of the cholesteryl ring. In the VLDL and both β-VLDL spectra the linewidth ratio of C3 (or C9) to C6 linewidth is 1.2 ± 0.1, while in LDL this ratio is ~ 2.0. Thus the motions of the cholesterol ring system in the cholesteryl esters of β-VLDL and VLDL were nearly isotropic, while in LDL these motions were significantly more anisotropic.

Early studies of the chemical composition of β-VLDL suggested that β-VLDL differs from LDL only in its higher triglyceride content: the ratios of cholesterol to protein and phospholipid to protein in β-VLDL are similar to those found for LDL when the relative proportions of lipids in β-VLDL are calculated, excluding triglyceride (*13*). In addition the apoprotein was found to be essentially all apoLDL (apoB) by a variety of criteria (*13, 14*). In subsequent studies on the apoproteins of β-VLDL, increased amounts of apoB, decreased amounts of apoC proteins, and an increased amount of an "arginine-rich apoprotein," relative to VLDL, were found (*15*). Moreover, the "arginine-rich apoprotein" (apoE) has been shown to consist of three components, one of which is missing in β-VLDL but not in VLDL (*16*).

Our ^{13}C NMR results suggest that the structural organization of the observed lipid components (particularly the cholesteryl esters) in β-VLDL resembles that found in normal VLDL, and differs substantially from that found in LDL.

The close resemblance of the ^{13}C NMR spectra of normal VLDL and the β-VLDL-1 indicate that the features of the high-resolution lipid spectra obtained for these lipoproteins, namely, the linewidths and relative intensities, are determined principally by the lipid composition and not by the nature of the apoprotein components. The ^{13}C NMR spectrum of the β-VLDL sample having a higher C-VLDL/triglyceride ratio (β-VLDL-2) shows an increased intensity in cholesteryl ester resonances but otherwise yields a spectrum similar to that for normal VLDL, while the ^{13}C NMR spectrum of normal LDL differs significantly from those for the normal VLDL and β-VLDL samples. Thus, the nature of the ^{13}C NMR spectra of β-VLDL lipids is not appreciably affected by the increased content of apoB in β-VLDL relative to normal VLDL. Furthermore, the rotational reorientation of the cholesterol ring of the cholesteryl esters observed in the ^{13}C NMR spectra of normal VLDL and β-VLDL is probably not highly anisotropic and is similar to that for cholesteryl ester dissolved in excess triolein. These results are compatible with the results of Sata *et al.* (*17*) which suggest that cholesteryl esters in both normal VLDL and β-VLDL occupy a triolein liquid core.

J. A. HAMILTON, N. J. OPPENHEIMER
R. ADDLEMAN, A. O. CLOUSE
E. H. CORDES

Department of Chemistry, Indiana University, Bloomington 47401

P. M. STEINER
C. J. GLUECK

Lipid Research Center, University of Cincinnati Medical Center, Cincinnati, Ohio 45267

References and Notes

1. J. A. Hamilton, C. Talkowski, E. Williams, E. M. Avila, A. Allerhand, E. H. Cordes, G. Camejo, *Science* **180**, 193 (1973).
2. J. A. Hamilton, C. Talkowski, R. F. Childers, E. Williams, A. Allerhand, E. H. Cordes, *J. Biol. Chem.* **249**, 4872 (1974).
3. F. A. L. Anet, *Topics NMR Spectrosc.* **1**, 210 (1975).
4. R. S. Norton, A. O. Clouse, R. Addleman, A. Allerhand, *J. Am. Chem. Soc.*, in press.
5. J. Morganroth, R. I. Levy, D. S. Fredrickson, *Ann. Intern. Med.* **82**, 158 (1975).
6. D. S. Frederickson, J. Morganroth, R. I. Levy, *ibid.*, p. 150.
7. G. Camejo, *Biochemistry* **6**, 3228 (1967).
8. Lipid Research Clinic Program, *Manual of Laboratory Operations* (Government Printing Office, Washington, D.C., 1974), vol. 1.
9. D. S. Fredrickson and R. I. Levy, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, 1972), p. 583.
10. J. A. Hamilton, thesis, Indiana University, Bloomington (1974).
11. H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, J. D. Roberts, *J. Am. Chem. Soc.* **91**, 7445 (1969).
12. J. A. Hamilton, N. Oppenheimer, E. H. Cordes, in preparation.
13. S. Quarfordt, R. I. Levy, D. S. Fredrickson, *J. Clin. Invest.* **50**, 754 (1971).
14. W. V. Brown, R. I. Levy, D. S. Fredrickson, *Circulation* **39** (Suppl. III-4) (1968).
15. R. J. Havel and J. P. Kane, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2015 (1973).
16. G. Utermann, M. Jaeschke, J. Menzel, *FEBS Lett.* **56**, 352 (1975).
17. T. Sata, R. J. Havel, A. L. Jones, *J. Lipid Res.* **13**, 757 (1972).
18. Supported by NIH grant GM 19631.

