ABSTRACTS OF CONTRIBUTED PAPERS*

ACHAR, S.B., and WEISMAN, R.A., Department of Biological Chemistry, School of Medicine, Wright State University, Dayton, Ohio

Effect of altered growth conditions on adenylate cyclase activity and cyst formation in Acanthamoeba castellanii

The effect of varying growth components and initial cell density on the pattern of adenylate cyclase (AC) activity and cyst formation was examined during the growth of *A. castellanii* (Neff). Adenylate cyclase activity remains low during the exponential growth period but rises sharply about 2-4 fold to form a peak at the beginning of the stationary phase. Whenever cell growth was decreased, either by lowering the concentration of glucose or proteose peptone in the growth medium, the AC activity peak still occurred at the stationary phase, but the activity was less than that of control cultures grown in complete medium. When the initial concentration of glucose was reduced from 1.5% to 0.3%, mature cysts were formed following the stationary phase. In contrast, when the proteose peptone concentration was reduced, bizarre cell forms rather than mature cysts were formed following the stationary phase. Growth conditions were also altered by varying the cell density of an initial inoculum of log cells from 10^4 cells/ml to 10^5, 10^6 or 10^7. The time to reach the AC peak at the stationary phase decreased from 110-120 hours with an inoculum of 10^4 cells/ml to 70-80 hours with 10^6. In the case of an initial cell density of 10^7, no further growth occurred but the cyclase peak still was not observed for 60-70 hours, suggesting some inhibitory effect of fresh growth medium on the AC activity increase normally observed at the stationary phase. These results are consistent with a model in which the AC peak depends both on parameters of cell growth and on the presence of a carbon source in the medium.

BOWERS, B., and OLSZEWSKI, T.E., Laboratory of Cell Biology, National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland

Stereological measurements on phagocytosing Acanthamoeba

In order to obtain quantitative information on vacuolar and surface membrane distribution and cell and vacuolar volume changes with phagocytosis, we have sampled phagocytosing *Acanthamoeba* at 0, 15, 30, and 45 min after incubation with saturating loads of particles (lipid-extracted yeast). Electron

*Arranged alphabetically according to name of first author

micrographs of the samples were analyzed with the coherent multipurpose test system described by Weibel and Bolender (Principles and Techniques of Electron Microscopy, Vol. 3, M.A. Hayat, ed.). The initial analysis excluded cytoplasmic membrane vesicles smaller than 0.1 um in diameter therefore the cytoplasmic membrane surface area may be underestimated in these results. Total vacuolar volume and total cell volume increased about 15% in cells saturated with particles. The surface area of the plasma membrane also increased indicating that lack of surface membrane is not the limiting factor in cessation of particle uptake. Total surface area of vacuolar membranes appears to decrease slightly with phagocytosis and may account for the increased plasma membrane surface. The surface area of the cell is more than 2-fold that calculated for a smooth sphere of the same diameter as the rounded cell. The quantitative relationships observed are consistent with the idea that cytoplasmic vesicles provide a pool of membrane that is exchangable with surface membrane.

BYERS, T.J., AKINS, R.A., KING, L.E., KUHNS, M.C., and NIES, E.A.,
Department of Microbiology and Developmental Biology Program,
Ohio State University, Columbus, Ohio

Drug-induced encystment in A. castellanii: Problems and models

Changes in drug sensitivity are an important problem in studies of drug-induced encystment. In 1973, ethidium bromide (EB), chloramphenicol (Cap), and erythromycin (Ery) inhibited cell multiplication and induced encystment in our line of Neff's clone I-12. Currently, they inhibit multiplication, but only poorly induce encystment in this line. Good encystment does occur in a recently isolated subclone. In 1971, hydroxyurea (Hu) induced encystment in I-12 without inhibiting ^{3}H-uracil incorporation into RNA (V. Rudick). Currently, Hu blocks ^{3}H-uracil incorporation and encystment is substantially reduced.

Drug-induced encystment in our unagitated cultures is very dependent on cell age and concentration of cells at the time of drug treatment. Actinomycin blocks encystment by log phase (LP) cells, but induces encystment in postlog (PL) cells diluted to LP concentrations. A number of drugs, such as 5-fluorodeoxyuridine, induce more encystment with diluted PL cells than with LP cells. A few drugs, including EB, Cap, Ery, berenil, and mitomycin C (MC), can induce high levels of encystment with both LP and diluted PL cells. Cycloheximide and emetime block encystment by cells of all ages.

