FAILURE OF SPLEEN FROM TUMOR-BEARING ANIMALS TO PRODUCE TUMORS

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In 1927 Blumenthal and Auler (1) reported that the injection of rats with emulsified spleen from those bearing Tumor B or Tumor E. A. was sometimes followed, from 10 to 35 days later, by the appearance of neoplasms which, though differing fundamentally in histology from those borne by the animals furnishing the splenic tissue, recurred after removal and proved to be transplantable. When the article was written, one such growth had reached its sixth generation.

As the two neoplasms in question are not widely known, a brief description of each was appended. Tumor B occurred originally in a rat treated with a mixture of lymph from a cancer patient and lymph-node extract from a rat with a transplantable neoplasm. Tumor E. A. followed the injection of a rat with pleuritic exudate from a patient with mammary carcinoma.

Experiments with other growths, including the somewhat more authentic Flexner-Jobling Carcinoma, were attended by no such result.

In a second communication, Blumenthal, Auler, and Solecka (2) pointed out that the hypothesis of a carcinogenic agent implied in the first paper would perhaps be rejected by other investigators on the ground that cancer cells might have been contained in the spleen emulsion introduced, even though no metastases had been discovered. Such a criticism, however, they regarded as unjustifiable in view of the comparatively large number of cells required for successful transplantation, while further proof against its validity was sought in the architecture of growths produced since the publication of
their first article by inoculating spleens from rats bearing the Jensen Sarcoma. These neoplasms had a distinctly alveolar structure showing no resemblance to the sheet of spindle cells composing this well known tumor; furthermore, they differed in their clinical behavior, growing more slowly than the Jensen Sarcoma and giving rise to metastases.

Eleven experiments were described with an equal number of spleens from rats bearing the Jensen Sarcoma, in which three tumors appeared. The number of successful results, which the authors regarded as small, was suggested as a reason for the long prevalence of the belief that mammalian tumors can be propagated only with living and intact cells.

In a third article (3) the results obtained with mouse tumors were discussed.

The extraordinary outcome of these investigations, so at variance with the ideas of most pathologists, has been confirmed by Teutschlaender (4), who said that in one experiment he had had a similar experience, though he gave no details. Tinozzi (5) also was able to produce tumors by inoculating certain of the organs from mice bearing Sarcoma 37, or Carcinoma 63; he was most successful with spleen, the lung yielding a tumor in but one case, and liver and brain in none. The tumors metastasized and proved to be transplantable, though they differed widely in both histology and clinical course from those borne by the animals furnishing tissues for injection. Tinozzi, like Blumenthal and his associates, suggested the presence of some etiological agent other than the cancer cell itself; with this inference Lewin (6) agreed.

Such an assumption is of the greatest importance, for if it could be substantiated our whole conception of malignant disease, acquired only after years of painstaking observation and laborious experiment, would have to be discarded at once or at the best completely revised. For this reason the work has been repeated with each of the 13 tumors under cultivation at the Crocker Institute, some of which were tested more than once.
The spleens employed came from animals with large, ulcerated tumors, and were emulsified and injected immediately after removal. It is impossible to tell how much material was introduced by the German investigators in their earlier experiments, but it may have amounted to from one-fourth to one-half a spleen. In the absence of any explicit statement, various quantities centering about 0.15 cc., the equivalent of half an average spleen, were tried (0.025, 0.05, 0.10, 0.15, 0.20, and 0.30 cc.) The remainder of the organ in each case was preserved, to be examined for microscopic metastases in case of a positive result.

Four experiments were done in this way, with 72 rats; in Experiments 1 and 2, spleens from rats bearing the Flexner-Jobling Carcinoma were used, in Experiment 3 those from animals with the Jensen Sarcoma and, in Experiment 4, spleens from rats bearing the Crocker Institute Sarcoma No. 8.

The animals were examined regularly every seven days; the inoculated tissue usually disappeared by the third week, and no tumors arose subsequently, although 57 of the rats survived for five weeks or more and many of them for from 12 to 30 weeks (Figs. 1 and 2). Samples removed and sectioned seven and 14 days after inoculation contained only degenerating spleen; in two cases the attempt was made to recover a 21-day inoculum for examination, but not enough of the injected material remained.

The somewhat different technic described by Blumenthal and his associates in their second paper was then substituted. They ground about one-sixth of a spleen in four cc. of physiological saline solution for 10 minutes, and divided the resulting preparation into two portions; to one was added five cc. of physiological saline solution, and to the other five cc. of physiological saline containing 0.2 per cent of glucose. The amount injected was one cc.

