fluid. Venoconstriction associated with increased perfusion pressure would lead to increased venous pressure. The close negative correlation between finger distensibility and venous pressure lend support to the above view. The experiments support the hypothesis that increased venomotor tone is the cause of decreased distensibility of fingers immersed in cold water.

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Effect of Glucan on Mouse Sarcoma 37.* (29508)

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Our earlier studies dealt with oncolytic effects of various fungi, including yeasts and yeast-like organisms, and their carbohydrate derivatives(1,3,4,6). Of these agents, the most effective in anti-tumor activity was hydroglucan(2), the active component of which was considered to be glucan, from which it was prepared by Nickerson(5). Glucan has been shown by Riggi and DiLuzio(8) to be a powerful stimulator of the reticulo-endothelial system. The supply of hydroglucan, which was available to us in experimental quantities only, is now exhausted. Before going ahead with the preparation of a fresh supply, it seemed advisable to test the source material, glucan, for possible oncolytic activity.

Material and methods. Samples of purified glucan were kindly supplied us by Standard Brands, Inc., New York City. Glucan, like hydroglucan and zymosan, is insoluble in water, physiological saline, or alcohol, and must be injected into animals in the form of a suspension, as proposed by Pillemer *et al* (7) for zymosan. Glucan (100 mg in 100 volumes of water) is maintained at boiling temperature in a water bath for one hour and then centrifuged at 4000 rpm for 30 minutes. The supernatant is discarded and replaced by an equal volume of distilled water in which the residue is resuspended. To avoid contamination, we autoclave the preparations for 30 minutes at 15 lb pressure in small lots just before using.

The tumor-destructive capacity of glucan was tested against Sarcoma 37 in solid and ascites form transplanted into ICR/albino mice (both males and females, avg wt 25-30 g); against mammary tumors arising spontaneously in C3HNIcr mice; and against a transplantable form of the mammary tumor derived in our laboratory and maintained in the same mouse stock. Treatment of subcutaneous implants of Sarcoma 37 was begun one week after implantation, when the vascular system was well established. In the case of ascites tumors, treatment was initiated as soon as abdominal swelling was discernible. Both spontaneous and transplanted mammary tumors were allowed to grow until they were approximately one centimeter at the base before starting treatment.

Tissues of responsive and resistant tumors and of host organs were preserved in a picro-formol-acetic mixture, sectioned in paraffin, and stained in hematoxylin and eosin. These preparations were examined by our staff pathologist.

Results. Activity against established tumors. The ability of glucan to induce regression of subcutaneously implanted Sarcoma 37 in comparison with hydroglucan is indicated

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TABLE I. Comparison of Effect of Glucan *vs* Hydroglucan on Subcutaneously Implanted Sarcoma 37.

Agent	Route of inj	No. mice	Sex	Dosage: No. doses	Dosage: Amt/dose, ml	Dosage: Total, mg	Mortality at 1 wk	Tumor regressions: No.	Tumor regressions: %
Glucan	I.V.	50	♂	5	.2	1.00	1	46	92
	"	50	♀	5	.2	1.00	0	49	98
Hydrog.	"	50	♂	5	.2	1.00	0	49	98
	"	50	♀	5	.2	1.00	0	48	96
Glucan	I.P.	20	♂/♀	5	.2	1.00	0	14	70
Hydrog.	"	20	♂/♀	5	.2	1.00	1	16	80
Glucan	Subcut.	50	♂/♀	5	.2	1.00	0	30	60
Hydrog.	"	50	♂/♀	1	1.0	1.00	0	28	56
None	—	100	♂/♀	—	Controls		0	3	3

TABLE II. Effect of Glucan Injection (1 mg/ml) Prior to Tumor Implantation on Growth of Sarcoma 37.

No. mice	Route of inj	No. doses	Dosage: Amt/dose	Dosage: Total, mg	Tumor implanted	Tumor takes	Regressions: No.	Regressions: %
30	I.V.	5	.2 ml in saline susp.	1.00	One wk post-treatment	30	24/30	80
30	Subcut.	5	.2 ml in mineral oil	1.00	*Idem*	30	20/30	66
30	"	5	.2 ml in saline susp.	1.00	"	30	13/30	43
30	I.M.	5	.2 ml in mineral oil	1.00	"	30	16/30	55
30	None	—	Controls		On same day as experimentals	30	0/30	0

in Table I. As was the case with hydroglucan(2,5) over 90% of the implants regressed following intravenous glucan injection. Single or multiple doses totaling, in terms of larger animals, approximately 6.5 mg/kg were effective by the intravenous route; intraperitoneal and intramuscular injection of equivalent amounts had the same effect as lowering the dose, with a corresponding reduction in the number of tumors responding. Again, the results were identical to those obtained with hydroglucan, *e.g.* the introduction of one milligram of glucan subcutaneously into a 50 g mouse resulted in 60% tumor regression; the same amount of hydroglucan by the same route caused 58% of the treated tumors to be sloughed. There was no significant difference in response of males *vs* females to either of these carbohydrates. Such slight differences as appear in the Table are probably referable to the higher incidence of spontaneous sloughing often noted in females.

Sarcoma 37 in the ascites form was not affected by injection of glucan and survival time was not increased over that of control mice. Neither spontaneous mammary tumors in C3HNIcr mice, nor the transplantable tumor derived from this tumor stock, responded to glucan. Since the results obtained with the tumors tested were indistinguishable from those with hydroglucan, it was not considered necessary to repeat the entire spectrum of tumors previously studied(2).

