ON THE ONCOGENIC EFFECT PRODUCED IN RATS BY TISSUE CULTURE PREPARATIONS, SUBJECTED TO THE ACTION OF HUMAN MAMMARY GLAND CANCER TUMOR EXTRACTS*

G. I. Deichman

Laboratory of Tissue Culture, Dept. of Etiology and Pathogenesis of Tumors
(Head – Active Member of the Academy of Medical Sciences of USSR,
Prof. A. D. Timofeevskii), Institute of Experimental and Clinical Oncology
(Director – Active Member of the Academy of Medical Sciences of USSR,
N. N. Blokhin), Academy of Medical Sciences of USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences of USSR A. D. Timofeevskii)
Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 2,
pp. 85-88, February, 1962
Original article submitted April 18, 1961

A combination of two techniques, tissue culture, for the isolation and accumulation of the virus, and inoculation of susceptible animals with culture preparations, helped to isolate the oncogenic virus of polyoma [8]. However, there have been only a few communications dealing with attempts to utilize this combination of methods in order to isolate other oncogenic viruses, especially from human tumors [4].

The present work has been an attempt to isolate a virus from cancerous tumors of the human mammary gland, using monkey kidney tissue culture, and inoculating culture fluids into newborn animals. The following communication presents some data obtained in the course of this work.

METHODS

In these experiments we have used three extracts from cancerous tumors of mammary glands of 4 woman who were operated at the clinic of the Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of USSR, during the period from February to May 1959. These extracts will be designated as No. 1, No. 2 and No. 3. Extract No. 3 was prepared from tumors of two patients. The extracts were homogenized in Tyrode's solution in the proportion of 1 : 10 at 5000 r.p.m. for 20 minutes. The supernatent fluid, after centrifugation at 2000 r.p.m., was used to infect monkey kidney cells. Extracts and tissue culture preparations of Macacus rhesus and Macacus cynomolgus kidney cells were mixed in test tubes in the porportions of 5 : 1 by volume, and the mixtures were placed in the refrigerator at 4°C for 18 hours. Then the cells were resuspended in a nutrient medium consisting of 0.5% calf serum and phosphate buffer solution; suspensions were placed into Carrel flasks.

Tissue culture preparations (culture fluids after 7-25 days of cultivation) were inoculated intraperitoneally in 0.2 ml amounts into newborn nonpedigreed rats, 2-18 hours after birth.

In each lot, $\frac{1}{2}$ - $\frac{2}{3}$ of the number of animals were inoculated with material from infected cultures, and the rest were either not inoculated at all or (usually) inoculated with control preparations (culture fluids from infected cultures heat-inactivated at 56°C for 30 min or fluids from uninfected cultures). Immediatedly after inoculation the animals were placed, each lot into a separate cage. Thus, the experimental and the control animals were in close contact from the moment of inoculation.

Simultaneously with these experiments, a group of rats from the same source were inoculated immediately after birth with fluid from a culture of human malignant cells (strain 558 M_1). Of 37 rats which died or were killed 11 to 23 months later, none had malignant growths. These animals served as controls to our experiment.

The results of morphological study of tumors and organs of rats, conducted by E. L. Prigozhina, will be published in a separate communication, as well as results of experiments on hybrid mice S57 × S3NA.

*Presented at a conference at the Department of Etiology and Pathogenesis of Tumors, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of USSR, on February 22, 1961.

RESULTS

Extract No. 1 was carried in tissue culture through 5 consecutive passages. Extracts Nos. 2 and 3 went through 3 consecutive passages in vitro. We did not note any degenerative changes in monkey kidney tissue cultures which could be regarded as due to a cytopathogenic effect.

TABLE 1. The Finding of Malignant Tumors in Experimental and Control Rats in "Pure" and "Mixed" Passaging Experiments

Kind of experiment	Animals	Finding of tumors	Total
"Pure" passaging	Experimental	9/48 (18.7%)	14/80 (17.4%)
	Controls	5/32 (15.6%)	
"Mixed" passaging	Experimental	2/35 (5.7%)	2/54 (3.7%)
	Controls	0/19	

Note: The numerators are the numbers of tumors which arose; denominators are the numbers of dead or killed rats.

Five to seven days after inoculation of tissue culture preparations into newborn rats, we noted a sharp (25% and more) reduction in weight and growth in some of the experimental animals. As a rule, such rats were not viable and died during the following 2-6 weeks, suffering from enteritis or pneumonia.

One of the rats, showing clearly defined cachexia, was killed on the 40th day after it had been inoculated with fluid from a culture, subjected to the action of extract No. 1 (1st passage). The blood serum and liver and spleen extracts of this rat were inoculated into newborn rats and into monkey kidney tissue culture for further passaging and for inoculation into newborn animals.

TABLE 2. Formation of Tumors in Rats in "Pure" and "Mixed" Passaging Experiments in Relationship to the Original Material and Passage Number

Kind of experiment	Animals	Finding of tumors					Total
		1	2	3	4	5	
"Pure" passaging	№ 1	0/4	1/2	1/6	3/15	2/11	7/38 (18.4%)
	№ 2	0/22	0/7	2/5	—	—	6/34 (17.6%)
	№ 3	0/5	—	1/3	—	—	1/8
"Mixed" passaging	№ 1	in vitro	in vivo	in vitro	in vitro		
		—	0/10	1/27	1/17	—	2/54 (3.7%)

Note: Same as for Table 1.

