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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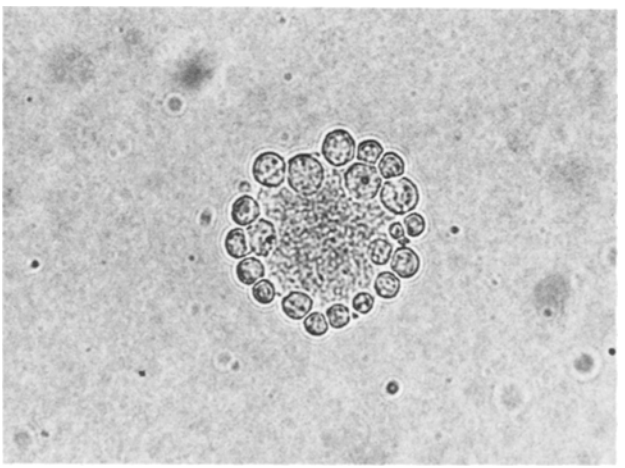
Interaction Between Mycoplasmas and Ehrlich Ascites Tumor Cells

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18–20 g) which had been implanted with 10^7 Ehrlich tumor cells (ETC) in their peritoneal cavity 5 days earlier, was withdrawn, separated from ascitic plasma and washed repeatedly with Hanks balanced salt solution (BSS) at low speed centrifugation to eliminate erythrocytes, and then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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tumor cells with mycoplasmas in suspension was firm under the experimental conditions. Repeated tests showed that the mycoplasmas in suspension (controls) did not show any appreciable fall in the titer over a 2 h incubation period at 37 °C. Thus the fall in the titer of the mycoplasmas in the mixture (Table) was evidently due to the adsorption rather than due to inactivation. The pattern of adsorption shown by the TT strain of *M. gallisepticum* was noteworthy. The mixture when sampled after 5 min showed marked adsorption of mycoplasmas. After 10 min some elution was observed, followed again by adsorption; this trend was reproducible. The other 2 test strains, unlike the TT strain of *M. gallisepticum*, did not show such an adsorption pattern with the tumor cells. However, a similar type of adsorption pattern for influenza virus on *Aerobacter aerogenes* spheroplasts was reported by BROWN et al.[9].

In order to study the receptor activity of the ETC, equal volumes of the tumor cells suspended in BSS (2×10^6 cells/ml) and purified neuraminidase-receptor destroying enzyme (RDE) from *Vibrio cholerae* (Behringwerke, West Germany) at different strengths were mixed and incubated at 37 °C for 45 min. The cells were then removed by centrifugation at 500 *g* for 10 min, washed 3 times in BSS and resuspended in BSS to produce the original concentration. The adsorption of these treated cells with mycoplasma colonies on agar was studied as usual at 37 °C. Controls included untreated cells and cells treated with heat inactivated RDE (100 °C for 2 min). Tumor cells treated with 50 units of RDE failed to participate in the adsorption phenomenon with all 3 test mycoplasma strains, whereas the adsorption was uninhibited when untreated tumor cells or ETC treated with heated RDE were used in the sytem. Thus it is clear that the interaction in the present experiments between the test mycoplasma strains and the tumor cells was dependent upon RDE-sensitive receptors on the ETC. It may be interesting to point out that the presence of sialic acid in the cellular membranes of Ehrlich ascites carcinoma cells has been reported[10,11]. Preliminary experiments have shown that, in vivo, pronounced inhibition of tumor growth can be observed with mice which were injected with mycoplasma-treated ETC.

Interaction of Ehrlich ascites tumor cells with mycoplasmas in suspension

Mycoplasma	Mycoplasma[a]-ETC mixture (time after mixing in min)					
	0	5	10	15	30	120
M. gallisepticum (Strain TT)	5.5[b]	4.0	4.9	3.7	2.2	1.6
M. gallisepticum (Strain PG 31)	5.3	5.2	4.0	3.1	2.9	2.4
M. pneumoniae (Strain FH-Liu)	5.0	4.8	3.8	3.0	2.8	2.6

[a] Initial titer of the mycoplasmas added in each case was 10^6 CFU/0.2 ml and repeated tests showed that there was no significant fall in the titer of control mycoplasma suspensions during the test period. [b] The figures represent the titers of the mycoplasmas in the supernatant at the indicated interval; titers expressed as log 10/0.2 ml.

Zusammenfassung. Es wurde gezeigt, dass die Adsorption von Mycoplasmen an Ehrlich Ascites-Tumorzellen an das Vorhandensein von neuraminidase-empfindlichen Zellrezeptoren gebunden ist.

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Radiation Effects on Embryonic Chick Tibiae

Radiation is known to affect the development of avian embryos in general and their long bones in particular. However the time at which the embryo is most radiosensitive is in dispute. MULLER and MORENG[1] indicated that chick embryos are most radiosensitive after 2 days of incubation, while other workers have shown that acute mortality is highest after irradiation at 8–10 days of incubation and that, using this criterion, there is no radiosensitive period after two days[2,3]. Using morphological developmental abnormalities and 'defective legs' as criteria, avian embryos are most radiosensitive around 50–55 h of incubation[4,5] with a secondary peak at about 8–9 days[5]. The effect of radiation on the growth inhibition of embryonic chick tibiae, reported in this paper, is an extension of these investigations.

Material and method. White Leghorn chicken eggs of approximately the same size (65 g/egg) were used in the experiments. The eggs were stored at 4 °C (never more than 4 days) prior to incubation, and were incubated for 17 days in a 'Humidaire automatic-turner' incubator at 37.5 ± 0.5 °C. The tibiae were dissected out of the 17 day embryos and measured with calipers.

The G.E. Maxitron X-ray machine was operated at 250 kVp, 30 mA with a Hvl of 1.2 mm Cu. There was 1/4 mm Cu + 1 mm Al added filtration and the tube to target distance was 125 cm. Under these conditions the dose rate was about 30 R/min. Dosimetry was carried out using a Victoreen R-meter placed at the same distance from the X-ray tube as the center of the egg. The dosimeter was laid on the perspex tray of a specially constructed irradiation chamber which was heated to 37 ± 2 °C, by means of an externally placed lamp, in order to

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