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EFFECTS OF IRRADIATION ON THE CILIA OF THE SYLVIAN AQUEDUCT

A scanning electron microscopic investigation

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The existence of a ciliated surface of the ependymal coating of the ventricular system of the central nervous system has been known for more than a hundred years although few investigations of the physiologic function of the cilia have been reported. Beating cilia in the brains of mammals were first revealed by PURKINJE in 1836. VALENTIN (1842) was the first to describe ciliary movements in the ventricular system of the human foetus and adult man. LUSCHKA (1855) confirmed these observations while they were contradicted by HASSALL (1852). The ependymal coating of the ventricles of the brain was then largely forgotten until the scanning microscope has again stimulated its examination. Most investigations on the ependymal cilia have been later than 1970 (KNIGGE & SCOTT 1970, TORACK & FINKE 1971, CLEMENTE & MARINI 1972, SCOTT et coll. 1972). The physiology of these cilia has also been the subject of much discussion. SCOTT et coll. stated: 'Cilia have traditionally been regarded as structures responsible for the movement of matter (e.g. mucus, fluids) through the lumen of the tubular organs'. That the cilia possess this power was demonstrated by KONNO & SHIOTANI (1956) and CATHCART & WORTHINGTON (1964) who used red blood corpuscles on the ventricular surface to demonstrate the stream caused by ciliary action. When the ciliary beating was stopped by epinephrine, the red blood

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corpuscles failed to move. A surface coated by cilia, due to its larger area, has a greater power of absorption. Microvilli, however, have also been described as being responsible for the function. This physiologic aspect has been established for surfaces covered by cilia in other parts of the body, for example in the trachea, nasal sinuses, nasal cavity and the ovarian duct.

This communication deals with the response of the cilia to ionizing irradiation in human subjects. It describes the appearances of the surface of the aqueduct of Sylvius in a patient who had undergone irradiation treatment for a temporal tumour and who died one year later. It is hoped that it will be of value to those who treat cerebral tumours and who have access to both scanning electron microscopy (SEM) and transmission electron microscopy to perform investigations of this type. It is obvious that the relatively restricted employment of this method necessitates collecting data from the isolated cases in different parts of the world in order to reach any conclusive results. It is important to establish a standardized method of approach as a basis for later comparisons.

There were two reasons for choosing the aqueduct of Sylvius for examination. First, it has been demonstrated that the aqueduct contains ciliated cells and since the tubulus produces a relatively high speed of ventricular fluid transportation, it was considered that the cilia had to be physiologically active. Secondly, the area received a large dose of irradiation so that abnormalities could be considered as being due to its effects.

Materials and Methods. A man, aged 75, who had died at the same time as the irradiated patient, was selected as a control. The sample was taken 21 hours after death in both subjects. The control had died from bronchopneumonia. The irradiated patient was a woman aged 62 who had had headache for a year. EEG, pneumography and angiography revealed a tumour of the right temporal lobe. Operation disclosed that the intracranial pressure was considerably raised with the gyri sulci levelled and destroyed. The corresponding upper posterior part of the temporal lobe fluctuated on palpation. One centimetre from the surface a cyst was emptied of a yellowish fluid. A reddish-blue solid tumour at the bottom of the cyst with the appearances of a malignant glioma infiltrated medially and upwards into the posterior part of the frontal lobe. Some of the mass was sucked out; microscopy revealed malignant glioma.

The condition of the patient markedly improved. It was the intention to give 5 600 to 5 800 rad within the area of the tumour and about 4 500 rad in the midline. Calculations indicated that this could be performed from only one side. As the tumor was so large, it was necessary that the field towards the site of the tumour should have a good margin. The patient was admitted to the radiation therapy clinic. A field 9 cm $\times$ 10 cm in size over the temporal lobe was mapped

