discharge to interrupted null motion is also expected from this scheme. The null movements produce brief bursts of firing in the excitatory cell, but these movements are in the preferred direction of the inhibitory cell, which is strongly driven, thereby inhibiting the LGN cell and preventing any escape discharge. The narrowness of the direction-sensitivity function is also a natural consequence of the inhibitory input. For example, movement at 90° to the preferred-null axis will activate both retinal inputs, resulting in balanced excitation and inhibition, and again no response from the LGN cell occurs. The cancellation experiment (Fig. 1, E and F) gives a negative result at the retinal level because inhibition by movement in the null direction is strictly localized to the region immediately ahead of the test target. However, the LGN neuron receives powerful inhibition from the retinal neuron which is active throughout the test-target's motion. Thus excitation and inhibition are opposed at the geniculate, and the evoked response is canceled.

While this scheme is sufficient to explain our experimental facts, more complex arrangements are not excluded. Each retinal input in Fig. 2, for example, may actually be a group of units with similar preferred directions. The number of similar units could be a factor in the relative weighting of excitation and inhibition, and the weighting does seem to vary somewhat from cell to cell. Another possible arrangement is to include inputs from the two retinal direction-selective groups with preferred directions at right angles to those shown in the figure, making a total of four inputs. These two extra inputs could be either excitatory or inhibitory, but, if present, their contribution is probably small.

We note as a final point, one other difference between the retinal and LGN direction-selective cells; namely, the LGN cells are more variable than retinal units in their responses to repetitions of a given stimulus. This observation has implications for the reliability of the LGN neuron as an information channel or may be related to the role of nonvisual inputs to the LGN (*11*).

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9. The stimulus for the direction-sensitivity functions was a rectangular raster on a CRT face. The raster could be driven across the screen in a direction perpendicular to the long axis of the rectangle. The whole CRT could be rotated around its center, thus changing the direction of movement. Action potentials were converted to standard pulses and counted during the traverse of the raster sweep.
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12. We thank L. M. Davies, Miss B. Ferguson, and R. M. Tupper for technical assistance. C.W.O. is a postdoctoral research fellow of Fight for Sight, Inc., New York, and E.T. is a postdoctoral fellow of the National Institute of Neurological Diseases and Stroke, U.S. Public Health Service.

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Viral Infection Across Species Barriers: Reversible Alteration of Murine Sarcoma Virus for Growth in Cat Cells

Abstract. *Infection of cat embryo cells by a centrifugally induced aggregate of murine sarcoma virus and feline leukemia virus gave rise to a defective, focus-forming virus which propagated in cat cells, but not in mouse cells. This virus, apparently enveloped with a feline leukemia virus coat, was later subjected to aggregation with murine leukemia virus, whereupon it regained the capacity for growth in mouse cells.*

The infection of mouse cell cultures with the Moloney isolate of murine sarcoma virus (MSV) is defective since superinfection with murine leukemia virus (MLV) is required for production of focal lesions and release of progeny virus (*1*). Titration patterns of various stocks of MSV revealed that such stocks contained, in addition to defective MSV and excess endogenous MLV, variable amounts of competent MSV, which could form foci on mouse embryo cultures without superinfection by the free endogenous MLV. The competent MSV appeared to consist of a temporary association of the sarcoma and leukemia viral genomes in a single effective particle having an exterior antigenicity similar to that of defective MSV or MLV (*2*).

Further investigations showed that competent sarcoma virus consists of an interviral aggregate of defective MSV and MLV which has an enhanced infectious capacity (*3*). Of particular interest was the observation that sedimentation of defective MSV and MLV in the preparative ultracentrifuge could lead to the formation of competent MSV. These findings raised the question whether MSV would form aggregates with other viruses, with alterations of the biological specificities of the combinations.

We now report the successful aggregation of defective MSV with feline leukemia virus (FelLV) which resulted in a focus-forming, defective virus capable of indefinite propagation in cat cells but not in mouse cells. This modified virus, tentatively designated MSV-FelLV, could after several passages in cat cells be restored for successful propagation in mouse cells by a similar aggregation with MLV.