Encystment-inducing drugs have multiple effects on cellular macromolecule synthesis. For example, Ery, Cap, MC and EB all reduce ^{3}H-thymidine incorporation into nuclear DNA, whereas, the first 3 transiently stimulate incorporation into mitochondrial

DNA. MC stimulates whole cell ^{3}H-leucine incorporation, whereas, Ery initiates a delayed degradation of protein.

Many of the drug effects we have observed can be explained if competence for encystment begins to develop in early log phase and increases during postlog phase. Undiluted PL cells do not encyst well in crowded conditions. Diluted PL cells prefer to multiply, but, probably because they have already completed some of the early steps in encystment, readily form mature cysts in any agent that inhibits multiplication without blocking cytoplasmic protein synthesis (on "80S" ribosomes). It will be suggested that only those drugs capable of inducing high levels of encystment in log phase cells are likely to affect the initiation mechanism and that they may exert their effects through the mitochondria.

CHAGLA, A.H., and BOWERS, B., Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland

Isolation and Characterization of the Phagosome Membrane of Acanthamoeba castellanii

We have undertaken to isolate phagosome membranes and to compare them with plasma membranes with special reference to protein composition and protein/lipid ratio in the two membranes. Phagosome membranes were isolated by the method of Wetzel and Korn (J. Cell Biol., 1969, 43, 90-104). Contamination of these membranes with other cell components was checked by three methods: 1) Electron micrographs showed membranes mostly in small closed vesicles without contamination by cell organelles, 2) Enzymatic analysis of the membranes using specific markers for various organelles revealed no detectable succinic dehydrogenase, RNA or thiamine pyrophosphatase activity. The washed phagosome membranes still retained some acid phosphatase, B-glucosidase and alkaline phosphatase activity, 3) To monitor general contamination of phagosome membranes, radioactive homogenate from cells that had not taken in beads was added to non-radioactive homogenate of cells from which phagosomes were to be isolated. Radioactivity in the purified phagosome membrane fraction showed 10-15% contamination of the phagosomal membranes by proteins from other sources. The protein/lipid ratio of the phagosome membranes was higher than that previously reported for Acanthamoeba plasma membranes. We are currently investigating protein/lipid ratio of plasma membranes from cells that had ingested beads.

CURSONS, R.T.M., DONALD, J.J., KEYS, E.A., and BROWN, T.J., Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand

Acanthamoeba - immunity, virulence and drug action

Diseases caused by Pathogenic Free-living Amebae (PFLA) can be divided into a swimming-associated acute meningo-encephalitis (caused by Naegleria fowleri) and non-swimming chronic diseases caused by pathogenic Acanthamoeba spp. Although pathogenic Acanthamoebae and Naegleria are comparatively widespread in the environment, the incidence of disease caused by them is much lower than for Naegleria. It is generally thought that Acanthamoeba are opportunistic pathogens, only occurring in defense-weakened patients or in parts of the body with low immunity (e.g. the eye). Although experimental animals such as mice are susceptible to intranasal or intravenous inoculations, primates are insusceptible unless on immunosuppressive treatment or via intrathecal or intracerebral inoculations. The finding of a neutralizing factor in fresh adult human sera (but not in hyperimmune rabbit or guinea-pig serum) capable of neutralizing 3×10^4 A. culbertsoni (A-1)/ml in Vero cell culture may explain this apparent low incidence of the disease in the human population.

The variance in susceptibility of experimental animals to isolated strains of pathogenic Acanthamoebae has also been investigated. It is thought that this may be due to the difference in production of phospholipase. We have shown A. culbertsoni (A-1) produces more phospholipase A than does non-pathogenic Acanthamoeba castellanii (1501). A similar result was found with N. fowleri and Naegleria gruberi.

Finally, the in vitro susceptibility of Acanthamoebae to chemotherapeutics has been investigated, the results of which are tabulated.

Effect of Various Chemotherapeutics on A. castellanii (1501) using 2×10^5 cells cm^{-3} after 96 hr.

Drug	Amebistatic ($\mu g\ cm^{-3}$)	Amebicidal ($\mu g\ cm^{-3}$)
Amphotericin B (AmB)	10.8	-
5-Fluorocytosine (5 Fc)	25	250
Rifampicin	500 encystment	
Tetracycline	1000 encystment	

We wish to acknowledge financial support from the New Zealand Health Department, the Medical Research Council and Roche Products Pty. Ltd.

