has taken place between the exposure of the frame from which the enlargement has been made and that now being examined. It is hoped that this method will enable the films to be examined with the required accuracy at a rate of about one frame every two or three minutes.

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TISSUE CULTURES OF MOUSE LENS EPITHELIUM

BY

IDA MANN

From the Laboratories of the Imperial Cancer Research Fund, London

IT has been pointed out by Kirby[1] that if an attempt is made to grow the lens in tissue culture the only cells which survive and multiply are those of the subcapsular epithelium. This is to be expected since the lens fibres are fully differentiated and cannot divide. Kirby's preparations were made with the lenses of chicks. In the present experiments an attempt was made to grow mammalian lens epithelium in tissue culture.

EXPERIMENTS

Mice were used, mostly of the inbred strain known as C3H. Lenses of young mice up to 10 days old were used first but proved difficult to plant out without infection. Lenses from embryo mice of the same strain were then tried, with greater success. The embryos used were in the later stages of the pregnancy and good results were obtained with full term embryos taken just before birth. With care uninfected cultures could be obtained in about 60 per cent. of the trials. About 70 cultures were made in all.

The culture medium which gave the best results had the following formula :—

Rat serum	... ...	1½ parts
Tyrode solution	... ...	1 part
Mouse embryo extract	...	1 part
Dist. water—1-2 drops per c.c.		

RESULTS

1. If the lenses were cultured free from all surrounding tissue and with intact capsules they survived for about 10 days unchanged, and no outgrowths occurred.

2. If the capsule was ruptured at the moment of planting it shrank and lens fibres and subcapsular epithelial cells were extruded. The lens fibres dissolved in the medium within the first 2-3 days. The capsule remained unchanged but the epithelium proliferated and grew away from it all round in a flat sheet of large globular clear cells. Fragments of the sheet tended to break away and float off, so that permanent preparations were hard to make. Most of the cultures were therefore photographed *in vivo*.

Differentiation could be seen in the cells of the culture around the tenth day, beyond which very few survived. The first stage of differentiation was shown by increase in the cytoplasm and movement of the nucleus to one side. In a few cells a further stage was reached, the voluminous clear cytoplasm apparently flattened so that the cell became bluntly pointed at both ends, the nucleus remaining applied to one side of the central part of the elongated portion of the cell. This movement of the nucleus to one side of the cell is the first stage in the process of normal differentiation of a lens epithelial cell into a lens fibre in the intact lens. It can be seen towards the equatorial region in horizontal sections of mouse and other mammalian lenses. The nucleus moves to the side of the cell next to the capsule, and the cell then elongates forming a lens fibre with the nucleus eventually equidistant from the two ends.

The accompanying figures are representative of the results obtained. Fig. 1 shows a stained preparation of a surviving intact mouse embryo lens at 6 days. The subcapsular epithelial cells stain deeply.

Fig. 2 shows a similar lens after 10 days survival. The staining is no longer uniform as most of the cells have disappeared over the anterior pole and the nuclei are pyknotic elsewhere.

Fig. 3 shows a mouse embryo lens growing in tissue culture at 3 days. The capsule was ruptured at the time of planting. Débris of lens fibres and beginning sheets of subcapsular epithelial cells surround the shrunken and wrinkled capsule.

Fig. 4 shows a culture at 6 days. The sheet of outgrowing epithelium is wider.

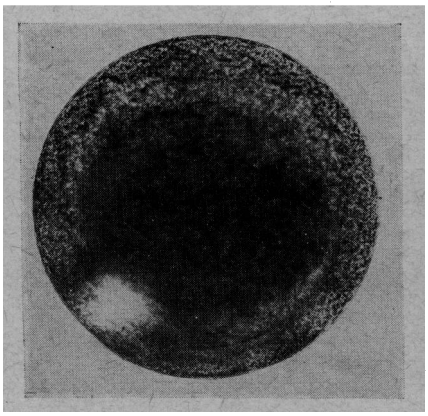

FIG. 1.

Intact surviving mouse embryo lens at six days, fixed and stained.

