the traumatic reaction adjacent to injection site. From this time on, this type of fluorescence became more intense and widespread. In the control animals, fluorescence was observed in the phagocytic cells adjacent to injection site.

Electron microscopic examination carried out on the fifth post-inoculation day revealed intracytoplasmic crystalloid structures made up of particles, the size and shape of which corresponded to those of poliovirus. These crystalloid particle aggregates were repeatedly observed in mononuclear elements of the inflammatory infiltrates as well as in the endothelial cells of the intraspinal blood vessels. Nothing was seen in the nerve cells, which could be taken to signify the presence of poliovirus[9,10].

Virological assays showed that both virulent and attenuated viruses multiplied rapidly in the lumbar region after intraspinal inoculation, the maximum titre being achieved within the first 24 h. From the second day, a decrease was observed which was more pronounced for the attenuated virus. In the other regions of the central nervous system, a rising titre of virulent virus became apparent from the 3rd day, whereas no virus was detected with the attenuated strain.

Our findings indicate that inflammatory cells including activated microglial cells and cells of the vascular walls show a close association with the poliovirus. The immunofluorescence of some of the inflammatory cells, the electronmicroscopic demonstration of poliovirus-like crystals and the virus assays, all indicate the potential capacity of some of the inflammatory cells to replicate the poliovirus.

The fluorescence in the control animals suggest that this finding in some phagocytic cells of poliomyelitic monkeys may signify another process, i.e. virus uptake ('viropexis'). Whenever pinocytosis of an infectious particle occurs, the inflammatory cell can serve as a 'vector', providing local spread of virus.

To understand the development of the virulent infectious process it is necessary to keep in mind not only the pathogenic activities of the individual inflammatory cells, but also the circumstances under which these activities take place. The whole process can be compared to an 'epidemic' within the greatly enlarged population of closely packed cells of the inflammed nervous tissue. The crowding and mobility of the majority of these cells thus facilitates the local spread of infection. The nerve cells, which make up a small fraction of this cell population, become infected in the course of this 'epidemic' together with other non-neural potential host cells. They probably do not participate significantly in the virus replication, because their intracellular metabolism is rapidly damaged by the viral genome, often before the virus replication can be initiated. Their pathogenic role appears rather to rest on their ability to trigger the 'secondary' inflammatory reaction. The inflammatory elements are then responsible for replication and spread of the virus.

The results allow the conclusion that the inflammatory reaction, apart from its defensive function[11], plays an important pathogenic role in the development of poliovirus infection. Both defensive and aggressive mechanisms run parallel within the heterogeneous cell population of the focus of infection but opposing one another. Their intensity and mutual relationship will determine the course of the infectious process.

Zusammenfassung. Das Studium der sich entwickelnden entzündlichen Reaktion auf mit attenuiertem und virulentem Poliovirus infizierten Affen zeigte, dass diese in der Pathogenese der Poliomyelitis eine defensive und eine aggressive Rolle spielt. Die erstere umfasst eine unspezifische, sekundäre Antwort auf die Nervenschädigung (Neuronophagie) und eine spezifische, primäre Reaktion auf das virale Antigen (lokale Immunreaktion). Die letztere ist vor allem dadurch gekennzeichnet, dass einige der mobilen entzündlichen Elemente das Poliovirus replizieren und weiter verbreiten können.

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[10] K. BLINZINGER, J. SIMON, D. MAGRATH and L. BOULGER, Science *163*, 1336 (1969).
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The Amphibian Lens: a Three Month Organ Culture[1]

Several years ago this laboratory reported that it is possible to maintain the ocular lens of the bullfrog (*Rana catesbeiana*) in organ culture for at least seven days[2]. We implied then that the culture system described would probably also permit cultivation for far longer periods. Data to be presented in the current account will show that this is so – that in fact one can maintain bullfrog lenses in culture for periods up to 3 months.

