procedure we would thus be able to locate the atmospheric "epicenter" of the impact point.

So far we have analyzed only a dozen signals in the detail shown for the case described here. But a survey of 8 years of visual drum recordings reveals a strong minimum of occurrence in the hours between midnight and dawn. These observations seem to match the 3 a.m. minimum shown in the diurnal distribution of meteor falls given by Heide (*3*); we realize, of course, that the existence of such a nocturnal minimum may in part be due to a nocturnal absence of observers. Heide explains this minimum (if real) as a meteor velocity effect due to the earth's orbital speed (about 30 km/sec). The high speed resulting from the combination of the approach of an early-morning meteor with the orbital speed of the earth would cause complete destruction of the meteor in the high atmosphere. Lower resultant speeds, especially for meteors arriving later in the day, would permit them to fall or at least to reach elevations low enough to shock the atmosphere, thus generating acoustic waves.

From this information and our analysis it appears that the objects we have detected belong to the class of more dense, meteorite-type bodies rather than to the class of "fluffy" or "dust ball" objects detected more commonly by photographic and radio means at elevations between 80 and 100 km. The acoustic method may thus provide a means for estimating the flux of sporadic, meteoritic material rather than that of the less dense upper-atmospheric meteoric bodies. For flux determinations, a grid of single-site acoustic detectors is necessary with one of the sites being tripartite to permit distinctions to be made between the meteoric signals and stationary artificial sources.

Finally, the dominant recorded energy for these wave trains lies within 0.3 to 3 hertz. The low-frequency cutoff is sharp, being a function of source parameters; the higher-frequency portion falls gradually, being a function of attenuation effects.

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Transformation of Chick Embryo Neuroretinal Cells by Rous Sarcoma Virus in vitro: Induction of Cell Proliferation

Abstract. *Neuroretinal cells from 7-day-old chick embryos are transformed and induced to proliferate after infection with Rous sarcoma virus in vitro. Susceptibility of neuroretinal cells to the virus is also dependent on the stage of development since infection of cells from 10-day-old embryos is uneffective.*

Interactions of Rous sarcoma virus (RSV) with permissive cells in vitro have been mainly studied in chick embryo fibroblasts. Cells from differentiated chick tissues, such as iris epithelium (*1*) and myoblasts (*2*), can also be transformed by RSV and release virus. However, no chick cells from a neural tissue have been shown to be susceptible in vitro to infection and transformation by RSV.

We report that RSV infection of neuroretinal (NR) cells from 7-day-old chick embryos in vitro results in morphological transformation and virus replication. The transformed NR cells were induced to proliferate up to 12 doublings. In contrast, NR cells from 10-day-old embryos were not transformed by RSV, supported only minimal virus replication and did not multiply. Neuroretina was selected for these experiments since this tissue is made exclusively of neural cells and has a low capacity for multiplication in vitro (*3*). Neuroretinas were dissected from leukosis-free flocks of brown Leghorn chick embryos after fertile eggs were incubated for 7 or 10 days at 37°C. The tissues were dissociated as described (*4*), and the yield of cells ranged from 4×10^6 cells (7-day embryos) to 4×10^7 cells (10-day embryos) per neural retina. Cultures obtained by this procedure consisted mainly of small, round cells with an average diameter of 6 μm and were entirely devoid of pigmented cells and fibroblasts. A high titer variant of the Schmidt Ruppin strain of RSV, subgroup D, was used in our experiments. The virus was produced and assayed on fibroblasts from brown Leghorn chick embryos of the C/O phenotype as judged by their susceptibility to the four main subgroups of avian oncornaviruses. Suspensions of single NR cells, in Eagle's minimal essential medium supplemented with 5 percent calf serum, 10 percent Bacto tryptose phosphate, and antibiotics were infected with RSV at a multiplicity of about one focus-forming unit (FFU) per cell, in the presence of diethylaminoethyl dextran (5 μg/ml) (*5*). Portions (2×10^6) of control or infected cells were seeded in 35-mm plastic petri dishes and kept at 37°C in a humidified atmosphere (5 percent CO_2 in air). Complete cell attachment occurred within 2 hours. The medium (2 ml per dish) was renewed the following day and every third day thereafter.

Table 1. Kinetics of RSV replication in NR cells from 7- and 10-day chick embryos. Infected NR cells (2×10^6) in 2 ml of Eagle's minimal essential medium containing 5 percent calf serum and 10 perecnt Bacto tryptose phosphate were seeded in 35-mm plastic petri dishes. Four hours later, attached cells were extensively washed to eliminate unadsorbed virus. Fresh medium (2 ml) was added and renewed at intervals as indicated. RSV was titrated by the focus-forming assay on chick embryo fibroblasts of the C/O phenotype.

Time after infection	Virus titers (FFU/ml) in NR cells from	
	7-day embryos	10-day embryos
Day 0*	2×10^6	2×10^6
Day 1	2×10^3	30
Day 3	7×10^4	9
Day 5	7×10^5	
Day 6	1.2×10^6	
Day 8	3×10^6	50

* Input virus.

