Retinoblastomas are rapidly growing tumors that frequently outgrow their blood supply resulting in extensive areas of necrosis. The characteristic microscopic features of retinoblastomas include the concentric arrangement of proliferating viable cells forming sleeves around blood vessels. At the periphery of the sleeves the cells of the retinoblastoma become necrotic. Published data based on various experimental and human neoplasms indicate that the thickness of the cuff of neoplastic cells is dependent on the maximum distance that oxygen can diffuse from the blood vessels before it is consumed by the tumor cells. Based on this model we hypothesized that in retinoblastomas the thickness of the sleeves of tumor cells should be a function of the growth rate of the cells of the retinoblastoma measured as the mitotic activity.

Materials and Methods

Two hundred retinoblastomas from the Registry of Ophthalmic Pathology at the Armed Forces Institute of Pathology were studied. Fifty of these tumors did not have the sleeve pattern of growth. From each of the 150 tumors that had the sleeve pattern, the most circular concentric cuff of neoplastic cells was selected. The following measurements were made on each selected cuff: (1) the shortest distance from the inner edge of the cuff to the first row of necrotic cells, (2) the diameter of the central blood vessel, and (3) the number of the mitotic figures in the cuff. The distances were measured using a calibrated reticule in the ocular of an American Optical light microscope (Reichert Scientific Instruments, Division of Warner-Lumbert Technologies, Inc., Buffalo, NY). Assuming that the cuffs were circular, the area was calculated using the standard formula for the area of a concentric ring. In each case the presence or absence of extraocular extension by the retinoblastoma was recorded. We defined extraocular extension as invasion of the retrolaminar optic nerve or episcleral tissues.

On the 15 most recent of the 150 retinoblastomas with the sleeve pattern we did additional measurements. From each tumor the thickness of ten cuffs was measured to determine the consistency of the cuff size in the tumors. To investigate further the relationship between the size of the cuff and mitotic activity (MA) in the cuff, the three most circular sleeves were selected, and the cuffs were subdivided into three concentric rings of equal thickness. The number of mitotic figures were counted in each of the three rings. The largest dimension of each tumor was measured.

We used regression and logistic regression to analyze the relationships among the different features that we measured on the 150 retinoblastomas.
Results

In 150 of the 200 retinoblastomas studied, the sleeve pattern was present. The most circular cuff in each tumor was measured. The mean thickness of the cuff was $98.7 \pm 11.9 \mu m$ (± standard deviation). The mean diameter of the central vessel (VD) was $57.5 \pm 18.6 \mu m$, and the mean area of the cuff (AC) was $49,199 \pm 13,970 \mu m^2$ (Figs. 1, 2). The mean number of mitotic figures per cuff was $8.8 \pm 3.7$.

Using regression analysis, we investigated the relationships among the thickness of the cuff and AC, VD, and MA in the cuff. The AC had a slightly better correlation with MA and VD than did the thickness of the cuff. The best model describing this relationship was one in which there was a positive correlation between AC and VD and a negative correlation between AC and MA. This model expressed as an equation is:

$$AC = 28,581 + 559 \text{VD} - 1315 \text{MA}$$

The two parameters in the model were significant ($P < 0.0001$), and the model's $r^2 = 0.70$. The lack-of-fit test indicated no significant lack of fit for this model ($P = 0.59$). To illustrate the negative relationship graphically between AC and MA (Fig. 3), we standardized all of the tumors to VD ($57.5 \mu m$) using this regression equation.