Experimental. Lenses were isolated from enucleated eyes by techniques that have been fully discussed elsewhere[3]. They were then cultured in either of 2 media. The first, 'A-199', consists of 88.3% water; 5% sodium bicarbonate (55 g/l); and 6.7% 10X 199[4] (Grand Island Biological Co., Grand Island, New York, USA). The second, 'R20', has the following composition: 5.3% 10X 199, 3.0% sodium bicarbonate (55 g/l), 71.7% H_2O and 20% rabbit serum (Pel-Freez Biologicals, Inc., Rogers, Arkansas, USA). Both fluids are approximately isosmotic with bullfrog aqueous humor (225–235 mOsm) and have a pH of 7.1–7.2. Penicillin, 50 units/ml, and streptomycin, 50 µg/ml, were added to the media prior to explantation. Each lens was placed in a silicone

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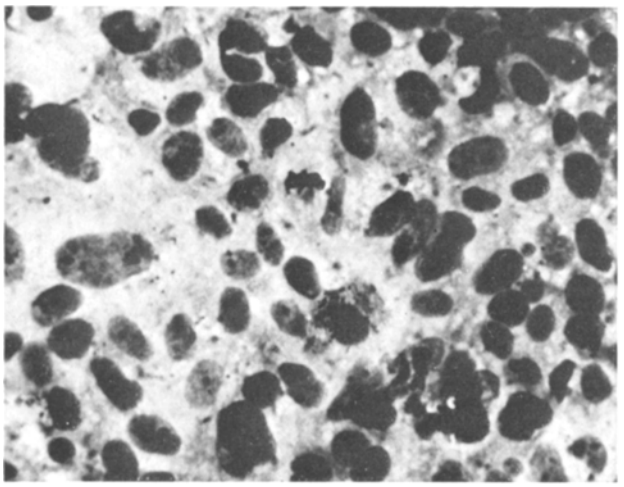

Fig. 1. Posterior capsule of a lens that was cultured for 84 days. This region normally has no cells. However, after explantation to 'R20' cells have migrated and can be seen dividing in this locale. Note the metaphase figure and also the range of nuclear sizes. Approximately. × 400.

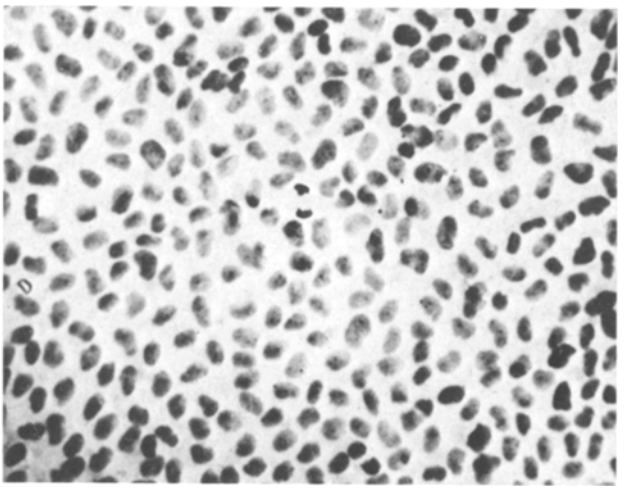

Fig. 3. Photomicrograph of the anterior capsule of a lens cultured for 30 days in 'A-199'. Notice that, although a mitotic figure is present, the cell population density is relatively light; it remains so for the longest culture periods that have been studied. × 250.

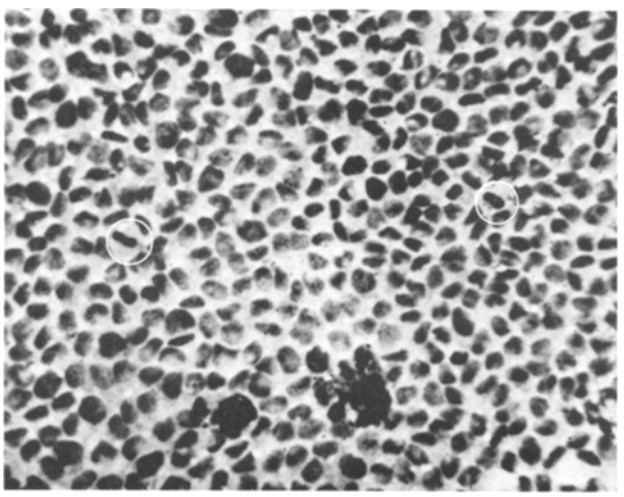

Fig. 2. Photomicrograph of the anterior capsule from a lens that was cultured for 84 days in 'R20'. The cell population density is heavy and 2 mitotic figures are apparent. The approximate density of cells shown in the photograph is usually achieved between 2–3 weeks post-isolation. × 250.

