Relation between Time of Conditioning of Host and Survival of Tumor Homografts in Mice.* (21025)

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Successful and progressive growth of homografts of a transplantable tumor has been induced in normally resistant mice of an inbred strain by pretreating the hosts with killed homologous tumor tissue, normal serum, or antiserums to the tissues(1-4). (The term *homograft,* as used here, denotes a graft between animals of the same species, but of differing genetic backgrounds.)

The present investigation, one of a series directed toward elucidating the processes underlying the abrogation of resistance, deals with a study of the necessary time relationships, between treatment (conditioning) of the otherwise resistant host and inoculation of the tumor homograft, for successful survival of the graft. It was motivated by the observation that tumor homografts often exhibit marked growth before regression.

Materials and methods. The transplantable tumor was Sarcoma I, which is indigenous to the A strain of mice. When inoculated subcutaneously, it grows progressively in 100% of these mice, killing the hosts within 3 to 5 weeks. Hosts were from the C57BL/6Ks and C57BR/aSn strains. They are genetically unrelated to the A strain, and also differ from each other so that transplantable tumors indigenous to one strain will not survive in the other. Sarcoma I rarely survives in untreated mice of these strains. All mice were approximately 2 to 4 months old at the start of the experiments. The hosts were conditioned by intraperitoneal injections of supernatant of a centrifuged homogenate of Sarcoma I prepared according to a method previously described(5). Whole tumor, stored in the frozen state (–23°C), was homogenized with an approximately equal weight of 0.85% NaCl in a high-speed blendor. The homogenate was then centrifuged at 4000 x g for 20 minutes at a temperature of 4°C. The supernatant was recentrifuged at room temperature for 20 minutes at an average RCF of 11,000 x g. The second supernatant was stored frozen (–23°C) until needed. The grafting of live tumor was done by trocar under aseptic conditions. A small bit of tumor was placed subcutaneously in the interscapular region. The course of growth of the grafts was followed by periodic palpation until the animals either died with a progressively growing tumor or showed no gross signs of a graft for a consecutive period of 2 months, at which time they were sacrificed as negative.

Experimental findings. The time of survival of homografts of Sarcoma I in the untreated host was first determined. This was done by excising the subcutaneous bed in the region of the tumor inoculum, and regrafting

TABLE I. Viability of Homografts of Strain A Tumor, Sarcoma I, in Untreated C57BL/6Ks and C57BR/aSn Mice as Determined by Regrafting into Strain A/Ks Mice.*

Elapsed time between homografting and excision for regrafting (days)	No. of viable homografts as shown by No. of A/Ks recipients dying with tumors received from†	
	C57BL/6Ks donors	C57BR/aSn donors
3	10/10	10/10
7	10/10	10/10
10	9/10	10/10
15	4/10	0/10
21	1/12	—

* Bits of live tumor inoculated subcutaneously by trocar into the foreign strains (C57BL/6Ks and C57BR/aSn Mice). Tumor bed excised at stated number of days after inoculation and regrafted into mice of the A strain to test for viability.

† Numerators represent total No. of mice dying with tumors; denominators, total No. of mice grafted. Each A/Ks recipient received a graft from one donor.

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this material subcutaneously into mice of the indigenous A strain. Regrafting was done under aseptic conditions by nicking the skin in the mid-dorsum with scissors to provide a point of entry, and placing the inoculum subcutaneously with a pair of forceps. One strain A/Ks host was used for each C57BL/6Ks or C57BR/aSn donor. Survival in the foreign host was determined by positive growth of the regrafted tumor in the strain A recipient. (This test for survival was suggested by a similar procedure described by Mitchison(6).)

It is clear that tumor cells will survive at least as long as 10 to 15 days, and probably longer in the untreated foreign hosts (Table I). Actually there must be a longer survival period than is here indicated since it has been determined(7) that there is a given minimum number of cells necessary in an inoculum to obtain growth even in mice of the indigenous strain.

The effect of varying the time of injection of tumor supernatant (with respect to the time of grafting of live tumor) on the survival of tumor homografts is shown in Table II. A single injection of one ml of tumor supernatant was given each mouse on the days indicated in the table. It is clear that the normal resistance of the host is abrogated only if the animals are conditioned prior to tumor grafting, or, for the C57BR/aSn mice, at most one day after grafting, even though the grafts can be assumed to survive for as long as 2 weeks or more (Table I).

