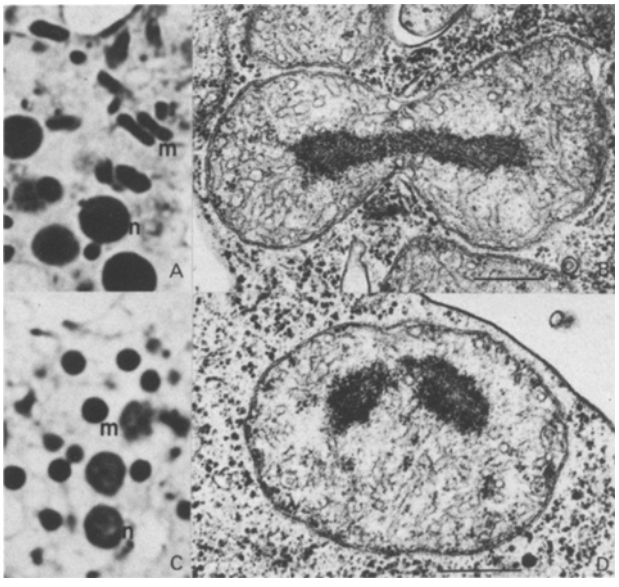

Light (A, C) and electron micrographs (B, D) illustrating nuclei (n) and mitochondria (m) in plasmodia fixed immediately after incubation for 3 h in a nutrient medium containing 50 µg/ml cytochalasin B (C, D) or in control medium (A, B). Micrograph B depicts the normal mitochondrial morphology. The cytochalasin B treated mitochondrion exhibits a large spherical configuration (C) and the mitochondrion contains a V-shaped nucleoid (D). Bar = 0.5 µm.

mitochondria contain contractile proteins[13,14] and they suggest that these proteins have an important role in mitochondriokinesis. An attempt to decorate the microfilaments in the interior region of the invagination by heavy meromyosin failed because they were unstable during extraction with glycerol.

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The fine structure of cloned cells from normal adult rat brain

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Summary. A cell clone was isolated from a normal adult rat brain culture and maintained in vitro for many passages. It possessed glial characteristics; in particular ultrastructural examination revealed astrocytic features including the presence of filaments 9–11 nm in diameter.

Brain tumours induced transplacentally by a simple nitrosamide, N-ethyl-N-nitrosourea (ENU), are gliomas of various types, the majority having a mixed population of astrocytes, oligodendrocytes and ependymal cells[2]. In these pleomorphic tumours – which develop most frequently adjacent to the lateral ventricles – astrocytes and their precursors are the predominant cells. ENU-induced gliomas can be studied both in vivo and in vitro: the cells retain their glial characteristics both on culturing and on reinjection into syngeneic hosts[3,4]. Moreover, cells removed and cultured from ENU-treated brains during the latent period – the interval between administration of the carcinogen and development of neurological signs of neoplasia – display features of malignant cells of glial origin[5–7].

It is therefore of utmost importance to establish a control cell line composed of normal glial cells. Here we report on the ultrastructural features of a glial cell clone isolated from a normal adult rat brain culture.

Material and methods. The original culture, ARBO, was initiated[20] from the periventricular region of normal adult rat brain (inbred BD-IX strain) using 0.05% collagenase as described previously[4–6]. ARBO C9 was one of the clones subsequently derived. It was maintained in Dulbecco's modification of Eagle's medium containing 15% foetal calf serum. To prepare samples for electron microscope examination the cells were removed by trypsinisation from the surface of the tissue culture containers, resuspended in complete medium and centrifuged. Surplus medium was removed by resuspension and centrifugation in phosphate-buffered saline prior to fixation in one-half strength Karnovsky fixative[8] for 15 min. Secondary fixation in 1% phosphate-buffered osmium tetroxide at pH 7.4 for 10 min was carried out after washing in buffer. At each stage cells were centrifuged then resuspended in the appropriate solution. All solutions were filtered through a 0.22 µm membrane filter to avoid contamination with particulate material. After a final wash in buffer the cells were suspended in 2% agar at 60 °C, centrifuged and then allowed to cool. Pelleted blocks of 0.5 mm^3 were cut from the tip of the resulting solid agar column, processed for electron microscopy and embedded in epon resin. Sections for electron microscopy were cut and stained with uranyl acetate and lead citrate before examination in an Hitachi HU12A electron microscope.

