ies of the red blood cell membrane (*5*), rat phrenic nerve (*15*), and the behaviors of simple antibiotic ionophores, such as valinomycin (*6, 14*) and gramicidin (*16*). Measurement of the appropriate membrane properties should enable this interpretation of the critical volume hypothesis to be examined in more detail.

KEITH W. MILLER
Harvard Medical School, Departments of Pharmacology and Anesthesia, Massachusetts General Hospital, Boston 02114

References and Notes

1. J. H. Hildebrand, R. R. Sayers, W. P. Yant, *Nature (Lond.)* **121**, 577 (1928); K. W. Miller, W. D. M. Paton, W. B. Streett, E. B. Smith, *Science* **157**, 97 (1967).
2. E. E. P. Barnard, in *The Effects of Pressure on Organisms*, M. A. Sleigh and A. G. MacDonald, Eds. (Cambridge Univ. Press, Cambridge, 1972), p. 343.
3. R. W. Brauer, R. O. Way, M. R. Jordan, D. E. Parrish, in *Underwater Physiology*, C. J. Lambertsen, Ed. (Academic Press, New York, 1971), p. 487; R. W. Brauer, S. M. Goldman, R. W. Beaver, M. E. Sheehan, *Undersea Biomed. Res.* **1**, 59 (1974); W. L. Hunter and P. B. Bennett, *ibid.*, p. 1; K. W. Miller, W. D. M. Paton, E. B. Smith, in *Troisième Journees Internationales d'Hyperbarie et de Physiologie Subaquatique*, X. Fructus, Ed. (Doin, Paris, 1970), pp. 31–34.
4. M. J. Lever, K. W. Miller, W. D. M. Paton, E. B. Smith, *Nature (Lond.)* **231**, 368 (1971); K. W. Miller, W. D. M. Paton, R. A. Smith, E. B. Smith, *Mol. Pharmacol.* **9**, 131 (1973).
5. P. Seeman, *Pharmacol. Rev.* **24**, 583 (1972); J. C. Metcalfe, P. Seeman, A. S. V. Burgen, *Mol. Pharmacol.* **4**, 87 (1968).
6. S. M. Johnson, K. W. Miller, A. D. Bangham, *Biochim. Biophys. Acta* **307**, 42 (1973); J. R. Trudell, W. L. Hubbell, E. N. Cohen, *ibid.* **291**, 335 (1973).
7. M. J. Halsey and D. W. Kent, in *Abstracts of Scientific Papers of the Annual Meeting of the American Anesthesiologists Association* (American Society of Anesthesiologists, Chicago, 1972), pp. 105–106.
8. K. W. Miller, W. D. M. Paton, E. B. Smith, *Nature (Lond.)* **206**, 574 (1965); K. W. Miller, W. D. M. Paton, R. A. Smith, E. B. Smith, *Anesthesiology* **36**, 339 (1972).
9. G. G. Power and H. J. Stegall, *J. Appl. Physiol.* **29**, 145 (1970).
10. G. Lundgren and H. C. Ornhagen, in *Proceedings of the Fifth Symposium on Underwater Physiology*, C. J. Lambertsen, Ed. (Academic Press, New York, in press).
11. J. R. Partington, *Advanced Treatise on Physical Chemistry* (Longmans, Green, London, 1949), pp. 677–679.
12. L. D. Proctor, C. R. Carey, R. M. Lee, K. E. Schaefer, H. van den Ende, *Aerosp. Med.* **43**, 867 (1972).
13. J. Chouteau, in *Troisième Journees Internationales d'Hyperbarie et de Physiologie Subaquatique*, X. Fructus, Ed. (Doin, Paris, 1972), p. 8.
14. S. M. Johnson and K. W. Miller, *Nature (Lond.)* **228**, 75 (1970).
15. J. C. Hsia and J. M. Boggs, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3179 (1973).
16. S. B. Hladky and D. A. Haydon, *Biochim. Biophys. Acta* **274**, 294 (1972).
17. K. W. Miller, unpublished data.
18. J. Walkley and W. I. Jenkins, *Trans. Faraday Soc.* **64**, 19 (1968).
19. Supported in part by the National Institute of General Medical Sciences (contract GM 25904-07) and the Office of Naval Research (contract N00014-73-A-0340-0001) with funds provided by the Naval Bureau of Medicine and Surgery. I thank J. Benson for some computational assistance.