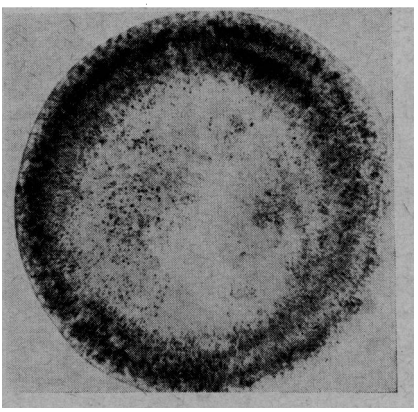

FIG. 2.

Intact mouse embryo lens in culture at 10 days. Fixed and stained. Many of the nuclei do not stain, many are pyknotic.

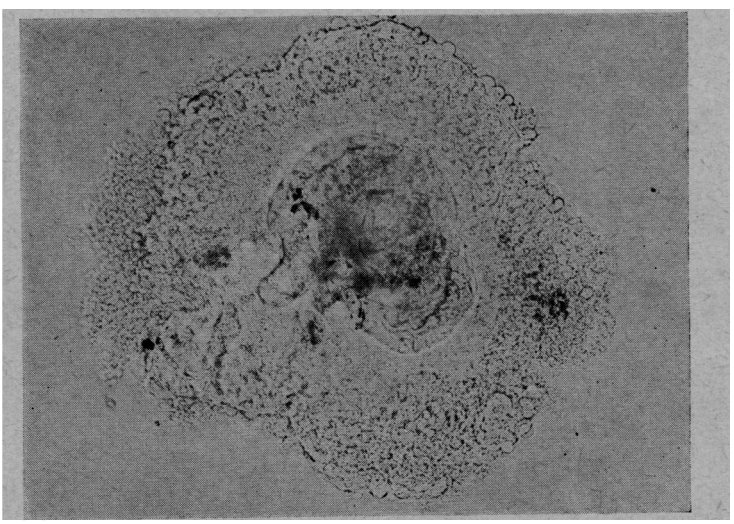

FIG. 3.

Mouse embryo lens with ruptured capsule in tissue culture at 3 days. Living preparation.

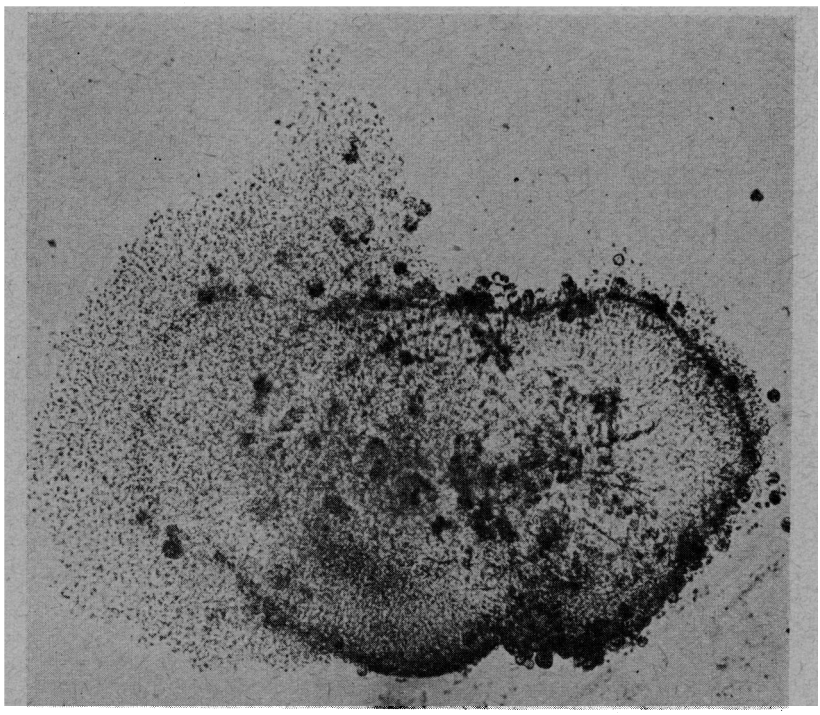

FIG. 4.
Mouse embryo lens with ruptured capsule in tissue culture at 6 days. Living preparation.

