Effect of Levan on Transmitted Mouse Leukemia and on Ehrlich Ascites Tumor Implants.* (22614)

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Evidence is accumulating that leukemia in mice may be caused by a filtrable, submicroscopic agent, presumably a virus(1). Pillemer *et al.* reported that the complement-properdin system neutralizes and inactivates some viruses(2), and that native levan and other high molecular weight polysaccharides alter properdin levels *in vivo*(3). Hestrin *et al.*(4,5) suggested that the infection-promoting activity (IPA) of native levan, injected intravenously or intraperitoneally into mice having an intraperitoneal infection, may result largely from an extraperitoneal interaction between levan and host. Davies and co-workers(6) suggested that this IPA is also related to an endothelium sealing activity (ESA) on the part of the large polysaccharide molecules, which, circulating in the blood, enter into the interstices of capillary endothelium and alter its sieving characteristics toward colloids in an area of inflammation.

Because of (a) the reported anti-viral activity of the complement-properdin system, (b) the IPA of native levan, and (c) the presumed viral etiology of mouse leukemia, the effects of a properdin-lowering substance, levan, on the course of transmitted leukemia was studied.

Based on one preliminary experiment(7) there is some indication that the lowering of the properdin level by high molecular weight polysaccharides may facilitate transplantability of the Ehrlich Ascites Tumor. In view of this and of the phenomenon of ESA, it was desirable to know if levan injected intravenously would either interfere with the passage of nutrients to a rapidly growing tumor, thus inhibiting the establishment of the tumor, or as Koprowski(7) has indicated, facilitate the establishment of the tumor. In addition, the direct action of levan on the Ehrlich Ascites Tumor cell was studied by observing the effect of injecting a mixture of levan and tumor cells subcutaneously or intraperitoneally.

Methods. (a) Leukemia: Male C58 mice, age 2 months, were used. Eleven mice (Group A) were injected intraperitoneally with 5 mg of levan† dissolved in 0.15 M NaCl solution. Group A and 11 control mice (Group B) then received intraperitoneally 0.3 ml of a suspension of lymph nodal cells 25,000 to 30,000/mm^3) from a leukemic C58 mouse. The cells were obtained from a mesenteric lymph node by teasing with forceps and probing needles. The cells were then suspended in a balanced physiologic buffer solution and the suspension was finally filtered through an electron microscope grid mesh. The mice of Group A received intraperitoneally 5 mg of the levan solution every fourth day until palpable peripheral lymph nodes were observed. Including the initial injection, 3 injections of levan were given.

(b) Ehrlich Ascites Tumor: Female or male CFW mice, generally 6 to 8 weeks old, were used,

I. Experimental mice received by tail vein 5 mg of the levan solution. Two to 5 hours later these mice and the controls received subcutaneously 0.3 ml of a suspension of Ehrlich Ascites Tumor cells. The tumor cells were obtained from the peritoneal cavity of mice 7 to 9 days after implantation. Thereafter, the experimental animals received one injection of levan 4 days later, one group by tail vein, another group, intraperitoneally. In the final experiment of this series one group of 11 mice received one levan injection by tail vein 4 days later and 10 mice of another group received the levan by tail vein daily for 1 to 4 days.

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† Levan ("Hestrin IV") was kindly donated by Dr. Louis Pillemer.

TABLE I. Effect of Levan on Establishment of Solid Phase Tumors in CFW Mice Receiving Ehrlich Ascites Tumor Cells Subcutaneously.*

Exp.	Controls†	Levan on 4th day‡ I.P. route	Levan on 4th day‡ I.V. route
1	4/4	6/6	Not done
2	6/6	6/6	5/5
3	7/7	6/6	5/5
		Levan by I.V. route	
		On 4th day	On days 1 and 4	On days 1, 2, 3, 4
4	12/12	11/11	3/3	7/7

* All mice initially given levan by tail vein, followed by subcut. inj. of tumor cells.
† Numerators = No. of tumors. Denominators = No. of animals in each group.
‡ No. of days after initial injection.

II. An aliquot of a suspension of the tumor cells was mixed with an equal volume of the levan solution and immediately injected into mice either subcutaneously or intraperitoneally. A second aliquot of the tumor cell suspension was mixed with the levan and, after 5 minutes, was similarly injected. Control mice received only the tumor cells, either subcutaneously or intraperitoneally. There were 5 mice in each of these 6 groups.

Results. (a) Leukemia: Palpable peripheral lymph nodes began to appear in both control and levan-treated mice 7 days after the injection of the suspension of leukemic cells. Two days later one levan-treated mouse was dead and all the remaining 21 mice had palpable peripheral lymph nodes. The levan-treated mice all died 9 to 13 days after the injection of cells, while the controls died 12 to 16 days after the injection of cells.

