6	8	14	1.99 ± .39	1.00 ± .70	50	103
Total	71	63	Avg	1.03 ± .44	.78 ± .50†	76	

* S.D. = Standard deviation.
† Total avg difference in tumor size is significant (P = <.005).

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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