Retinoblastoma treated by freezing

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The effect of cryotherapy on intraocular neoplasms is disappointing, except in the case of retinoblastoma, which is readily destroyed by freezing and in which cures have been reported by Lincoff, Maclean, and Long (1967) and Rubin (1969). This communication reports the effect of cryotherapy on the second eye in a case of bilateral retinoblastoma, in which complete histological examination of the treated eye by serial sections was carried out after death, which was caused by metastases from the grossly involved first eye.

Case report

A 3-year-old boy of Cape Coloured descent underwent enucleation of a blind staphylomatous left eye on January 16, 1970.

At this time the right fundus was normal on indirect ophthalmoscopy and he was discharged from hospital 3 days later to be followed-up as an outpatient. Routine histological examination of the enucleated eye revealed a tumour filling the globe and extending into the cut end of the optic nerve stump. The child did not return to the clinic, and after letters had produced no result the police took a message to the family and the child was readmitted on March 1, 1970, which was 6 weeks after the enucleation.

Examination on second admission

The left socket was healthy and no mass could be detected by digital examination. General examination revealed a cheerful well-nourished child.

The right eye showed a large mass of tumour in the superior and superotemporal areas of the fundus with obvious vitreous seedlings (Figs 1 and 2, opposite).

Special investigation

X ray examination of the whole skeleton including the optic foraminae and the lung fields showed no abnormality, and the haemoglobin level, white cell count, and erythrocyte sedimentation rate were all within normal limits.

Therapeutic possibilities

Great reluctance was felt at removing the second eye, particularly as the spread of the tumour from the first eye rendered the prognosis as to life very poor.

The tumour seedlings were too anterior and extended too far into the vitreous for the technique of applying radioactive sources to the sclera to be used (Stallard, 1948). We therefore considered the heroic manoeuvre of inserting a radioactive tantalum wire into the vitreous near the tumour, but planning for this treatment showed that it was impossible to deliver the 10,000 r needed to destroy anoxic vitreous seedlings without causing a cataract, so this approach was abandoned.

Intra-arterial cytostatic agents would only have reached the vitreous seedlings if given in large doses which would cause blindness from retinal fibrosis.

FIG. 1 Plan of fundus, showing retinoblastoma (hatched), area frozen (outlined - - - -), and areas removed to allow entry to fixative (dotted)

FIG. 2 Fundus photograph, showing tumour deposits in vitreous

The only form of local therapy available therefore was to freeze the whole tumour including the vitreous seedlings. This entailed the freezing of about one-fifth of the whole volume of the eye and required an iceball of at least 6 mm. radius in order to include all the tumour deposits in the vitreous. Moreover, this volume of freezing had to be produced without repeated cycles of freezing and thawing in order to avoid severe haemorrhage.

Fortunately a suitable cryoexy machine was available. Originally designed for retinal detachment work and for use with CO₂ gas as a refrigerant, this machine had a 4.5 mm. diameter probe which fell to a temperature of $-86^{\circ}\text{C}.$ and could form an iceball 10 mm. in diameter, when liquid nitrous oxide from an anaesthetic gas cylinder was used as the refrigerant (Molteno & Luntz, 1968).

Treatment

Irradiation of both orbits, optic nerves, and chiasmata was started, supplemented by intramuscular triethyl melamine (TEM) and preceded by cryotherapy to the lesion of the right eye (Fig. 3).

FIG. 3 Radiotherapy plan, showing equal irradiation of both orbits

FREEZING TECHNIQUE

Under general anaesthesia the upper half of the globe was exposed *via* an incision in the superior fornix. The probe was applied to the full-thickness sclera. It was positioned by observing indentation with a direct ophthalmoscope and then cooled until the iceball ceased to grow after between 2 and 2½ minutes.

A total of five overlapping applications was given; after this procedure there was considerable vitreous haze in the vicinity of the tumour through which small haemorrhages were visible, but the appearance of the tumour was otherwise unaltered.