(b) Ehrlich Ascites Tumor: I. From Table I it can be seen that the injection of levan did not inhibit the formation of tumors: All mice, control and levan-treated, developed tumors at the site of inoculation of the Ehrlich Ascites Tumor cells. All of these animals were kept 2 weeks or longer. No tumors were observed to regress. In addition, there was no apparent enhancement of tumor formation.

II. The direct mixing of levan and the tumor cells and their subsequent injection, immediate or delayed, did not prevent tumor growth in any of the mice injected either by the subcutaneous or intraperitoneal routes. All subcutaneously injected mice developed tumors at the site of inoculation. One of the levan-treated mice died 7 days after the injection. It had a small tumor at the site of inoculation. The remaining 14 mice were sacrificed 54 days after the injection and no tumor was observed to have regressed. Of the 15 mice receiving the intraperitoneal injection, 14 developed ascites and died from 9 to 26 days after the injection, which is not uncommon among CFW mice used for serial transfer of this tumor. One mouse had been killed 7 days after the injection and its tumor cells served as seed for serial transfer. In Table II are the survival times of the mice of the 3 groups.

Discussion. (a) Leukemia: In this laboratory the transmission of leukemia in C58 mice has always been 100% successful. Since mice injected with the same suspension of leukemic cells may succumb to leukemia over a period of 7 days or more, the slight difference in the times of death of the controls and levan-treated animals is not considered significant. From this experiment it is apparent that the course of the leukemia was not altered by the levan.

(b) Ehrlich Ascites Tumor: This tumor had been implanted by intraperitoneal injection 59 times in CFW mice prior to the beginning of these studies. It was introduced in this laboratory by one of the authors (W.B.W.) after it had been maintained for several years in the Stocker strain of Swiss Albino mice. After subcutaneous injection into CFW mice the ascites cells give rise to solid phase tumors at the site of inoculation. During the course of these experiments 126 mice, including the 78 listed in Table I, were injected subcutaneously with Ehrlich Ascites Tumor cells. One hundred and fourteen (90%) mice developed tumors, while 12 mice failed to develop tumors. None of these tumors was observed to regress. Since tumor establishment was not inhibited by the levan, whether injected independently of or after direct contact with the tumor cells, it would seem that the postulated ESA of the levan had no effect on tumor development in this study. The effect of repeated levan injections on the properdin level is unknown. From Table I it is seen that the results were

TABLE II. Time of Death, after Intraperitoneal Injection, of Mice Bearing the Ehrlich Ascites Tumor.

Mouse	Days after inj.
Control	7*
"	9
A	15
Control	16
A	16
A	16
B	16
B	16
B	18
A	18
B	20
B	20
Control	20
A	21
Control	26

* Killed. Used as seed for serial transfer.
A = Levan-cell mixture inj. immediately.
B = " " " " after 5 min.

the same with 2 to 5 injections (including the initial injection) of levan. The incidence of successful intraperitoneal transmission of the Ehrlich Ascites Tumor in CFW mice is greater than 98% in this laboratory: Of 271 mice used for its serial transfer not more than 3 unsuccessful transfers have been encountered and none since the forty-sixth transfer. Because of this high incidence of successful transfers, this form of the tumor was considered satisfactory for study of the effect of levan on tumor cells after direct mixing. Since survival times of the mice in the 2 experimental groups listed in Table II were not prolonged as compared to the controls, it would appear that the levan did not prevent the establishment of the ascites form of the tumor.

Summary. Injections of levan intraperitoneally into C58 mice did not alter the course of transmitted leukemia. Levan injected by tail vein or intraperitoneally failed to inhibit and did not apparently enhance establishment of a solid phase tumor at the site of inoculation of Ehrlich Ascites Tumor cells. The direct action of levan on the tumor cells injected immediately or after a 5 minute delay did not result in inhibition of either solid phase tumors or the ascites tumor.

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Platelets XVIII. Relationship of Platelets to Activity of 5-hydroxytryptamine Creatinine Sulfate.* (22615)

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It was originally thought that 5-hydroxytryptamine creatinine sulfate (5-HT) is an intimate constituent of platelets(1,2). This concept has more recently been revised to show that, like other factors(3), 5-HT may be only absorbed to platelets(4). However, the exact role of platelets in the metabolism of 5-HT remains controversial.

We have recently shown that 5-HT has a powerful local venopressor effect. Thus, measurement of local venous pressure is a simple and reliable method, detecting as little as 0.3 γ/5-HT administered in 1 minute time (5). In an effort to elucidate the relationship

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