increased in the dark. Fig. 3 shows that the major cortical response elements (identified also in Fig. 1) seem to correlate poorly with increasing artifact area and total stimulus charge. Smaller responses were produced by the higher charges and associated artifacts.

The sites of electrical excitation of the retina are not known. One would expect most retinal cells to respond at some current level. Ogden and Brown localized electrically stimulated responses to both the outer and inner nuclear layers. Brindley and Knighton concluded that best sensitivity probably lies in the region of the photoreceptor synaptic terminals. But Potts and Inoue have succeeded in evoking visual experiences in persons with blinding diseases of the outer retinal layers. If it was known that receptors were not present in these patients, the expected excitability of the inner retina would be verified. Future work with the chronically implanted retina will attack the contributions of the receptors.

From the Departments of Ophthalmology and Physiology, University of Florida, Gainesville, Fla. This research was assisted by grant B NS 75-20147 from the National Science Foundation. Submitted for publication Jan. 21, 1977. Reprint requests: Dr. William W. Dawson, Department of Ophthalmology, Box J-284, J. Hillis Miller Health Center, University of Florida, Gainesville, Fla. 32610.

Key words: electrical excitability, retina, light adaptation, phosphenes, chronic electrodes.

REFERENCES


Calcification in retinoblastoma. JOHN D. BULLOCK,* R. JEAN CAMPBELL, and ROBERT R. WALLER.**

Review of 40 cases of retinoblastoma revealed that histologic evidence of calcification was noted in 38 of the 40 tumors, whereas only three of 16 roentgenograms were positive for calcium. Calcification detected by roentgenograms and histopathologic examination correlated poorly with quantitative determination for tumor calcium. Compared to control eyes, however, eyes with retinoblastoma contain large amounts of calcium (1.2 vs. 218 micromoles/mg, ash). This calcification, though frequently not observed in standard roentgenograms, should be detected by the newer diagnostic modalities such as hypocycloidal polytomography, computerized transaxial tomography, ultrasonography, and radionuclide scintigraphy with technetium diphosphonate, a bone-scanning agent.

Roentgenographic evidence of calcification is considered one of the most useful signs in the diagnosis of suspected retinoblastoma in patients who present with leukokoria. The standard roentgenographic examination, however, is not uniformly successful in establishing the correct diagnosis. The reported rates of calcification on roentgenograms in patients with known retinoblastoma have been as high as 80 percent in some series but as low as 50 percent in others. These figures are important because retinoblastoma is frequently misdiagnosed. In patients with suspected unilateral retinoblastoma, Howard found that 12 percent had no malignant disease, whereas Kogan and Boniuk found 30 percent without malignancy.

Recent technological advances have facilitated the in vivo localization of calcium in tissue. These include hypocycloidal polotomography, computerized transaxial tomography, ultrasonography, and radionuclide scintigraphy with technetium diphosphonate. The possibility of using these new techniques to enhance the physician's diagnostic acumen prompted a study of calcification in retinoblastoma.

Materials and methods. A study of 40 eyes with retinoblastoma was undertaken to identify and quantify the degree of calcification present within the tumors. Roentgenograms of the skull and orbit, when available, were reviewed to...
Fig. 1. Roentgenogram of retinoblastoma patient, showing calcification within left orbit.

Fig. 2. Left, Gross specimen of retinoblastoma with obvious flecks of calcium. Right, Roentgenogram of globe showing obvious calcific densities.
determine the presence of calcium. Microscopic sections were examined, and tissue blocks were x-rayed and then ashed for quantitative analytic studies.

Results.

Standard roentgenographic findings. Roentgenograms were taken of only 16 of the 40 tumors, and only three of the 16 showed evidence of calcium. Of those three patients, one had positive findings in both the skull and the orbital views (Fig. 1), one had a positive skull view but the orbital view was not done, and the third had a negative skull view and a positive orbital view. This demonstrates the importance of obtaining the orbital view in the diagnosis of retinoblastoma.

In one case, the gross specimen of retinoblastoma (Fig. 2, left) contained multiple flecks of calcium that were readily detected on the roentgenogram of the globe (Fig. 2, right) but were not seen on the orbital view before enucleation.

Histopathologic findings. Of the 40 retinoblastomas, 38 (95 percent) contained histologic evidence of calcium. Calcification was readily detected on sections stained with hematoxylin and eosin and was confirmed with Von Kossa and alizarin red stains.