TABLE II. Relationship between Time of Conditioning of the Host and the Survival of Tumor Homografts.

Interval between inj. of tumor supernatant and grafting of tumor*	No. of mice dying with progressively growing tumors†	
	C57BL/6Ks hosts	C57BR/aSn hosts
Days before grafting		
7	10/11	6/10
5	6/11	3/ 9
3	5/11	5/10
1	6/11	15/20‡
0	—	2/10
Days after grafting		
1	0/11	3/19‡
4	0/11	0/ 9
7	0/11	0/10

* Tumor supernate, derived from homogenate of Sarcoma I, administered as a single intraperitoneal injection.

† Numerators are No. dying; denominators, total No. of mice in each group.

‡ Data from 2 exp.

TABLE III. Time of Duration of Conditioning as Measured by the Growth of Strain A Tumor, Sarcoma I, in C57BL/6Ks Mice.

Time interval between inj. of tumor supernatant and grafting of live tumor	No. of C57BL/6Ks mice dying with tumors	
	Treated	Uninj. controls
10 days	19/20	1/10
6 wk	8/10	1/10
10	8/10	0/ 6
14	12/12	0/ 6
19	10/10	0/ 6
23	6/11	0/ 6
28	7/10	1/12
32	9/12	0/ 6
36	5/10	0/ 6
40	5/10	0/ 6

The duration of the conditioning effect (Table III) was determined in C57BL/6Ks mice by inoculating different groups of mice with live tumor at increasing time intervals after treatment with tumor supernatant. The mice were from a group of 300 animals that had been used to produce antiserum to Sarcoma I incidental to other experiments to be reported elsewhere. In April 1953, they had received 4 intraperitoneal injections per animal of 0.5 ml each of tumor supernatant given over a consecutive period of 2 weeks. A group of 20 of these mice received grafts of live Sarcoma I 10 days after the last injection of the tumor supernatant, and thereafter groups of 10 to 12 mice each were grafted at approximately monthly intervals. Tumor was grafted simultaneously into untreated control mice of comparable age. At the time of this writing, progressive growth of homografts is evident in 6 of 11 mice which had been inoculated with live tumor 45 weeks after being injected with the tumor supernatant.

Discussion. Our data indicate that the normal immune reactions to the tumor homografts are negated only if the animals are conditioned prior to tumor inoculation. Since the homografts survive at least for as long as 7 days in all the untreated mice, tumor super-

natant injected as recently as one day before tumor grafting would have a sufficient period to initiate the processes (which may require some time for their development) leading to abrogation of resistance in the host. In this sense, the conditioning process resembles the time relationship observed in the preparation of animals to produce immune sera. The extremely long period over which the effects of conditioning persist is of interest in this connection. It is hardly likely that tissue antigens would survive in the host over so long a time (at least 45 weeks in these experiments). If an immune reaction is involved in conditioning (and at present this is a moot point), the inoculation of the live tumor graft may elicit an anamnestic response.

Summary. In previous studies, it has been shown that in many instances the normal resistance of mice of an inbred strain to homografts of a tumor can be abrogated by injecting the host with an extract of tumor tissue. It is here shown that such an injection must be given prior to tumor inoculation, or at most one day after inoculation, to obtain loss of resistance. The effects of such a procedure persist for long periods, as shown by progressive growth of tumor homografts in 80-100% of mice grafted approximately 5 months after receiving injections of tumor extract, and in at least 50% of the mice grafted up to 10 months after treatment with extract.

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Vitamin B_{12} Activity of Plasma and Whole Blood from Various Animals.* (21026)

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Numerous reports have recently appeared concerning the vit. B_{12} activity of whole blood (1-4) and blood plasma or serum(5-8) in a variety of animal species. With the exception of the study by Yamamoto *et al.*(9) which indicated that dog erythrocytes contain no microbiological B_{12} activity, information concerning the distribution of vit. B_{12} between the erythrocytes and plasma of blood is lacking.

It seemed of interest, therefore, to study the distribution of vit. B_{12} activity between the cellular fraction and plasma of blood from a variety of animals including mammals, birds and reptiles. The results of this investigation are presented in this report.

Materials and methods. Blood was obtained from the human, rabbit, dog, calf, chicken and alligator in oxalated or heparinized containers. The samples were divided into two portions and one of the portions was

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