Effect of X-Rays on the Transmissibility of Fowl Sarcoma in Its Nonfilterable Phase*

B. Miszurski,** M. Pikovski, G. Goldhaber, and L. Doljanski

[From the Department of Experimental Pathology, and the Radiological Department, The Hebrew University (Cancer Laboratories), Jerusalem, Palestine]

(Received for publication February 8, 1945)

It is a recognized fact that the filterable fowl tumors are remarkably resistant to roentgen radiation (13, 23, 24, 28, 30, 31). This resistance is due to the extreme refractoriness of the causative agent to x-rays. There is, however, no information as yet concerning the radiosensitivity of virus-induced fowl tumors during the period in which they have temporarily lost their filterability. The following experiments were designed to determine the effect of x-ray doses lethal to the cells upon the sarcoma during this so-called nonfilterable phase.

MATERIAL AND TECHNIC

Tumor material.—We used a strain of Rous sarcoma, kindly placed at our disposal by Dr. A. Fischer, of Copenhagen, that has been kept in vitro since 1923 (9). Inoculation into young chickens is successful in almost 100 per cent. The tumor generally leads to the death of the fowl in 4 to 8 weeks, and metastases are of regular occurrence.

Extraction and filtration.—The filtrates used in the present experiments were prepared in the following way: Three to five grams of tumor, freed of necrotic areas, was finely minced, thoroughly ground with sterile sand, and extracted with 20 cc. of 5 per cent NaCl for 1 hour. Eighty cubic centimeters of distilled water were then added, the suspension was centrifuged at 3,000 r.p.m. for 15 minutes, and the supernatant fluid drawn off and passed through a filter. Two types of filters were used: (a) paper-pulp-sand filter, and (b) Berkefeld “N” candle. The filtration was performed at a pressure of 20 mm. of mercury.

Irradiation.—Irradiations were carried out with a demountable x-ray tube working at 35 kv. on a current of 15 ma. The tube had a copper anticathode, and a window of aluminum foil 30μ in thickness. Absorption analysis showed that the rays penetrating through the window foil and the 0.03 mm. mica coverslip were mainly the characteristic x-rays of copper. Radiation was administered at a target-object distance of 3.7 cm.; the x-ray intensity at the distance of the irradiated object was about 80,000 r/min. for a tube current of 15 ma.

The tumor fragments (30 to 50 mgm.) were placed on a depression slide, covered with a mica coverslip, and sealed in with paraffin. The material was irradiated with 50,000 r, this being 10 times the dose necessary to prevent further multiplication of the cells (7).

Inoculation.—White leghorns, 2 to 6 months old, were used. The irradiated fragments were deposited in a pocket made in the muscle of the breast or of the leg. The filtrates were injected intramuscularly, 1 cc. being used for each injection.

Design of experiments.—Ten series of experiments with 10 different tumor samples were carried out. Hard, yellowish, slowly growing tumors were generally selected, and Berkefeld and paper-pulp-sand filtrates were made from each. In addition, small pieces of the growth were irradiated. The irradiated material and one or both filtrates were inoculated into the same chicken. The activity of the original tumor material was also tested in every case.

RESULTS

The results (Table I) may be summarized as follows:

(a) Berkefeld filtrate gave the most irregular results, only 3 out of 10 tumors having yielded active filtrates. Paper-pulp-sand filtrates were more effective, positive results having been obtained in 5 out of 10 experiments.

(b) Grafts of irradiated tumor gave positive results more frequently than either of the filtrates. In Experiments 1, 5, and 10 both Berkefeld and paper-pulp-sand filtrates were inactive, whereas irradiated tumor gave rise to growths.

(c) In some cases the irradiated tumor failed on inoculation. Thus in Experiments 3 and 6 not only both filtrates but the irradiated material, too, was found to be innocuous.
Tumor no. | Chicken | Length of life, days | Results of inoculation with | X-rayed tumor | Paper-pulp-sand filtrate | Berkfeld filtrate |
--- | --- | --- | --- | --- | --- | --- |
1 | A | 65 | ++ | - | - | - |
| B | 66 | ++ | - | - | - |
| C | 38 | + | - | - | - |
2 | A | 24 | - | ++ | - | - |
| B | 23 | + | - | - | - |
| C | 60 | ++ | - | - | - |
| D | 22 | + | - | - | - |
3 | A | 53 | - | - | - | - |
| B | 53 | - | - | - | - |
| C | 26 | - | - | - | - |
| D | 45 | - | - | - | - |
4 | A | 11 | ++ | - | - | - |
| B | 27 | + | ++ | - | - |
| C | 23 | + | + | - | - |
| D | 20 | + | - | - | - |
| E | 26 | ++ | - | - | - |
5 | A | 44 | + | - | - | - |
| B | 16 | + | - | - | - |
| C | 16 | - | - | - | - |
| D | 39 | + | - | - | - |
6 † | A | 61 | - | - | - | - |
| B | 70 | - | - | - | - |
| C | 55 | - | - | - | - |
| D | 36 | - | - | - | - |
7 | A | 28 | + | - | - | - |
| B | 23 | + | - | - | - |
| C | 186 | + | - | - | - |
| D | 27 | ++ | + | - | - |
8 ‡ | A | 42 | - | - | - | - |
| B | 25 | + | ++ | ++ | - |
| C | 54 | - | ++ | - | - |
| D | 48 | + | + | + | - |
| E | 14 | + | + | + | - |
9 | A | 23 | + | - | - | - |
| B | 31 | ++ | + | - | - |
| C | 104 | + | + | + | - |
| D | 18 | + | - | - | - |
| E | 40 | ++ | + | - | - |
10 | A | 43 | - | - | - | - |
| B | 78 | - | - | - | - |
| C | 78 | - | - | - | - |
| D | 97 | - | - | - | - |
| E | 97 | - | - | - | - |
| F | 32 | ++ | - | - | - |

