tween seedlings labeled in the light and in the dark, but the differences are not large enough to justify a definite conclusion.

There are two possible explanations for our results. The chloroplast DNA may simply be replicating faster than the nuclear DNA. This would be consistent with the hypothesis that the chloroplasts themselves are replicating, but does not prove it. On the other hand, chloroplast DNA may be turning over. From autoradiographic studies on tobacco root tips, Mourad has suggested (*18*) that there is turnover of cytoplasmic DNA, but she did not localize this DNA in a specific organelle. There have also been reports of the isolation of a rapidly labeling DNA fraction from a number of plant tissues (*19*), but unfortunately no precautions against, or assays for, bacterial contamination were reported in either paper.

Somewhat similar results have been reported by Shipp *et al.* (*10*). Using a different tobacco system, they detached small secondary leaves from the plant and labeled them with P^{32} in nutrient solution. They found almost all of the P^{32} label in a peak which formed bands in CsCl at the same density as unlabeled chloroplast DNA. They found approximately the same numbers of bacteria in their preparations as we did in ours, but they did not culture the bacteria to see if the buoyant density of the resulting DNA was the same as that of the chloroplast DNA and the majority of the P^{32} label. Considering the difficulties involved in eliminating the bacterial problem even when the seeds are sterilized with bromine water, it seems highly unlikely that leaves detached from the plant and labeled for periods up to 2 weeks would be any less likely to contain highly labeled bacterial DNA. However, the proportion of label in the chloroplast peak is decreased after 2 weeks of labeling, which would not be the case if bacterial DNA were being labeled. The ratios of the specific activities of chloroplast and nuclear DNA reported by Shipp *et al.* are much higher than those we found. In one experiment where the leaves were labeled for 6 days, approximately 80 percent of the label is in the chloroplast DNA, but it cannot be detected optically. If chloroplast DNA accounts for 2 percent of the total DNA by weight, by our calculations the ratio of specific activities for their experiment is 196. In an experiment where immature leaves were labeled for 12 hours, the ratio was 930. These differences may be a result of the differences between our two systems. The conclusion drawn from the experiments with both systems is essentially the same, namely, that chloroplast DNA in immature tissues is replicating faster than the nuclear DNA.

BEVERLEY R. GREEN
MILTON P. GORDON
Department of Biochemistry, University of Washington, Seattle

References and Notes

1. E. H. L. Chun, M. H. Vaughan, A. Rich, *J. Mol. Biol.* **7**, 130 (1963); R. Sager and M. R. Ishida, *Proc. Nat. Acad. Sci. U.S.* **50**, 725 (1963); D. S. Ray and P. C. Hanawalt, *J. Mol. Biol.* **9**, 812 (1964); G. Brawerman and J. M. Eisenstadt, *Biochim. Biophys. Acta* **91**, 477 (1964); M. Edelman, C. A. Cowan, H. T. Epstein, J. A. Schiff, *Proc. Nat. Acad. Sci. U.S.* **52**, 1214 (1964); N. Kislev, H. Swift, L. Bogorad, *J. Cell Biol.* **25**, 327 (1965); D. J. L. Luck and E. Reich, *Proc. Nat. Acad. Sci. U.S.* **52**, 931 (1964); M. Rabinowitz, J. Sinclair, L. De Salle, R. Haselkorn, H. H. Swift, *ibid.* **53**, 1126 (1965).
2. C. K. Schildkraut, J. Marmur, P. Doty, *J. Mol. Biol.* **4**, 430 (1962).
3. A. Gibor and S. Granick, *Science* **145**, 890 (1964).
4. D. S. Ray and P. C. Hanawalt, *J. Mol. Biol.* **11**, 760 (1965); M. Edelman, C. A. Cowan, H. T. Epstein, *ibid.*, p. 769.
5. J. T. O. Kirk, *Biochem. Biophys. Res. Commun.* **14**, 393 (1964); H. G. Schweiger and S. Berger, *Biochim. Biophys. Acta* **87**, 535 (1964).
6. D. J. L. Luck and E. Reich, *Proc. Nat. Acad. Sci. U.S.* **52**, 931 (1964).
7. J. M. Eisenstadt and G. Brawerman, *J. Mol. Biol.* **10**, 393 (1964).
8. S. Granick, in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic Press, New York, 1961), vol. 2, p. 489.
9. B. R. Green and M. P. Gordon, *Fed. Proc.* **24**, 539 (1965).
10. W. S. Shipp, F. J. Kieras, R. Haselkorn, *Proc. Nat. Acad. Sci. U.S.* **54**, 207 (1965).
11. J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).
12. N. Kislev, personal communication.
13. D. S. Ray and P. C. Hanawalt, *J. Mol. Biol.* **9**, 812 (1964).
14. B. R. Green and M. P. Gordon, unpublished observations.
15. B. R. Green, thesis, University of Washington, 1965.
16. We thank Dr. H. Douglas for identifying this bacterium.
17. We thank Dr. P. C. Hanawalt for H^3-DNA from *E. coli*.
18. E. B. Mourad, *J. Cell Biol.* **24**, 267 (1965).
19. K. Šebesta, J. Bauerová, A. Šormová, *Biochem. Biophys. Res. Commun.* **19**, 54 (1965); M. Sampson, A. Katoh, Y. Hotta, H. Stern, *Proc. Nat. Acad. Sci. U.S.* **50**, 459 (1963).
20. Supported by USPHS (grant AI 03352-05), the NSF grant G 23002, and the National Research Council of Canada. We thank Dr. H. Neurath and R. Wade for assistance with the ultracentrifuge.