Tissue block roentgenograms. The tissue blocks of the specimens were then x-rayed. Of the 37 blocks that were available, only 26 (70 percent) were positive for calcium. The findings varied from highly positive for calcium to complete absence of calcification (Fig. 3).

Quantitative analytic studies. Eight blocks were ashed and quantitatively analyzed for calcium (Table I). The quantities of calcium measured were then correlated with the presence or absence of calcium on the histologic sections and on roentgenograms of the blocks.

The two specimens that contained no histologic evidence of calcification averaged 221 μg (± 7 S.D.) of calcium per milligram of ash, whereas the six specimens in which calcium was
The patients were less than 15 years of age.

dence of calcification averaged 211 /ig (± 19 gram of ash, but the ashed control eyes averaged only 1.2 /ig (Table I).

Table I. Calcium content

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Ca content (μg/mg ash)</th>
<th>Histologic section</th>
<th>Roentgeno-gram of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma eyes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>220</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>226</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>190</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>193</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>259</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>195</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>248</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>216</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>218 ± 26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control eyes (all autopsy eyes; no pathology):

<table>
<thead>
<tr>
<th></th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>2.8</td>
<td>0.1</td>
<td>1.8</td>
<td>0.2</td>
<td>1.2 ± 1.3</td>
</tr>
</tbody>
</table>

Calcification

histologically evident averaged 218 μg (± 30). This difference was not statistically significant.

The calcium content of the three specimen blocks that contained no roentgenographic evidence of calcification averaged 211 μg (± 19 S.D.) of calcium per milligram of ash, whereas the five with calcium averaged 223 μg (± 30). This difference was not statistically significant.

Control series. A series of 24 patients was reviewed in whom enucleation was performed for reasons other than retinoblastoma or trauma. The patients were less than 15 years of age. Block roentgenograms of 24 specimens revealed only four (17 percent) with evidence of calcium. Lesions that are most commonly confused with retinoblastoma were consistently negative for calcium. These included glaucoma (six cases), Coats' disease (five cases), endophthalmitis (three cases), persistent hyperplastic primary vitreous (three cases), and microphthalmos, retinal dysplasia, and detachment (one case each). However, there was a notable absence of retrolental fibroplasia, which frequently contains calcium. 7

Positive roentgenographic evidence of calcium was found in the globe of a 5-year-old child who had microphthalmos with cyst and in the globes of three children with phthisis bulbi who were 4, 6, and 8 years old. The differentiations in these cases were made clinically, and therefore the roentgenograms were unnecessary.

The calcium content of the eyes with retinoblastoma was significantly (p < 0.001) higher than that of the control eyes. The retinoblastoma specimens averaged 218 μg of calcium per milligram of ash, but the ashed control eyes averaged only 1.2 μg (Table I).

Comment. Our review showed that calcium is present in large amounts in retinoblastomatous eyes, as compared with controls, yet standard roentgenograms are not reliable in detecting the calcification, a view which is commonly held.

In detection, distribution of calcium is important because a localized nidus would be detected by roentgenogram, yet diffuse calcification might not be seen. The newer diagnostic modalities such as hypocycloidal polytomography, computerized transaxial tomography, ultrasonography, and radionuclide scintigraphy with technetium diphosphonate* are more sensitive and should help in the clinical differentiation of retinoblastoma.

Jenifer Jowsey, Ph.D., performed the quantitative analytic studies of calcium.

Key words: retinoblastoma, ocular calcification.

From the *Wright State University School of Medicine, Dayton, Ohio, and **Mayo Clinic and Mayo Foundation, Rochester, Minn. Presented at the meeting of the Retina Section, Association for Research in Vision in Ophthalmology, Sarasota, Fla., April, 1976. This study was supported in part by the Heed Ophthalmic Foundation (Dr. Bullock). Reprint address: Dr. J. D. Bullock, 60 Wyoming St., Dayton, Ohio 45409.

*Technetium diphosphonate is a radionuclide with a great affinity for calcium hydroxyapatite, which has been shown to be present, along with other calcium compounds, within retinoblastomas (J. D. Bullock and H. C. W. Skinner: unpublished data). The ability of this agent to detect osseous calcification is presently being investigated in the rabbit by one of us (J. D. B.). In addition, radioactive calcinum and strontium are being studied for their use as radioactive labels in the diagnosis of retinoblastoma.

REFERENCES