Thus, two parallel series of experiments were simultaneously conducted with extract No. 1. In the first, all the passages were made in vitro, and the respective preparations were inoculated into animals ("pure" passaging), and in the second series, the 1st passage was made in vitro, the 2nd through a newborn rat, and the 3rd and 4th passages again in vitro ("mixed" passaging).

Eleven months after inoculation, the first rat died, with a sarcoma of the mesentery. At the time there were 332 rats under observation. Since then, 134 rats died, aged 11 to 20 months, among which, 16 malignant tumors were found.

The morphological structure and localization of malignant tumors, according to E. L. Prigozhina, were quite similar: 10 animals had reticulosarcoma of the mesentery, 3 had reticulosarcoma of lungs, 2 had sarcoma of subcutaneous tissue and one had osteofibrosarcoma.

As seen in Table 1, in "pure" passaging experiments, when newborn rats were inoculated with culture preparations, tumors arose in 17-18% of all cases, and approximately with the same frecuency among experimental and control rats. It is to be noted that in the control group of animals, 3 out of 5 tumors arose in intact (uninoculated) rats among 9 which died. In the "mixed" passaging experiment, when the original material was passaged through tissue culture, rat, and again through tissue culture, the number of tumors which arose in rats was considerably smaller (3.7%). These differences between the frequency of formation of tumors in "pure" and "mixed" passaging experiments were statistically significant (P > 95).

Table 2 shows the data on the formation of tumors in the "pure" and "mixed" passaging experiments in relationship to the original material and number of passage. Because there were no significant differences between the experimental and the control animals, we have divided the animals into groups, depending on the composition of preparations inoculated (modifications of the experiment), and have grouped control animals with the corresponding experimental ones.

When newborn rats were inoculated with preparations from monkey kidney tissue cultures subjected to the action of extracts from human mammary gland cancerous tumors, the frequency of formation of tumors in experimental rats, as well as in control rats which have been kept in close contact with the experimental ones, became increased. The following facts bear witness to this.

1. The statistically significant differences in the frequency of tumor formation in two similar groups of rats. Thus, when tissue culture preparations treated with extract No. 1 were inoculated, in the "pure" passaging experiment in vitro, tumors arose in 18.4% of the cases. When this material was passaged through the rat and subsequently passaged in vitro, the frequency of tumor formation became 5 times lower (3.7%).

2. A total absence of malignant tumors in rats of the same age, which were inoculated with preparations of cultures of human cancer cells at the same time when the animals used in this work were inoculated.

Two hypotheses may be offered regarding the possible causes for the formation of tumors in rats, after they had been inoculated with preparations from tissue cultures. The oncogenic effect could have been produced by: 1) the virus, being passaged in vitro, from human mammary gland cancers, or 2) by a release into the culture medium by the monkey kidney cells of a substance (possibly the spontaneous monkey virus), which may have an oncogenic effect in rats.

The differences in the frequency of tumor formation between the "pure" and the "mixed" passaging experiments support the first hypothesis. However, the almost identical frequency of tumor formation, following the inoculation of preparations from different extracts and from different in vitro passages, supports the second hypothesis. In this connection it is of interest to note that in experiments of Axelrod et al. [1] there was noted an early formation of subcutaneous sarcomas in most control animals (hamsters), which were inoculated with preparations from uninoculated cultures of monkey kidney tissue.

The formation of tumors in control animals, including the intact ones, which were kept in close contact with the experimental ones, could be a result of their infection from the experimental animals. This hypothesis appears logical to us, both in the light of this investigation, and from recent literature on the contagiousness of many oncogenic viruses [2, 3, 5, 6, 7].

Of course, these hypotheses require further experimental proof.

SUMMARY

In administering to newborn rats preparations from the monkey kidney tissue cultures subjected to the action of the extracts from cancer tissues of the human mammary gland, sarcoma occurred in 17-18% of the experimental animals at the age of 11-20 months, as well as in control animals in direct contact with them. Much more rarely (in 3.7% of the cases) the tumors occurred in rats in an experiment in which the initial material was passaged both in vivo and in vitro (mixed passage). Tumors did not appear in another comparable group of rats inoculated at the same period with the preparation from tissue cultures of human origin. A question is discussed on the appearance of tumors in rats and a possible connection of this phenomenon with the material investigated or the monkey kidney tissue cultures.

LITERATURE CITED*

1. A. A. Axelrod, E. A. McCulloch, and A. F. Howatson, et al., J. nat. Cancer Inst., Vol. 24, p. 1095 (1960).
2. B. R. Burmester, Ann. N. Y. Acad. Sci. Vol. 68, p. 487 (1957).
3. B. R. Burmester, A. K. Fontes, and W. G. Walter, J. nat. Cancer Inst., Vol. 25, p. 307 (1960).
4. J. T. Grace and E. A. Mirand, Quart. Rev. Pediat., Vol. 15, p. 150 (1960).
5. L. Kilham, Am. J. Hug. Vol. 61, p. 55 (1955).
6. L. Kilham and H. T. Dalmat, Ibid., p. 45.
7. S. E. Stewart, B. E. Eddy, and A. M. Gochenour, et al., Virology, Vol. 3, p. 380 (1957).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

---

*No reference is made in the literature cited to the work of Eddy et al. [Proc. Soc. exp. Biol. (N. J.), Vol. 107, N 1, p. 191], since the author was not acquainted with these data at the time the article was handed in for publication.