DE JONCKHEERE, J., Laboratorium voor Hygiëne, Katholieke Universiteit Leuven, Belgium

Cytopathic effect in Vero cell culture in relation to virulence in mice within the genus Acanthamoeba

Reference strains of six different species of the genus Acanthamoeba i.e. A. astronyxis Ray, A. castellanii Neff, A. culbertsoni A-1, A. palestinensis Reich, A. polyphaga P23, A. rhysodes 1534 CCAP, and different isolates belonging to these species have been investigated for cytopathic effect (CPE) in Vero cell culture and virulence in mice after intranasal (IN) and intracerebral (IC) inoculation.

The reference strains, except A. culbertsoni A-1, did not grow at 37°C in axenic medium but they did at 30°C. These five strains showed no CPE at 37°C, but, except for A. astronyxis, were cytopathic at 30°C. Amoebae could be reisolated from mice brain with three of these five reference strains 3 weeks after IC inoculation. Acanthamoeba strains that killed mice, IN or IC, showed CPE at 37°C while strains that did not kill mice but where amoebae could be reisolated from the brain, only showed CPE at 30°C. Strains that did not kill mice and where amoebae could not be reisolated from the brain, however, showed sometimes CPE at 30°C. Most of the strains, which were isolated at 37°C, killed mice after IC inoculation.

The use of Vero cells may provide a useful tool for screening virulence in Acanthamoeba isolates since virulence in mice of a given strain may fluctuate considerably from one test to another.

DUMA, R.J., HELWIG, W.B., and MARTINEZ, A.J., Division of Infectious Diseases and Neuropathology, Medical College of Virginia, Virginia Commonwealth University, and Department of Pathology, Riverside Hospital, Newport News, Virginia

Meningoencephalitis and Brain Abscess Due to a Free-living Amoeba

A 47 year old diabetic female developed fatal meningoencephalitis due to a free-living amoeba. The responsible organism appeared to be neither Naegleria nor Acanthamoeba-Hartmannella. Both acute and chronic (granulomatous) inflammatory reactions of the brain were present, and both cysts and trophozoite forms were readily visualized. It should be realized that a variety of free-living amoebae exist in Nature which potentially can produce meningoencephalitis in humans, and that none of these organisms should be labeled or considered as "avirulent" or "non-pathogenic" until proven otherwise.

Assimilation of bacteriae is well demonstrated by A. culbertsoni or A. castellanii both of which possess large vacuoles and thus penetration and lysis of bacteriae are easily seen by microscope.

We want to insist especially on this resistance of bacteriae to amoebae enzymes. One has to consider differently phagocytosis of, on one hand, germs favoring multiplication of amoebae, for ex. Aerobacter aerogenes, which is easily and entirely phagocytised, as also other Enterobacteriacae, and, on the other hand, some mycobacteriae who resist and live on indefinitely in a cyst.

Nevertheless, one must not take for granted that all negative gram germs are completely lysed, as, quite often, even after the use of an antibiotic capable of destroying the germ, one cannot completely rid the amoebae of the phagocytised germ. We may thus conclude that these exists a partly diastasic action.

In 1973, at the Congress of Tropical Medicine and Malaria, in Athens, Eilersten showed for the first time some cases of cutaneous ulcers and cervical adenitis on young men who had been swimming in a pool in Bergen, Norway and from whom Mycobacterium marinum or M. Balnei had been isolated.

We went to Bergen, and isolated from water in pools, also from sand filters - not only A. castellanii, but also mycobacteriae (Jadin 1974). Three years later, water from these swimming pools, kept at room temperature ($\pm$ 20°C) in a sterile flask, still held cysts, which, on culture, still contained mycobacteriae. After this research, we came to consider amoebae as vectors, of mycobacteriae.

Other research work encouraged this view as for ex. isolation by J. & G. Viaillier (1973) of mycobacteriae in stream water in France and the fact that more mycobacteriae were found in filter water from a sanatorium after passing through a sand filter than before (Taquet et al. 1973). We must keep in mind that Cerva showed in 1971 that sand filters host amoebae, and thus multiply amoebae in filtered water.