Results. Ultrastructurally the cells were essentially similar to one another but showed a variation in the number and morphology of processes (figure 1) ranging from short, bulbous protrusions to long, slender, microvillus-like projections. The latter are probably the microvilli observed with the scanning electron microscope[9]. The cells displayed

irregular nuclei containing evenly distributed chromatin with peripheral heterochromatin clumped in places. The cytoplasm was abundant and of moderate electron density. It contained mitochondria, rough endoplasmic reticulum (whose cisternae were often somewhat dilated containing a flocculent material of moderate electron density), free ribosomes, lipid droplets, occasional microtubules and dense inclusion bodies. In addition to these cytoplasmic organelles many of the cells showed numerous filaments of 9–11 nm diameter (figure 2). They were distributed throughout the cytoplasm and showed an apparent uniformity in diameter, indicating a single type of filamentous structure. The general ultrastructural features and the dimensions of the filaments are consistent with those described for astrocytes in vivo[10].

Discussion. Although the morphological criteria for the identification of astrocytes in vivo have long been established, there are no absolute biochemical markers by which these cells can be positively identified in vitro[11]. Ultrastructural examination still provides an indispensable approach to the characterisation of astrocytes.

Very little is known about the fine structure of cultured normal astrocytes. Although cultures of normal human glia were described as predominantly astrocytic, they had a limited lifespan in culture[12]. The fine structure of normal astrocytes was compared with that of neoplastic astrocytes revealing that the differences were quantitative rather than qualitative[13,14]. In sparse proliferating cultures there were no ultrastructural features by which glioma cell lines could be distinguished from normal glial cells[14,15]. Both normal and malignant astrocytes contained glial fibres and the same cytoplasmic organelles. In another comparative study of normal and neoplastic astrocytes, both fibrillary and protoplasmic types were found in cultures of a normal adult human brain[16]. Several studies using more readily available tumour lines have revealed ultrastructural features of neuronal and glial cells in these cultures[4,17,18].

ARBO C9, a cloned line from normal adult rat brain, has been established in continuous culture[20]. However, it was not malignant in syngeneic rats[19,20] and did not show several properties exhibited by glioma cells: growth in agar[19,20], high fibrinolytic activity[19] and survival on implantation into the chick limb bud[21]. In addition, scanning electron microscopy showed that, though the cells possessed some microvillus-like projections, they lacked the number and variety of surface structures revealed on glioma cells[9]. The present study shows that ARBO C9 cells have ultrastructural features, particularly the presence of 9–11 nm filaments, suggesting astrocytic differentiation. It is known that certain cells possess more than 1 system of filaments and that filaments approximately 10 nm in diameter may be present in neurones (neurofilaments), glia (glial filaments) and in epithelial cells (tonofilaments)[22]. However, the presence of a population of filaments of uniform 9–11 nm diameter and other ultrastructural features provide convincing morphological evidence that the cells possess astrocytic characteristics.

Marked inducibility of glycerol phosphate dehydrogenase by hydrocortisone has been demonstrated in some glial cell lines[3,23]. Although ARBO C9 showed little or no activity in early investigations, at a later passage some induction (37.5%) was detected (Claisse and Roscoe, unpublished observations). The cells have also been found to contain 2′,3′-cyclic nucleotide 3′-phosphohydrolase activity (0.33 μmoles/min/mg protein)[24] which is comparable to that found in other glial lines[25]. Although these enzymes are more characteristic of oligodendrocytes than astrocytes[11,23,25], it appears that cloned cultures often possess the properties attributed to both cell types making definitive identification difficult[11]. It is, however, clear that ARBO C9 displays some distinctive glial features. Further characterisation of these cells may be achieved by immunological, biochemical and cytochemical studies of the clone.

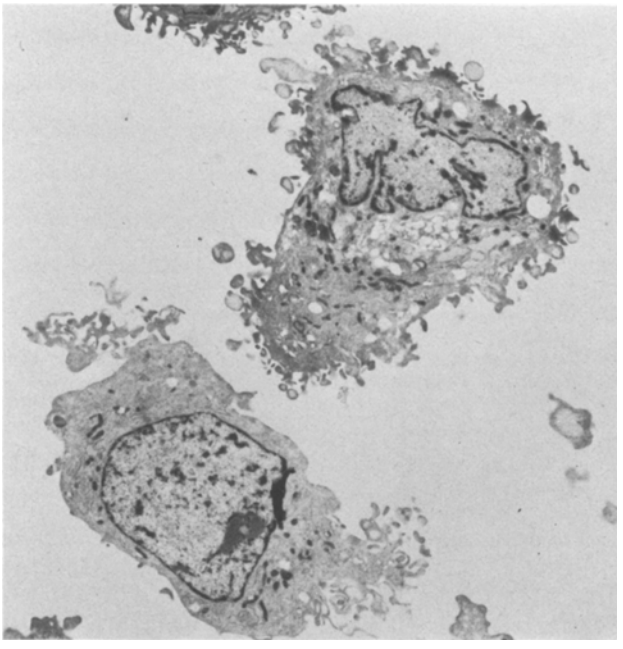

Fig. 1. Cells of astrocytic differentiation showing indented nuclei with evenly distributed chromatin and cytoplasmic processes of various sizes. × 3200.