Feline embryo fibroblast cultures, derived from approximately 30-day-old embryos, were established, and all virus infections were done between the third and the tenth tissue culture passages (*4*). The feline leukemia virus and MSV were used as tumor extracts in 0.05*M* sodium citrate (*5*). The MSV had a titer on Swiss mouse embryo cells of about 1×10^6 focus-forming units (FFU)/ml, of which 15 percent were competent (*2*). Briefly, the aggregation technique (*3*) consists of a tenfold dilution of MSV in Dulbecco's phosphate-buffered saline without calcium or magnesium and an initial cycle of centrifugation for 15 minutes at 5,000 rev/min. The supernatant contained defective MSV devoid of competent MSV. This defective MSV in the supernatants was sedimented at 25,000 rev/min (Spinco-39L rotor, 45 minutes); the resulting viral pellet was resuspended and contained 17 percent of competent MSV. The defective su-

pernatant MSV was also centrifuged at high speed, with the addition of 1 ml of a 10 percent extract of feline leukemia virus. After each centrifugation step, infectious virus content was determined on Swiss mouse embryo cells. Cat embryo cells (2×10^5 cells) were plated on 20-cm² plastic dishes, and 24 hours later were infected with three types of MSV preparations. The first consisted of low-speed MSV supernatant containing 3.2×10^3 FFU of pure defective MSV per dish, the second was the MSV pellet, obtained at high speed, which contained 4.0×10^3 FFU per dish, and the third set consisted of the MSV-FelLV interviral pellet derived from 4.0×10^3 FFU of MSV. Feline leukemia virus (0.1 ml of a 1:5 dilution of the 10 percent extract) or 2×10^5 leukemia virus helper units (LVHU) of MLV (*4*) was added to alternate dishes in each of the three sets of cat cells infected with MSV. Control plates of cells were also infected with pure FelLV or MLV. Cat cells were infected as described for the MSV assay (*4*). The maintenance medium was replaced every 3 days. Plates of cat cells infected with either defective or partially competent MSV were negative up to 20 days after infection whether or not MLV or FelLV were added at the time of MSV infection. Cat cells infected with either the interviral pellet alone or with the interviral pellet and added FelLV showed discrete foci of viral transformation 6 days after infection. The cells comprising the focus were fusiform, round, and very highly refractile. Elements of both cell destruction and proliferation were observed. Cells on plates exhibiting discrete foci were harvested, and the cell-free lysates were very infectious for fresh cat cells, showing confluent transformation (resembling cytopathic effect) at low dilutions and discrete foci in 3 to 5 days at high dilutions. The apparent titer of this focus-forming MSV-FelLV in cat cells was about 2×10^4 FFU/ml. Two passages of the cell-free virus in cat-cell tissue cultures rapidly increased its titer to $> 1 \times 10^5$ FFU/ml. At this time, the host range of the focus-forming virus was restricted to cat cells. Thus, more than 1×10^4 FFU of MSV-FelLV per dish of Swiss mouse cells did not produce foci or other signs of viral transformation despite added MLV or FelLV in alternate sets of dishes.

When MSV-FelLV is titrated in cat cells, the results indicate that the virus infection is defective (Fig. 1). The addition of adequate feline leukemia virus converted the "two-hit" pattern to a "one-hit" pattern. A small competent virus-like component is indicated by the titration pattern at high dilutions of the virus. The amount of FelLV required to express all of the defective MSV-FelLV was determined by a helper assay where the components were those of the feline system. When the increase of foci was plotted against the dilution ($\log_{10}$) of helper virus (Fig. 2), a typical leukemia virus helper assay was observed. The amount of FelLV could thus be obtained by the application of the standard derivation for helper activity (*4*).

The infection of cat cells with only FelLV did not result in any morphologic alterations for more than 2 months after the initial FelLV infection. In view of the known interference of murine sarcoma and leukemia viruses (*6*) and in order to obtain an insight into the nature of the MSV-FelLV coat we infected with MSV-FelLV (7.3×10^3 FFU per dish) normal cat cells and cat cells infected with FelLV for 30 days. An optimum amount, 1×10^5 LVHU of FelLV, was added to alternate dishes of cat cells to express all defective MSV-FelLV. Table 1 shows that, in normal cat cells, MSV-FelLV was readily expressed as foci, and exhibited a defective titration pattern which could be corrected by FelLV addition. Interference was manifest if the cat cells were previously infected with FelLV. No foci were observed up to 20 days after the addition of MSV-FelLV. These findings suggest that MSV-FelLV emerging from cat cells is apparently coated with FelLV.

