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INDUCTION OF AN EXPERIMENTAL TUMOUR OF THE LENS*

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It is a curious fact, hitherto ignored by pathologists, that neoplasms of the lens are unknown to ophthalmologists. It is often stated by students of cancer that any tissue or organ capable of cell division may be the site of a malignant neoplasm, yet the lens, as far as we know, in spite of the fact that mitoses occur in the subcapsular epithelium throughout life, never produces a cancer. This immunity may theoretically be due to various causes. It might be that the cells of the lens vesicle are inherently from the beginning resistant to malignant change. This would be strange since, if it were true, the lens would stand practically in a class by itself. It might also be that the presence of the capsule inhibits new growths of the lens, either by the tension it exerts or by its properties of a semipermeable membrane, preventing access of the required stimulus to the lens cells. That this is not the case is obvious, since lenses with ruptured capsules never become malignant. A third possibility is that the lens owes its immunity

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to its lack of blood supply. This presupposes the possibility of a blood borne cause of cancer. It would therefore seem of interest both ophthalmologically and pathologically to discover whether the cells of the subcapsular epithelium (the only ones capable of dividing after birth) could be induced experimentally to undergo malignant change.

Pathological techniques for the induction of experimental cancer include the use of chemical carcinogenetic agents and the investigation of inbred strains of animals. By a combination of these methods applied to the lens it can be shown that, when treated with a chemical carcinogen *and* provided with a blood supply the subcapsular epithelium will produce an epithelioma of extreme malignancy. There is, therefore, no inherent intracellular immunity of the lens to cancer and it is likely that its escape is due to its avascularity. The chemical agent chosen for the experiments was methylcholanthrene. This is known to induce tumours in many species of mammals (including rabbits and mice) when a minute quantity of it is placed in or in contact with the organ under investigation. Some strains, even of the same species of animal, are more resistant than others to this agent and in all species there is a latent interval of weeks, months or years (according to average length of life) before the tumours appear.

The first experiments were done on rabbits. A droplet of 0.4 per cent. solution of methylcholanthrene in liquid paraffin was injected with a fine needle into the lens cortex. The minute puncture of the capsule sealed completely and the lens remained clear, so that slit-lamp observation was possible. The oil could be seen lying in globules along the needle track. It showed a purple fluorescence in the slit-lamp beam, owing to the presence of the methylcholanthrene. The eyes were observed at weekly and then monthly intervals for 10 months, but no change in the lens occurred. Transparency was maintained and the droplets did not change their position in the lens, though a few minute crystals separated out within them. The eyes remained quiet throughout. It is possible that the animals have not yet been observed sufficiently long, but so far no effect has been produced by injecting the lens *in situ*.

It therefore seemed advisable to attempt to transfer the lens to a position where it could acquire a blood supply and there to treat it with the methylcholanthrene. For this purpose inbred strains of animals are necessary, and mice were chosen, both for their relative rapidity of reaction and because inbred strains showing a high natural cancer incidence were available.

Homozygous strains of mice are produced from a single pair by brother-sister matings through many (at least 30 and preferably

100) generations. When successful this inbreeding produces a pure strain in which all the mice resemble each other, all react in the same way to the same stimuli and all can be grafted with tissues (both normal and malignant) from each other without the production of immunological reactions leading to the death of the graft. Many of these strains exist and one of them, the C3H strain, was chosen as it was naturally prone to develop cancer and was known to react readily to methylcholanthrene.

The method was suggested by the work of Peyton Rous and Smith¹, who showed that when embryo tissue of pure line strains was grafted intramuscularly in adult related mice together with a trace of methylcholanthrene, malignant teratomas developed. These could be maintained indefinitely by transplantation from mouse to mouse of the same strain.

The lenses were dissected out from the eyes of young mice and implanted with the carcinogen under the skin of the flank of adult related mice. Three cancers of subcapsular epithelium were obtained.