stoppered specimen jar to which was added 30 cm³ of medium. This medium was renewed once each week after the initiation of culture. Gassing was found to be unnecessary; the pH did not vary by more than 0.1–0.2 units. The tissue was fixed in Carnoy's fluid at a number of intervals varying from 1 week to 3 months post-isolation. Whole mounts of the epithelium were prepared as described previously[3] and were stained with (3:1) Harris hematoxylin for 15 min.

Results and discussion. The lenses that were cultured in 'R20' (the serum containing medium) retained relatively good transparency for the first week or so before small opacities started to appear in some. Turbidity at the equatorial zone was also common. Mitotic activity was heavy beginning $3^1/_2$ days after explantation. It continued at varying levels for the entire culture period (3 months). By about 2 weeks after isolation the cells on the anterior surface of the lens begin to migrate toward the posterior. This is of some interest since there are usually no epithelial cells on the posterior surface. During their movement to this position the cells assume a fibroblastic appearance, the nuclei become elongated and division figures materialize (Figure 1). After 1 month the cell density on the anterior surface has become greater (Figure 2). In contrast to the 'R20' culture, the lenses in 'A-199' showed a very low level of mitotic activity. This was reflected in both the relative numbers of mitotic figures observed in each type of system at a given time and also by their cell population densities (Figure 3). However, in the 'A-199' material the cells also migrated from the front to the back of the lens and many of them underwent mitosis. The cells maintained in 'A-199' did not survive for more than 2 months.

The longest period for which the lens of any vertebrate has previously been cultured is 46 days. This work was done by Bito and Harding[5] with rabbit tissue. Medium 199 was used in these studies. In the present report it has been shown that frog lenses can be maintained for more than 56 days in what is, essentially, a diluted 199, and, for at least 84 days in a serum containing medium. It is of interest that Bito and Harding also observed a migration of anterior epithelial cells to the posterior side. Another important parallel is that serum containing media stimulate heavy mitotic activity in both mammals[6] and amphibians[2]. Thus 'A-199' is, basically, a maintenance medium while 'R20' supports growth.

When lenses are injured in the in vivo situation the cells are also stimulated to divide[7]. Preliminary results in our laboratory suggest that it may be possible to follow the healing process in lenses that have been wounded in vivo and then immediately transferred to 'A-199'. Clearly, the 'R20' would not be ideal for this

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type of study because one proliferative response would be superimposed upon another. Years ago, Bakker[8] undertook this sort of investigation, using a peritoneal exudate as a culture fluid. The composition of that fluid is, of course, unknown. Our culture technique should allow an analysis of some aspects of wound healing as it occurs in an organized tissue living in a completely defined culture medium.

Zusammenfassung. Mit zwei verschiedenen Medien ist es erstmals gelungen, Augenlinsen von *Rana catesbeiana* mehr als 6 Wochen zu kultivieren. Die Wanderung und Teilung der Epithelzellen wird beschrieben.

