An Experimental Study of Necrosis in Tumors*

WILLIAM E. SCHATTEN
(The Feinst Research Laboratories, Piedmont Hospital, Atlanta, Georgia)

SUMMARY

Factors that regulate the amount of viable tumor an animal can support have been studied in rats bearing Walker carcinoma 256. There is a maximum amount of viable tumor an animal can support, but continued growth of tumor resulting in increase in the amount of necrotic tumor occurs until death of a rat bearing Walker carcinoma 256. The number and location of tumors in an animal do not significantly influence the percentage of the total amount of tumor tissue that is necrotic except in instances in which tumors are very small. In rats with Walker carcinoma 256 there is a positive relationship between the percentage of the total amount of tumor tissue that is necrotic and the percentage of host weight occupied by tumor.

Extensive necrosis occurred in Walker 256 tumors in rats placed under a barometric pressure of 8 inches of mercury for 5 hours. There is rapid growth of tumor that remains viable if the tumor-host equilibrium is disturbed by decreasing the amount of viable tumor by placing a tumor-bearing rat under reduced barometric pressure.

Central necrosis is a common consequence of a neoplasm's growth. In a previous study, the author and co-workers (5) demonstrated that availability of oxygen to tumor cells was important in the production of central necrosis. The influence of systemic factors on the development of necrosis in tumors was implied. The present report is a study of factors that regulate the amount of viable tumor an animal can support.

MATERIALS AND METHODS

Female, albino Sprague-Dawley rats, weighing approximately 250 gm., were housed in individual suspended basket-type cages and fed Purina Laboratory Chow and water ad libitum. Each rat was weighed twice weekly. Only those animals which had gained weight at comparable rates for 2 weeks were used for the experiments. Transplants of pieces of Walker carcinoma 256 were introduced into the flexor muscles of the thighs or into the subcutaneous tissues of the flank by trocar technic.

Tumor-bearing animals were sacrificed at different intervals of time following transplantation. Each tumor to be studied was dissected from surrounding tissue, weighed, and the entire tumor was sliced to permit separation of necrotic tissue from viable tissue by sharp dissection. The necrotic and viable portions were weighed separately. Necrotic and viable portions of Walker 256 tumors are sharply demarcated and can be identified easily, since viable tissue is firm and gray and necrotic tissue is soft and reddish-brown (Fig. 1).

One group of tumor-bearing rats was subjected to a low oxygen environment at normal atmospheric pressure. These rats were placed in desiccator jars into which a 95:5 helium:oxygen mixture flowed continuously. After 5 hours of exposure to this 5 per cent oxygen mixture, rats were returned to their cages. These animals were sacrificed 24 hours after treatment.

Another group of tumor-bearing rats was subjected to a low oxygen environment by being placed under a reduced barometric pressure of 8 inches of mercury. At this negative pressure, oxygen tension in the inspired air is approximately equal to that in a 95:5 helium:oxygen mixture. Approximately 20 minutes are necessary to reduce the barometric pressure slowly to 8 inches of mercury; if this is done, rats tolerate this reduced pressure without difficulty. After 5 hours in a vacuum jar, rats were returned to their cages. These animals were sacrificed 24 hours after treatment. Another group of rats were treated in a

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The data on proportion of necrotic tumor and per cent of host weight occupied by tumor were analyzed according to formal analysis of variance and covariance procedures.

RESULTS

An animal can support only a certain amount of viable tumor, even though the total amount of tumor continues to increase until death of the animal (Chart 1). After a tumor comprised approximately 12 per cent of the weight of its host, further growth of tumor resulted in increase in the amount of necrotic tumor. There was no greater amount of viable tumor in animals with 75 gm. of tumor than in animals with 30 gm. of tumor. With the increase in each per cent of host weight occupied by tumor, the mean increase in the amount of necrotic tumor was $2.26 \pm 0.22$ gm.

Chart 2 shows that the number and location of tumors in an animal do not significantly influence the percentage of total tumor tissue that is necrotic except in instances in which tumors are very small. In rats with tumors occupying 24-72 per cent of host weight the percentage of necrotic tumor was significantly greater in those bearing two tumors than in those bearing five tumors ($P < 0.001$). In rats with larger tumors there was no significant differences between the percentages of total tumor tissue that was necrotic in animals bearing two tumors and those bearing five tumors ($P < 0.1$).

Chart 3 shows the relationship between the percentage necrosis in individual tumors in rats bearing five tumors and the per cent of host weight occupied by the total amount of tumor. The wide range in the percentage of necrosis in each of five tumors in the same rat is seen in this scatter graph. However, in these rats with five tumors there is a positive relationship between the percentage of the total amount of tumor tissue that is necrotic and per cent of host weight occupied by tumor (Chart 4). With the increase in each per cent of host weight occupied by tumor, the mean increase in the percentage of necrotic tumor was $3.60 \pm 0.57$.