This method was followed explicitly, except that in order to have sufficient material to inoculate a large number of animals, half a spleen was used, and the amount of fluid to
Experiment 1. March 7, 1928. Nos. 1-12 received subcutaneously 0.20 cc. of spleen emulsion from rats with growing FRC. Average weight, 102 gm. Nos. 13-24 received 0.10 cc. Average weight, 103 gm.

Experiment 2. April 26, 1928. Nos. 1-12 received subcutaneously 0.30 cc. of spleen emulsion from rats with growing FRC. Average weight, 86 gm. Nos. 13-24 received 0.05 cc. Average weight, 76 gm.

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**FIG. 1.**

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**Experiment 1**

| Week | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

**Experiment 2**

| Week | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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be added was multiplied by three accordingly. The remaining half of each spleen was preserved for histological examination in the event of tumors arising in the treated animals.

When spleen is thus ground in saline solution there results a clear reddish fluid with considerable detritus which rapidly sinks to the bottom of the vessel. Care was taken to have this solid material shared as equally as possible among the inoculated rats, though the article of the German investigators contains no instructions on this point.

Twelve experiments were carried out in this way, four with rats and eight with mice. Ninety-six rats were injected with spleens from animals bearing Sarcoma 39, the Flexner-Job-
ling Carcinoma, Carcinoma 9, or Sarcoma 10; and 192 mice with those of mice bearing Sarcoma 180, Sarcoma 37, Carcinoma 63, Carcinoma 48, Carcinoma 206, Carcinoma 11, or the Twort Carcinoma. Each experiment comprised 24 animals, half of which were injected with the plain saline preparation while the remaining half received the glucose-saline extract. Two experiments were done with Sarcoma 180.

Ninety-five of the 96 rats survived five weeks or more; of the 192 mice, 169 lived for at least five weeks. None of the groups has been under observation for less than 20 weeks, but in no case has there been the slightest suspicion of a tumor.

A final attempt to confirm Blumenthal’s observation was made with the spleens of animals in which tumors had been massaged, a procedure which Tyzzer (7) discovered to be effective in increasing greatly the number of metastases to the lung from a transplantable carcinoma of the Japanese waltzing mouse.

For the present purpose the Flexner-Jobling Carcinoma was chosen, as Knox (8) found that the number of pulmonary metastases rose from 20 per cent in controls to 57 per cent where this tumor had been manipulated. It was hoped that a few cells might reach the spleen and give rise to neoplasms when this organ was inoculated into other rats, thus affording an explanation of Blumenthal’s results that could be correlated with prevalent conceptions of the nature of malignant disease.

Flexner-Jobling tumors were massaged gently for half a minute every other day; at the expiration of two weeks, i.e., after the seventh massage, the host was killed, one-half of the spleen ground in saline or glucose-saline as before, and the other half preserved, with the lungs, for serial sections in case these should be required. Two such experiments were carried out with two spleens, each experiment comprising 24 rats. Forty-seven of these 48 animals were living 10 weeks later, but no tumors had appeared.

Young animals were used throughout, since these are well
known to offer the best soil for the growth of transplantable tumors.

**DISCUSSION**

It is impossible to reconcile the appearance of neoplasms in Berlin rats after the introduction of spleen from tumor-bearers, with the consistently negative results obtained with mice and rats in New York.

Blumenthal and his associates have not disposed entirely of the chance that the spleens used in their experiments contained metastatic deposits; for unfortunately there is no way of searching microscopically the identical material introduced. Inoculation of one part of the spleen and examination of the remainder can never supply rigid proof of either the presence or absence of secondary tumors in the injected portion. It is not entirely impossible, therefore, that the tumors employed by the German investigators are metastasizing at present in their animals, though not in the strains available at the Crocker Institute.

The most probable explanation is always the preferable one, and in the judgment of the writer this is the most probable explanation. At any rate, the total failure to duplicate in New York the work of Blumenthal and his colleagues shows that an outcome such as they described is not to be expected in all cases.

**CONCLUSIONS**

In 18 experiments comprising 13 different transplantable carcinomas or sarcomas, and 408 rats or mice of which 369 lived for five weeks or more after injection, there could be found not the faintest indication of a neoplasm following the inoculation of spleen from tumor-bearing animals.

**REFERENCES**