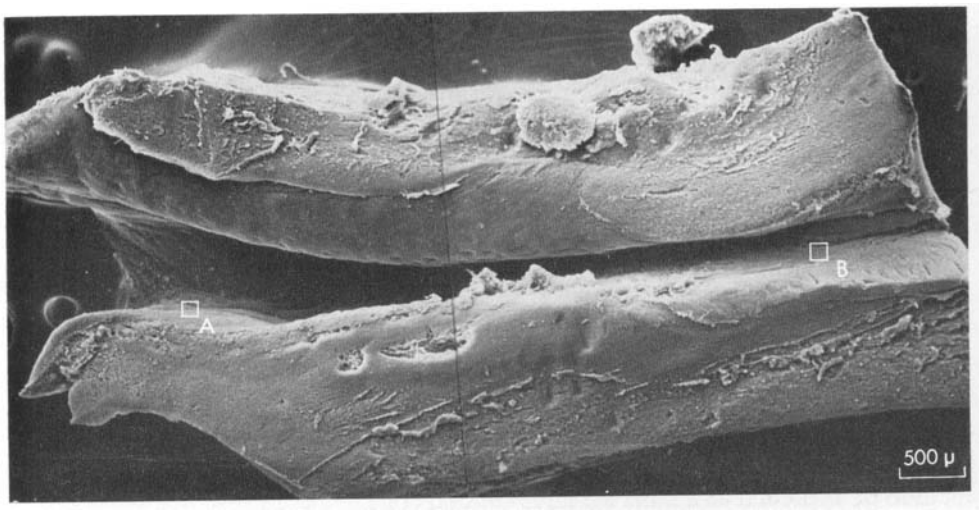

Fig. 1. Scanning electron microscopy of sectioned aqueduct of Sylvius. Cranial part to the left. ×17.5.

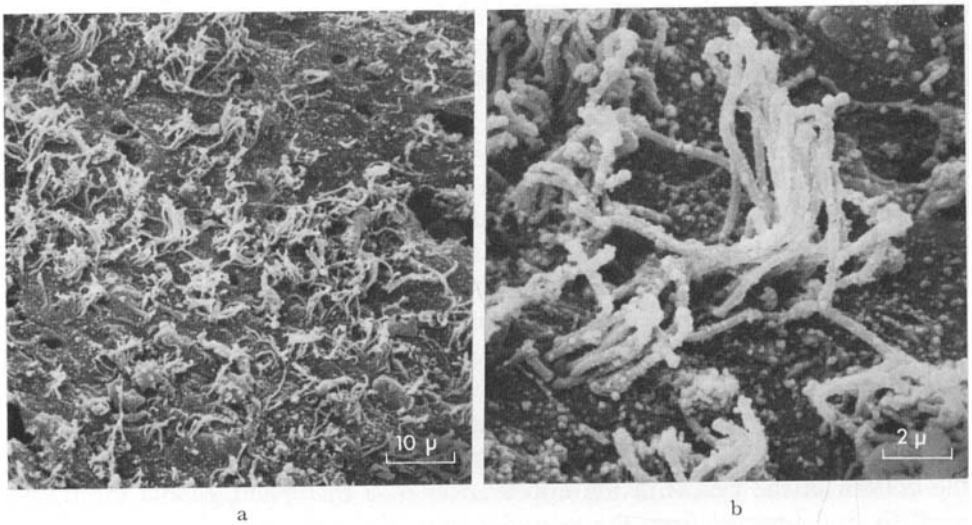

Fig. 2. Scanning electron microscopy from area A in Fig. 1 with groups of cilia. a) ×890. b) ×4 440.

out from the angiography with the aqueduct of Sylvius in the midline and near the midpoint of the field, and Betatron irradiation with a 33 MV dose of 1 625 rad (19 days, 10 fractions, 5 days a week) applied. As the field was large, only 75 rad were given at the beginning and successively raised to 225 rad. This treat-

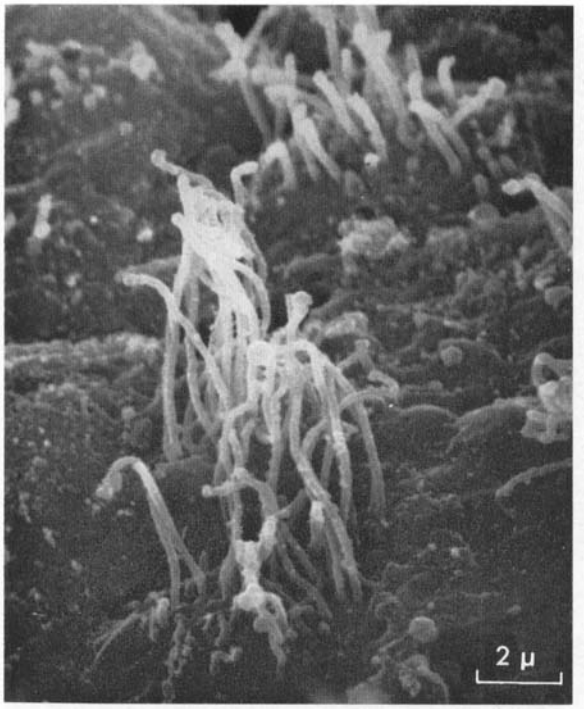

Fig. 3. Scanning electron microscopy from area B in Fig. 1 with groups of cilia. ×5 520.