3 June 1974

Antibody to Leukemia Virus: Widespread Occurrence in Inbred Mice

Abstract. *Mice from a wide variety of inbred strains produce immunoglobulin G antibody against murine leukemia virus. This is contrary to the common view that the mouse is immunologically tolerant to its endogenous leukemia virus.*

Until relatively recently it has been a widely held opinion that the mouse is immunologically tolerant to its endogenous leukemia virus (MuLV) (*1*). The first demonstration of autogenous immunity to MuLV was in NZB mice, where it was thought that the immune response to virus-associated antigens was actually a manifestation of the NZB autoimmune syndrome (*2*). New evidence (*3, 4*) now indicates, however, that autogenous immunity to MuLV exists also in certain other mouse strains (such as AKR and RF), and that immune responsiveness to MuLV-associated antigens might be more prevalent than was originally considered.

We report here studies that substantiate the view that the mouse is immunologically competent in respect to MuLV. With a sensitive radioimmune precipitation (RIP) assay we have found immunoglobulin (IgG) antibody to MuLV in mice of virtually all inbred mouse strains. These findings indicate that immune responsiveness of the mouse to MuLV might be the rule, rather than the exception.

The RIP assay used for the detection of mouse antibody against MuLV was modeled after the technique of Ihle *et al.* (*4*). In brief, MuLV from the

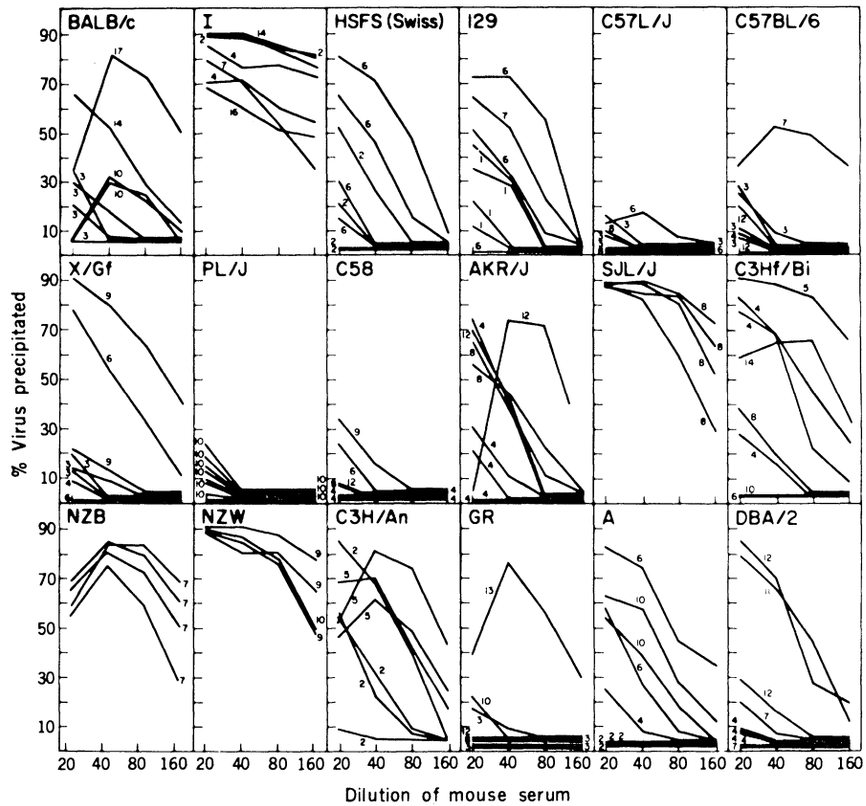

Fig. 1. Radioimmune precipitation (RIP) assay with serum from mice of various inbred strains. Each panel represents the results of mice from a single inbred strain (indicated in the upper left corner of the panel). Each line within a panel represents the titration curve of serum from a single mouse. The age of the mouse (in months) is indicated by the number located immediately next to the titration curve. Prozones of precipitation (for example, as uniformly observed in NZB mice) were considered the result of generally elevated IgG levels in certain individual mice; in support of this hypothesis was the observation that dilution of the antigobulin (goat antiserum to mouse 7*S* gamma globulins) produced analogous prozone effects with all mouse serums.