On introducing mycobacteriae in axenic cultures of Acanthamoebae, one may note how they multiply in amoebae vacuoles and quite often overrule completely. So that one may consider amoebae as a good media to mycobacteriae, who do not develop in a synthetic, or semi-synthetic medium.

It is thus that M. leprae, taken from leper glands, grow in amoebae vacuoles.

This being established, we asked the help of leprosy workers in Africa, Zaire, Ivory Coast, Senegal to place some nasal mucosity from lepers on petri dish spread with agar-bacteriae. From these we isolated Acanthamoebae and other amoebae of mycobacteriae, M. Vaccae obuense and M. chelonei.

From these, we supposed free living amoebae could be vector to mycobacteriae (Jadin 1975). We find these same amoebae and their mycobacteriae in the soil of leper huts. Remember that leprosy disappears where man no longer lives in direct contact with soil. In Europe, one still finds leprosy in places where man lives, as in central Africa, close to the soil.

We would like to add that perhaps Acanthamoebae may disperse viruses. It is a fact that these can develop inside of cell, and amoebae are living cells.

Serological examination of hepatitis patients shows that 40% of them possess anti-Acanthamoebae antibodies, while only 18% are found on same subjects. Origin of hepatitis A is often found in pollution of drinkwater (Jadin et al., 1972). It should be noted, that drinkwater is very rarely free of amibae. In 30 cities all over the world, only four were found without them (Jadin 1973).

All this shows the importance of Acanthamoebae and free living amoebae in dispersion of bacteriae and mycobacteriae. In fact, the dramatic effect of Acanthamoeba in meningo-encephalitis is only a small part of the role played by this spread protozoa.

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KAUSHAL, D.C., SHUKLA, O.P., and KRISHNA MURTI, C.R., Division of Biochemistry, Central Drug Research Institute, Lucknow-226001

Excystment of Hartmannella culbertsoni

Axenically produced cysts of Hartmannella culbertsoni hatch into trophozoites in presence of an autoclaved extract of a number of bacteria, fungi and even peptone and other proteinaccous substances. Crowding of cysts and dilution of bacterial extract adversely affect the excystment. Continuous presence of the factors in the medium is essential for excystment.

The fractionation of Escherichia coli extract has revealed that most of the excystment promoting activity is associated with a fraction rich in amino acids, glutamic acid being the principal excystment agent both in the qualitative and quantitative sense. Studies on the structure activity relationship of excystment agent suggest that γ-aminobutyric acid or glutamic acid, with an amino group, a carboxyl group and an intervening chain of 3-carbon atoms, appear to possess the optimum structural requirements for an excystment agent.

Studies on macromolecular changes and effect of metabolic inhibitors suggest that RNA and protein synthesis as well as initial events of excystment are not dependent on DNA synthesis. Energy transducing mechanisms, protein synthesis and sulfhydryl groups (SH) are, however, found to be essential. Excystment of H. culbertsoni is accompanied by secretion of two proteases, cellulase and chitinase. No enzyme is detected in the dormant

cyst, the activity appears early and persists even when excystment is over. One of the extracellular proteases has a molecular weight around 21,000 and is sensitive to inhibition by phenylmethylsulfonylfluoride, suggesting it to be a serine protease. The combined action of all the three partially purified enzymes has been demonstrated to alter the morphology of cysts.

LASMAN, M., Department of Zoology, The Hebrew University of Jerusalem, Jerusalem, Israel

Encystment of Acanthamoeba palestinensis. Factors influencing cyst formation

The effect of osmotic pressure, different electrolytes and organic compounds on cyst formation has been tested. The optimal osmolarity for encystment was about 150 milliosmolar, which was very close to that of the growth medium. Iso-osmotic solutions of KCl, $MgCl_2$, $CaCl_2$, glycine and sucrose led to maximum cyst formation during 48 hours of incubation at 30°C. The cysts were uninucleate with nucleolar-like bodies in the nucleus of every cyst.

Growth medium supplemented with $MgCl_2$ or KCl, 150 milliosmolar, yielded relatively large amount of cysts, which were frequently multinucleate. Each of the nuclei in these multinucleates have nucleolar-like bodies. The involvement of various agents in the induction of encystment is discussed.