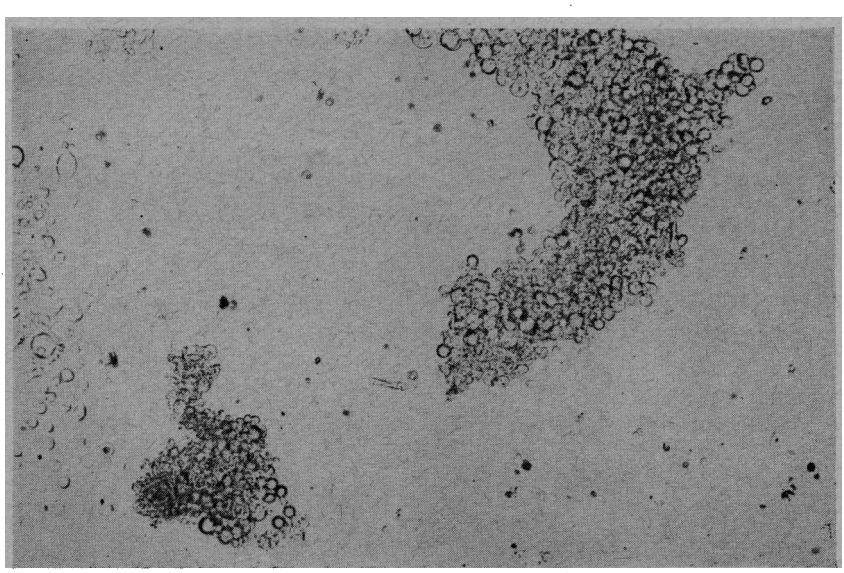

FIG. 5.
Portion of detached epithelial sheet from lens seen in Fig. 4. Living preparation.

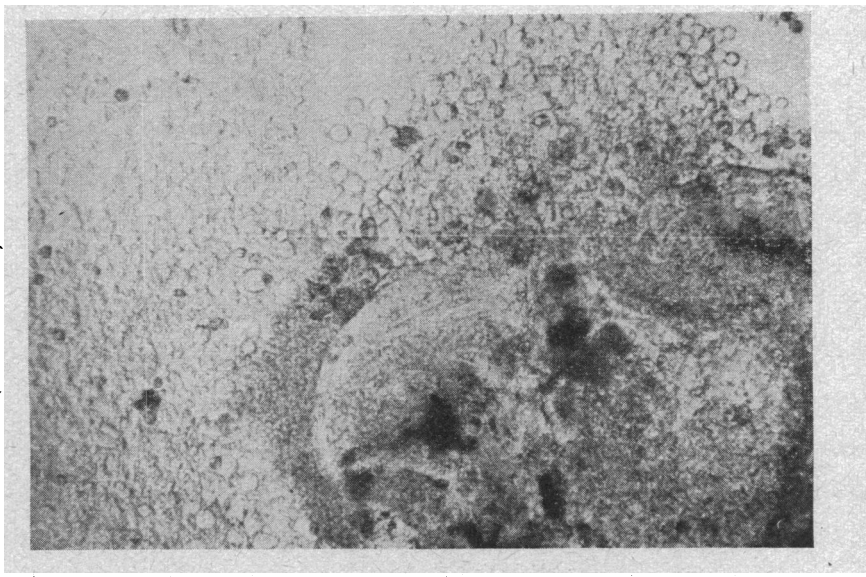

FIG. 6.

Full term mouse embryo lens growing at 11 days. Living preparation.