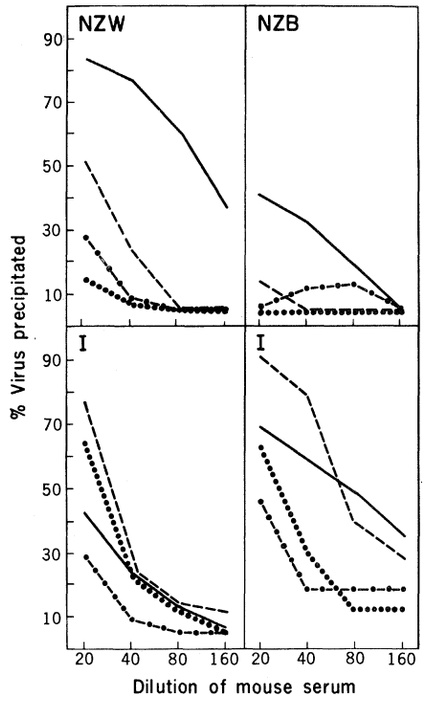

Fig. 2. Identification of immunoglobulins in normal mouse serum to MuLV. Each panel represents RIP assays with serum from a single mouse (strain indicated in the upper left corner of the panel). Each line within the panel represents a titration curve of serum with a different antiglobulin. Antibodies to mouse immunoglobulins were examined by immunoelectrophoresis and immunodiffusion to assure monospecificity; (———) IgG_1; (- - -) IgG_2; (· · · ·) IgA; and (- · - · ·) IgM.

continuously producing AKR cell line 130 (provided by M. G. Hanna, Oak Ridge National Laboratory) was labeled with ^3H in vitro by the addition of [^3H]uridine (20 μc/ml) and ^3H-labeled mixed amino acids (10 μc/ml) to the tissue culture medium. Virus was harvested 12 and 24 hours after labeling and purified by sucrose density-gradient centrifugation. The virus band (in sucrose) was then divided into small samples and stored frozen at −70°C. The specific activity of virus in these studies was 2.2×10^7 disintegrations per minute (dpm) per milligram of viral protein. In the RIP test, 50 μl (6000 dpm; 0.37 μg) of virus preparation was initially incubated with 200 μl of diluted mouse serum for 1 hour at 37°C. Mouse immunoglobins were then precipitated by the addition of 200 μl of undiluted goat antibody to mouse 7*S* gamma globulins (Hyland Laboratories) for 1 hour at 37°C, and then for 2 hours at 4°C. Precipitates were collected by centrifugation at 1000*g* for 10 minutes; residual radioactivity was measured in 300 μl of supernatant (*5*).

The results of RIP assays from the serums of normal mice are presented in Fig. 1. Each line represents a titration curve of a serum sample from a single mouse. It is apparent that antibody to MuLV was detected in mice of almost all strains examined. Mice of some strains (I, SJL/J, NZB, and NZW) produced high titers of antibody (precipitating 90 to 94 percent of the total radioactivity), while mice of other strains showed either intermediate titers (DBA/2, C3H/An, HSFS, 129, GR, and AKR) or relatively low titers (C58, C57L/J, and PL/J) of antibody to virus. Antibody titers were related to (but not dependent on) the age of the mouse. In general, older mice showed higher titers of antibody; however, there were many exceptions. In some cases antibody was found in mice younger than 4 weeks of age. This was considered the result of residual maternal antibody that was still present in the serums of these mice.

The finding of antibodies against MuLV in mice of different genetic backgrounds suggested that the viral antigens in question were group specific. This possibility was further considered since it was likely that the ^3H-labeled MuLV used in our RIP tests was actually a mixture of several antigenically distinct leukemia viruses (*6*).

The specificity of the precipitin reactions observed in the RIP assay were confirmed by examination of the antigen and antibody reagents. (i) The viral nature of the antigen was confirmed by polyacrylamide gel electrophoresis of ^{14}C-labeled antigen (^{14}C-labeled mixed amino acids) isolated from the AKR line 130 by density gradient centrifugation in a manner similar to that described above; more than 90 percent of radioactivity in the antigen migrated with proteins characteristic of MuLV. (ii) The precipitation of virus by mouse serum was not inhibited by prior absorption of the mouse serum with fetal calf serum, an indication that the mouse antibody detected in the RIP assay was not antibody to heterophile bovine antigen or a natural antibody with fetal specificity. (iii) In immunoelectrophoresis against whole mouse serum the antiglobulin (goat antiserum to mouse 7*S* gamma globulins) precipitated only 7*S* gamma globulins.

