ON THE FILTERABLE AGENT OF
MALIGNANT TUMORS

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That there is a filterable agent concerned in the growth of malignant tumors of mammals has been indicated for the living animal by the work of Burrows (1, 2) and of Biscglie (3, 4). The former, working with rats, found in a small proportion of his attempts that rat embryo tissue of 15 and 16 days development, comminuted and inserted into adult rats of the same descent after being immersed in the filtrate from the Jensen rat sarcoma, took the form of sarcomatous growth. In a much larger percentage there was evidence of stimulated proliferation without, however, the development of malignant tumor. Biscglie carried on similar experiments with chick embryos of 2 to 5 days development, which, comminuted and injected into adult chickens along with the filtrate from a mouse carcinoma, developed into sarcoma. He also has found evidence that the preliminary injection of tumor filtrates greatly enhances the malignancy of inoculated neoplasms. In addition, Rhoda Erdmann (5) has reported the development of transferable tumors in animals which had been injected with tumor filtrate after disturbance of the reticulo-endothelial system by the injection of India ink. Similar tumors developed in a few of her control animals without the preliminary treatment. Her work was done with filtrate of the Flexner-Jobling tumor, on closely inbred rats of apparently high susceptibility to that tumor (6).

The present work was undertaken in an effort to determine the possible presence, and the character, of reaction to tumor filtrates by tissues of post-embryonic origin. In the belief that such action would be most likely to take place in tissues already proliferating from other causes, the action of the filtrate was studied on induced connective tissue growth in a relatively early
stage of fibroblastic development. To induce this with as few complicating factors as possible, kieselguhr was selected as the irritating agent. Some preliminary experiments on white mice had shown that with the kieselguhr used, the simple injection of this suspended in salt solution resulted in a very friable growth of connective tissue, which did not lend itself to some of the manipulative procedures contemplated. To avoid this the expedient was adopted of working up the kieselguhr with white of egg into a doughy mass, and coagulating and sterilizing this in the autoclave. This material was then inserted in the form of dice of about 3 mm. diameter, into subcutaneous pockets. It was found that in rats the connective tissue growth caused by this procedure took the form of a definite capsule almost free from kieselguhr, with little organization of the central mass. A study of this capsular tissue at three day intervals indicated that the growth was at its height six days after the insertion of the kieselguhr "omelette," after which time the connective tissue began to assume an obviously more mature type.

The tumor filtrate used in this work was derived from rat sarcoma No. 10 of the Institute of Cancer Research, obtained through the kindness of Dr. Francis C. Wood. This is a very rapidly growing spindle-cell sarcoma, which ordinarily attains a size sufficient to kill its host in about four weeks, and which shows a very high inoculability into most strains of laboratory rats. With rats of Wistar Institute stock, the percentage of successful inoculations observed here was as a rule about 90. However, some rats of unknown strain obtained from a St. Louis dealer were much more resistant, giving only one successful inoculation in ten attempts. That this was not due to loss of virulence was shown by the transfer of the tumor from this one successful inoculation to ten rats of Wistar stock, with which only one failure resulted. To prepare the filtrate, the tumor was excised from the freshly killed rat, ground up in a mortar with powdered carborundum, and taken up with an equal volume of physiological salt solution. This was centrifuged at high speed from 1½ to 2 hours, and either filtered directly through a Mandler filter, or preferably first run twice by
<table>
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<tr>
<th>Exp. No.</th>
<th>Procedure</th>
<th>After 1 Week</th>
<th>After 2 Weeks</th>
<th>After 3 Weeks</th>
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<td>V</td>
<td>3 rats. Granuloma excised, comminuted, soaked overnight in ice box in sterile Locke’s sol., reinserted in fresh site in same animal.</td>
<td>Palpation impossible, because of adherent colloidion.</td>
<td>Nothing palpable.</td>
<td>1 palpable (?)</td>
<td>Nothing palpable.</td>
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suction through a fine fritted glass filter, which greatly expedited its passage through the Mandler. The filtrates were tested culturally for bacterial contamination, with negative results. Using the filtrate so prepared in connection with the six day growth of capsular connective tissue, the experiments shown in Table I were performed. Throughout this work rats of Wistar Institute stock were used except when otherwise stated.

