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Induced Resistance in Inbred Homozygous Rats to a Lymphosarcoma Autogenous to the Strain*

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In previous studies with mouse sarcoma 180[1] it was found possible to induce a resistant state in hybrid mice by implants attenuated by specific doses of radiation. The results of these studies may be briefly summarized as follows:

A dose of about 60,000 r in air was found to be required to prevent the proliferation of the sarcoma cells in tissue culture, *i.e., in vitro.* Later it was also shown that this dose was destructive for their immunizing action *in vivo.* Implants of the same type of tumor irradiated with doses of about 5,000 r in air, although failing to produce a detectable tumor *in situ,* nevertheless rendered the animal immune to further viable implants. Tumor grafts irradiated with doses smaller than about 5,000 r in air produced tumors following inoculation.

The present study was concerned with the question as to whether or not this phenomenon can be applied to tumors of a known genetic origin, grown in an inbred strain of animals. The importance of this question is realized when one considers the problem of inducing resistance to spontaneous tumors. Accordingly, animals of an inbred strain were used and a type of tumor which originated spontaneously in the same strain. These were obtained from Dr. Halsey J. Bagg with the following data:

The rats were bred by continuous brother and sister mating for more than 15 years and hence could be considered homozygous. The tumor, which originated in this strain of rats, and had been diagnosed by the late Dr. James Ewing as a reticulum cell type lymphosarcoma, had reached the 75th generation of transfer at the start of this experiment. The tumor takes were practically 100% in young rats. Spontaneous regression very rarely occurred among these animals.

Experimental. A rat bearing a 10-day-old lymphosarcoma at its 75th generation of transfer and 8 normal male rats 6 weeks old were supplied by Dr. H. J. Bagg at the start of these experiments. The tumor was removed from the rat under strictly aseptic conditions and cut into small fragments with sharp scissors. Six male rats were implanted by means of a trocar, each receiving one tumor fragment placed under the right axilla. All 6 rats developed tumors 8 to 10 days following implantation. The tumors grew progressively during the next 8 days, thus establishing 100% takes and progressive growth of the tumor in a new environment. Later quantities of rats were periodically supplied by Dr. Bagg. Then the experiment proper was started.

The technical procedure used here was the same as in the previous experiments with mouse sarcoma 180[1] of which a concise summary will be given here.

For each experiment a 10- to 12-day-old tumor, removed from the rat under strictly aseptic conditions was used. This was cut into small fragments with very sharp scissors. Portions of the tumor fragments were spread on a No. 1 round coverslip which had previously been attached to a square mica sheet, and were covered with a Maximow slide and sealed with paraffin. To avoid any evaporation of the tumor-tissue water, a fragment of moist filter paper was placed in the concavity of the Maximow slide. Physical factors for irradiation were: 200 KV, 20 ma, 0.5 mm Cu, 1.0 mm. Al and a H.V.L. equivalent to 0.9 mm Cu. Immediately after irradiation the tumor fragments were implanted into a certain

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1 Goldfeder, Anna, *Radiology,* 1942, **39**, 426.

TABLE I.
Effects of Filtered X-rays on Implants of Bagg's Reticulum Cell Type Lymphosarcoma (200 KV 20 ma ½ mm Cu + 1 mm Al. H.V.L. equal to 0.9 mm Cu).

Exp. No.	r in air*	Takes, %	Latent period, days	Initial tumor size, mm	Max. tumor size, mm	Remarks†
1	500	100	8	9x9x6	32x28x19	
2	1000	100	8-10	8x8x5	29x26x17	Max. size of tumor designates size tumor reached 2 weeks after first measurement.
3	1500	100	9-10	7x6x4	18x16x12	
4	2000	100	14	7x4x4	16x12x8	With increase of X-ray dose applied to implants, an increase of latent period and a decrease of max. tumor size occurred.
5	2500	60	18	6x6x4	12x9x6	
6	3000	0	0	0	0	

* r in air—roentgen units in air.
† Each experiment consisted of 6 to 8 rats.

TABLE II.
Resistance Induced in Homozygous Rats to a Lymphosarcoma Autogenous to the Strain.

Exp. No.	r in air*	No. of implanted rats	No. of takes	No. of re-implanted rats	No. of resistant rats	Remarks
1	2200	10	7	3	3	One of re-implanted rats produced a tumor which regressed after having reached a size of 12x10x7 mm. This rat became likewise immune.
2	2300	8	6	2	2	
3	2400	8	6	2	2	
4	2500	8	5	3	3	One of re-implanted rats produced a tumor which regressed after having reached a size of 15x19x8 mm. This rat became also immune.
5	2600	8	2	6	5	Two of the re-implanted rats produced tumors which eventually regressed, rendering the animals immune.
6	2300	8	5	3	3	

* r in air—roentgen units in air.

number of rats by means of a trocar. Only one tumor graft was implanted into each rat. Since the bore of the trocar was about 2.0 mm, the tumor grafts were approximately of the same size for each rat. The time elapsing from the removal of the tumor from the animal and implantation of the irradiated tumor fragments was about one hour. The same technical procedure was used for control animals except that these were implanted with non-irradiated tumor grafts.