POSTOPERATIVE COURSE

For the first week there was considerable conjunctival chemosis and the child could not bear close examination, but 8 days after the operation adequate examination of the fundus was possible. This showed clear media and a greatly shrunken tumour with numerous small discrete haemorrhages on its surface. By the 14th day most of the tumour had disappeared to leave a finely pigmented choroido-retinal scar. A yellow area of thickened retina some four disc diameters across and situated three disc diameters above the disc together with a small cluster of vitreous seedlings (which included the tip of the stalactite like deposit shown in Fig. 2) remained.

Re-operation

3 weeks after the first operation the residual tumour was treated by freezing. The superior rectus muscle was detached to allow access to the mass near the disc, while the area frozen was extended forward to include part of the ciliary body and any tumour deposits hidden amongst its processes. By indenting the sclera more deeply, the iceball was increased to include the remaining vitreous seedlings. Eleven overlapping applications were given, taking care to avoid freezing any area of tissue more than three times by slightly offsetting adjacent rows of applications.

POSTOPERATIVE COURSE

The residual tumour again showed scattered small haemorrhages during the first week but by the end of the second week all signs of tumour had disappeared, leaving an oval pupil displaced supero-temporally, a localized equatorial lens opacity, a large area of fine pigmentary disturbance in the fundus, and macular oedema which resolved during the third week after operation.

The child appeared to see well, played normally, and continued to receive cobalt⁶⁰ radiation (supplemented by three doses of 0.97 mg. intramuscular TEM) to a total dosage of 3,510 r.

Appearance of metastases

On May 3, 1970, a mass developed in the left socket to be followed shortly by widespread bony metastases and irregular enlargement of the liver. All active treatment was discontinued apart from opiates used to control pain. The metastases enlarged rapidly until death ensued on July 15, 1970.

The child could see and grasp accurately small objects offered to him 3 days before death. Immediately after death the fundus was examined by direct ophthalmoscopy and showed clear media with no signs of tumour. The eye was then enucleated, injected with buffered glutaraldehyde formalin solution, and placed in a container of the same fixative.

Autopsy findings

Both orbits and their contents were removed and the intracranial portions of both optic nerves and the chiasma were examined histologically at sixteen different levels.

The final diagnosis was that of a poorly differentiated fungating retinoblastoma of the left orbit with infiltration of all orbital structures. Spread into the subarachnoid space adjacent to the optic chiasma had occurred, with widespread metastases to the skeleton

(including the calvaria, rib cage, vertebral bodies, and right femoral neck, para-aortic lymph chain, liver, pancreas, and suprarenal bodies.

LEFT EYE

The histological appearance of the left eye, the left orbital recurrence, and all the secondary deposits examined was similar: an ill-differentiated tumour made up of medium-sized cells with deeply staining basophilic nuclei, and scanty cytoplasm; approximately six mitotic figures were present in each high-power field; areas of necrosis were present in the larger deposits but true rosette formation was not seen. Sections through the intracranial portions of the optic nerves and chiasma showed tumour cells in the subarachnoid space next to the left optic nerve and the left side of the chiasma, but the nerve tissue itself was not invaded.

RIGHT EYE

After fixation the eye was opened by cutting small windows just posterior to the insertions of the medial and lateral rectus muscles to allow entry of the paraffin wax.

After imbedding, the eye was cut into serial sections; these were stained with haematoxylin and eosin, except for every 50th section for which Verhoeff's connective tissue stain was used. The sections (715 in all) were examined under $\times 25$ and $\times 250$ magnification. An oil immersion lens with $\times 1,000$ magnification was used to examine areas of special interest.

Histological findings

The frozen area showed marked thinning of retina and choroid and atrophy of the ciliary body and iris. The extent of these changes was measured on every 50th slide in order to construct the diagram (Fig. 1). The section along the plane A-B is shown in Fig. 4.

FIG. 4 Section along plane A-B of Fig. 1, showing effects of freezing on iris, ciliary body, and retina extending posteriorly to a point 4 disc diameters above the optic nerve. Haematoxylin and eosin. $\times 1.6$

The vitreous formerly occupied by tumour tissue showed fine eosinophilic fibrils irregularly and loosely arranged with a total absence of cellular elements (Figs 5 and 6, overleaf).

