

THE IMMUNOLOGICAL RELATIONS OF THE ROUS CHICKEN SARCOMA.¹

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(Received for publication, April 22, 1915.)

The tumor² used throughout these experiments was the first chicken sarcoma reported by Dr. Peyton Rous,³ to whose courtesy the laboratory of the Crocker Fund is indebted for the fowl from which the transplants were made.

This growth, according to Rous's description, is composed of loose bundles of spindle cells, crossing in every direction and separated from the smaller blood vessels only by endothelium. Inter-cellular fibrils can be demonstrated with Mallory's phosphotungstic acid stain, though they are rare in the more cellular portions of the tumor. Areas of necrosis are present, dependent, in general, upon insufficient vascularization. The sarcoma shows a marked tendency to invade the surrounding tissues; furthermore, it metastasizes, generally by way of the blood stream and most commonly in the lungs, although secondary nodules in the heart, liver, and spleen are not rare. It possesses, accordingly, many of the characteristics of the transplantable tumors of the mouse and rat, but differs fundamentally from these in being transmissible in the form of a Berkefeld filtrate or of dried tissue.

When the present investigation was started, the immune reactions associated with this growth had not been fully investigated;

¹ Read before the American Association for Cancer Research, St. Louis, April 1, 1915.

² The employment of such terms as "sarcoma" and "tumor" throughout this paper is to be looked upon rather as a concession to convenience than as indicating the possession of any definite idea regarding the nature of the material in question.

³ Rous, P., *Jour. Exper. Med.*, 1910, xii, 696.

thus, it was not known whether fowls can be rendered resistant to its inoculation by previous treatment with fowl tissue, after the manner in which mice can be made refractory to mouse tumors with the normal tissues of their species.

The injection of 0.05 gm., or even less, of mouse spleen, kidney, embryo, or blood corpuscles will confer a resistance to the subsequent implantation of mouse tumor in from 70 to 100 per cent of treated animals, which sets in by the third day, reaches its height about the tenth, and persists for approximately three months.

It has been suggested by Pitzman⁴ that the refractory condition so evoked is due solely to a bacterial infection set up at the time when the immunizing material is introduced. If this were true, it should be possible to elicit resistance by preliminary treatment with the tissues of animals other than the mouse; the majority of observers, however, deny that this can be accomplished. In serious conflict with such an hypothesis, furthermore, is the observation of Woglom⁵ that the highest degree of resistance is procured by treatment with embryo skin, although, as both aerobic and anaerobic cultures made in this laboratory show, this is the tissue, of all those used to induce the refractory condition, which is certain to be sterile. It is highly probable, therefore, that the presence of an artificial immunity to tumor implantation represents the completion of a specific reaction.

A preliminary communication⁶ described unsuccessful attempts to duplicate this reaction in fowls by treatment with ten day chick embryos, five to forty days before tumor inoculation. The small size of some of the growths in fowls thus injected thirty-two or forty days before introduction of the sarcoma suggested the possibility that a much longer time might be required for the development of complete immunity than the ten days necessary in mice. Hence, the period was extended to 100 days.

Details of individual experiments are to be found in the accompanying table.

The fowls which it was sought to immunize were injected in the

⁴ Pitzman, M., *Ztschr. f. Krebsforsch.*, 1914, xiv, 57.

⁵ Woglom, W. H., *Jour. Exper. Med.*, 1912, xvi, 629.

⁶ Woglom, *Proc. N. Y. Path. Soc.*, 1914, N. S., xiv, 202.

left breast with from 1 to 10 cc. of fresh hashed chicken embryo, and at periods varying from 5 to 100 days afterward were inoculated with intact grafts (0.02 gm.) of tumor in the right breast,

TABLE I.

Experiment No.	Interval between treatment and tumor inoculation.	Dose of embryo emulsion.	No. of chickens.	Tumors.	No tumors.
	<i>days</i>	<i>cc.</i>			
I	5	5.0	10 controls*	10	0
			8 treated	5	3
II	5	10.0	6 controls	6	0
			6 treated	6	0
III	10	1.0	15 controls	15	0
			17 treated	16	1
IV	12	5.0	11 controls	11	0
			10 treated	10	0
V	14	2.0	5 controls	5	0
			9 treated	9	0
VI	14	4.0	2 controls	2	0
			5 treated	5	0
VII	25	5.0	10 controls*	10	0
			11 treated	10	1
VIII	28	2.0	3 controls	3	0
			3 treated	3	0
IX	32	5.0	5 controls	5	0
			3 treated**	2	1
X	40	5.0	11 controls	11	0
			7 treated**	7	0
XI	70	5.0	22 controls	21	1
			20 treated	18	2
XII	100	5.0	16 controls	15	1
			18 treated	18	0

* Same controls used for both experiments.

** More small tumors than among controls.

together with an equal number of normal controls. Three weeks after implantation of the tumor the fowls were autopsied.

117 treated and 106 control fowls lived long enough to come to autopsy. Of the treated, 109 (93 per cent) proved receptive for the tumor, and among the controls 104 (98 per cent) developed growths.

The difference between the treated fowls and their controls is slight enough to warrant the statement that immunity to the sarcoma in question can not be produced by preliminary injection with chicken embryo in the amounts administered. The number of tumors in each group is approximately the same, and, with the exception of Experiments IX and X, the growths in the treated fowls were fully as large as those in the controls.

Although three treated chickens out of eight in Experiment I did not develop tumors, a repetition of the experiment with double the amount of embryo emulsion gave a clean cut result, none of the treated chickens being found resistant. These, as well as the other five instances in which fowls previously injected with embryonic material failed to develop tumors, are, therefore, referable in all probability to natural resistance rather than to an artificial immunity consequent upon the preliminary treatment, a view strengthened by the fact that Rous and Murphy⁷ also have recently recorded their failure to immunize against this growth with normal tissue. The immune fowls in the treated series are partially offset, moreover, by two controls in which the grafts failed to proliferate.

The absence of any resistance 70 and 100 days after attempted immunization completely nullifies the suggestion contained in a preceding paragraph, that a period much longer than the 10 days requisite in mice might be necessary for the development of complete immunity in the chicken.

The failure to produce resistance can not be ascribed to insufficient dosage. A mouse weighing 15 grams can be made refractory to transplantable tumors by preliminary injection with 0.05 cc. of normal mouse tissue, an amount representing about 1/300 of its body weight, and 5 cc. of chicken embryo, when injected into a fowl weighing 1,500 grams, is roughly equivalent to this quantity.

The absence of immunity is not an unanswerable argument against the neoplastic nature of this tumor; for, in the first place, it is not known that the immunological reactions characteristic of the mouse have their counterpart in the chicken, and, secondly, a tumor is occasionally found, even in the mouse, against which no resistance can be produced. At most it can be said only that the outcome of these experiments is a warning against the unreserved acceptance of this growth, at present, as a true tumor.

CONCLUSION.

The injection of chicken embryo, in amounts of from 1 to 10 cc., confers no resistance against the Rous chicken sarcoma, when this is inoculated from 5 to 100 days after the preliminary treatment.

⁷ Rous, P., and Murphy, Jas. B., *Jour. Exper. Med.*, 1914, xx, 419.