Experiments and details of technique

Thirty-eight experiments were performed and 26 tumours resulted. Of these, three were carcinomas of the subcapsular epithelium and the remainder various types of sarcoma of the host mouse or epitheliomas of its skin. The three lens tumours occurred in the subcutaneous tissue of the flank of male mice, which rules out any possibility of derivation from mammary gland tissue of the host. The lenses were obtained from the eyes of mice of various ages (new born, two days, three days, four days, seven days and nine days old), but tumours were induced only with seven day and nine day old lenses. They were inserted under the skin of the flank of mice of the same inbred strain, together with methylcholanthrene. In some cases the hyaline capsules were ruptured and the lenses mixed with a few crystals of methylcholanthrene. In others the unruptured lenses coated with a solution of the carcinogen in oil were used. In others again teased lenses were mixed with methylcholanthrene in solution in equal parts of soft and liquid paraffin. The exact method employed did not appear to be important, two of the tumours being obtained with a solution of 1 mg. of methylcholanthrene per 0.5 c.c. of a mixture of soft and liquid paraffin, and the third tumour with crystals only. In all the successful experiments the lens capsules were ruptured. The tumours appeared between two and three months after implantation.

FIG. 1.

Tangential section through the subcapsular epithelium of the mouse lens at the anterior pole.

FIG. 2.

Subcapsular epithelium and equatorial region of lens of seven day old mouse.

FIG 3.

Section through lens tumour. Nuclei showing mitosis and typical arrangement of chromatin.

The tumours obtained were anaplastic carcinomas possessing certain characteristics of structure suggestive of their origin from the subcapsular epithelium (Figs. 1 and 3). They were investigated by stained sections, by smear preparations, by passing them through other mice of the same strain and by observing their behaviour in tissue culture.

The first tumour obtained (known as M8.134) can be taken as the type and described in full.

Protocol of experiment M8.134

The lens of a seven day old C3H mouse was mixed with a 0.2 per cent. solution of methylcholanthrene in equal parts of soft and liquid paraffin and inoculated into the subcutaneous tissues of the right flank of a male C3H mouse one month old. The small lump resulting from the inoculation remained for three—four weeks and finally disappeared. A tumour appeared at the site fairly suddenly about two and a half months after inoculation and grew rapidly, being ready for transplantation a fortnight later. The mouse was killed and the tumour examined and transplanted into six C3H mice. It has retained its characteristics through eight

passages so far and continues to grow rapidly, no failure being noted in any of the transplants.

Characteristics of lens tumour M8.134.—The tumour tissue is greyish and translucent. The grafts grow rapidly and form large tumours filled with necrotic tissue and blood. Sections of the periphery of the tumour show large cells with voluminous or oval

FIG. 4.

Smear preparation of lens tumour. The typical chromatin arrangement and the displacement of some of the nuclei to one side can be seen.

FIG. 5.

Tissue culture of lens tumour at 48 hours. Living preparation.

nuclei, prominent nucleoli and a reticular or punctate arrangement of chromatin. They resemble the normal cells seen in Fig. 1. Mitoses are frequent. In the young cells the nucleus is central, in the older cells it moves to the edge of the cell just before becoming pyknotic, as does the nucleus of the normal subcapsular epithelial cell before it differentiates into a lens fibre (Fig. 2). Large areas of necrosis and liquefaction occur in the centre of the tumour. Metastases occurring in the inguinal lymph nodes show the same

FIG. 6.

Extending flat sheet of cells at edge of tissue culture of living lens tumour at four days.

structure, but with less necrosis. The tumour is soft and the cells can be well seen in smear preparations (Fig. 4).

