Reports

Resistance of Mice Infected with Moloney Leukemia Virus to Friend Virus Infection

Abstract. *Mice inoculated with the Moloney strain of mouse lymphoid-leukemia virus showed marked diminution of spleen weight response to infection with Friend leukemia virus given 3 to 4 weeks later.*

The successful use of viral interference for detection of fowl visceral lymphomatosis virus (*1*) has stimulated efforts to develop a comparable system with the mouse leukemia viruses. Although some strains of mouse lymphoid leukemia viruses propagate in tissue culture (*2*), no viral interference has been reported in infected cultures, and demonstration of viral growth has continued to depend exclusively on induction of leukemia in animals, a procedure requiring at least 3 to 4 months. Since the Friend mouse leukemia virus (*3*) produces leukemia after a very short incubation period and can be quantitated with precision by measuring the change in spleen weight in response to infection (*4*), studies were made to determine if an animal interference or inhibition procedure could be used to detect mouse leukemia viruses, based on inhibition of response to Friend virus.

Groups of eight weanling mice (13 to 15 g) were inoculated intraperitoneally with 0.1 ml of test or control material; 21 to 28 days later, all mice were challenged by intravenous inoculation of 0.2 ml of a diluted suspension of Friend virus from infected mouse spleen known to produce, in normal mice, 1-g enlargement of the spleen at 14 days (*4*). At this time all mice were sacrificed and spleens were weighed. Several control groups inoculated with saline solution alone were interspersed throughout each test; additional controls included groups inoculated with suspensions of normal mouse tissue from low-leukemic mouse strains, control tissue-culture fluids, fluids from cultures inoculated with normal mouse tissues, and various mouse viruses. Experiments in infant mice (4 to 48 hours old) were carried out similarly, except that the initial inoculation was 0.04 ml intraperitoneally plus 0.03 ml intracerebrally, and the challenge was made at 28 days.

Initial experiments in suckling and weanling non-inbred Swiss mice indicated that prior infection with the Moloney strain of mouse lymphoid-leukemia virus (*5*) rendered mice significantly resistant to the challenge of Friend virus, as indicated by diminution of spleen weight response; a number of experiments were made to attempt to develop a reproducible system, to identify the factor responsible for the inhibition as the leukemia virus, and to determine the possible usefulness of the system for detection of naturally occurring mouse leukemia viruses. However, with the Swiss mouse there was no consistently reproducible inhibition, and the other objectives could not be definitively obtained. Significant inhibition of spleen weight response occurred in the majority of tests of Moloney leukemic-mouse tissues (7 of 9 tests) or Swiss mouse-embryo tissue-culture fluids (13 of 15 tests) infected with the Moloney virus. Although tissues of spontaneously leukemic AKR, C58, and C3H mice gave positive inhibition in 7 of 18 tests, the results of repeat tests were not consistent, and the magnitude of inhibition was generally less than that produced by the Moloney agent. Negative results were obtained with control tissues (11 tests), control tissue-culture fluids (16 tests), two human leukemic sera, tissue-culture fluids from five serially cultivated human leukemic tissues, and with polyoma virus, reovirus type 3, K virus, mouse thymic agent, and the C3H mammary tumor agent.

The experiments in Swiss mice showed that an interval of greater than 2 weeks between inoculation of Moloney virus and challenge with Friend virus was necessary in order to establish resistance to the latter.

In more recent experiments, the use of BALB/cN female weanling mice provided a sharper and more reproducible inhibition system for the Moloney virus. Table 1 shows results of a representative test, designed to elucidate the effect of dosage of challenge virus and of concomitant infection with the lactic dehydrogenase (LDH) agent, which is a frequent contaminant of mouse-tumor materials (*6*). The data show that response to both dosages of Friend virus was inhibited by prior infection with the Moloney virus-infected tissue-culture fluid, that the lactic dehydrogenase agent affected neither the challenge infection nor the inhibition by Moloney virus, and that tissue of a leukemic mouse infected neonatally by nursing a mother infected with Moloney virus (*7*)

Table 1. Representative Friend virus inhibition test in BALB/c mice. All mice were challenged with Friend virus 22 days after the initial inoculation. METC, mouse embryo tissue culture fluid; ID_{50}, 50-percent infective dose; LDH, lactic dehydrogenase.

Initial inoculum	Spleen weight 14 days after challenge		
	Median (mg)	Range (mg)	Mean $\log^{10}$ ± S.E.* (mg)
Challenged with 10^0 Friend virus			
Control METC fluid, 1:10	1625	900–2050	3.17 ± 0.04
Control METC fluid, 1:10, + $10^{2.5}$ ID_{50} of LDH agent	1700	1600–2060	3.24 ± .02
Moloney-infected METC fluid†, 1:10	330	225–525	2.52 ± .04
Moloney-infected METC fluid, 1:10, + $10^{2.5}$ ID_{50} of LDH agent	320	250–950	2.57 ± .07
Challenged with 10^{-1} dilution of Friend virus			
Control METC fluid, 1:10	900	650–1250	2.95 ± .03
Moloney-infected METC fluid, 1:10	205	160–220	2.29 ± .02
Leukemic C3H f/Lw, infected with Moloney virus by nursing; 5% tissue suspension	185	150–310	2.29 ± .04
Saline	935	665–1150	2.96 ± .04

* Although standard errors are presented here, it should be noted that the spleen weights were not necessarily normally distributed. †Third tissue culture passage, harvested 7 days after inoculation.

also produced marked inhibition of response to Friend virus. Other experiments in BALB/c mice have shown that the inhibiting factor is present in fluids of tissue cultures infected with Moloney virus at titers of no more than 10^3 infectious doses per milliliter, and that the Gross passage-A mouse-leukemia virus (*8*) does not induce resistance.

