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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ensure that any observed effects were not due to a change in temperature within the eggs.

Results and discussion. The first experiment was designed to determine the time at which the developing chick tibiae were most radiosensitive and to test whether the radiosensitivity observed by other workers at 2 and/or 9 days of incubation was a general phenomenon reflected in growth inhibition as well as mortality. In the experiment approximately 15 eggs were irradiated with a fixed exposure of 375 R on each day of incubation up to the 16th day. This exposure was chosen as it is not sufficiently high to kill any of the embryos. The results of the experiment are portrayed in Figure 1. Control bone lengths from 28 embryos are shown and all points are plotted with their standard errors. 2 batches of eggs were sham irradiated on the 3rd and 14th days of incubation to demonstrate that there was no growth inhibition due to a change in temperature within the eggs (open circles in Figure 1). The tibiae weights varied in a similar manner to the lengths.

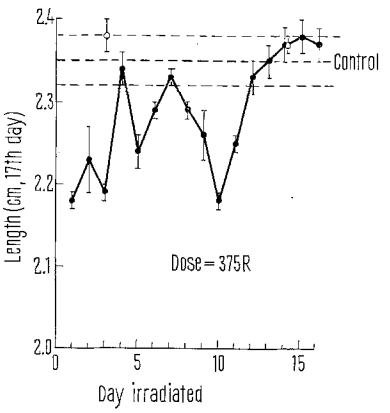

Fig. 1. The effect of 375 R on the length of embryonic chick tibiae as a function of the day of irradiation.

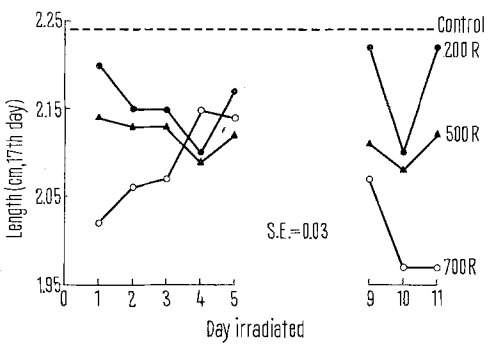

Fig. 2. The length of embryonic chick tibiae as a function of 3 radiation exposures and the day of irradiation.

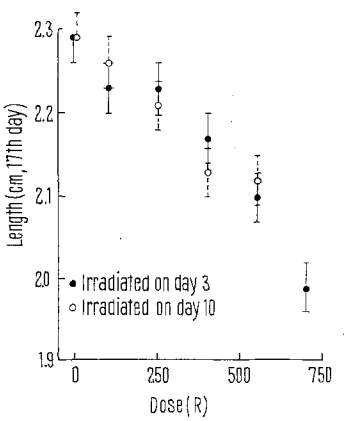

Fig. 3. Dose-effect curves, for 3- and 10-day-old embryonic chick tibiae in terms of length.

Two general periods of radiosensitivity were observed. The first was during the first 3, and possibly 5, days of incubation and the second was from days 9–11 with a definite maximum at day 10.

A second experiment was undertaken to confirm and compare these 2 radiosensitive periods and to seek to more clearly establish which of the incubation ages during the first 5 days was most radiosensitive. Radiation exposures of 200 R, 500 R, and 700 R were used to ensure that any observed effect was not a function of a specific exposure. 12 eggs were irradiated on each of days 1–5 and 9–11 of incubation. The results of the experiment are shown in Figure 2. We observe that: 1. The length of the bone is related to the radiation exposure. 2. After 700 R the first 3 days appear more radiosensitive than days 4 and 5. 3. The results obtained on day 10 indicate a decrease in bone length after irradiation at least equal to observed decreases at any other time.

Finally dose-effect curves were established for eggs irradiated on the 3rd and 10th days of incubation. Each point represents the mean of about 24 bone lengths and standard errors are plotted. There is no difference in tibia radiosensitivity at these 2 developmental times (Figure 3).

A 3-day-old embryo weighs approximately 20 mg whereas a 10-day-old embryo weighs about 2.4 g[6]. This difference in weight is an accurate reflection of the difference in organization and differentiation of the 2 embryos. Their similarity in radiosensitivity is therefore somewhat surprising. However similar tibiae radiosensitivity need not to be synonymous with equal embryo sensitivity in terms of mortality nor in terms of the mechanism of radiation damage. Investigations are proceeding to determine whether the mechanism of radiation damage to developing avian long bones is similar at 3 and 10 days of incubation[7].

Zusammenfassung. Hühnerschienbeine zeigen 2 strahlensensitive Perioden während der embryonalen Entwicklungszeit. Wachstumshemmung durch ionisierende Strahlen wirkt sich am stärksten während der ersten 3 Tage sowie am 10. Tag der Inkubation aus. Strahlendosis-effektkurven zeigen eine vergleichbare Strahlenempfindlichkeit am 3. und 10. Tag, wenn Schienbeinlänge und -gewicht als Dosiseffekt gemessen werden.

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