Failure of Hyaluronidase to Increase the Invasiveness of Neoplasms*

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In recent papers (1, 5) it was suggested that invasion of the adjacent normal tissues by malignant tumors may be facilitated by the presence of a spreading factor such as hyaluronidase. In support of this hypothesis, it was reported that some human carcinomas have been found to contain a spreading factor. Further support would be given to this hypothesis if it could be shown that invasiveness of neoplasms is increased by injection of hyaluronidase into or near a neoplasm, since the connective tissue barrier would presumably be weakened, and penetration of tumor cells facilitated. Accordingly, injections of hyaluronidase were made into tumors of two kinds, a transplantable mouse sarcoma and the Shope rabbit papilloma.

**Experiments with a Transplantable Mouse Tumor**

Mouse tumor 241 is a rapidly growing and metastasizing sarcoma, originally produced by a chemical carcinogen, transplantable in nearly 100 per cent of C57 black mice, and usually killing them in about 3 weeks. Since this tumor does not itself produce hyaluronidase, any effect of injected hyaluronidase would not be masked.

Forty-eight mice were injected subcutaneously in the flank with tumor 241, and transplants became established in all animals. Subsequently twice a day, 0.25 mgm. of hyaluronidase in 0.2 cc. balanced salt solution was injected into or near the tumors in 24 mice, while the same number of control animals were injected with salt solution alone.

At the end of 19 days, many of the mice had succumbed and the rest, in poor condition, were sacrificed. At autopsy, all the tumors showed fixation to the skin and extension into the thoracic wall, without obvious differences between experimental and control animals. Microscopic sections of the lungs revealed metastases in 8 of the mice injected with hyaluronidase and in 6 of the controls.

In a second series of animals, 36 mice were injected in the flank with tumor 241, and subsequently 18 of the animals were injected 6 times a week with hyaluronidase, the remaining mice with salt solution alone.

Half the animals were sacrificed after 14 days, and the tumors were exposed in order to estimate fixation to the chest wall or other underlying structures. Of 9 animals injected with hyaluronidase, 8 showed fixation whereas in 9 animals injected with salt solution alone, the tumor was fixed in only 5. This difference is not statistically significant.

The tumors were then dissected out and their volumes determined by measuring in a graduated cylinder the amount of liquid which they displaced. For tumors injected with hyaluronidase, the average volume was 2.2 cc.; for tumors injected with salt solution alone, 1.7 cc. The difference proved not to be statistically significant.

A histologic section was prepared from the lungs of each mouse, and the metastatic tumors, if any, were counted. Of animals treated with hyaluronidase, 5 of 9 showed lung metastases and the total metastases were 7. The metastatic growths ranged in size from 2 mm. in diameter to minute lesions. The control animals showed the same incidence of pulmonary metastasis, i. e., 5 of 9 animals, and the total number of metastases was 6. It is concluded from these observations that significant differences

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3 Differences of this magnitude or greater would be expected to occur by chance in more than 10 per cent but less than 20 per cent of such series.

4 Equal or greater differences would occur by chance in 10 to 20 per cent of such series of experiments.

5 As only one section was examined from each pair of lungs, it is likely that some minute metastases were missed.

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1 As shown by failure of extract of this tumor to reduce the viscosity of hyaluronic acid (unpublished experiments) when tested by a method recently described (5).
in the 2 series of mice were not present 2 weeks after inoculation.

The remaining mice were sacrificed at intervals up to 24 days after inoculation. At autopsy, the tumors were adherent to deep structures in all except one animal in each series, and thus no difference in local invasiveness was evident. The average volume of the tumors was, for animals injected with hyaluronidase, 3.4 cc.; for control animals, 4.7 cc. All animals (9 in each series) had pulmonary metastases. The total number of pulmonary metastases in animals injected with hyaluronidase was 38; in control animals, 37. Metastases ranged in size from 4 mm. in diameter to less than 1 mm.

Thus from these experiments no evidence was obtained that repeated injection of hyaluronidase increased either local invasiveness or pulmonary metastasis.