Of the 200 retinoblastomas 95 had extraocular extension. In 50 of the 95 tumors with extraocular extension, the sleeve pattern was not present. All of the 105 tumors without extraocular extension had areas with the sleeve pattern of growth. Of the 45 cases with extraocular extension and the sleeve pattern, all had this pattern in the intraocular portion of the tumor. Only four had the sleeve pattern in the extraocular portion of the tumor. Logistic regression analysis of the 150 retinoblastomas with the sleeve pattern indicated that extraocular extension was positively correlated with VD ($P < 0.0001$); however, it was not significantly correlated with MA ($P = 0.88$). When VD was included in the logistic model, neither the thickness of the cuff nor AC were significantly correlated with extraocular extension ($P > 0.6$).
Analysis of the subgroup composed of the 15 most recent retinoblastomas provided additional information. Comparison of the thickness of the ten cuffs measured in each tumor indicated that the thickness of the sleeves in a tumor was less variable than the variability between tumors (F = 13.5, P < 0.0001). The number of mitotic figures in each of the three concentric rings was inversely related to the distance from the central blood vessel. The mean number of mitotic figures for the inner, middle, and outer rings were 6.2, 2.8 and 0.5, respectively. Because the areas of the rings were not equal we also calculated the number of mitotic figures per 10,000 μm² (Table 1). We did not observe any mitotic figures more distant than 73 μm from the blood vessel. None of these 15 tumors had extraocular extension. The mean largest dimension for the 15 tumors was 16.1 ± 6.2 mm. There was no significant correlation between the size of the tumors and the thickness of the cuff (P = 0.8).

Discussion

The sleeve pattern was a frequent finding in the 200 retinoblastomas we reviewed, being present in 75% of the cases. All of the 105 tumors without extraocular extension had the sleeve pattern, but the sleeve pattern of growth is not specific for retinoblastoma. In ocular tumors, we noted the sleeve pattern in cases of oat cell carcinoma of the lung metastatic to the choroid and rarely in cases of uveal melanoma. Moore et al7 studied 96 squamous cell carcinomas from bronchus and found the sleeve pattern in 33 tumors (34%). They also studied 140 squamous cell carcinomas of the uterine cervix, and 19 of them (14%) had the sleeve pattern. The sleeve pattern seems to be more related to the rate of growth and ability of the tumor to induce neovascularization than to histologic type.

The thickness of the sleeve of viable retinoblastoma cells surrounding blood vessels was very consistent in the 150 tumors (98.7 ± 11.9 μm). Shipper1 reported in his thesis that the cuff of viable retinoblastoma cells ranged in thickness from 90–110 μm, results almost identical to ours; however, he did not investigate this observation further. We found that the sleeve thickness was even more consistent when it was measured on multiple cuffs in the same retinoblastoma. The area and thickness of the cuff were related to the MA in the cuff and the VD.

Moore et al7 found that the mean thickness of the cuff of neoplastic cells in 52 squamous cell carcinomas was 104 μm. Tannock2 studied rapidly growing experimental tumors and found that the thickness of the sleeves of viable cells ranged from 60–120 μm. He showed that the thickness of the cuff is the result of the radial diffusion of oxygen from tumor blood vessels. Oxygen deficiency was the major factor causing necrosis of the cells because of its high rate of consumption and low solubility in the neoplastic tissue. In this radial diffusion model the distance from the blood vessels to the necrotic cells is a function of the rate at which oxygen tension drops to zero.

The distance that oxygen can diffuse (the oxygen diffusion radius) is dependent on a number of factors. These include: (1) the blood oxygen tension, (2) the coefficient of diffusion of oxygen in the tissue, and (3) the rate of oxygen consumption by the neoplastic cells. Of these three factors oxygen consumption by the cells of the retinoblastoma and blood oxygen tension are the ones that most probably account for the variability in the thickness of the cuffs that we observed between different tumors. We suspect that VD is related to the blood oxygen tension, and the MA in the cuff is related to the metabolic activity of the tumor cells.

The observed relationship between VD and thickness of the cuff suggests a positive correlation between VD and blood oxygen tension. Fenton et al9 studied four different types of experimental tumors and found a complex relationship between the oxygen tension and VD that was dependent on the location of the vessel in the tumor and the type and size of the tumor. Only one of the four types of tumors had a consistently positive correlation between VD and blood oxygen tension.