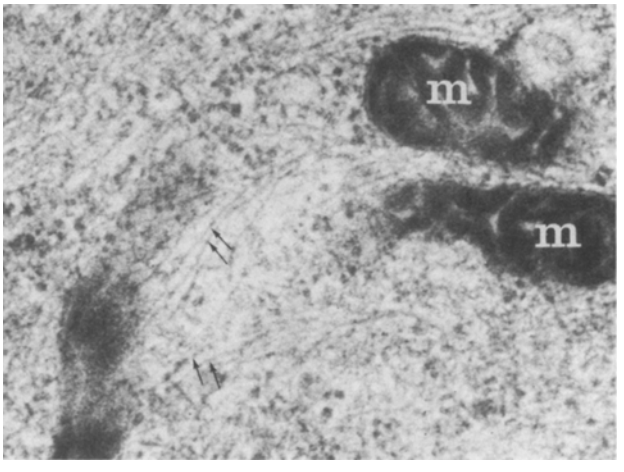

Fig. 2. Filaments of 9–11 nm (arrows) are seen together with mitochondria (m) in the cytoplasm of an astrocyte. × 64,500.

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Lysozyme in eggs of the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae)

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Summary. Lysozyme exhibiting bacteriolytic activity was found in boll weevil eggs at a concentration of 2.22 ng of lysozyme per boll weevil egg.

Fleming first described in 1922 the bacteriolytic activity of lysozyme in hen egg white[1], and lysozyme was subsequently found to be widely distributed in various cells, tissues and secretions of many species[2,3]. Insect lysozyme was recently purified from the eggs of the Dipterous *Ceratitis capitata* and found to have a mol.wt of 23,200[4].

The enzyme lysozyme functions in host defense by its ability to lyse the cell walls of many bacterial species with gram-positive bacteria being more sensitive to lysozyme than gram-negative bacteria[5]. Lysozyme hydrolyzes glycosidic bonds between amino sugars of the bacterial cell wall murein layer leading to dissolution or lysis of the rigid cell-wall structure[6].

The boll weevil eggs were collected from 2-day-old wax-coated food pellets. After removing the wax coat along with any frass, the food pellets containing the eggs were expelled through a sterile 10-ml syringe having no needle into a beaker containing a 100% saturated NaCl solution. The food debris was allowed to settle and then was aspirated from the bottom of the beaker. The eggs, which floated on top of the saturated NaCl solution, were washed 5 times with the saturated NaCl solution and then 3 times with sterile distilled water. The eggs were counted after they had settled to the bottom of the beaker of distilled water. A total of 185 eggs (0.1 ml volume) were homogenized in 0.2 ml of 0.85% sterile saline. No extraneous particulate material was present. Lysozyme and protein determinations were then done on the egg homogenate.

Lysozyme concentration in the boll weevil eggs was determined according to the enzyme assay described by Wardlaw[7] using a lysozyme test kit (Kallestad Lab, Chaska, Mn.). In this test, triplicate wells cut in an agarose culture of *Micrococcus lysodeikticus* bacteria were filled with the boll weevil egg homogenate. After 18 h of incubation at room temperature, the cleared ring diameters around the wells due to the lytic action of lysozyme were measured in mm with a calibrating viewer (Kallestad Lab, Chaska, Mn.). The concentration of boll weevil egg lysozyme was then determined from a graph on 2 cycle semi-logarithmic paper against purified primary human urine lysozyme reference standards of known concentrations done at the same time (Kallestad Lab, Chaska, Mn.).

The total boll weevil egg lysozyme concentration for 185 eggs was found to be 0.411 µg. This would be 2.22 ng of lysozyme per boll weevil egg. The total boll weevil egg protein concentration for 185 eggs using the Lowry method[8] and egg albumin as the standard was found to be 4.73 mg. This would be 25.6 µg of protein per boll weevil egg. 1 boll weevil egg would thus contain 2.22 ng lysozyme/25,600 ng protein, meaning that 0.009% of the total protein of a boll weevil egg is lysozyme. The presence of lytic activity due lysozyme would attest to its importance in protection of the boll weevil egg against bacterial infection.

Lysozyme activity has also been found in boll weevil hemolymph and frass[9]. A mean lysozyme activity peak of 7.0 µg/ml was found in boll weevil hemolymph at 48 h following inoculation with heat-killed *Serratia marcescens*. Mean lysozyme activity of 3 µg/ml was also demonstrated in the hemolymph of uninoculated boll weevils.

The cotton boll weevil is a common insect pest to cotton production in the USA. Thus, the presence of lysozyme in boll weevil eggs and also in hemolymph and frass would serve a protective function against infection by the various bacteria found in the boll weevils' plant and soil environments.

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