EXPERIMENTS WITH THE SIOPE RABBIT PAPILLOMA

In contrast to the invasive character of the mouse tumor used in the preceding experiments, the rabbit papilloma is essentially benign. That it can become invasive, however, has been demonstrated (7). Therefore, by injecting hyaluronidase into these tumors, it was hoped to increase the incidence and rate of invasiveness and malignant transformation, if hyaluronidase is capable of producing these effects.

Papillomas were induced by applying Shope virus to the dorsal skin of 29 rabbits of mixed breed, several drops of the virus preparation5 being rubbed into a scarified area of about 1 sq. cm. In from 15 to 19 days, papillomas appeared in all the animals. Fifteen rabbits were subsequently injected every day in the tumor area with 1 cc. of balanced salt solution containing 0.5 mgm. of hyaluronidase. The control group of 14 rabbits was injected with salt solution alone.

The experiment was terminated after 30 weeks, at which time 8 of the 15 rabbits which had received hyaluronidase showed complete regression of their papillomas, 6 had died of intercurrent infection between the first and 30th week with papillomas still present, and one survived with papilloma apparently regressing. When the rabbits whose papillomas had regressed were sacrificed, the sites of the tumors were no longer discernible and there were no metastases. In the 6 rabbits dying of intercurrent infection, autopsy revealed papillomas limited to the skin. There were no demonstrable metastases. Microscopically there was no invasion by the tumor of the underlying connective tissue.

Of the control series, in 5 rabbits the papillomas regressed and disappeared during the first 8 weeks, and the others were sacrificed or died at various times. None showed local invasiveness or metastasis. From these experiments it is evident that injection of hyaluronidase into the rabbit papilloma did not cause invasiveness or metastasis nor otherwise apparently influence the growth of the tumors.

DISCUSSION

The two kinds of tumors selected for studying the effect of hyaluronidase on invasiveness differed greatly in their rate of growth, and in their tendency to invade locally and to metastasize, the mouse tumor 241 being highly malignant, the Shope rabbit papilloma usually benign but potentially malignant. It was hoped that one or the other grade of malignancy would be appropriate for demonstrating whether hyaluronidase could enhance invasiveness. That this proved not to be the case may indicate (a) that hyaluronidase has no power to increase invasiveness or (b) that appropriate tumors were not selected. A third explanation is possible, that is, that anti-hyaluronidase may have been formed as the result of repeated injection of hyaluronidase and that this inhibiting factor prevented any spreading effect or tendency of hyaluronidase to increase invasiveness (4).

The effect of spreading factor upon the rate of growth of tumors, rather than upon their invasiveness, has been studied by others. Duran-Reynals (2) reported inhibition of growth of the Brown-Pearce epitheloma of the rabbit when testicular extract was added to the tumor cell inoculum, but suggested the possibility that dilute testicular extract may act as a stimulant to tumor growth. Tanzer (8) found that of several transplantable mouse tumors some were inhibited by testicular extract while others were not affected. Similar results are reported for tumors in the rat, guinea pig, and rabbit by Prime and Haagensen (6). The subject has been reviewed recently by Duran-Reynals (3).

SUMMARY

Earlier experiments had suggested the possibility that invasiveness by malignant tumors may be faci-
itated by the presence of a spreading factor such as hyaluronidase. It was thought that injection of this substance into or near a neoplasm might so alter the surrounding connective tissues as to permit more ready penetration by tumor cells.

This possibility was tested by repeated injection of hyaluronidase into: (a) 2 series of mice (C57 blacks) bearing tumor 241; and (b) into a series of rabbits bearing Shope papilloma. Injections were made into the tumors or their immediate vicinity. In each series control animals were injected with balanced salt solution alone.

All the mouse tumors grew rapidly and soon became locally invasive; many formed metastases in the lungs. There was no significant difference in local invasiveness, in size of tumors, or in number of pulmonary metastases between animals injected with hyaluronidase and those injected with saline alone.

In the papilloma-bearing rabbits, the tumors regressed in some, persisted in others, but in no instance gave evidence at autopsy of local invasiveness or metastasis. No difference was observed between rabbits injected with hyaluronidase and those receiving saline alone.

It is concluded that in these experiments, hyaluronidase did not promote local invasiveness or metastasis. Either hyaluronidase lacks the property of doing so, or the tumors selected were not appropriate, or anti-hyaluronidase may have been formed and prevented the expected results.

REFERENCES
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