Since the radiosensitivity of this type of tumor was not known, the first task was to determine the lethal and sublethal doses for the proliferation of tumor-grafts *in situ*. The same criterion for radiation effects were used here as in previous experiments,[1] namely: The latent period (which designates the time elapsing between the implantation of the tumor fragments and the first occurrence of a detectable tumor in the animal); the percentages of takes and size of tumor, initial and maximum. The time of 2 weeks elapsing from the first measurements of the tumors was arbitrarily

designated the maximum size. This was done because the lymphosarcoma type of tumor is fast growing and after 2 weeks proliferation in the animal, it starts to break through the skin.

Results. In Table I are recorded the effects of filtered X-rays on implants of the reticulum cell type lymphosarcoma. Since the first task was to determine the approximate sublethal, and lethal dose of irradiation, the exposures were given within ranges of 500 r in air. As can be noted in this table up to a dose of 2,000 r in air applied to the implants resulted in 100% of takes. The effects of irradiation started to be apparent with a dose of 2,000 r in air. Although 100% of takes were still produced by implants exposed to the above mentioned dose, however, the latent period was extended to 14 days instead of 8 to 10 days as occurs among control animals.

With the increase of the X-ray dose applied to the implants a sudden decrease in percentages of takes occurred with a corresponding increase of the latent period. Thus a dose of 2,500 r in air resulted in 60% of takes with a latent period of 18 days, while implants exposed to 3,000 r in air failed to produce a detectable tumor in the animals. From the results recorded in Table I it seems reasonable to assume that the threshold dose for this type of tumor would be between 2,200 and 2,600 r in air. Further experiments carried out within the above range of roentgens are recorded in Table II.

An inspection of Table II reveals the following: In Experiment 1 with a dose of 2,200 r in air 7 out of 10 rats developed tumors. The remaining 3 which failed to produce tumors after a period of 28 days were reimplanted with freshly excised viable tumor grafts. One of the 3 reimplanted rats produced a tumor which eventually regressed after having reached a size of 12x8x4 mm in diameter. This rat became likewise immune. All 3 rats are still alive and proving to be resistant to the reticulum cell type lymphosarcoma.

Experiments 2 and 3 gave similar results. Two rats of each experiment (of which the implants of Experiment 2 were exposed to 2,300 r in air and the implants of Experiment 3 were exposed to 2,400 r in air) failed to produce tumors, became refractory to further re-inoculations of viable tumor grafts.

In Experiment 4 of 8 rats which were implanted with tumor grafts exposed to 2,500 r in air 5 produced tumors. The remaining 3 rats were reimplanted. Only one of the reimplanted rats produced a tumor which later regressed after having reached a size of 15x10x8 mm in diameter. This rat became likewise resistant to fresh viable tumor grafts. All 3 rats are still alive and proving to be immune to the reticulo-cell type lymphosarcoma.

In Experiment 5 with a dose of 2,600 r in air, 2 out of 8 rats produced tumors. The remaining 6 rats were reimplanted with fresh viable tumor grafts 27 days following the first implantation of the irradiated tumor grafts. Three out of the 6 reimplanted rats produced tumors within 15 days following reimplantation of fresh viable tumor grafts. In 2 of these rats the tumors later regressed rendering both refractory to further viable tumor implants. Thus Experiment 5 resulted in 5 resistant rats.

In order to test the reproducibility of these results, an experiment with a dose of 2,300 r in air was repeated as shown in Experiment 6, Table II. In this case 3 of 8 rats became resistant to further viable tumor grafts.

The question is apparent why in each experiment only a certain percentage of animals could be made resistant although they were all implanted with tumor grafts attenuated with the same dose. This question may be explained by the following: not each tumor graft contained cells of a uniform viability. It is known for example that sensitivity of cells to a given dose of irradiation varies with the stage of mitotic activity; on the other hand, it may be assumed that the amount of implanted tumor grafts was insufficient to produce a resistant state in the animal. This assumption is supported by the fact that rats in which the tumor grew and later regressed, all became refractory to further tumor grafts. Recent tests not included in the table on 4 rats which became refractory to further viable tumor grafts 7 months ago revealed a persistent resistance. Thus, from these experiments the possibility of inducing a resistant