Table 1. Infection of normal and FelLV-infected cat embryo cells by FSV; FSV denotes the third passage of MSV-FelLV in cat embryo cells.

Dilution of input FSV	Foci on normal cat embryo cells		Foci on cat embryo cells preinfected with FelLV	
	FSV per se	FSV + FelLV	FSV per se	FSV + FelLV
1 : 10	Confluence	Confluence	0	0
1 : 40	Confluence	Confluence	0	0
1 : 160	Confluence	Confluence	0	0
1 : 640	28,24 (8.3×10^4)	111,121 (3.7×10^5)	0	0
1 : 2,560	2,2 (2.6×10^4)	40,23 (4.0×10^5)	0	0
1 : 10,240	0,0 ($< 1.5 \times 10^4$)	8,5 (3.3×10^5)	0	0

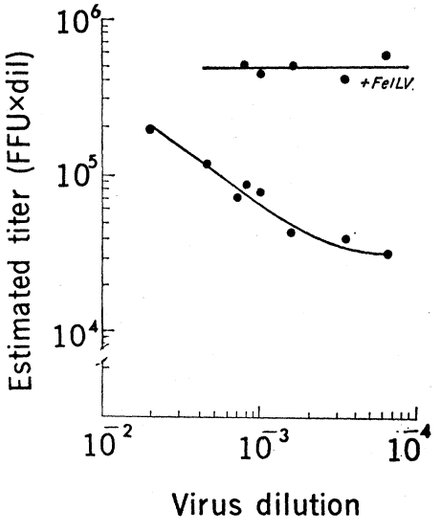

Fig. 1. Titration pattern of a high-titer stock of MSV-FelLV on feline embryo cells, relating virus dilution to the estimated virus titer. Alternate dishes of infected cells also received 0.1 ml of FelLV as a 1:8 dilution of a 10 percent feline lymphoma extract at the time of MSV-FelLV infection. In all these infections of cat cells, foci were read on day 5 after infection.

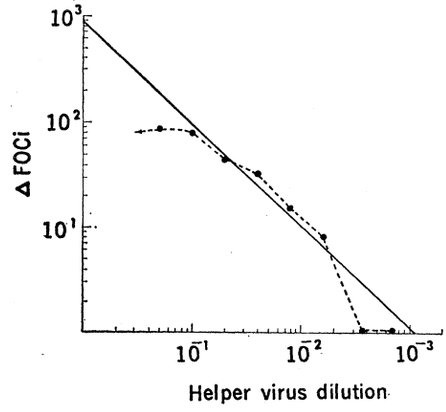

Fig. 2. A leukemia virus helper assay for FelLV. Defective MSV-FelLV was used as indicator virus for all plates at a dilution giving an average of eight foci in control plates. Serial dilutions of FelLV, 0.1 ml per dish, were added at the time of MSV-FelLV infection. The maximum number of foci expressed per plate was 102. The projection to zero dilution and calculation of virus titer according to the standard derivation gave a titer of 8.3×10^6 LVHU/ml (*4*).

At all passages of MSV-FelLV in cat cells, cellular susceptibility was restricted to cat cells and produced no foci in mouse cells. The new, specific host range of the virus obtained by aggregation of MSV with FelLV prompted attempts at reentry of MSV-FelLV back into the murine cells by a second aggregation procedure now combining MSV-FelLV and Moloney leukemia virus. The MLV (1×10^7 LVHU) was sedimented with 2×10^5 FFU of MSV-FelLV in saline. The resulting interviral pellets were then used to infect mouse cells insusceptible to pure MSV-FelLV. After 6 days of infection, typical foci were seen in Swiss mouse embryo cells, and viral progeny of these foci did not transform murine cells chronically infected with MLV. The sarcoma focus-forming genome apparently became reenveloped with an MLV coat.