ment was immediately followed by 8 MV photons over 23 days (total of 11 fractions with 225 rad at each treatment except the last two that were each of 250 rad). This scheme was interspersed with the administration of electrons (over 41 days), from 200 rad at the beginning up to 275 rad. The patient received a total calculated maximum dose of 5 800 rad to the tumour over 48 days and died 12 months later.

Both the cranial end and the caudal part of the aqueduct lay within the field of the 'full dose' which was calculated to be 4 800 rad in the midline (at a depth of 7 cm).

The aqueduct of Sylvius of the control was cut out in a rectangular cube measuring 7 mm × 7 mm × 17 mm, and after pre-preparation a part of it was fixed in glutaraldehyde 2.5 % in phosphate buffer (MILLONIG 1961), Ph = 7.4 for two hours. After rinsing in pure phosphate buffer, the specimen was divided in its midline and dehydrated in acetone, amylacetate and liquid carbon dioxide, and finally dried by the critical point method of ANDERSSON (1951). The pieces were then attached to a frame and coated with gold-palladium, after which they were examined microscopically with a Cambridge—Mark II Stereoscan scanning electron microscope.

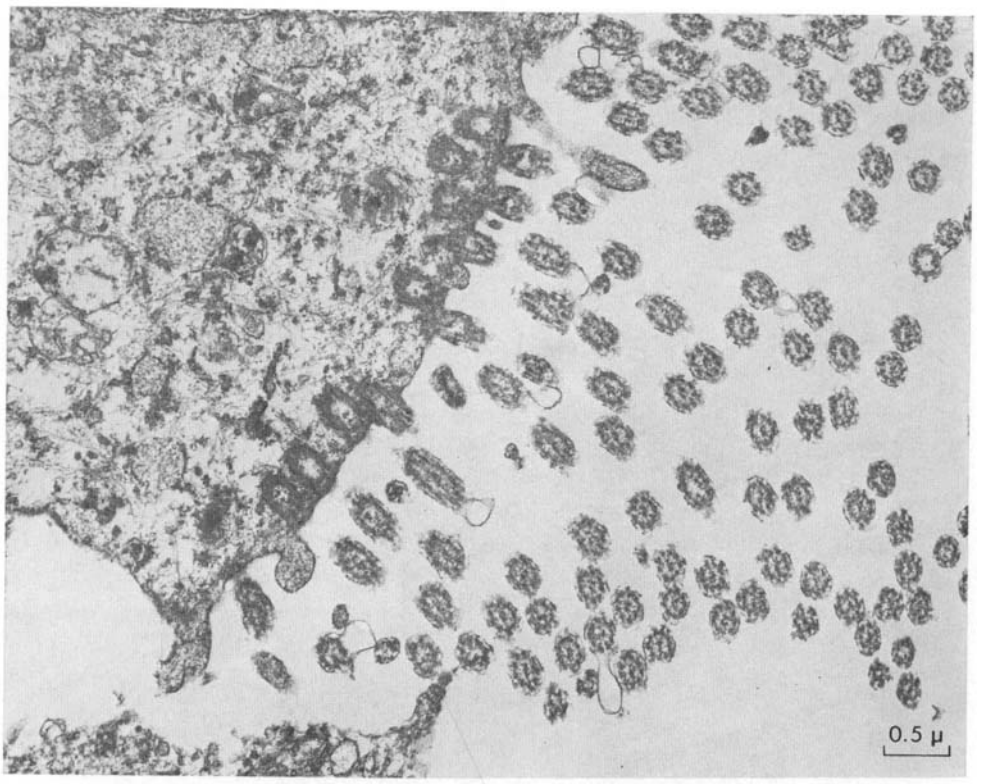

Fig. 4. Transmission electron microscopy of the ependymal ciliated cells in length and cross-sectioned 9 + 2 cilia. ×17 500.