The nature of the immunoglobulins in mouse serum to MuLV was examined further by the use of monospecific antibodies to mouse immunoglobulins (antiglobulin). Goat antiserums against mouse IgG_1, IgG_2, IgM, and IgA were purchased from Meloy Laboratories; a monospecific reagent against the Fc portion of mouse IgG_1 was provided by U. Hammerling (Sloan-Kettering Institute). All serums were first examined by immunoelectrophoresis to confirm their expected range of specificities. When these serums were used as antiglobulins in the RIP assay, it was found that mouse antibodies to MuLV were distributed among all four classes of immunoglobulins (Fig. 2). In most cases, however, the highest titer antibodies were of the IgG_1 and IgG_2 classes.

In summary, we have found that mice of almost all inbred strains naturally produce antibodies against MuLV. These antibodies are predominantly of the IgG class and indicate at least a secondary immune response to MuLV. Since infectious virus can be isolated from mice of certain strains where there also exists circulating antibody (such as AKR), it is probable that MuLV is carried in these mice as infectious virus-antibody complexes, analogous to that observed in chronic lymphochorionic meningitis disease (*7*). A further question raised by our findings is whether the immunization observed was the result of MuLV that was horizontally transmitted from mice of high leukemic strains to other mice of low leukemic strains, or whether antibody in low leukemic mice was stimulated by infrequent spontaneous activation of endogenous MuLV. In either case, the detection of antibody to virus both in mice of nonproducer (low leukemic) strains as well as producer (high leukemic) strains is of particular significance in respect to human leukemia, where it is of interest to determine evidence of virus infection in the absence of overt virion production.

Note added in proof: Since this report was prepared, we have learned that Aaronson and Stephenson (*8*) have found the widespread occurrence of antibody in the mouse that neutralized the xenotropic variant of MuLV.

ROBERT C. NOWINSKI
SUSAN L. KAEHLER
McArdle Laboratory for Cancer Research and *Immunobiology Research Center, University of Wisconsin, Madison 53706*

References and Notes

1. L. J. Old, E. A. Boyse, G. Geering, H. F. Oettgen, *Cancer Res.* **28**, 1288 (1968); R. J. Huebner, P. S. Sarma, G. J. Kellof, R. V. Gilden, H. Meier, D. D. Myers, R. L. Peters, *Ann. N.Y. Acad. Sci.* **181**, 246 (1971).
2. T. Aoki, E. A. Boyse, L. J. Old, E. deHarven, U. Hammerling, H. A. Wood, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 569 (1970); R. C. Mellors, T. Aoki, R. J. Huebner, *J. Exp. Med.* **129**, 1045 (1969); R. C. Mellors, T. Shirai, T. Aoki, R. J. Huebner, K. Krawczynski, *ibid.* **133**, 11e (1971).
3. T. Aoki, E. A. Boyse, L. J. Old, *Cancer Res.* **26**, 1415 (1966); M. B. A. Oldstone, T. Aoki, F. J. Dixon, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 134 (1972); M. G. Hanna, Jr., R. W. Tennant, J. M. Yuhas, N. K. Clapp, B. L. Batzig, M. J. Snodgrass, *Cancer Res.* **32**, 2226 (1972); V. W. Hollis, Jr., T. Aoki, O. Barrera, M. B. A. Oldstone, F. J. Dixon, *J. Virol.* **13**, 448 (1974).
4. J. N. Ihle, M. Yurconic, Jr., M. G. Hanna, Jr., *J. Exp. Med.* **138**, 194 (1973).
5. Controls for each test included (i) ^{3}H-labeled MuLV incubated with goat antiserums to mouse 7*S* gamma globulin alone, and (ii) ^{3}H-labeled MuLV incubated with buffer alone. Incubation of MuLV with only the antiserum to immunoglobulin resulted in precipitation of approximately 10 percent of ^{3}H-labeled virus; this was probably the result of heterophile antibody to mouse antigen since complete RIP tests, which included mouse serums in the intermediate step, did not show the background precipitation.
6. T. Aoki, R. B. Herberman, P. A. Johnson, M. Liu, M. M. Sturm, *J. Virol.* **10**, 1208 (1972).
7. M. B. A. Oldstone and F. J. Dixon, *J. Exp. Med.* **129**, 483 (1969).
8. S. A. Aaronson and J. R. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1957 (1974).

