testine, as proposed by Starling (*2*). Such reabsorption includes the movement of low-molecular-weight solutes, primarily ions. If large amounts of ions are reabsorbed in this way, the need arises for an extrarenal avenue for electrolyte excretion, and such a mechanism exists in the form of the nasal salt gland of birds and reptiles. The possible relation of this gland to cloacal function was previously discussed by Schmidt-Nielsen *et al.* (*13*), who suggested that it may be an evolutionary prerequisite necessary for fully exploiting the potential of uric acid excretion for water conservation.

Water movement due to the plasma colloidal osmotic pressure does not exclude other possibilities, such as active transport of ions followed by passive movement of water. Skadhauge (*14*), for example, has demonstrated active sodium reabsorption in the chicken cloaca and lower intestine, and Junqueira *et al.* (*15*) have similarly implicated sodium transport in snakes. It should be noted, however, that all our results are compatible with the conclusion that the plasma proteins alone can provide the necessary force for water transport in the cloaca, and that none of our evidence suggests the necessity to invoke active transport or other alternate mechanisms.

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10. The control group was deprived of food and water for 2 days before testing because their cloacal contents would otherwise have had too much liquid for the wick method. After 2 days the readings were consistent, although the lizards were not sufficiently dehydrated to increase significantly the plasma colloidal osmotic pressure.
11. The measured colloidal osmotic pressure of 5.0 percent bovine albumin at *p*H 7.4 was 268 ± 3 mm-H_2O (S.D.).
12. The force acting on the cloacal contents can also be regarded as being that which constitutes the sum of the force provided by the colloidal osmotic pressure of the interstitial fluid and the negative hydrostatic pressure of the interstitial spaces, as described by A. C. Guyton [*Circ. Res.* **12**, 399 (1963)]; Scholander (*6*); and S. B. Strømme, J. E. Maggert, P. F. Scholander [*J. Appl. Physiol.* **27**, 123 (1969)].
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Demonstration of Biological Activity of a Murine Leukemia Virus of New Zealand Black Mice

Abstract. *Although New Zealand Black mouse embryo and adult tissues show evidence of murine leukemia viral particles and antigens, efforts to demonstrate biological activity of a murine leukemia virus by standard methods have proved negative. Cocultivation of tissues of these mice with non-virus-yielding hamster cells transformed by Moloney sarcoma virus, however, has resulted in the rescue of a pseudotype sarcoma virus, presumably carrying the New Zealand Black mouse leukemia virus coat. This virus has an unusual host restriction, producing foci of cell alteration only in rat cells.*

New Zealand Black (NZB) mice and their F_1 hybrids with New Zealand White (NZB/NZW) mice develop a spectrum of autoimmune phenomena, including Coombs-positive hemolytic anemia and immune-complex type nephritis, resembling human systemic lupus erythematosus (*1, 2*). Abundant "C-type" particles characteristic of murine leukemia virus have been described in these mice (*3*), and the times of appearance of circulating G+ murine-leukemia-virus antigen and its antibody have been correlated with the times of onset of anemia and nephritis, respectively (*4*).

The great majority of murine leukemia virus strains propagate on either BALB/c or NIH Swiss mouse embryo cells (*5*), with production of the murine leukemia virus group-specific complement-fixing antigen (*6*), and formation of syncytia on cocultivation with XC cells (a line of rat cells infected with Rous sarcoma virus) (*7*). High-leukemic mouse strains, such as AKR and C58, contain the group-specific murine leukemia virus antigen in secondary mouse embryo cultures and adult tissues, which consistently release infectious murine leukemia virus and form syncytia when cocultivated with XC cells (*8*). New Zealand mouse embryo tissue cultures and adult organs contain naturally occurring murine leukemia virus antigen in amounts comparable to the high-leukemic strains. However, neither recovery of infectious virus nor formation of XC cell syncytia has been observed, indicating the absence of murine leukemia virus biological activity using conventional methods despite the presence of antigens and "C-type" particles in NZB tissue.

Another approach to the demonstration of murine leukemia virus biological activity involves the use of a hamster tumor cell line (HT-1) transformed by the Moloney strain of murine sarcoma virus (M-MSV). These cells lack the murine leukemia virus group complement-fixing antigen and show no evidence of virus production by bioassay or electron microscopy (*9*). Upon cocultivation with mouse leukemia virus–infected mouse or rat cells, the sarcoma virus genome is rescued from HT-1 cells, and enveloped in the type-specific coat of the murine leukemia virus used for the rescue (*9*). The rescued pseudotype sarcoma virus is recognized in tissue culture by the production of foci of transformation. Since NZB cells contain murine leukemia virus antigen, a rescue experiment was attempted by cocultivating HT-1 cells with NZB cells in tissue culture. Murine leukemia virus growth has been shown to be subject to host range restrictions (*5*); accordingly, the cocultivation fluids were assayed on BALB/c, NIH, and NZB mouse embryo cells. In addition, cells derived from rats were used, since these cells, in contrast to mouse cells, can detect

M-MSV and some of its pseudotypes directly, without requiring murine leukemia virus as helper virus (*10*).

