Technical methods

A quick method for the isolation of glomeruli from human kidney

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The glomerular basement membrane antiserum has been used to induce nephrotoxic nephritis (Lannigan, Barabas, Peters, and Hardwicke, 1969) in experimental animals, and to study the changes in the glomerular basement membrane in various diseases (Gallo, Hudson, and Potter, 1967; Nagasawa and Shibata, 1970) by applying fluorescent antibody techniques on frozen sections of renal tissue. To raise a good antiserum it is important to have a pure glomerular preparation. The techniques previously described are either time-consuming or do not give a pure preparation of decapsulated glomeruli. To overcome this difficulty we followed a method based on the principle introduced by Spiro (1966) for the isolation of bovine glomeruli. The present technique describes a quick method for the uniform isolation of decapsulated glomeruli from human kidney.

Method

A fresh normal human kidney was obtained from a young man who died in a road traffic accident. Its capsule was stripped off the cortex. The medulla was dissected away and the cortical tissue was cut into small pieces which were pushed through an 80-mesh metal sieve using a spoon spatula. The kidney mesh was suspended in about 100 ml of cold sterile normal saline. It was spun at 1 000 rpm for three to four minutes and the supernatant was removed with a pasteur pipette. The wet glomerular pellet was then passed through a 150-mesh metal sieve and resuspended in about 50 to 75 ml sterile normal saline. The emerging suspension was rich in glomeruli most of which were without Bowman's capsule. The suspension was spun again at 1 000 rpm for three to four minutes and the supernatant was removed with a pasteur pipette. The centrifugate was resuspended in normal saline and left standing in a refrigerator for about 10 minutes. The supernatant was removed gently using a pasteur pipette and the deposit, which consisted of decapsulated glomeruli (Figure), was resuspended in sterile normal saline. A small drop of the suspension was put on a slide, stained with toluidine blue, and examined under the microscope.

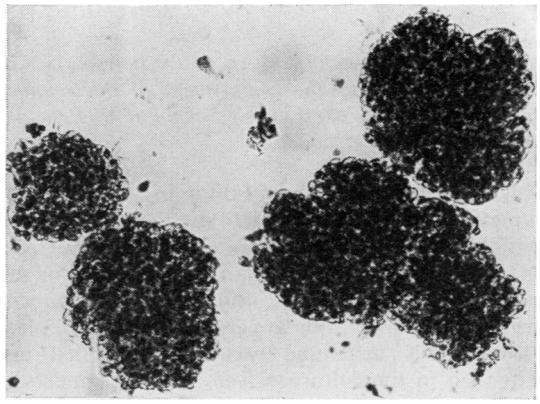

Fig. *Preparation of decapsulated glomeruli. Note the absence of tubules and interstitial cells. Toluidine blue.* × *150.*

This preparation was broken down by a two-stage method using an ultrasonicator (Soniprobe, type 7530A, Dowe Instruments Ltd). In order to liberate cells from the glomeruli, the sonicator was operated at a very low voltage for 10 seconds. The suspension was allowed to stand for 10 to 15 minutes at 4° C. The cell-rich supernatant was drawn off and the glomerular deposit was further broken down into small fragments by selecting a higher voltage on the sonicator for about 10 seconds. The preparation was centrifuged at 6 000 rpm for 10 minutes at 4°C. The sediment was washed four times in sterile normal saline. The final preparation was greyish-brown in colour and was stored at −20°C.

The above procedure is easier and much shorter than those described by Krakower and Greenspon (1951) and Spiro (1966) to isolate glomeruli from animal tissue. The resulting preparation by this method was completely free from tubules and almost completely free of cellular components. Similar preparations were obtained from rat and rabbit kidneys using 150 and 200 size sieves.

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Cultivation of human embryonic liver cells in disposable microplates

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Zuckerman, Tsiquaye, and Fulton in 1967 described a method for the cultivation of monolayers of differentiated parenchymal human embryonic liver cells in glass tissue culture plates made especially for this purpose. The apparatus utilized and the precise method of implantation are given in detail by Fulton (1960). This technique was later modified and extended to the culture of very small fragments of human adult liver obtained by needle biopsy for diagnostic purposes (Taylor, Zuckerman, and Farrow, 1970). The present communication describes the adaptation of these methods to the growth of human embryonic liver cells in commercially available disposable plastic microplates and the need to manufacture special glass tissue culture vessels is thus eliminated. This adaptation also facilitates the cultivation of tissue when only very limited amounts are available and renders the method more suitable for wide-scale use by utilizing readily available apparatus.

Human embryo livers 8 to 16 weeks old were obtained from the Tissue Bank of the Royal Marsden Hospital and a suspension of cells was prepared by disaggregation of the tissue for 10 minutes in 0·2% trypsin (Zuckerman *et al*, 1967b). Four cycles of trypsinization were utilized. Dispersed cells were removed after each cycle and kept at 4°C until trypsinization was complete. The trypsin was inactivated with 20% calf serum and a pellet of cells obtained by centrifugation at 700 rpm for five minutes. Cells were washed once in growth medium consisting of Eagle's minimum essential medium *p*H 7·2 containing 10% foetal calf serum, 100 units penicillin per ml, 100 μg streptomycin per ml, and 50 units nystatin per ml.

Modifications of the equipment and methods described by Fulton (1960) consist of the use of Perspex plates approximately 1 in. square with nine divisions, and disposable Falcon plastic microtest plates which contain 60 wells and come already sterilized with an easily manipulated lid. Discs, 3·5 mm in diameter, were cut from polythene film[1], 150 gauge (0·0015 in.) using a metal punch. The diameter of the discs was slightly smaller than that of the wells. The cells were implanted on the sterilized polythene using clotted mouse plasma as an adhesive. After implantation, excess fluid was not removed from beneath the discs by inverting the Perspex plate on top of sterilized filter paper and applying gentle pressure to it, because it was found that any pressure exerted on the disc resulted in injury to the cells. Instead, the Perspex plates were simply allowed to

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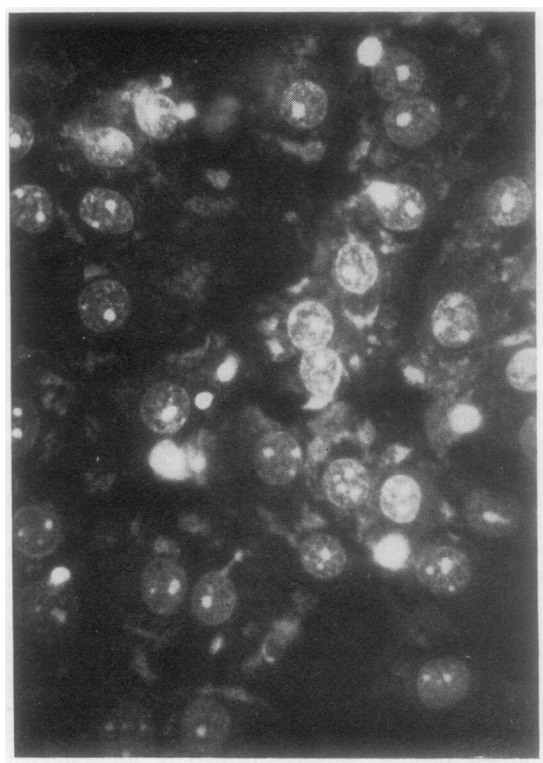

Fig. 1 *The typical appearance of human embryo hepatocytes after four days in culture* × *900.*

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