Parts of the epithelium of the aqueduct of Sylvius were then fixed in OsO_4 1 % in phosphate buffer, Ph = 7.4 for two hours, after which they were rinsed in pure buffer, dehydrated in alcohol and imbedded in Vestopal W with styrene. One micron thick section was dyed in Richardson's azure II. Ultra-thin slices were cut out on an LKB-Ultrotome and contrasted in lead acetate-uranyl-acetate or in a cube with uranylacetate 0.5 %. They were then examined by microscopy with a Philips EM 300 electronmicroscope.

Results

One half of the sectioned aqueduct of Sylvius appears in Fig. 1. The ependymal cells covering the aqueduct were ciliated (Figs 2 a, 3). The cilia lay in groups and appeared to be evenly distributed along the entire aqueduct. The small, round formations resembling microvilli in Fig. 2 b probably represent postmortem

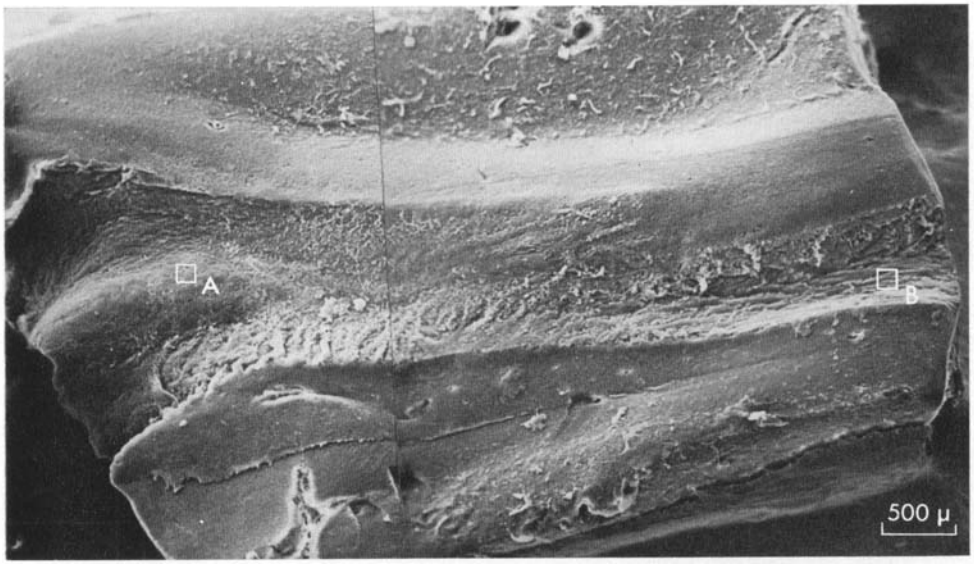

Fig. 5. Scanning electron microscopy of sectioned aqueduct of Sylvius from irradiated patient. Cranial part to the left. ×19.5.

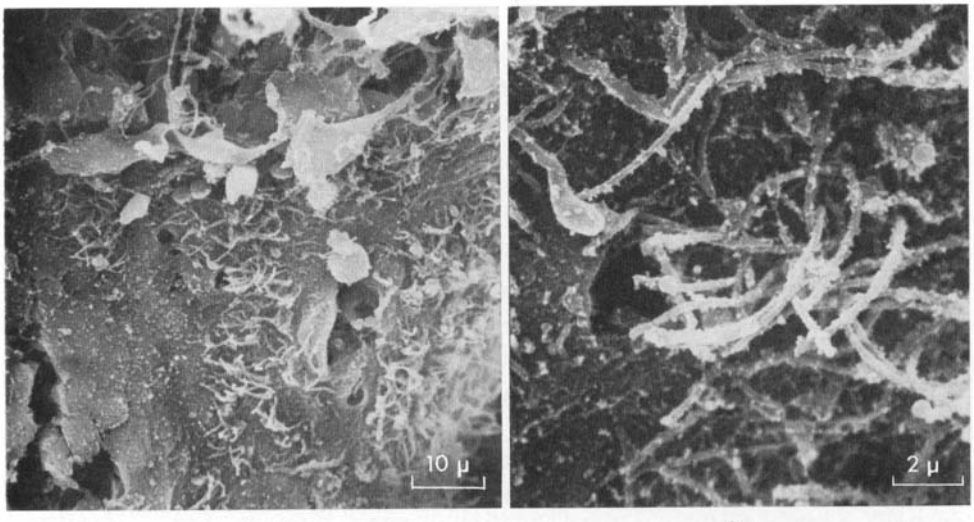

Fig. 6. Scanning electron microscopy from area A in Fig. 5 with groups of cilia. a) ×935. b) ×4 680.