MARKOWITZ, S.M., SOBIESKI, T., MARTINEZ, A.J., and DUMA, R.J., Department of Medicine, Division of Infectious Diseases, and Department of Pathology, Division of Neuropathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Experimental Acanthamoeba Infections in Mice Pretreated with Methylprednisolone or Tetracycline

Human infections due to free-living amebas of the genus *Acanthamoeba* have been reported sporadically, occasionally in individuals with underlying diseases. To determine if such infections may be considered opportunistic, groups of laboratory mice were pretreated with either methylprednisolone or tetracycline and inoculated intranasally with 1.075×10^4 *Acanthamoeba castellanii* isolated from a natural fresh water well. Results were compared with controls receiving either drug or amebas alone, and controls receiving saline injections with and without amebas. The mortality rate for those animals receiving methylprednisolone

Amoebae were grown axenically in Neff's optimum growth medium at 30 C on a gyratory shaker. Cultures were treated with drug (20.0 and 2.0 mcg/ml) during mid-logarithmic growth (4-6 X 10^5 cells/ml) and were monitored by hemacytometer counts. Cultures of A. castellanii treated with 20 mcg/ml of drug exhibited a marked decrease in growth rate in less than 6 hours. Cells washed free of drug after 24 hr were not capable of recovery when transferred to fresh medium. The cultures died without encysting. Treatment with 2.0 mcg/ml of drug caused a decrease in growth rate followed by pre-mature encystment. Drug-treated cells (24 hr) recovered when transferred to fresh medium but cysts were not viable. Similar results were observed for drug-treated A. culbertsoni cultures. Exceptions included no pre-mature encystment and a longer response time to the drug.

PAULE, M.R., DETKE, S., D'ALESSIO, J., and SPINDLER, S.R., Department of Biochemistry, Colorado State University, Fort Collins, Colorado

DNA-Dependent RNA Polymerases from Encysting Acanthamoeba castellanii: Catalytic Properties and Subunit Structures

The three classes of eukaryotic DNA-dependent RNA polymerase have been purified to homogeneity from A. castellanii (Neff strain) by low ionic strength extraction followed by precipitation of the enzymes with polyethyleneimine (PEI), differential elution from PEI, batchwise treatment with DEAE-cellulose, phosphocellulose chromatography, column chromatography on heparin-Sepharose (I and III) or DEAE-Sephadex (II) and sedimentation on glycerol gradients. Milligram quantities of all three enzymes can be obtained from a single kilogram of cells. The polymerases have the subunit compositions ($M_r \times 10^{-3}$): (I) 185, 133, 41.5, 37, 35, 22.5, 17.5, 15.5, 13.3, < 10; (II) (193, 178), 152, (39-41), 22.5, 18.5, 15.5, 14, 13.3, 12.5, 12.0, < 10; (III) 169, 138, 82, 52, 37, 34, 30, 28.5, 22.5, 17.5, 15.5, 13.3, < 10. The two high molecular weight subunits of polymerase II sum to a stoichiometry of one suggesting two forms of the enzyme. Polymerases I and III both have subunits of M.W. 37,000, 22,500, 17,500, 15,500 and 13,300 and polymerase II have subunits of 22,500, 15,500 and 13,300 suggesting that these enzymes share a common pool of these subunits. Neither the number of molecules, the catalytic properties nor the subunit structure of RNA polymerase I is altered during the first 10 h of synchronously induced encystment (Neff's E.M.) suggesting that the cessation of ribosomal RNA synthesis occurring early in encystment does not result from major RNA polymerase I modifications.

PAULS, K.P., and THOMPSON, J.E., Department of Biology, University of Waterloo, Waterloo, Canada

Electron Spin Resonance Studies of Microsomal Membranes from Acanthamoeba castellanii

Spin labelled fatty acids have been used to compare the physical properties of lipid in rough and smooth microsomal membranes from trophozoites and cysts of Acanthamoeba castellanii. Two stearic acid spin labels were used - ($I_{(1,14)}$), stearic acid bearing a paramagnetic nitroxide group on carbon 16; ($I_{(12,3)}$), stearic acid bearing a paramagnetic nitroxide group on carbon 5.