The retina and choroid layers were fused together with loss of rod and cone outer segments and of ganglion cell nuclei at the periphery of the frozen area, but centrally the retina was markedly thinned and reduced to a feltwork of glial fibres containing a few nuclei resembling those of fibroblasts

FIG. 5 Higher-power view of Fig. 4, showing changes in iris, ciliary body, and adjacent vitreous. Haematoxylin and eosin. $\times 18$

FIG. 6 Higher-power view of Fig. 4, showing changes in peripheral retina and adjacent vitreous. Haematoxylin and eosin. $\times 18$

together with scattered uveal and neuroepithelial pigment containing cells. The retina in these areas was firmly fused with pigment epithelium which was irregularly thickened.

The choroid showed marked thinning with disappearance of the choriocapillaris and many of the medium-sized blood vessels. Bruch's membrane was not prominent in any of the sections, but the artefactual separation of the pigment epithelial layer from choroid around the periphery of the frozen areas demonstrated that it was probably intact, while more centrally, where it had been maximally frozen and where tumour tissue had been situated, the presence of uveal pigment-containing cells in the retina demonstrated that it had been breached either by excessive freezing or by the tumour (Figs 4 to 9, above and opposite).

The iris and ciliary body showed marked stromal atrophy and complete disappearance of the neuroepithelial layers where frozen (Fig. 5).

In the lens the localized equatorial opacity noted during life could not be detected histologically.

The remainder of the eye which had not been treated by freezing showed histologically normal retina, fovea, choroid, ciliary body, iris, drainage pathways, and optic nerve, with the exception of a minute area of retinoblastoma situated nasally and quite separate from the treated area. This retinoblastoma *in situ*, which showed typical histological features of the tumour, appeared to be originating from the inner nuclear layer and was confined to the retina except for three minute vitreous seedlings.

The only unusual findings were an almost complete lack of mitotic figures (1 to 8 per section), vacuolation, and hyperchromicity of nuclei in certain areas. These changes, probably caused by radiotherapy and TEM, were interspersed with areas of morphologically normal tumour cells; there was nothing to suggest that necrosis or shrinkage of the tumour had occurred (Figs 10 and 11, overleaf).

FIG. 7 Centre of frozen area, showing fusion of retina and choroid with destruction of normal architecture but no recognizable tumour. Haematoxylin and eosin. $\times 190$

FIG. 8 Heavily frozen area not involved by tumour, showing loss of rod and cone outer segments and of ganglion cells, but retention of traces of normal architecture. Haematoxylin and eosin. $\times 190$

FIG. 9 Posterior margin of frozen area, showing minimal changes: loss of ganglion cells and damage to rod and cone layer which is fused to pigment epithelium. Haematoxylin and eosin. $\times 190$

Discussion

The tumour in this case was of the anaplastic type and behaved aggressively, spreading rapidly and widely, but did not secrete significant amounts of vanillyl mandilic acid even when metastases had become clinically obvious.

The tumours of the right eye were almost certainly independent primaries, as the first appeared 3 months before any other secondaries, and the second was confined to the peripheral retina – a most unusual site for a blood-borne tumour metastasis.

The persistence of apparently viable tumour in the right eye (10 weeks after completion of a full course of Cobalt⁶⁰ and TEM therapy), the massive early recurrence in the left

FIG. 10 *Retinoblastoma in situ. Haematoxylin and eosin. $\times 18$*

FIG. 11 *Margin of retinoblastoma in situ, showing origin from inner retinal layers. Haematoxylin and eosin. $\times 375$*

orbit, and the clinical behaviour of the frozen tumour strongly suggest that cryotherapy was the only effective therapeutic agent in this case.

Summary

- (1) A case of bilateral retinoblastoma is described, together with the techniques used to freeze the tumour tissue in the second eye.
- (2) The autopsy findings are reported, including the histological examination of the treated eye by means of serial sections.

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