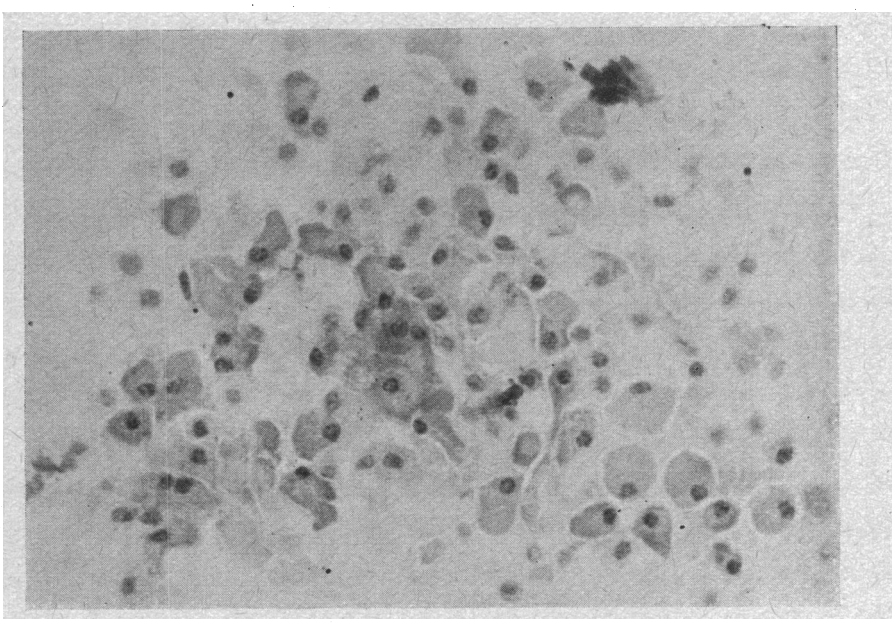

FIG. 7.

Epithelial sheet from mouse embryo lens tissue culture at 10 days. Fixed and stained with haematoxylin and eosin.

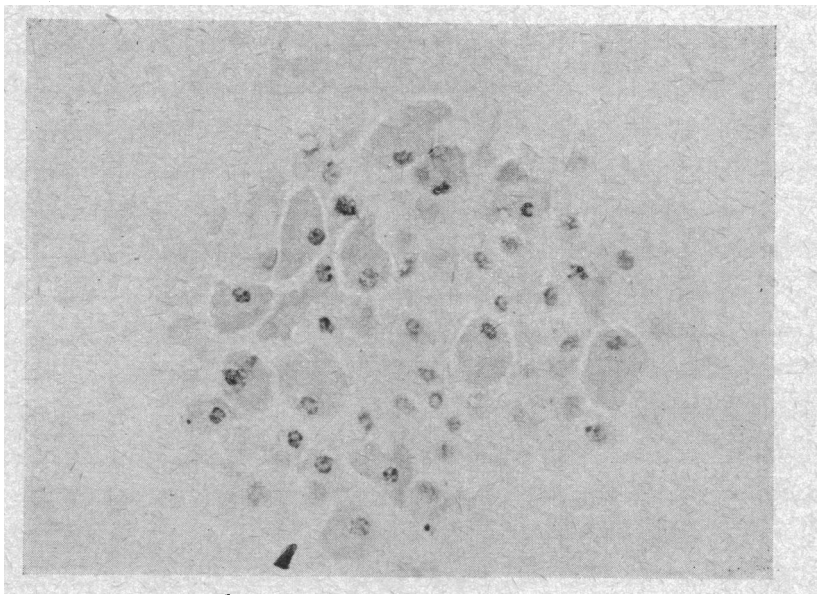

FIG. 8.

Similar to Fig. 7, but showing slightly more cellular differentiation.

Fig. 5 shows a portion of the epithelial sheet which has broken off from the explant and is floating free in the medium.

Fig. 6 shows a higher power view of the epithelial cells growing out from the capsule at 11 days. The embryo in this case was full term.

Fig. 7 shows an outlying portion of the epithelial sheet fixed and stained at 10 days. The position of the nuclei at the side of the cell and the large amount of clear cytoplasm is well seen.

Fig. 8 shows another portion of a similar sheet in which two at least of the cells show an attempt at elongation at both ends away from the nucleus. No culture remained alive after 12 days and no further stage of differentiation was observed.

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