Cultures of mouse embryo and Fisher rat embryo fibroblasts were prepared by standard methods (*5*). HT-1 cells (cloned in agar) and a continuous line of normal Osborne-Mendel rat kidney (NRK) cells (*11*) have been maintained in this laboratory. In cocultivation experiments, 10^5 HT-1 cells were grown with 3×10^5 secondary NIH, BALB/c, or NZB mouse embryo cells as well as with cells derived from 2-month- and 8-month-old NZB kidneys. Cultures were maintained on Eagle's minimal essential medium (Microbiological Associates) with 10 percent unheated fetal calf serum, glutamine, and antibiotics (*6*). Fluids were changed every 2 to 3 days, and cells and fluid were harvested at 8 days, and frozen at −60°C for later assay for sarcoma focus-formation. These assays were conducted on the three types of mouse embryo cells as well as on rat embryo and NRK cells, with McCoy's 5a medium with 5 percent calf serum (heated for 30 minutes at 56°C) and antibiotics (*5*). Prior to testing, all specimens were thawed and passed through a 0.45-nm filter (Millipore Corporation).

As indicated in Table 1, HT-1 cells alone, cocultivated with mouse cells, or infected with the Rauscher strain of murine leukemia virus (R-MLV) did not release a focus-forming virus. When R-MLV was added to the cultures containing both HT-1 and mouse cells, a pseudotype virus, M-MSV(R-MLV), was produced which included foci in both mouse and rat cells. The filtered supernatants from 1-week-old cultures of HT-1 cells cocultivated with the three types of NZB cells also yielded focus-forming virus, which could be detected only in rat cells. Assays on BALB/c, NIH, and NZB mouse embryo monolayers were consistently negative. The titer of this NZB-rescued sarcoma virus ranged from 5 to 100 focus-forming units per 0.5 ml of cocultivation fluid. If fresh NZB mouse embryo cells were added to the HT-1 NZB mixed cultures after 1 week, the virus yield was raised five- to tenfold. The rescue of virus by cocultivation with NZB cells has been obtained in all of eight different experiments, with five lots of NZB cells.

Since the NZB cells contain the group-specific murine leukemia antigen, it is presumed that the murine sarcoma virus has been rescued in an NZB leukemia virus coat [M-MSV(NZB)]. The lack of foci in mouse cells could result from a host range restriction (*5*), inhibition by too much NZB murine leukemia virus (*12*), or a lack of sufficient NZB leukemia virus to serve as "helper" for the NZB pseudotype. Addition of a dose of R-MLV which gives optimal helper activity with other pseudotypes did not lead to focus-formation in NIH mouse embryo cells.

Table. 1. Murine sarcoma focus formation after cocultivation of various cells. ME = mouse embryo tissue culture cells; RE = Fisher rat embryo cells; NRK = normal rat kidney cells (*11*); HT-1 = hamster tumor cells, transformed by Moloney strain of murine sarcoma virus; R-MLV = Rauscher strain of murine leukemia virus.

Filtered supernatant from cultivated cells*	Foci in NIH-ME, BALB/c-ME, NZB-ME	Foci in RE and NRK
HT-1 only	0	0
HT-1 + NIH-ME or BALB/c-ME	0	0
HT-1 (R-MLV)	0	0
HT-1 + NIH-ME (R-MLV)	200	200
NZB-ME	0	0
HT-1 + NZB-ME	0	50 to 100
HT-1 + NZB 2-month kidney	0	10
HT-1 + NZB 8-month kidney	0	5

* Plus sign (+) denotes cocultivated cells. In parentheses are cells superinfected with virus.

Foci in NRK cells induced by the M-MSV(NZB) virus have been established as transformed cell lines. They do not release any focus-forming virus detectable in mouse or rat cells. If only small amounts of NZB murine leukemia virus were present in the original filtered cocultivation fluid, this "nonproducing" state would be expected (*10*). When the transformed NRK cells are cocultivated with fresh NZB mouse embryo cells or superinfected with R-MLV, a focus-forming sarcoma virus is again detected. In the former case, an NZB pseudotype virus is produced which induces foci only in rat cells; in the latter case, the Rauscher pseudotype, M-MSV(R-MLV), is found which produces foci in both mouse and rat cells as expected (*10*). NZB pseudotypes are not neutralized by antiserums which neutralize M-MSV, indicating that the sarcoma virus rescued is not serologically similar to the virus which originally transformed the HT-1 cells.

The "C-type" particles in NZB mice, therefore, have biologic activity similar to other murine leukemia viruses in being able to rescue the sarcoma genome from HT-1 cells. The NZB murine leukemia virus differs from previously described naturally occurring murine leukemia virus strains in the complexity of demonstration of its biological activity and the host range restriction of the murine sarcoma virus pseudotype to rat cells only. Although biological activity has been demonstrated by these rescue experiments, it has not been possible thus far to establish the virus in serial passage in vitro.

It remains unknown whether the NZB murine leukemia virus initiates autoimmune processes, or is a passive agent in the development of immune-complex renal disease, as in the case of lymphocytic choriomeningitis virus (*13*). "C-type" particles are seen in virtually all mouse strains (*14*), though generally in lesser amounts than in New Zealand mice. The unusual presence of both G+ antigen and antibody in the lifetime of a single mouse may reflect the immunological hyperreactivity of these mice to many antigens, including autoantigens (*2, 15*). Propagation of the NZB murine leukemia virus is needed to further define its possible role in the pathogenesis of autoimmune disease.

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