Effect of pre-injection on subsequent growth of tumor implants. When mice were injected with glucan prior to implantation of Sarcoma 37 (Table II) the subsequent implants grew at the same rate as controls for approximately one week. At this time growth was slowed in a large percentage of tumors, varying with route of injection. Up to 80% regression was obtained in mice injected intravenously (5 injections of 0.2 mg each). Subcutaneous injection of glucan also caused tumors to be sloughed (66% when glucan was suspended in mineral oil, as opposed to 43% in saline solution). Subcutaneous and intramuscular pre-injections were about equally effective.

TABLE III. Toxicity of Glucan to Non-Tumor-Bearing Mice.

No. mice	Avg wt, g	Dosage: No. doses	Dosage: Amt/dose, ml	Dosage: Total, mg	Amt/g of body wt, mg	Lethality: No.	Lethality: %
20	25	1	1.00	1.00	.08	20/20	100
20	20–25	1	1.00	1.00	.04–.05	20/20	100
30	30	1	1.00	2.00	.06	15/30	50
20	30	1	1.00	1.50	.05	4/20	20
20	30	1	1.00	.75	.025	0/20	0
20	30	5	.20	1.00	.03	0/20	0
20	50	5	.50	2.50	.05	0/20	0

Toxicity. There was no evidence, either macroscopic or microscopic, of toxicity at tumor-necrotizing levels (0.005-0.006 mg/g of body weight). The occasional deaths immediately following injection, *e.g.* 2 in 390 mice shown in Table I, are believed to be accidents of injection. In an experiment involving 100 tumor-bearing mice, 4 with unresponsive tumors died in the 5 weeks following cessation of treatment, but only one mouse that had sloughed its tumor succumbed in the same period. Five tumor-free mice in a single cage died 2 months later. However, none of these mice had evidence of disease other than pneumonitis, and it is presumed that they died of a respiratory infection not necessarily related to glucan injection. Sixteen of the remaining tumor-free survivors were set aside for periodic examination. One mouse died at 13 months post-treatment and the remainder were sacrificed for microscopic study. No tissue changes arising from glucan treatment could be observed with certainty.

A large series of non-tumor-bearing ICR/albino mice was tested for toxic response to varying amounts of intravenously injected glucan. Some of the results are presented in Table III, where it will be noted that the amount that could be tolerated was closely related to the body weight of the mouse. For example, a total dose of 0.05 mg/g of body weight was lethal for all 20-g mice, but killed no mice weighing 50 g. For mice weighing 30 g, which is the average for 6-week-old mice ordinarily employed in our experiments, the L.D. 50 was established at 0.06 mg/g, *i.e.* 10-fold the tumor-destructive dose. Death following a lethal dose of glucan occurred within 5-15 minutes after intravenous injection and was accompanied by convulsions and spinning reactions usually associated with cerebral changes.

Microscopic observations. The response of tumor tissues was similar to that following injection of hydroglucan(1). The changes were non-specific without evidence of hemorrhage.

Although the almost instant death of mice that had received large doses of glucan, together with convulsions and behavior that might indicate brain changes, suggested mechanical blockage of capillaries with attendant anoxia, microscopic evidence for this was lacking. Even in very small mice, there was no distention of vascular walls and no clumping of granules could be observed; in fact, glucan particles could not be visualized in the tissues of treated mice. In the case of hydroglucan, the irregular-shaped particles had a tendency to clump *in vitro*, and occasionally mice treated with hydroglucan developed small lung abscesses presumably associated with local deposition of the carbohydrate substance(2). Glucan particles appeared to be more uniform in size and more evenly dispersed in suspension. Whether or not particle clumping is related to lung pathology, it is true that only 2 cases of microabscesses were observed in lungs of glucan-treated mice.

Tissues were examined from mice that had sloughed their tumors following glucan injection and had either died or been sacrificed at 2 weeks, 3 weeks, 1 month, 2 months, 6 months, 9 months and 1 year following cessation of treatment. No changes that could be related to treatment were observed. Lungs of mice that had died at varying intervals following loss of tumor were lacking in specific disease changes, though there was pneu-

monitis, lung congestion or generalized hyperemia in most cases. However, these changes were commonly observed in control mice also. Amyloid deposition was noted occasionally in liver and spleen. In 2 animals bloody cysts were encountered in association with enlarged lymph nodes; and in 3 others inflammatory lesions were found in the region of the aorta. Whether these represent sites of localization of glucan particles is not known. There is no evidence, therefore, that the deaths at various times following tumor-necrotizing doses of glucan were related to response of the hosts to the carbohydrate, and no light is thrown on the immediate response to lethal doses through microscopic evaluation of the tissues.

Summary. Hydroglucan, a carbohydrate substance derived by Dr. W. J. Nickerson of Rutgers University, from glucan (a component of the yeast cell wall) had previously proven to be an effective oncolytic agent against certain experimental mouse tumors. In the present studies, glucan is shown to be equally effective against the same tumors, and to have no observable toxic effects at tumor-destructive levels.

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Effect of Macrophages on Primary Immunization of Lymph Node Cells Against Homografts in Diffusion Chambers.* (29509)

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Primary immunization in a partially isolated system has been obtained by incubating normal rabbit lymph node fragments with homologous skin in standardized Millipore diffusion chambers maintained in an indifferent host(1). The immunized cells, on injection intradermally into the original skin donor, were found to produce a localized graft-*vs*-host lesion, the *transfer reaction,* comparable to that produced by cells immunized *in vivo*(2,3,4). This lesion is specific and provides a measure of the degree of homograft immunity achieved. The cells, whether immunized in chambers or *in vivo,* may act by transferring information to cells of the animal in which the lesion is actually elicited. This is suggested by several findings: that their reactive capacity is destroyed by RNase(5), that crude RNA prepared from them can endow normal rabbit lymph node cells with specific reactivity(6), and that the principal cell type participating in lesion formation is derived from the host and not from the transferred cells(7).

It has been frequently suggested, on purely morphologic grounds, that primary im-

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