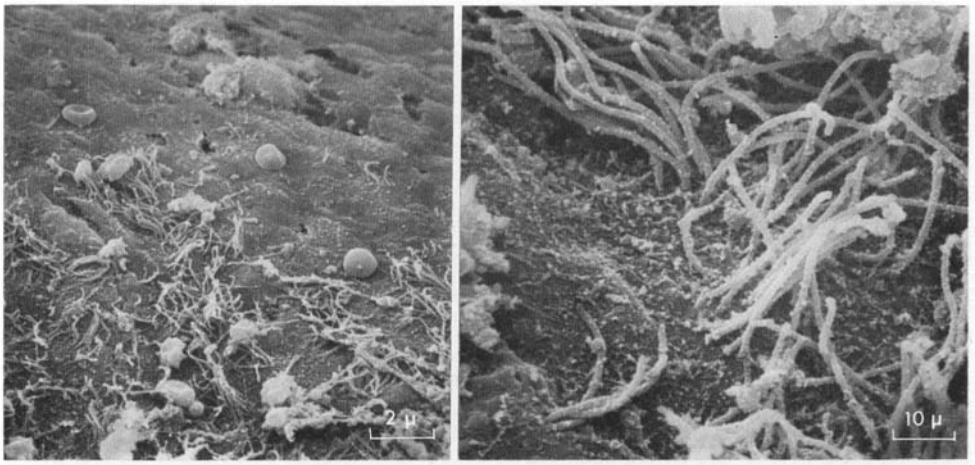

Fig. 7. Scanning electron microscopy from area B in Fig. 5 with groups of cilia. a) ×835. b) ×4 170.

changes. Fig. 4 is a section through the ependymal cells; they belong to the 9 + 2 type of cilia, the motile ones. Fig. 5 represents one half of the sectioned aqueduct of Sylvius taken from the irradiated patient, where the ependymal cells are also ciliated, but where areas with a lesser number of cilia and also parts having no cilia at all are present (Figs 6 a, 7 a). It is not possible to ascertain any differences in the appearance and thickness of the cilia. The ependymal cells (Fig. 7 b) also have surface formations resembling microvilli, and are again probably postmortem changes.

Discussion

The presentation although only of a single case would appear to be justified by introducing radiation biology, and more specifically the therapy of tumours of the central nervous system, into more or less virgin domains. Virgin, as the instruments necessary for the examinations have not previously been available although not unknown, for knowledge about the cilia has existed for a very long time.

The scanning electron microscope findings might indicate a generally reduced number of ciliary cells and it is assumed that this might have been the consequence of the radiation therapy; the circulation of the ventricular fluid might thus have been affected.

No further conclusions may be drawn about the effects of irradiation on the cilia in the current case. The cilia in the whole of the aqueduct of Sylvius had received 4 800 rad, but did not appear to differ much from those from the control patient. Experience with ciliary surfaces of the type in, for example the trachea and the bronchi, have indicated that cilia have an enormous regenerative capacity, so that those in the present examinations may have been newly developed cilia, unaffected by irradiation given 12 months earlier. A much larger material is necessary before any statements can be made regarding the effects of irradiation on the dynamics of the cerebrospinal fluid space.

SUMMARY

Scanning electron microscopy has been used to examine the surface of the aqueduct of Sylvius in a patient irradiated following operation for a malignant temporal glioma. The number of cilia covering the surface were reduced in comparison with those of a non-irradiated patient. The effect upon the circulation of the ventricular fluid is discussed.

ZUSAMMENFASSUNG

Skanning-Elektronenmikroskopie wurde verwendet, um die Oberfläche des Aquaeductus Sylvii bei einem Patienten zu untersuchen, der nach einer Operation wegen eines malignen Temporalglioms bestrahlt worden war. Die Zahl der Zilien, die die Oberfläche decken, war im Vergleich zu der eines nichtbestrahlten Patienten vermindert. Die Folgen für die Zirkulation der Flüssigkeit des Ventrikels werden diskutiert.

RÉSUMÉ

La microscopie électronique à balayage a été utilisée pour examiner la surface de l'aqueduc de Sylvius chez un malade irradié après opération pour un gliome temporal malin. Le nombre de cils couvrant la surface était diminué par rapport à un sujet non irradié. Les auteurs examinent l'effet de cette lésion sur la circulation du liquide ventriculaire.

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