Results. Table 1 summarizes the serum bacteriostatic and cidal levels of 13 laboratory workers against 2 strains of 4 different organisms. Levels varied from organism to organism and person to person. No activity was found against the 2 *Staphylococcus aureus* strains. This finding prompted the subsequent testing of 12 strains of *S. aureus* against sera from 21 patients. Again, absolutely no static or cidal activity was detected against the *S. aureus* strains. Table 2 summarizes serum static and cidal levels of 30 hospital patients vs 12 different organisms. The lack of cidal activity against gram positive organisms was evident. Activity vs gram negative organisms varied somewhat. There was no significant difference between serum cidal levels from laboratory personnel and hospital patients with the unexplainable exception of *Pseudomonas aeruginosa* No. 5904. Table 3 notes the differences in serum cidal activity of 3 patients against 12 bacteria. Various sera retested after 2–7 days storage at 4 °C lost significant amounts of activity. Inocula were found to be stable, regarding the number of viable organisms/ml, for 5 days when held at 4 °C.

Discussion. Various soluble humoral substances are apparently responsible for the bactericidal activity of serum. Among these substances are complement, properdin, lysozyme, phagocytin, antibodies against various bacteria, fatty acids, acid hydrolases, alkaline polypeptides and perhaps components of the myeloperoxidase system found in lysosomes. The extent to which each of these, and possibly other factors, contribute is unknown but likely varies among people and different factors may inhibit or kill different organisms. The common lack of post-infection immunity to staphylococcal infections may be related to the absence of serum cidal activity. In this study, serum from a given individual exhibited various activity against different organisms, suggesting that different mechanisms may be involved in the destruction of different organisms.

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Inhibition of *Physarum* mitochondrial division by cytochalasin B

T. Kuroiwa and H. Kuroiwa

Department of Cell Biology, National Institute for Basic Biology, Okazaki 444 (Japan), 7 May 1979

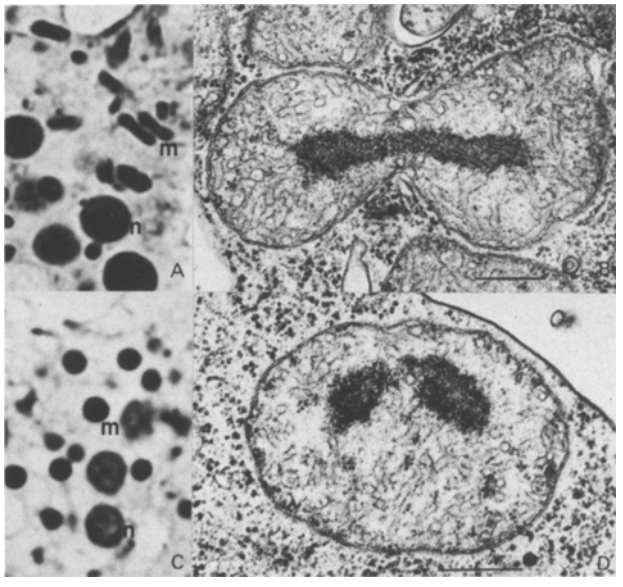
Summary. The mitochondrial division of *Physarum* is inhibited by cytochalasin B. Dumbbell-shaped dividing mitochondria become spherical bodies by this inhibitor. These results suggest that contractile proteins are essential for the mitochondrial division.

Physarum mitochondria contain an electron dense nucleoid, which consists of a large amount of DNA[2], RNA[3], and protein[2–4]. In the division of *Physarum* mitochondria, it is possible to observe 2 separate processes, the separation of the daughter mitochondrial nucleoids and mitochondriokinesis (mitochondrial division excluding nucleoidal division).

Materials and methods. Mitotically synchronized plasmodia of *Physarum polycephalum* were prepared by fusion of microplasmodia as reviewed by Guttes and Guttes[5]. Surface plasmodia between the 2nd postfusion mitosis (MII) and the 3rd postfusion mitosis (MIII) were used in these experiments. The 2nd synchronous nuclear division occurred 18 h after fusion, and the 3rd division at 28 h, while semi-synchronous mitochondrial division occurred within 5 h after the mitosis[6,7]. Cytochalasin B was employed according to the method of Axine and Peaven[8]. Small explants in diameter about 3 mm from the plasmodium at 1 h after MII were incubated for 3 h in a nutrient medium[5] containing 50 µg/ml cytochalasin B (Serva) or in control medium. These explants were harvested from 0–10 h later at intervals of 2 h after cessation of cytochalasin B treatment, fixed in Champy's solution[6] or in 6% glutaraldehyde and 1% OsO_4 solution[6] and prepared for light or electron microscopy by standard methods. An attempt to decorate actin filament with heavy meromyosin was done according to the procedures described previously[9]. Ultrathin sections were stained with both saturated uranyl acetate and lead citrate[6] and examined with a Hitachi-11E electron microscope.

Results and discussion. Compared with the mitochondrial division of higher eukaryotes, *Physarum* mitochondrial division is very simple. A spherical mitochondrion elongates, becomes ovoid and grows into a dumbbell-shaped mitochondrion (figure, A and B). The dumbbell-shaped mitochondrion divides semi-synchronously during mid S to form 2 spherical daughter mitochondria[10]. At that time, the dumbbell-shaped nucleoid also divides and separates into daughter mitochondria[6]. The dividing mitochondria of control preparations can be seen in plasmodia during late S fixed in Champy's solution (figure, A) or glutaraldehyde-OsO_4 solution (figure, B). When the ovoid mitochondrion elongates in a longitudinal direction and reaches a length of about 3.0 µm, a limiting membrane begins to invaginate in the equatorial region of the mitochondrion (figure, B). On the other hand, when a small explant of the plasmodium was exposed to cytochalasin B at 50 µg/ml for 3 h before fixation, a large number of mitochondria exhibited the large spherical or ovoid configuration (figure, C). The large spherical or ovoid mitochondria contain a V-shaped mitochondrial nucleoid (figure, D) or 2 nucleoids. Since dumbbell-shaped nucleoid in *Physarum* dividing mitochondrion was readily bent in the middle when isolated dumbbell-shaped mitochondrion was swelled and transformed into a spherical body[11], it appears that the large spherical or ovoid mitochondria containing V-shaped nucleoid may be in the division stage.

Cytochalasin B is well known as a specific inhibitor of the function of microfilaments[12]. Therefore, the simplest explanation for the absence of dumbbell-shaped mitochondria is that cytochalasin B has disrupted the function of contractile proteins so that the mitochondrion fails to become dumbbell-shaped. Our observations are consistent with the previous biochemical data which suggested that



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

G.J. Pilkington, P.L. Lantos and J.P. Roscoe[1]

The Bland-Sutton Institute of Pathology, School of Pathology and Department of Neurological Studies, The Middlesex Hospital Medical School, London W1P 8AA (England), 30 April 1979

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