9. This work was supported in part by PHS grant CA-07175 and NIH grant 72-2022.

12 March 1974; revised 22 May 1974 ■

Language in Man, Monkeys, and Machines

Rumbaugh *et al.* (*1*) claim to have demonstrated language use—reading and sentence completion—in a chimpanzee named Lana. Since numerous investigators are now studying language use in infrahuman organisms, we should keep under continuing review the criteria for evaluating claims that an infrahuman organism is using language. We propose the following: (i) A strong criterion and a weak criterion of language use in nonhumans can be articulated, the choice of criterion depending on the inferences the investigator wishes to make. (ii) By the strong criterion, only *Homo sapiens* presently uses language; by the weak criterion, man, computers, and some chimpanzees use language. The distinction is based on process and product comparisons, respectively. (iii) Lana has not been shown to use language by any criterion strong enough to exclude rats, worms, and any other conditionable animal.

Since man is the only species whose language utilization is unquestionable, man provides the reference point for judging the equivalence of animal performance with language use. The weak criterion asserting weak equivalence requires only that some of the behavioral products of man and nonhumans are apparently similar. For example, if a convincing case can be made that a chimpanzee behaves in a way that requires labeling, syntax, and semantics, the animal can be said to use some language, by the weak criterion, regardless of how the behavior was induced. Strong equivalence, in contrast, requires that the linguistic performance of nonhumans be accomplished by mechanisms similar to those of men. This criterion entails a far heavier burden of evidence; that is, it must be shown that the organism learns its language by mechanisms similar to those of men, makes similar errors, shows a similar developmental pattern, effects its language use by similar neurological structures, and demonstrates any pattern that can be shown to be true of all human languages (that is, linguistic universals). The appropriate criterion must be chosen by reference to the intent of the scientist. If he is interested only in the symbolic capacity of a particular species such as the chimpanzee, the weak criterion suffices and the term "language" functions as a useful metaphor. However, if the scientist wishes to relate the animal's performance to that of humans, the strong criterion must be met.

The weak criterion of equivalence is the only one that has heretofore been met in the comparative study of language, because highly structured, carefully controlled training procedures must be introduced to overcome the chimpanzee's lack of vocalization and spontaneous linguistic behavior, shortcomings sometimes characterized as trivial. The most successful effort has been that of Premack (*2*), who has trained his chimpanzee Sarah by means of operant techniques. Such training procedures themselves preclude the strong criterion; they are totally unlike the circumstances under which the human child learns language. They require that production and comprehension of symbols and symbol strings be carefully shaped. The animal is reinforced with 100 percent consistency; it is presented with only well-formed strings; and only the well-formed strings for a particular phase of training receive reinforcement. In contrast, human children are inconsistently reinforced; they are presented with ill-formed strings; and their ill-formed productions are often rewarded, especially if they are factually correct (*3*). The training procedure also precludes the opportunity for an animal to make errors similar to those of the human child acquiring language, as well as the opportunity to show the developmental sequence that is universal among human children. However, the weak criterion can be met with nonhumans, and Sarah appears to have met it. Premack gives sophisticated evidence of labeling, syntax, and semantics in Sarah's behavioral repertoire. While this is an impressive accomplishment, it does not warrant generalizations to human language use. The measures necessary to overcome Sarah's linguistic shortcomings are too heroic for useful comparisons to be made. A logical equivalent would be verbally instructing a human to swing through trees with the aid of cables, harness, and nets in an effort to study the ontogeny or phylogeny of tree-swinging in simians.

Rumbaugh *et al.* have failed even to meet the weak criterion; they give no convincing evidence of any language use in Lana. There is no evidence that Lana labels. Her performance of different response sequences for different rewards might be called labeling if the rewards obtained were shown to be appropriate to her known drive states (which they were not). But if this is labeling, then rats that discriminate between the response sequences necessary for food and water in a T-maze can be said to be labeling the sides of the maze as "the food side" and "the water side." Similar labeling could be attributed to any lower animal whose responses correlate with its drive states. Second, there is no evidence that Lana uses syntax. A knowledge of syntax implies the capacity for linguistic productivity; the obvious way to test for its presence in Lana would be to teach her a new lexigram—such as *raisin*—and see if she generates the novel string *Please/machine/give/piece/of/raisin* without shaping. Premack's chimp Sarah has apparently performed successfully in such a test; however, the present authors do not report even attempting it. Correct insertion of the new item in the appropriate string could also be used to