Conclusions about the mechanism of the inhibition of Friend virus response cannot be made from present data. Possible mechanisms include cross immunization, viral interference, immunologic rejection of altered cells (*9*), or less likely, a change in physiological state such as elevation of corticosteroid levels (*10*).

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Microtus: A Simple Method of Recording Time Spent in the Nest

Abstract. *A prairie vole,* Microtus ochrogaster, *was tagged with a radioactive label, a survey meter was placed over its nest, and the presence or absence of the animal in the nest was recorded on tape.*

Vertebrate animals have been tagged with isotopes and their movements have been traced by survey meters (*1*) to determine distances moved and area traversed. We have now devised a technique for measuring automatically the amount of time that the prairie vole spends in its nest or resting site.

A large male prairie vole (*Microtus ochrogaster*) was tagged subcutaneously with a 0.7 by 2.5 mm piece of alloy wire containing approximately 55 μc of Co^{60}. The animal was released at the point of capture and traced immediately into a burrow with a Victoreen Thyac II, model 489 survey meter and a scintillation probe (*2*). The next few days revealed that the animal spent most of the time underground in this same spot, presumably in a nest. A means of automatically recording the presence or absence of the animal in the nest was then devised.

Atomic Accessories model 463-1 GM survey meter was sufficiently sensitive to indicate the presence of the animal in the nest. The earphones were detached, and one end of a 200-foot length ($\sim$ 61 m) of single-conductor, shielded cable (*3*) was fitted with an appropriate connector and attached to the meter in place of the earphones. The meter with cable attached was enclosed in a plastic bag and placed on the ground directly over the nest. The other end of the cable was plugged into the input jack of a Wollensak model T-1500 tape recorder in a nearby building. With this equipment, the audio output of the survey meter could be recorded on tape. The recorder was energized by a recycling timer set to turn the current on every 4 minutes and 40 seconds and to keep it on for 20 seconds, thus giving a record every 5 minutes. During the 20 seconds that the recorder was running, some 14 seconds elapsed during which the recorder was warming up and not recording; this period provided a marker to separate consecutive recordings. Recording was done at a speed of 3¾ inches per second (9.5 cm/sec), playback at 7½ (19.5 cm/sec). At these speeds, in 2 minutes of playing time, one can hear the results of a full hour of recordings.

A 1200-foot tape ($\sim$ 376 m) will record for more than 12 hours, so the recorder needed attention only twice a day. A set of batteries (five size D cells) for the meter lasted more than 24 hours.

An advantage of this method of obtaining and recording data is that the parts are readily available, relatively inexpensive, and easily and quickly assembled and put in operation. A major disadvantage is that it is not known if the radioactive tag affects the behavior of an animal, and it is most difficult to use untagged animals for controls.

Figure 1 presents data recorded at 5-minute intervals from 8:00 P.M., 6 February, to 8:00 P.M., 7 February 1963. Temperature ranged from 31° to 46°F (−1° to +8°C), and the weather was almost continuously foggy with occasional drizzle. A black line indicates time at the nest, and blank areas indicate time away. A solid line does not necessarily mean that the animal was continuously at the nest; it may have left and returned between consecutive recordings. It does mean, however, that the animal did not spend as many as five consecutive minutes away from the nest at any time during continuation of the line.

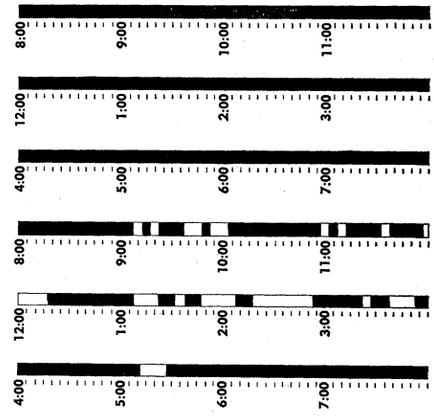

Fig. 1. From left to right and top to bottom, the chart indicates time in the nest (solid black) and time away from the nest (blank) of *M. ochrogaster* from 8:00 P.M., 6 February, to 8:00 P.M., 7 February 1963. Determinations were made at intervals of 5 minutes.

During the 24-hour period, the animal spent approximately 175 minutes out of the nest, divided into 15 activity periods with an average duration of 11.7 minutes, all during the daylight hours. *Microtus ochrogaster* is known to be active at night in summer, but little data are available on its activities in winter.

Twenty-two full days of recordings have been accumulated on two species, and the behavior pattern in Fig. 1 appears to be typical of *M. ochrogaster* during inclement weather.

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References and Notes

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2. I thank the Faculty Research Fund Committee of the University of Kentucky for the survey meter and scintillation probe.
3. Shielded cable is unnecessary. A completely adequate signal may be transmitted on several hundred meters of field telephone wire.

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