The production of focus-forming MSV-FelLV was readily reproducible by the centrifugation procedure. In contrast, attempts at transspecies rescue of defective MSV genome from MSV-induced hamster tumors, by a cultivation of cat cells, hamster tumor cells, and a superinfection with FelLV were negative (*7*). Partial purification of MSV-FelLV by sedimentation and by sucrose density gradient banding revealed that, at equilibrium, the peak of MSV-FelLV focus-forming activity is localized where the buoyant density is 1.16 g/cm^3. The apparently easy crossing of the species barrier by the fusion techniques in vitro may have been facilitated by common antigens found between the mouse and the cat leukemia viruses (*8*). Indeed, we have on occasion observed that a coinfection of cat embryo cells with competent MSV and FelLV could, after a second tissue culture passage, spontaneously produce limited amounts of MSV-FelLV. The scope of this aggregation technique is under investigation with reference to crossing of other species barriers where appropriate C-type leukemogenic viruses are available. Modifications of this technique may eventually provide delivery systems for the introduction of various genetic messages into receptive cells.

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Bird Migration: Influence of Physiological State upon Celestial Orientation

Abstract. *By means of photoperiod manipulation, the physiological states of spring and autumn migratory readiness were induced in indigo buntings. The orientational tendencies of these two groups of birds were tested simultaneously in May 1968, under an artificial, spring planetarium sky. Birds in spring condition oriented northward; those in autumnal condition, southward. These results suggest that changes in the internal physiological state of the bird rather than differences in the external stimulus situation are responsible for the seasonal reversal of preferred migration direction in this species.*

Migration, by definition, refers to a two-way journey. For most birds residing in North Temperate areas, this migration consists of a southward trip each autumn followed by a northward return the following spring. Distances covered in such round-trip journeys are considerable, frequently exceeding 4000 to 6000 miles (6400 to 9600 km) (*1*).

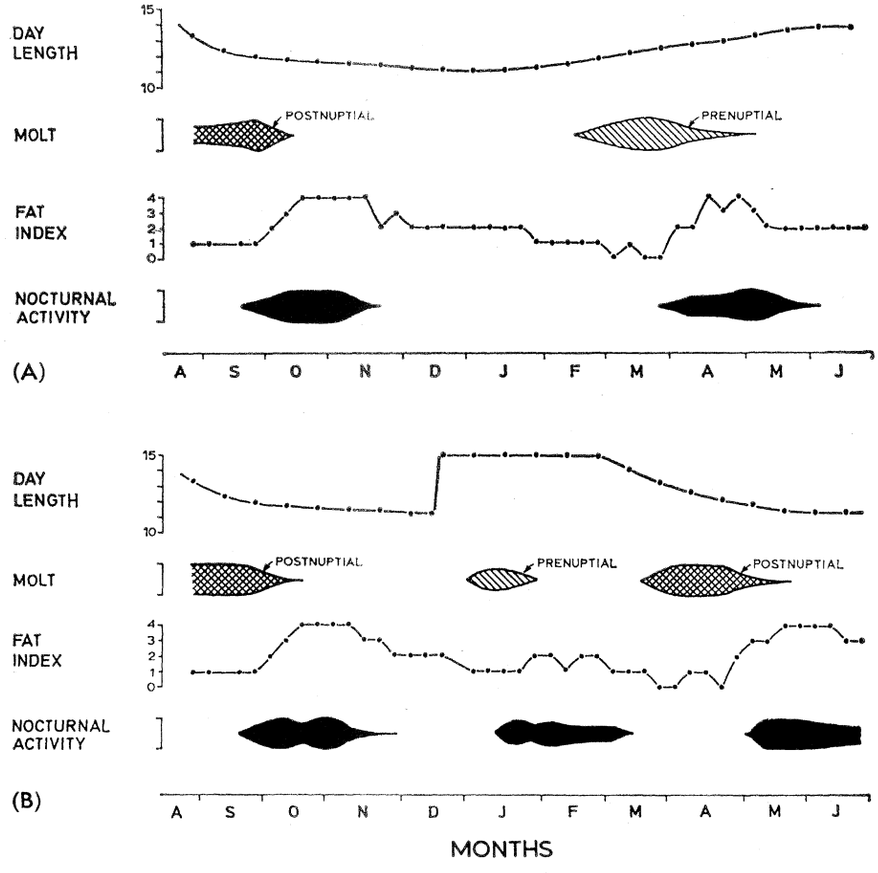

Fig. 1. The occurrence of molt, fat deposition, and nocturnal activity in indigo buntings (*7*). (A) Control birds on a natural photoperiod. (B) Experimental birds exposed to an accelerated photoperiod regimen.