28 June 1976; revised 29 September 1976

HLA Antigens and Corticosteroid Response

Abstract. *Compared with normal individuals, patients with primary open-angle glaucoma have increased prevalences of HLA-B12 and B7 antigens and are more responsive to glucocorticoids. Lymphocytes from both ocular normotensive and glaucomatous individuals with the HLA-B12 antigen require significantly ($P < .02$) lower concentrations of prednisolone to inhibit phytohemagglutinin-induced transformation.*

Significantly increased prevalences of HLA-B12 (55 percent) and HLA-B7 (48 percent) antigens are found in both black and white patients with primary open-angle glaucoma (POAG) when compared with similar subjects with normal intraocular pressure (20 percent B12 and 18 percent B7) (*1–3*). Of clinical importance, the presence of HLA-B12 or B7 in glaucoma suspects with increased intraocular pressure increases the risk of development of glaucomatous damage to the optic nerve (*3*). Furthermore, a family history of glaucoma is more prevalent among those glaucoma suspects as well as POAG patients with B7 or B12 antigens, as compared to comparable patients who lack these antigens (*4*). From the epidemiologic point of view, populations such as the Australian aborigines with no HLA-B12 or B7 antigens (*5*) are found to have no POAG (*6*).

POAG patients are more responsive to topical ocular application of corticosteroids than are other normotensive individuals (*7*). POAG patients demonstrate significantly greater reduction of plasma (in vivo) cortisol when given 0.25 mg of dexamethasone than do normal subjects (*8*). Similarly, in vitro, phytohemaggluti-

Table 1. HLA-B 12 and B 7 antigens and prednisolone inhibition of lymphocyte transformation. Probabilities for compared values are indicated by superscript letters: [a], $P < .001$; [b], $P < .02$; and [c], $P < .02$

	Primary open-angle glaucoma		Ocular normotensive	
	Patients (No.)	Pred I_{50}*	Patients (No.)	Pred I_{50}*
Total	25†	37 ± 12[a]	30‡	76 ± 20[a]
With B12	13 (52 percent)	32 ± 10[b]	7 (23 percent)	60 ± 7[c]
Without B12	12	42 ± 9[b]	23	81 ± 20[c]
With B7	14 (56 percent)	37 ± 10	6 (20 percent)	71 ± 16
Without B7	11	38 ± 13	24	77 ± 21

*The concentration prednisolone (nanograms per milliliter) for 50 percent inhibition of phytohemagglutinin-induced lymphocyte transformation (mean ± standard deviation). †18 white, 15 female, 64 ± 8 years old. ‡21 white, 18 female, 55 ± 11 years old.

nin-induced transformation of lymphocytes from POAG patients is inhibited by lower concentrations of prednisolone than in nonglaucomatous patients (*9*).

In an attempt to correlate corticosteroid responsiveness with the presence of HLA antigens, 25 POAG and 30 ocular normotensive patients were chosen at random from the population of the Glaucoma Center of Washington University and subjected to histocompatibility antigen typing and in vitro prednisolone inhibition of lymphocyte transformation (*2*, *9*). The POAG patients demonstrate the increased prevalences of B12 and B7 antigens previously described (Table 1) (*2*), and also the greater responsiveness of their lymphocytes to prednisolone (*9*) ($P < .001$). Whether from patients with POAG ($P < .02$) or from patients with ocular normotensive ($P < .02$), lymphocytes of individuals with HLA-B12 antigen respond to significantly lower concentrations of prednisolone than do those without this antigen. No apparent differences in prednisolone response are noted in either group of patients related to the presence of the HLA-B7 antigen. Relevant to these observations may be the recent finding that HLA-B12 differs from other A and B antigens in having valine instead of arginine in position 6 of the NH_2 terminal sequence (*10*).

The role of HLA-B12 antigens in the corticosteroid response of not only glaucomatous but also normal patients may be of importance. An analogous correlation of tissue antigens and glucocorticoid responsiveness is found in mice. The thymus cells of male mice with the $H2^a$ haplotype are reported to be more sensitive to the lytic action of corticosteroids in vivo and in vitro than are those mice with the $H2^b$ haplotype (*11*).