The tumour was grown in tissue culture* and Figs. 5, 6 and 7 show some results. Fig. 5 shows the explant after 48 hours. Tumour cells and a few fibroblasts are beginning to wander out. By four days (Fig. 6) the tumour cells have arranged themselves in the flat sheet typical of tissue cultures of carcinomas, as distinct from sarcomas, which show a scattered arrangement. If the culture is continued the sheet of cells tends to break away from the tumour explant and large and small islands of cells float free in the medium. Fig. 7 shows this and also demonstrates clearly the epithelial character of the cells. A high power photomicrograph of a portion of the sheet in Fig. 7 is seen in Fig. 8. The preparation is fixed and stained. Mitotic figures and completed mitoses are

* The best medium was found to be one part of rat serum, two parts tyrode solution and one part mouse embryo extract. Sometimes a few drops of distilled water were added.

FIG. 7.

Five day tissue culture of lens tumour M8. 134. The explant is on the left, the new sheets of cells are breaking away on the right. Living preparation.

FIG. 8.

Fixed and stained preparation of five day tissue culture of tumour M8. 134. Note characteristic nuclei and presence of mitotic figures.

seen. The similarity of the cells to those of the tumour seen in Figs. 3 and 4 is apparent. In some of the tissue cultures of the tumour the extending edge showed fibrillar outgrowths somewhat similar to those described by Kirby² in tissue cultures of chick lenses. These are seen in Fig. 9. This fixed preparation of a five day tissue culture of the same tumour also demonstrates the attempted differentiation by movement of the nucleus to the side of the cell before pyknosis sets in.

The other two tumours resembled M8.134 in their structure and behaviour. They were slightly slower in growth and one of them, although showing the same characteristic epithelial cells, also contained sarcomatous areas, probably derived from the host.

The main points which show these tumours to be derived from the implanted lens epithelium are their carcinomatous character, their growth in tissue culture in friable sheets and the displacement of the nuclei and their degeneration in the course of attempted differentiation.

Since these tumours are derived from the lens epithelium which is the anlage of the lens fibres, one might expect them to resemble these somewhat in chemical composition and therefore to contain

FIG. 9.

Growing edge of five day tissue culture of lens tumour M8. 134 showing fibrillar processes from the cells and the attempt at differentiation shown by the movement of the nucleus to the side of the cell. Some of these displaced nuclei are pyknotic.

the organ specific protein α crystallin. Although this is by no means the only protein in the adult lens, its universal presence has led to the idea that the lens, unlike all other organs, has very little or no species specificity. If this were true (which is doubtful) these lens tumours might be expected to grow when grafted into other strains of mice, or even into other species of animals. Experiments to decide whether this was the case were therefore undertaken. The tumours, being known to grow well when grafted into mice of the same homozygous strain, were grafted into mice of other inbred strains, some showing a high and some a low incidence of spontaneous cancer. The high cancer lines, Strong 3 and R3 and the low, C57 black and the S strain, were used. The tumour failed to grow in all of these. It is not, therefore, even chemically common to mice as a whole.

The work of Greene³ on the growth of heterologous tumours in the anterior chamber of the rabbit's eye suggested that the mouse lens tumour might be tried in this way. Two of the three lens tumours were planted out on to the iris of rabbits, and the course of events carefully watched with a slit-lamp. A slight inflammatory reaction occurred around the tumour, shown by a gelatinous exudate and a few haemorrhages. The graft then shrank, became buried by the iris stroma and disappeared. In 10 days its site was only apparent as a small puckered scar-like area on an otherwise normal iris. Lens tumours do not, therefore, exhibit organ specificity.

In conclusion I wish to thank the Council of the Imperial Cancer Research Fund for their assistance in this research.

Summary

The statement has been made by general pathologists that any tissue or organ capable of cell division may be the site of a malignant neoplasm. Ophthalmologists, however, agree that carcinoma of the lens is unknown, although mitoses occur in the lens epithelium throughout life. The possible reasons for this are discussed. Experiments showing the induction of cancer of the lens in mice are described, using pure line strains and chemical carcinogens. These demonstrate the ability of the lens epithelium to become malignant under certain conditions, and throw some light on the immunity of the organ in the body.

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