Chart 5 shows the results of exposing rats with bilateral intramuscular tumors to a 5 per cent oxygen mixture for 5 hours. This treatment did not effect a significant increase in the percentage
of necrotic tumor except in the group of rats with tumors occupying 7\(\frac{1}{2}\)-12\(\frac{1}{2}\) per cent of host weight (P < 0.001 for this group).

Treatment of rats bearing bilateral intramuscular tumors by placing them in a vacuum jar with a negative pressure of 22 inches of mercury for 5 hours significantly increased the percentage of necrotic tumor in all groups (P < 0.001) for all groups (Chart 6). Treatment of rats bearing five small tumors in a vacuum jar also significantly increased the percentage of necrotic tumor (P < 0.001, Chart 7), but it can be seen by comparison of Charts 6 and 7 that small tumors were not affected by the reduced barometric pressure nearly as much as were larger tumors.

There was rapid growth of tumor that remained viable following treatment of rats with bilateral intramuscular tumors by placing them under reduced barometric pressure (Chart 6). The percentage of necrotic tumor decreased significantly between 24 and 96 hours after treatment of tumors comprising 2\(\frac{1}{2}\)-7\(\frac{1}{2}\) per cent of the host weight (P < 0.01) and tumors comprising 7\(\frac{1}{2}\)-12\(\frac{1}{2}\) per cent
of the host weight ($P < 0.001$). In the former group the percentage of necrotic tumor 96 hours after treatment was still significantly greater than in control rats ($P < 0.005$). However, in the latter group the percentage of necrotic tumor 96 hours after treatment was no greater than that in untreated rats.

**DISCUSSION**

These data demonstrate that there is a maximum amount of viable tumor an animal can support; but continued growth of tumor resulting in increase in the amount of necrotic tumor occurs until death of a rat bearing Walker carcinoma 256. There are two determinants of the amount of necrotic tumor in an animal: (a) systemic factors that govern the amount of viable tumor that can be supported and (b) the growth potential of the tumor. Walker carcinoma 256 has a dominant growth potential. Regressions are extremely rare, and the growth rate is reliable, reproducible, and rapid (7).

Local factors played a significant role in determining the percentage of the total amount of tumor that was necrotic only when there were very small tumors. Studies of the blood supply of experimental neoplasms by Algire and Legallais (1) and of human neoplasms by Bierman and co-workers (2) show solid malignant tumors to have an abnormal vascular system. The major arterial supply to transplanted tumors has been shown to be peripheral and to be distributed circumferentially to capillaries that penetrate the globular mass. Nutrients are therefore available to centrally located cells in very small tumors but not in larger tumors. This explains the findings in this study that small tumors in rats bearing five tumors had less necrosis than larger tumors in rats bearing two tumors when the total amount of tumor in each group was approximately equal. Also, these small tumors were not affected as much by treatment in a vacuum jar as were larger tumors. The vascular pattern developed by an individual tumor plays a role in the determination of the amount of necrosis in that particular tumor, as evidenced by the wide range in the percentage of necrosis in individual tumors in rats bearing five tumors. However, in those rats with multiple tumors the total amount of necrotic tumor is determined by systemic factors regulating the amount of viable tumor the rat can support and by rate of growth of the tumor.

Goodman (3) has shown that mice with multiple tumors live the same length of time as mice with...
single tumors, and at death the total tumor mass is
the same in both groups. Also, excision of one of
two tumors in a mouse increases the growth rate
of the remaining tumor. The present report
defines this concept further by demonstrating that
an animal can support only a certain amount of
viable tumor regardless of the number and location
of implants. Also, there is rapid growth of tumor
that remains viable if the tumor-host equilibrium
is disturbed by decreasing the amount of viable
tumor by placing a tumor-bearing rat under re-
duced barometric pressure. These data show that,
one a host has been altered or conditioned so that
it will support a certain amount of viable tumor,
removal of a portion of this tumor results in its
rapid growth until it equals the pre-existing viable
tumor mass. The author (4) has demonstrated
previously that there is enhancement of growth of
metastases following removal of a primary tumor.

Sundstroem and Michaels (6) demonstrated
that necrosis of tumors was greater in animals sub-
jected to negative pressures than in animals sub-
jected to low oxygen environments at atmospheric
pressure. The findings presented in this paper
demonstrate that exposure to an environment of
5 per cent oxygen at atmospheric pressure which
results in decreased oxygen tension and alters dif-
fusion of oxygen in the blood does not cause nearly
as much necrosis in tumors as does exposure to
negative pressures. Urbach and Noell (8) demon-
strated there is a poor response of tumor tissue
pO2 to the administration of 100 per cent oxygen,
and results of this study indicate that there is
probably little decrease in the normally low tumor
tissue pO2 when blood pO2 is decreased. The
mechanism of action of negative pressures in en-
hancing necrosis in tumors is unexplained. The
physiology of flight has been studied extensively,
and work is now being carried out in this labora-
tory to elucidate the effects of low pressures on
tumors.

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FIG. 1.—This photograph demonstrates the demarcation
between necrotic and viable portions of Walker 256 tumor.
The outer rim of viable tissue is easily distinguished from the
necrotic core.
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William E. Schatten


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