+ = slowly growing tumor.
++ = rapidly growing tumor.
- = no tumor.

† Tumor No. 6 developed in a resistant fowl that had failed to respond to 2 previous inoculations of tumor.
‡ Tumor No. 8 was derived from tumor No. 1, which yielded inactive Berkfeld and paper-pulp-sand filtrates.

**DISCUSSION**

The filterability of chicken tumors is subject to considerable fluctuation (14, 32), varying not only with different strains, but also within an individual strain. Even those strains that are known to be easily filterable (e.g., Rous sarcoma I) pass through non-filterable phases, in which their filterability is diminished or temporarily lost.

There is a considerable bulk of evidence to indicate that the distinction between filterable and nonfilterable fowl tumors is to a great extent an artificial one. By the use of appropriate filters and adequate methods of...
pretreatment it is possible to improve the effectiveness of filtration considerably (2, 10, 15, 18, 35, 36). But the fact still remains that even when tested with better methods of extraction and filtration tumors often yield filtrates that are unaccountably inactive.

In order to study more closely the problem of the cell-free transmissibility of fowl tumors, Cramer and Foulds (6) undertook experiments to test for the presence of the causative agent by a method other than filtration. These authors investigated the transmissibility of fowl tumors that had previously been subjected to repeated freezing and thawing, and were able to show that the failure of cell-free transmission in the nonfilterable phase is not caused by the failure of the filtration method alone. The frozen tissue displayed variations in infectiveness similar to those of filtrates prepared from the same tumors, and sometimes it was completely inactive.

The present work represents an attempt to gain some further information on the cell-free transfer of a virus-induced fowl sarcoma in its nonfilterable phase, by investigating the transmissibility of tumor material previously irradiated with x-ray doses lethal for the cells. The results were compared with those obtained by inoculation of filtrates from the same tumors.

The application of irradiation as a method for separating the causative agent from living cells (12, 13, 16, 28, 31, 33) is based on the observation that a wide gap exists between the doses necessary for inactivation of the causative agent and those that prevent a further multiplication of cells. It has been established that 5,000 r ("delayed lethal dose") suffice to arrest any further multiplication of cells in tissue culture (7); on the other hand, many millions of r are required for complete inactivation of the neoplastic viruses (8, 11, 24, 41, 42). Irradiation appeared to be a particularly appropriate method for demonstrating the causative agent in tumor tissue because it involves no danger of retention of the agent by ultrafilters, and safeguards it in its original concentration.

Besides, it is doubtless more reliable than freezing, since mammalian tumors have often been found readily transmissible after having been subjected to this treatment (3, 19-22, 25, 39), but their transmission is never successful after exposure to x-ray doses ranging from 1,000 to 6,000 r (1, 17, 29, 34, 40).

Our experiments reveal that the irradiation method makes possible the cell-free transfer of a number of Rous sarcomas that cannot be transferred with Berkefeld and paper-pulp-sand filtrates; yet cases remain in which no evidence for an agent separable from the cells can be found, even with the method of irradiation.

We should like to point out that this absence may be apparent rather than real. Considering the experiments of Murphy and his co-workers (26, 27), of Sittenfield, Johnson, and Jobling (37, 38), and of Claude (5), as well as of Carr (4), the possibility should not be disregarded that the transmitting agent may still be present in the irradiated material, its activity being counteracted by an associated inhibitor, or by an antibody. Furthermore, it may well be that the causative agent is present, though in such minute quantities that with our only test object, the susceptible fowl, its presence cannot be detected. Whether the observations made here have any possible bearing upon the significance of the nonfilterable phase of virus-induced fowl sarcomas is thus problematical.

SUMMARY

Five out of 10 slowly growing Rous fowl sarcomas were found to be nontransmissible by both Berkefeld and paper-pulp-sand filtrates. After irradiation with doses lethal for the sarcoma cells, but practically harmless to the causative agent, 3 out of these 5 tumors could be transmitted, while the remaining 2 could not. The significance of these findings for the understanding of the so-called nonfilterable phase of the fowl tumors is discussed.

REFERENCES


Effect of X-Rays on the Transmissibility of Fowl Sarcoma in Its Nonfilterable Phase

B. Miszurski, M. Pikovski, G. Goldhaber, et al.

Cancer Res 1945;5:422-425.

Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/5/7/422.citation

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.