Tannock1 found, using tritiated thymidine labeling in a mouse mammary tumor, that the fraction of cells that undergoes cell division (growth fraction) is inversely related to the distance from the blood vessels. The growth fraction in the mouse mammary tumor was 100% for cells located less than 30 μm from a blood vessel. For cells located between 30–60 μm from the blood vessel the growth fraction was 80%, and for cells situated greater than 60 μm, the growth fraction was 50%. He observed no differences in the cell cycle time or in the length of G1, S, and G2 with distance from the blood vessel. In his experiments, the decrease in mitotic activity with distance from the blood vessel could be directly correlated with the decrease in growth fraction. The loss of cells from the

Table 1. Mitotic activity (± standard deviation) within concentric rings of retinoblastoma cells surrounding blood vessels*

<table>
<thead>
<tr>
<th>Ring of neoplastic cells</th>
<th>Number of mitotic figures per ring</th>
<th>Number of mitotic figures per 10,000 μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner third</td>
<td>6.2 ± 2.7</td>
<td>7.7 ± 3.7</td>
</tr>
<tr>
<td>Middle third</td>
<td>2.9 ± 1.6</td>
<td>2.1 ± 1.2</td>
</tr>
<tr>
<td>Outer third</td>
<td>0.6 ± 0.6</td>
<td>0.3 ± 0.3</td>
</tr>
</tbody>
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* The thickness of each ring equals one third the thickness of the neoplastic cuff.
proliferative population would result in a slowing of the growth rate of the tumor as it becomes larger.

Our observations on MA in the perivascular cuffs of retinoblastomas parallel those of Tannock.3 We found that the mitotic rate decreases with distance from blood vessels (Table 1). This finding suggests that retinoblastomas, like experimental tumors in animals, do not grow exponentially.10 The growth rate of retinoblastomas is more dependent on the ability of the tumor to induce neovascularization than on the inherent growth rate of the neoplastic cells.

The pattern of growth in the retinoblastomas with sleeves of viable cells around central blood vessels was observed less frequently when there was extraocular extension. In the 95 tumors with extraocular extension, the sleeve pattern was absent in the orbital portion in 91 cases and was missing in both the intraocular and orbital portions in 50 cases. We believe that multiple factors may be related to these findings. First, based on the fact that all 105 retinoblastomas without extraocular extension had the sleeve pattern, we suspect that most of the retinoblastomas without the sleeve pattern probably had this pattern before they developed extraocular extension. In tumors without the sleeve pattern, the original intraocular portion of the tumor with the perivascular cuffs probably was replaced by tumor cells able to recruit new vessels from sources other than the central retinal artery. Second, we observed that the vascular pattern in retinoblastoma growing in the orbit is different than that in the eye when there is no extraocular extension. In the eye the tumor has large dilated vessels that are widely separated. In the orbit the tumor grows with numerous small capillaries interspersed between the tumor cells. Third, until the tumor invades the choroid all of the blood supply to the tumor must come from the central retinal artery. This may limit the amount of neovascularization of the tumor. Fourth, the vitreous has been observed to have an inhibitory effect on vascular proliferation.11 Fifth, in contrast with orbital invasion, central nervous system metastases of retinoblastoma frequently have the sleeve pattern of growth. These observations suggest that the orbital tissues provide a better environment for neovascularization that does the retina and central nervous system.

The sleeve pattern of growth in retinoblastomas probably does not have prognostic significance beyond its correlation with extraocular extension,12 but it may have clinical implications. It has been observed that the radiosensitivity of neoplastic cells is dependent on oxygen tension.2 This suggests that the radioresistance of cells of the retinoblastoma is a function of their distance from the central blood vessel in the cuffs of neoplastic cells. The cells most likely to survive after radiation are those in the periphery of the cuff.

Key words: retinoblastoma, blood vessel, oxygen, growth pattern, mitotic activity

References