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Failure of Homozygous Embryo Skin to Prevent Growth of Autogenous Tumor-Grafts in the Rat.*

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A question of considerable interest was brought to my attention on the subject of induced resistance. The question was whether induced resistance can be produced by normal tissues. This concerns the relationship between the immunizing agent and the genetic antecedent of the host. For example, a number of investigators[1-11] were able to induce a resistant state to malignant growths including leukemia in hybrid animals by injecting normal homologous tissues, such as liver, spleen, blood, and embryo skin. On the other hand, similar results were not obtained[12-14] when pure lines of inbred strains of animals were used. From the two sets of observations, it was concluded that the degree of induced resistance which can be produced depends upon the genetic relationship between the host and the immunizing agent, and that no resistance to tumor growth can be produced either with normal or with malignant tissues in animals of a pure inbred line.

However, the author has reported[15] that there is a possibility of inducing immunity in animals of a pure line to a tumor which originated in the same strain, provided the tumor grafts had been previously attenuated *in vitro* with specific doses of X-rays.

It was of interest to investigate whether or not the same phenomenon could be demonstrated by the use of normal embryo skin of the same pure inbred strain of rats, for it has been shown[6,8,11,14] that embryo skin is the most effective agent among normal tissues in this respect.

The technic employed here was similar to that employed by previous investigators[14] and consisted of the following: the skin of rat embryos was removed under strictly aseptic conditions, cut with sharp scissors into minute particles and a concentrated suspension prepared in a small amount of 0.85% saline solution. Portions of 0.3 cc of this suspension were injected subcutaneously in the right side of a rat which had been previously shaved and cleaned with alcohol. Twelve days later, the left sides of the same rats were implanted by means of a trocar with grafts weighing about 3-4 mg of the reticulum cell type lymphosarcoma which originated in the same strain. As controls 6 rats of the same strain were implanted with grafts of the same tumor. All the rats of these experiments, both experimental and control, were males. The same criteria were used in the evaluation of the

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TABLE I.
Tumor Takes in Rats Inoculated with Embryo Skin (of the same strain) Prior to Tumor Implantation.

No. of rats	Latent period, days	Initial tumor size, mm	Maximum tumor size, mm	Remarks
1	10	8x7x6	35x18x14	Died 24 days following implantation of tumor.
2	10	5x4x4	30x16x14	Died 24 days following tumor transplantation.
3	10	6x5x4	33x23x20	" 26 " " " "
4	10	6x6x5	28x20x19	" " " " " "
5	10	7x6x5	36x25x18	" " " " " "
6	12	6x6x4	35x24x20	" 28 " " " "
7	12	4x4x3	38x25x24	" " " " " "
8	12	6x4x4	28x25x29	Killed for tumor transplantation.
9	12	5x4x4	29x21x18	All these rats died within 30 to 40 days following implantation of the tumor.
10	14	6x5x4	32x26x22	
11	14	4x4x3	28x24x16	
12	14	5x5x4	30x25x20	
13	14	6x4x4	28x22x18	
14	14	6x5x4	32x25x19	
15	No growth occurred			This rat was resistant to the primary and to subsequent tumor grafts.

results as in the previous experiments,[15] namely: the percentage of takes, the latent period of the takes, and the initial and maximum tumor size.

In Table I are recorded the observations made on the rats treated with homologous embryo skin prior to tumor grafting and in Table II those of controls. A comparative analysis of the data recorded in these tables reveals the following: Of the 15 experimental rats in Table I 14 developed tumors following a latent period from 10 to 14 days, while among the 6 control rats recorded in Table II all developed tumors following a latent period of 12 days. Judged by the initial and maximum tumor sizes of both experimental and control experiments, the tumors developed at approximately the same rate until the death of the animals.

No significance can be attributed to the fact that one of the 15 rats treated with embryo skin was refractory to the tumor grafts, or to the extended latent period of 14 days among some of these rats (Table I) instead of 10 to 12 days occurring among controls (Table II) in regard to the immunizing effect of the embryo skin. Such single instances were repeatedly observed among control animals of previous experiments. This observation holds particularly true where larger numbers of animals are being used. Rat 15, which proved to be refractory to the first tumor graft, was found likewise to be refractory to subsequent tumor implants. Such a phenomenon has been repeatedly observed among rats of previous experiments. If the first graft fails to produce a tumor, the animal remains refractory to subsequent tumor grafts.

It seems safe to conclude, from the ob-

TABLE II.
Tumor Takes in Control Rats of the Same Strain.‡

No. of rats	Latent period, days*	Initial tumor size, mm	Max. tumor size, mm†
1	10	5x5x4	31x18x15
2	10	7x5x4	35x22x17
3	10	6x4x4	34x19x15
4	12	5x5x4	28x20x16
5	12	5x4x4	34x30x19
6	12	7x5x4	38x29x22

* Latent period designates time elapsing between implantation of tumor grafts and first appearance of a detectable tumor.
† Maximum tumor size reached at death of animal.
‡ All rats died within 25 to 40 days following implantation of tumor grafts.

servations recorded in the Tables I and II, that inoculations of embryo skin into rats of the same genetic constitution, fail to elicit a resistant state to grafts of a tumor autogenous to the strain. This observation is in accord with findings of previous investigators who likewise failed to elicit immunity in a pure line of animals with embryo skin of the same genetic line.[14]

Discussion. There is no intention here to discuss generally the genetic principles involved in the problem of induced resistance to malignant growth. As indicated in the previous communication[15] the results obtained are specifically concerned with the reticulum cell type lymphosarcoma, originating in a pure line of rats of Dr. Bagg. It was suggested that the type of tumor may be responsible for the specific immune action in an inbred strain of rats. The results obtained from the experiment described in the present paper brought further evidence, in this case, that the induced resistance is specific to the type of tumor and independent of the genetic inter-relation between the host and the immunizing agent, inasmuch as embryo skin of the same strain proved to be ineffective in eliciting the same phenomenon.

On the other hand the results of the present paper are in full agreement with those obtained by other investigators, showing that no resistance to tumor grafts can be elicited in animals of a pure line with embryo skin of the same strain.

Summary. Rats of a pure inbred line treated with embryo skin of the same strain failed to produce a resistant state to grafts of a tumor autogenous to the strain.

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Shock Produced by the Application of Tourniquets to the Hind Limbs of Rats.*

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The tourniquet method has been accepted as a valid means of producing experimental shock and numerous investigators have used some variety of tourniquets on laboratory animals, including albino rats. Some investigators have used rubber bands,[1,2] cord tied tightly or wrapped in numerous turns about the leg,[3-8] and compression from clamp devices to cut off the circulation to a limb.[9,10] We were interested in standardizing the tourniquet method so that graded and reproducible degrees of trauma could be effected. Animals shocked by this method were to be used in making biochemical studies for comparison with similar studies[11,12] on rats shocked by the

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