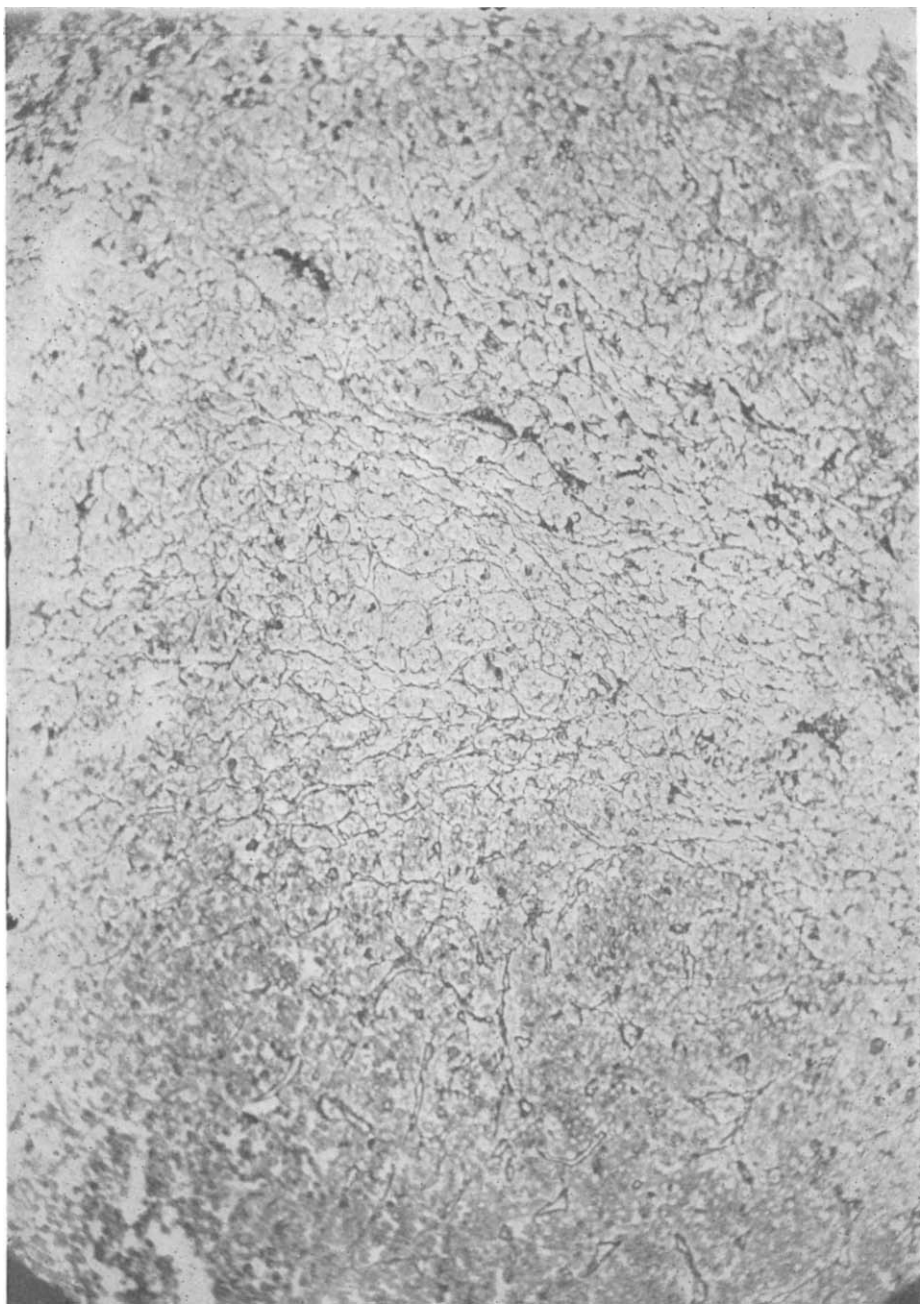

FIG. 1.
Reticulum cell type lymphosarcoma of rat. Silver stained. × 153.

state in a pure line of rats to autogenous type of tumor has been demonstrated.

It was regarded unnecessary to include a separate table of the records of the control animals, because of the fact that all the experiments gave 100% takes. In these animals the latent period varied from 8 to 10 days.

Further experimentation with a larger num-

ber of rats is in progress. A feature of this is the testing of rats which were successfully treated with X-rays on their possible immunity.

Discussion. An abundant literature has accumulated on the problem of a possible immunity to malignant growth since the classic observations of Clowes and Baeslack[2] on the existence of tumor resistance. These investigators observed that mice in which an actively growing tumor regressed spontaneously, or in which a transplantable tumor failed to "take" in the first instance, became resistant to malignant growth. However, the results obtained by subsequent investigators in pursuance of this phenomenon were confusing and contradictory.