Arrhenius plots of rotational correlation times (Tc) calculated from the spectra for $I_{(1,14)}$ incorporated into microsomes showed an abrupt discontinuity in slope for membranes from both trophozoites and cysts. The temperature at which this discontinuity occurred was significantly higher for rough microsomes than for smooth, but the differences between cysts and trophozoites were not significant for either type of membrane. The value of Tc at 29°C, the culturing temperature, in effect scores fluidity of the lipid matrix in the region being probed. Comparisons of these values showed no significant change as a result of encystment for either rough or smooth microsomes, although for both cysts and trophozoites the smooth surfaced membranes were more fluid than corresponding rough surfaced membrane. Arrhenius plots of order parameters (S) calculated from spectra of $I_{(12,3)}$ incorporated into the membranes showed no discontinuities in slope. Nor were there changes as a result of encystment in the value of S at 29°C for either type of membrane, although again there were differences between the two types of membrane.

The data are consistent with previous reports to the effect that there is more mobility in the tails of the fatty acid side chains than in regions of the chains close to the phospholipid headgroups. However, there appears to be little change in the fluidity of these membranes accompanying encystment.

PRZELECKA, A., SOBOTA, A., HREBENDA, B., and JANOSSY, A., Nencki Institute of Experimental Biology, Warsaw, Poland

Calcium binding at plasma membrane of Acanthamoeba castellanii

In trophozoites of Acanthamoeba castellanii fixed in glutaraldehyde, supplemented with $CaCl_2$ electron-dense deposits (EDD) appear at the cytoplasmic side of the plasma membrane. After 15 min fixation their diameter was about 560 Å, after 24 hrs - it reached up to 700 Å. The distance between the EDD was about 3000 Å. Elemental analysis of EDD was performed with the

use of an electron microscope (JEM 100 B) combined with an energy dispersive X-ray analyser (EDAX). The results showed that EDD contained high amounts of P and Ca, with a small addition of Mg, the corresponding values of weight percent for these elements being 4.2, 2.9 and 0.4, respectively. The P:Ca atomic ratio was about 1.89, whereas the P:(Ca+Mg) atomic ratio was 1.55.

In the cells pretreated for 30 min with metabolic inhibitors (DNP, KCN, IAA, NaF, N_2) the deposits appeared as well, even in larger amounts. The elemental composition of the deposits did not change after action of the inhibitors. However, some deviations in atomic ratios between the elements were observed; the P:Ca atomic ratio ranged from 1.65 (after DNP+IAA) to 2.09 (after KCN).

On the other hand, the formation of EDD was prevented by extracting the cells with glycerol, by a short pretreatment with Triton X-100, and by freeze-thawing or mild homogenization, leading to disruption of the cell membrane-cytoplasm contact. The above observations indicate the presence of a calcium sequestering system active at the plasma membrane of Acanthamoeba. Various small molecular weight phosphate compounds (like ATP, ADP, and inorganic phosphates) contained in the cytoplasm seem to be involved in capturing the excess of calcium ions entering the cell.

SEILHAMER, J.J., and BYERS, T.J., Developmental Biology Program and Department of Microbiology, Ohio State University, Columbus, Ohio

Erythromycin-, chloramphenicol-, and oligomycin-resistant mutants in A. castellanii

Spontaneous mutants of A. castellanii resistant to 500 ug erythromycin/ml (Ery^R), 2.5 mg chloramphenicol/ml (Cap^R), and 15 ug oligomycin/ml (Oli^R) were isolated. Their resistant character was phenotypically stable and drug-specific. Multiple mutants could be obtained by stepwise selection. Approximately one in 10^5 amebas were able to form colonies by 12 days in multi-well plates in any of the three drugs. By 40 days, the frequency increased 100-fold for Ery and Cap, but no significant increase was seen for Oli. We were unable to obtain spontaneous mutants resistant to cycloheximide, emetine, FUdR, or ethidium bromide. It is suggested that Ery^R, Cap^R, and Oli^R could be mitochondrial mutants. All efforts to cause gene exchange between mutants using polyethylene glycol-induced cell fusion have failed due to toxicity of the PEG.

SOBOTA, A., and GLAZUNOV, V.V., Nencki Institute of Experimental Biology, Warsaw, Poland

Influx of thallium and rubidium ions and their influence on growth and encystment of Acanthamoeba castellanii

Potassium is the main cation of cells and it takes part in different cellular processes. The K^+ action in cellular metabolism may be followed by substituting this ion with its analogs - Rb^+ and Tl^+.

Tl^+ and Rb^+ influx and their influence on cell growth and differentiation was studied in Acanthamoeba castellanii (Neff strain). The cells were cultured axenically in Neff growth medium without shaking or aeration in darkness at 27°C. The transport of Tl^+ and Rb^+ was measured by means of ^{204}Tl and ^{86}Rb radioisotopes.