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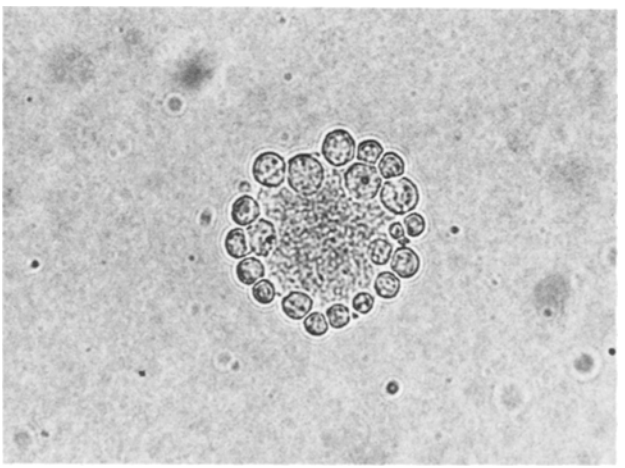
Interaction Between Mycoplasmas and Ehrlich Ascites Tumor Cells

Moore and Diamond[1] demonstrated that when Ehrlich ascites carcinoma cells or sarcoma 180 cells were mixed either with influenza virus or Newcastle disease virus and immediately inoculated into mice, pronounced inhibition of tumor growth resulted. Colonies of several mycoplasma species possess the property to adsorb erythrocytes[2–4]. In addition, the adsorption of human and bovine spermatozoa[5], tissue culture cells including Hela cells[4,6,7] has also been reported. This communication describes the nature of interaction between the mycoplasmas and the Ehrlich ascites carcinoma cells and the in vivo fate of these tumor cells after exposure to mycoplasmas.

Material and methods. Two different strains of *M. gallisepticum* and one of *M. pneumoniae* were used in the present study. For growing the mycoplasmas, the media (PPLO broth and PPLO agar) were prepared according to the method of Chanock et al.[8] except that in place of 10% unheated horse serum, 10% GG-free horse serum (Grand Island Biological Co. New York) was employed and the pH of the agar medium was adjusted to 6.5. The ascitic fluid from mice (NMRI strain, 18–20 g) which had been implanted with 10^7 Ehrlich tumor cells (ETC) in their peritoneal cavity 5 days earlier, was withdrawn, separated from ascitic plasma and washed repeatedly with Hanks balanced salt solution (BSS) at low speed centrifugation to eliminate erythrocytes, and then resuspended in either phosphate buffered-saline (PBS) adjusted to a pH of 7.2 or BSS to a concentration of 2×10^5 cells per ml. Experiments were carried out to study the adsorption of ETC to colonies of mycoplasmas grown on agar plates, to sheets of mycoplasmas attached to the bottom of plastic Petri dishes and to examine the interaction of tumor cells with mycoplasmas in suspension.

The techniques for studying the adsorption phenomenon with mycoplasma colonies and mycoplasma sheets were essentially those developed by Manchee and Taylor-Robinson[3,6]. For studying the interaction between the tumor cells and the mycoplasmas in suspension, the mycoplasma suspensions containing 10^6 colony-forming units (CFU)/0.2 ml were prepared from 3–4-day-old broth cultures of the respective mycoplasma strains, washed twice with BSS before resuspending to original concentration in BSS. 5.2 ml portions of this suspension were then mixed with 0.8 ml of the tumor cell suspension adjusted to a concentration of 2×10^7 cells per ml and prepared also in BSS. After mixing the contents, a sample was removed immediately for mycoplasma titration and the mixtures were then incubated at 37 °C. Suitable controls to test the survival of tumor cells and mycoplasmas in the suspending medium over a 2 h period at 37 °C were also included. At timed intervals 1 ml of the sample was removed from the mixture, the cells sedimented at 900*g* for 10 min and the titer of the mycoplasmas determined in the supernatant. The amount of cell-associated mycoplasmas at a given time was determined by difference between the titer of the mycoplasmas found in the supernatant and the titer of the original inoculum.

Results and discussion. It was observed that the tumor cells became adsorbed to the colonies (Figure) as well as the sheets of the test mycoplasma strains. It could be noted that the 3 mycoplasma strains adsorbed the tumor cells onto the surface of their colonies when the tests were carried out at 37 °C. The adsorption was also observed at 4 °C but the process was very slow and fewer tumor cells participated in the interaction. The interaction of the



Adsorption of Ehrlich ascites carcinoma cells to a colony of *M. pneumoniae* (Strain FH-Liu). × 400.

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