10 November 1965

Murine Leukemia Viruses: Antigenic Studies by Quantitative Complement Fixation

Abstract. *The murine leukemia viruses of Rauscher and Friend, derived from plasma of infected Balb/c mice, was purified. Their antigenic relationship was studied by quantitative complement-fixation reactions with the virion antigen and homologous antiserums. The complement-fixation curves observed in cross-reactions indicated close antigenic similarity between these two leukemia viruses. Highly purified viral preparations contained detectable amounts of host antigens.*

The interrelations of certain murine leukemia viruses have been studied by a number of investigators. On the basis of viral ultrastructure or pathology of induced leukemias these agents appear to represent a family of related viruses. Some of these agents seem to be distinct in certain immunological respects. Thus, Old *et al.* (*1*) demonstrated by cytotoxic tests that leukemias induced by Friend (*2*), Moloney (*3*), and Rauscher (*4*) (FMR) viruses are related, whereas Gross virus (*5*) leukemias showed no evidence of antigenic similarity. Moloney (*6*) demonstrated cross-neutralization between Friend and Moloney viruses but found no immunological relationship with Gross virus. On the other hand, results of Gross (*7*) seem to indicate neutralization of Moloney and Gross viruses by rabbit antiserum to Gross virus. Furthermore, immunological relations between the FMR and Gross leukemia agents were suggested by the demonstration of a common complement-fixing antigen (*8*).

We chose a highly sensitive, quantitative complement-fixation test (*9*), previously applied to the differentiation within type I polioviruses (*10*) to compare the antigenic character of Rauscher and Friend leukemia viruses purified by density gradient centrifugation. The antigens were derived from the plasma of Balb/c mice 3 to 4 weeks after intraperitoneal infection with Rauscher (*11*) or Friend virus. Mice were inoculated with extracts of spleens from infected Balb/c mice; control animals

received normal spleen extracts. The plasma was subjected to differential centrifugation and concentration (*12*). The pellets were resuspended in 0.153*M* potassium citrate (*p*H 6.75), clarified by low-speed centrifugation (2 minutes at 10,000g), and were then centrifuged for 3 hours at 105,000*g* on a linear sucrose density-gradient barbiturate buffer, (*p*H 7.4, density 1.05 to 1.30). The resulting single band at a density of 1.16, on electron microscopic examination, showed a homogeneous preparation of virus particles of typical morphology (*13*). The virus band was removed by lateral puncture of the centrifuge tube with a syringe and was dialyzed at 4°C against 400 volumes of barbiturate buffer, *p*H 7.4. In some experiments the purified virus was subjected to a second cycle of sucrose density-gradient centrifugation.

Homologous antiserums were prepared by immunization of Balb/c mice with formalin-inactivated (0.1 percent HCHO) 10-percent extracts of virus-infected or normal Balb/c spleens. The mice were inoculated intraperitoneally with 0.5 ml of a mixture consisting of equal parts of inactivated spleen extract and complete Freund's adjuvant. Three weeks later a booster dose of 0.25 ml without adjuvant was administered subcutaneously, and the animals were exsanguinated after 8 to 10 days. The antiserums to virus (*14*) had a homologous neutralization index of 10^3 or greater. Antiserums to normal Balb/c spleen or plasma were prepared by hyperimmunization of guinea pigs. All serums were inactivated at 56°C prior to use.

The quantitative complement fixation test was carried out as follows: Isotonic barbiturate buffer, *p*H 7.4, containing 0.1 percent of bovine serum albumin was used as diluent for all reagents. In test tubes (13 by 100 mm) submerged in an ice bath, 0.2 ml of a predetermined optimum dilution of antiserum and 0.2 ml of guinea pig complement, containing 1.1 to 1.2 50-percent hemolytic units as determined by prior titration, were mixed. To this mixture was added 0.2 ml of varying concentrations of antigen. After an incubation of 18 to 20 hours at 4°C, 0.4 ml of sensitized sheep erythrocytes was added, and the mixture was incubated at 37°C in a water bath for 1 hour. The reaction was stopped by chilling the tubes in an ice bath.