Attempts were made to induce immunity in animals by a procedure of intracutaneous injection of tumor material. Some investigators[3,4] found this method effective, while others[5,6] failed to obtain positive results. The method of intracutaneous injection found a wide application in the work of Besredka, Gross *et al.*[7,8] These investigators reported that intracutaneous injection of Brown-Pearce carcinoma into rabbits rendered the animals, following regression of the tumors, immune to subsequent inoculation of the same type of tumor. These animals showed also to be refractory against intratesticular tumor transplants. Similar observations were made by the use of this method with Rous chicken sarcoma. However, the results obtained by Besredka *et al.* have not been generally substantiated. Several investigators[9,10,11,12] failed to produce immunity against Brown-Pearce carcinoma by the use of the intracutaneous method. On the other hand, several reports by various investigators substantiated the findings of Besredka *et al.*[13,14,15]

It is generally thought that the use of hybrid animals and transplantable tumors of unknown genetic origin might be responsible for the lack of uniformity among the results of the multitude of reported investigations. Space does not permit a comprehensive review of this problem. (The reader is referred to the bibliography.[16,17]) However, there are a few pertinent facts which are worthy of discussion at this point, and to which the results of this paper are relevant.

The viewpoint that the use of genetically unknown material was responsible for the contradictory results has received support in recent literature.[18,19] In these investigations it was shown that the induced resistant state bears a genetic relation to the type of living cells used and to the host. These authors used normal tissue cells, such as blood or embryo skin as the immunizing agent. Negative results as regards immunization against tumor growths were obtained in most instances. Inasmuch as the immunizing material used was derived from a pure line of animals, it was believed not possible to induce resistance in a pure strain of animals against tumors originating within their own strain. However, the experiments reported in this paper demonstrate the possibility of inducing such re-

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sistance in a genetically pure strain of rats.

These divergent results raise the question as to whether the *type* of immunizing material used is not a more critical factor. It may be mentioned here that the author has been working, also, with another type of tumor, a mouse mammary adenocarcinoma which arose spontaneously in C57 "Y" black mice (also a pure strain of Dr. Bagg) in which, thus far, no immunity has been produced. It is interesting to note that the investigators[18,19] also used an adenocarcinoma. Consequently, we are faced with the possible phenomenon that immunity is possible in a pure line of animals when a reticulum cell type lymphosarcoma is used, whereas no such resistance can be produced with an adenocarcinoma.

Another experiment[20] has been reported in which immunity was successfully induced in a pure strain of C3H mice. Herein a mouse sarcoma produced by methylcholanthrene was used as the immunizing agent. A question may be raised as to whether a tumor produced by an extrinsic factor (chemical carcinogen) may be classified with spontaneous tumors in this respect. If so, then this work lends further amplification to the idea that the type of tissue used for immunization is a significant factor.

It is possible that this idea may have some bearing on the relative recurrences of different types of human malignancies. It is well known that certain cases, which either have had spontaneous regressions or have been successfully treated, suffer no further recurrences, whereas many cases, following apparently successful treatment, develop further malignant growth in later years. It is not unreasonable to infer that in the former cases the particular type of tumor suffered was capable of inducing a self immunity.

It is obvious, however, that much more and extensive work is necessary to resolve the question of immunity to cancer, particularly to spontaneous tumors. The method herewith described for inducing immunity appears to offer a good means of standardizing work along this line. If the thesis discussed here, that tumors may be biologically classified into those which can and those which cannot induce immunity, is ultimately to be validated, such standardization is necessary. Attempts have been previously made to induce immunity by implants attenuated by irradiation, for example, [21,22,23,24]. However, this work was done before the X-ray unit was quantitatively established and precluded accurately reproducible results. This obstacle has been overcome by the establishment of precise physical factors in the attenuating procedure and, consequently, as has been shown, results may be reproduced at will.

In general, it may be stated that standardization of procedures is a desideratum definitely to be hoped for, and which should greatly facilitate the solution of the cancer problem as a whole.

Summary. Rats of an inbred line, which were 100% susceptible to a reticulum cell type of lymphosarcoma arising spontaneously in the same strain, could, in an appreciable percentage of cases, be rendered resistant to the same tumor. The method consisted of implanting tumor grafts which were previously attenuated with specific doses of X-rays. In most cases the resistant state followed inoculation without the appearance of any visible tumor. In certain cases a tumor did appear but regressed after having reached a certain size. These likewise became immune. Hence this procedure eventually resulted in immunity to an autogenous tumor. Out of 58 control rats, only one spontaneous regression was observed. This rat likewise proved to be refractory to further viable tumor grafts of the same type.

The results of these experiments give evidence, apparently for the first time, that it is possible to render animals of a pure line immune against an autogenous tumor.

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