The maximal influx of Tl^+ and Rb^+ was about 3 nmoles per gram dry weight per min, with K_m of 0.05 and 0.25 for Tl^+ and Rb^+ respectively. The influx was temperature-dependent; Q_{10} equalled 2.0. The influx of ions was strongly inhibited in the cells treated with NaCN and NaN_3, while 2,4-DNP, NaF, oubain, SHAM appeared to be uneffective in this respect.

Tl^+ added to the growth medium of a 2-3 day-old culture in concentration 0.05-1.0 mM progressively reduced the multiplication rate of Acanthamoeba cells and induced their encystment. The maximal effect of growth inhibition was observed at 0.6-1.0 mM Tl^+; in 72 hrs nearly 100% of the trophozoites differentiated into cysts. A further increase of Tl^+ concentration up to 5 or 10 mM caused a surprisingly rapid multiplication of the cells, during the first two hours, followed by a stationary-like phase. However, at this thallium ion concentration no cyst could be observed.

On the basis of these results the suggestion arises that thallium ions play some role in switching on either the differentiation or multiplication process in Acanthamoeba cells.

TYNDALL, R.L., WILLAERT, E., and STEVENS, A.R., Zoology Department, University of Tennessee, Knoxville, Tennessee and VA Hospital, Gainesville, Florida

Isolation of Acanthamoeba from Cultured Mammalian Cells

Acanthamoeba were isolated from established cell cultures of human choriocarcinoma (BeWO), mouse sarcoma (KBALB) and rat hepatoma (H35). All three isolates were cytopathogenic for

cultured mammalian cells. Serologic and immunochemical analysis showed that the amoeba isolated from the human cell line is a new species of Acanthamoeba, i.e. A. royreba sp. n., whereas those isolated from the rodent cells were found to be A. culbertsoni. The new species did not induce meningo-encephalitis in mice on intranasal inoculation whereas the A. culbertsoni isolates were encephalitic. Mammalian cell DNA was altered or destroyed in cultured mammalian cells infected with the Acanthamoeba as indicated by loss of methyl green staining moieties in the nucleus. All three amoeba isolates had high levels of histochemically detectable esterase and alkaline phosphatase activity. The esterase profiles in acrylamide electrophoresed cell extracts of most known species of Acanthamoeba showed a distinct, reproducible pattern for each species that may be a useful tool in speciating these amoeba.

VISVESVARA, G.S., JONES, D.B., and HEALY, G.R., Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia

The Spectrum of Disease Caused by Acanthamoeba spp. and the Susceptibility of Certain Isolates to Chemotherapeutic Agents

To date, approximately eight human cases of amebic meningoencephalitis caused by Acanthamoeba spp. have been described, and the amebae have also been implicated in fatal disease of domestic animals. The organisms have been isolated from: 1) the nasopharynx of patients suffering from upper respiratory tract distress; 2) ear discharges; 3) bronchial secretions; 4) and from stools of individuals suffering from gastritis and diarrhea, as well as from the respiratory tracts of normal individuals. Antibodies to Acanthamoeba spp. have been found in the sera of patients suffering from upper respiratory tract distress (Elridge and Tobin, 1967), optic neuritis and macular disease (Schlaegel and Culbertson, 1972), as well as in sera from normal persons (Cursons et al. 1977). Since Acanthamoeba spp. are ubiquitous and can be easily isolated from various environments including air and fresh or brackish water, infections of the eye and respiratory tract are probably more common than appreciated.

Jones et al. (1975) and Nagington et al. (1974) repeatedly isolated trophozoites and cysts from the corneas of several patients suffering from eye diseases. Immunodiffusion test results showed that the serum of one of these patients contained precipitin antibody against A. polyphaga.

Studies of in vitro interaction with monkey kidney tissue culture (Vero line) and pathogenicity to mice were done on one of the isolates of Jones et al. (1975). Results indicated that its virulence was low. A. culbertsoni and several of the

Acanthamoeba spp. isolated from the eyes of the patients mentioned above were susceptible, *in vitro*, to paromomycin, clotrimazol and thiabendazole, but refractory to 5 fluorocytosine, trifluorothymidine, adenosine arabinoside, trimethoprim, and sulfamethoxazole.