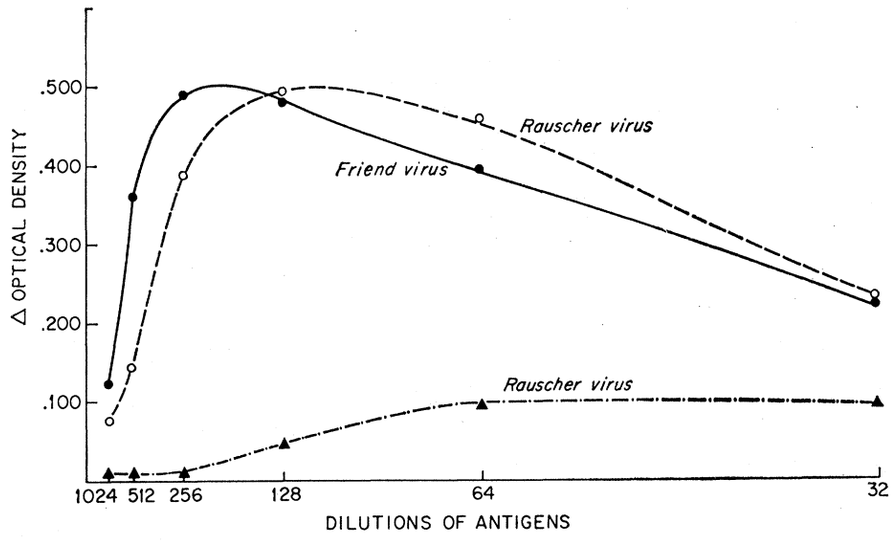

Fig. 1. (Upper curves) Complement fixation with antiserum to Friend virus 1:160, and homologous (Friend) and heterologous (Rauscher) murine leukemia viruses. (Lower curve) Reactions between Rauscher virus and antiserum to normal mouse plasma.

Each tube then received 2.0 ml of diluent, and the nonhemolyzed cells were sedimented by centrifugation. The optical density (OD) of the hemoglobin in the supernatants was measured with a Beckman BD specrophotometer at 413 mμ. The degree of complement fixation was expressed as the difference (ΔOD) between the OD of the antibody control and the antigen-antibody mixtures. Duplicate sets of controls for the hemolytic system, antigens, and antiserums were included in each test.

The purified preparations of each virion readily fixed complement (Fig. 1). The curves for the cross-reaction between antiserum to Friend virus and Friend and Rauscher antigens are similar. The reciprocal reactions with antiserum to Rauscher virus also demonstrated the similarity of these antigens. In all cases the optimum dilution of antiserum was determined by preliminary checkerboard titrations with the homologous antigen. None of the mouse antiserums contained antibodies to normal spleen or plasma, and neither of the viral antigens reacted with serums from mice immunized with normal spleen or plasma. However, the presence of small amounts of host component could be consistently demonstrated (bottom curve) in all preparations of purified antigens even after two cycles of density-gradient centrifugation. This finding is not unexpected. Dalton *et al.* (*13*) demonstrated budding of murine leukemia viruses from the plasma membrane of target cells, a suggestion of the possible incorporation of host material by the virus during maturation. Furthermore, de Thé (*15*) has shown the presence of enzymes associated with the external structures of the Rauscher and Moloney viruses isolated from mouse plasma.

PAUL GERBER
TIMOTHY E. O'CONNOR
SUZETTE M. BIRCH
Division of Biologic Standards and
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland

References and Notes

1. L. J. Old, E. A. Boyse, E. Stockert, *Nature* **201**, 777 (1964).
2. C. Friend, *J. Exp. Med.* **105**, 307 (1957).
3. J. B. Moloney, *Proc. Amer. Assoc. Cancer Res.* **3**, 44 (1959).
4. F. J. Rauscher, *J. Nat. Cancer Inst.* **29**, 575 (1962).
5. L. Gross, *Proc. Soc. Exp. Biol. Med.* **76**, 27 (1951).
6. J. B. Moloney, *Fed. Proc.* **21**, 19 (1962).
7. L. Gross, *Proc. Soc. Exp. Biol. Med.* **119**, 420 (1965).
8. J. W. Hartley, W. P. Rowe, W. I. Capps, R. J. Huebner, *Proc. Nat. Acad. Sci. U.S.* **53**, 931 (1965).
9. E. Wasserman and L. J. Levine, *J. Immunol.* **87**, 290 (1961).
10. P. Gerber and S. M. Birch, *ibid.* **95**, 70 (1964).
11. Obtained from the John L. Smith Memorial Laboratory of Chas. Pfizer & Company under contract with National Cancer Institute.
12. J. B. Moloney, *J. Nat. Cancer Inst.* **24**, 933 (1960).
13. A. J. Dalton, L. W. Law, J. B. Moloney, R. A. Manaker, *ibid.* **27**, 747 (1961).
14. Antiserums to Friend virus were furnished by Dr. Mary Fink, NIH.
15. Guy de Thé, paper presented at the Conference on Murine Leukemia, Philadelphia, Pa., October 1965; monograph to be published by the National Cancer Institute.

8 March 1966