Morphological transformation was evident in NR cells from 7-day embryos within 36 hours after infection by RSV and simultaneously affected most of the

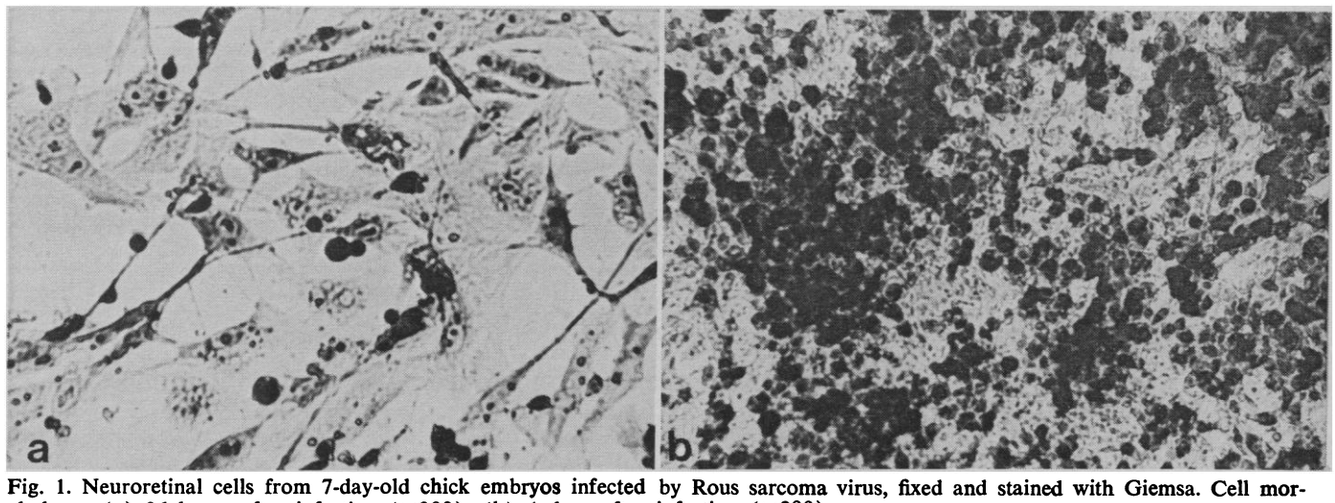

Fig. 1. Neuroretinal cells from 7-day-old chick embryos infected by Rous sarcoma virus, fixed and stained with Giemsa. Cell morphology: (a) 36 hours after infection (×300); (b) 4 days after infection (×200).

cells. Early changes induced in NR cells were different from those observed in chick embryo fibroblasts infected with the same virus strain. Transformed NR cells were considerably enlarged, polygonal, or elongated with abundant cytoplasm and numerous extensions (Fig. 1a).

Transformed cells started readily to multiply, and by day 4 the dishes were filled with cells proliferating in multilayers (Fig. 1b). They were transferred at a split ratio of 1 : 3 in 75-cm^2 plastic flasks in Eagle's basal medium containing 10 percent fetal calf serum and were then subcultured eight times at confluency, over a 2-month period corresponding to about 12 cell doublings (cell destruction was not taken into account). Cell division stopped at about the ninth passage. In contrast, cultures of uninfected cells did not show evidence of multiplication and could not be propagated in vitro.

RSV replication was also studied in infected NR cells. Virus production could be detected in the medium at 24 hours and titers of about 10^6 FFU/ml were reached by day 5 or 6 (Table 1). In addition, RSV was continuously released in the medium during subcultivation of transformed NR cells.

Under similar conditions NR cells from 10-day embryos were also infected with RSV. No morphological transformation was seen, only minimal virus replication was detected after 8 days (Table 1), and there was no cell multiplication. On the other hand, fibroblasts from the same 7- and 10-day-old embryos were equally susceptible to RSV.

The experiments reported here show that the lifespan of neuroretinal cells from 7-day chick embryos is increased after RSV infection; transformed cells were induced to proliferate for up to 12 generations, whereas uninfected cells did not grow in vitro. These results, reproduced in several successive experiments, are different from those reported on the growth changes after RSV infection in cells that are capable of multiplying in vitro. The lifespan of transformed chick fibroblasts is reduced in comparison to that of control cells (*6*), and in order to demonstrate the induction of proliferation in chick fibroblasts after transformation by RSV, it is necessary to use experimental conditions that restrict the growth of uninfected cells (*7*). Therefore, NR cells from 7-day chick embryos appear to be an interesting system for studying the effects of RSV on cell multiplication. It may be noted that NR cells from 7-day embryos do multiply in vivo; hence, RSV might restore a cell function (or functions) required for multiplication which is lost in vitro. In addition, the fact that NR cells from 10-day chick embryos were not transformed by RSV and supported only minimal virus replication shows that susceptibility of NR cells is also dependent on the stage of development.

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