The foregoing experiments, although performed in each case with a very limited number of animals, served as an indication of the type of procedure most likely to give positive results. Of the various methods, that adopted in Exp. I appeared to be the most promising. Excision of the granuloma with reinsertion after immersion in the filtrate did not apparently result in the continued growth of the connective tissue, unless, as in Exp. IV, it had been subjected to previous extraction.
To study the results of Exp. I in more detail, this experiment was repeated with additional controls. For these, the granulomas were injected either with ½ cc. of sterile 50 per cent normal rat serum, or with the tumor filtrate modified by one week's storage in the ice box. Nine rats, five of Wistar Institute stock and four of the St. Louis stock, were injected with the fresh filtrate, four being killed one week later, the remainder two weeks after the injection. Of four rats injected with serum, two were killed after one, two after two weeks. The four rats injected with the stored filtrate were handled similarly. Immediately after killing the rats the granulomas were excised, fixed in Zenker-formol-osmic acid, and sectioned after paraffin embedding. The same procedure was followed with eighteen white mice, six of which were injected with fresh filtrate, six with
the stored filtrate, and six left with the granulomas untreated. Smaller masses of the kieselguhr omelette were used here, and only 0.1 cc. of the filtrate was injected. Half of each of these series were killed after one week, the others a week later.

On microscopic examination of these granulomas, there was an observable difference, although of varying degree, between those which had been injected with the fresh filtrate and those which had not received this treatment. One week after the injections those which had received the fresh filtrate showed a capsular mass ordinarily much greater than in the controls, and formed almost entirely of recent fibroblasts, the individual cells being in large part still ovoid, with comparatively little interstitial material. A number of them showed nuclei in mitotic division. There was no indication of malignant hyperplasia; the individual
cells were of fairly uniform type, and they were arranged in a definitely concentric course. In the control material this overgrowth of young connective tissue was either wholly absent or very inconspicuous. The cells here were for the most part drawn out into long spindles, and formation of interstitial material was relatively far advanced. Mitotic figures were not observed, and while they may have been present, were certainly of much rarer occurrence than in the other material. In the specimens removed at the end of the second week, both those which had been injected with fresh filtrate and the controls showed progressive development of the capsular tissue, more advanced, as would be expected, in the case of the controls. There did not appear to be any evidence of a continuation of the increased proliferation seen in the one week material injected with fresh filtrate.

With the mouse granulomas, the general findings were similar to those of the rats. The evidence of capsular proliferation following the injection of fresh filtrate was somewhat obscured in the one week material by occasional central organization in the controls, which took the form of ingrowth of immature fibroblasts. If this was disregarded, and the capsular tissue alone considered, there appeared to be the same response of stimulation to the injection of fresh filtrate.

In both rats and mice the injection of a freshly prepared filtrate of the rat sarcoma used in this work therefor appeared to be followed by an increased proliferation of already developing connective tissue. This stimulation was of temporary duration and limited extent, and in none of the animals observed did it take the form of malignant hyperplasia.

The limitations of the action of the filtrate are open to at least two explanations. Either it may be counteracted or neutralized by agencies present in the normal animal body, or the effect may be arrested by removal of the injected material by the circulation. Experiment IV was conducted with too few animals to be decisive, and its results were not studied histologically, but to the extent that it warrants attention, it would indicate that the first explanation is correct, and that these neutralizing
agencies are already present to some extent in fibroblasts of the period used in the experiment, and are removable by extraction. Weightier evidence to this effect is furnished by the work of Burrows already cited. The fact that in a very small proportion of his animals the stimulation of embryonic tissue by tumor filtrates took the form of malignant hyperplasia, would almost certainly indicate that in those animals there was a deficiency of restraining agencies normally present.

REFERENCES