BERNARD BECKER
DONG H. SHIN
PAUL F. PALMBERG
STEPHEN R. WALTMAN
Department of Ophthalmology, Washington University School of Medicine, St. Louis, Missouri 63110

References and Notes

1. S. R. Waltman, P. Palmberg, W. Newton, B. Becker, *Lancet* **1975-I**, 927 (1975).
2. D. H. Shin, B. Becker, S. R. Waltman, P. F. Palmberg, C. E. Bell, *Arch. Ophthalmol.*, in press.
3. D. H. Shin and B. Becker, *Am. J. Ophthalmol.*, in press.
4. D. H. Shin, M. A. Kass, B. Becker, *ibid.*, in press.
5. H. V. Bashir, J. M. MacQueen, D. B. Amos, J. J. Guinan, J. M. Johnston, B. Brotherton, B. Boettcher, G. J. Ashton, in *Histocompatibility Testing 1972*, J. Dausset and J. Colombani, Eds. (Munksgaard, Copenhagen, 1973), p. 311.
6. I. Mann and R. Roundtree, *Am. J. Ophthalmol.* **66**, 1020 (1968).
7. B. Becker, *Invest. Ophthalmol.* **4**, 198 (1965).
8. _____, S. M. Podos, C. Asseff, D. G. Cooper, *Am. J. Ophthalmol.* **75**, 73 (1973).
9. J. F. Bigger, P. F. Palmberg, B. Becker, *Invest. Ophthalmol.* **11**, 832 (1972).
10. J. Bridgen, D. Snary, M. F. Crumpton, C. Barnstable, P. Goodfellow, W. F. Bodmer, *Nature (London)* **261**, 200 (1976); C. Terhorst, P. Parham, D. L. Mann, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 910 (1976).
11. M. Pla, J. Zákány, J. Fachet, *Folia Biol.* **22**, 49 (1976).
12. This work was supported by grants EY 01167 and EY 00336 from the National Eye Institute, Bethesda, Md.

30 July 1976

Bovine Lymphosarcoma: Development of a Radioimmunologic Technique for Detection of the Etiologic Agent

Abstract. *A highly sensitive and specific radioimmunoassay has been developed for the major structural protein of an oncornavirus etiologically associated with bovine lymphosarcoma. This test can be used to identify cattle which have been exposed to the bovine leukemia virus and may thus develop or transmit the disease. Analysis of randomly obtained serums indicates that infection with this virus is widespread among cattle.*

A viral etiology to naturally occurring cancer has been established in several species including the chicken, mouse, cat, and gibbon ape (*1*). In these animals leukemia-causing type-C RNA viruses are transmitted as infectious agents. In recent years, evidence has accumulated that lymphosarcoma and leukemia of domestic cattle also have a viral etiology. Virus particles have been detected in lymphoid cells (*2*) and milk of leukemic cattle (*3*). Moreover, persistent lymphocytosis can be transmitted to newborn calves (*4*) and even sheep (*5*) by intravenous inoculation of cell-free filtrates prepared from tissue culture fluids of virus-producing cells. That the virus is horizontally transmitted under natural conditions has been suggested by the occurrence of a large number of lymphosarcoma cases in certain "high incidence" herds (*6*). Because this disease poses serious economic problems as well as an undefined hazard to humans, several countries have initiated disease eradication campaigns (*7*).

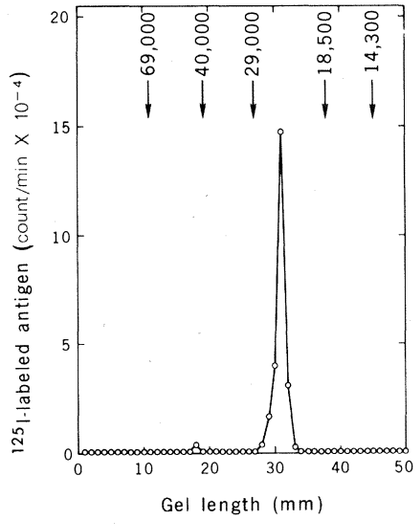

Fig. 1. Electrophoretic analysis of ^{125}I-labeled BLV p24. Samples of ^{125}I-labeled p24 [with a radioactivity of about 200,000 counts per minute (cpm)] were subjected to electrophoresis on 60 mm of 12 percent SDS-polyacrylamide gel at 1.0 ma per gel for 4 hours. After electrophoresis, the samples were either stained with Coomassie blue or sliced into 1-mm fractions and tested for radioactivity in a Searle gamma counter model 1285. Molecular weight standards used for calibration included bovine serum albumin (69,000); aldolase (40,000); carbonic anhydrase (29,000); β-lactoglobulin (18,500); and lysozyme (14,300).

A major problem in studying the epidemiology of bovine leukemia virus has been the lack of sensitive methods for detecting animals that have been exposed to the virus. Recently, immunofluorescence (*8*), immunodiffusion, and complement-fixation (*9*) tests for antibody to bovine leukemia virus (BLV) have been developed. However, clinical criteria are to a large extent still relied upon for the detection of infected animals, despite the obvious problem that such methods may detect only the small fraction of infected animals that develop overt disease (*10*, *11*). Radioimmunologic techniques have markedly increased the sensitivity with which viruses can be detected in a number of systems. In the present report we describe a radioimmunoassay for the detection and quantitation of serologic reactivity against the major structural protein of BLV. The results obtained with this method provide support for the etiologic association of BLV with lymphosarcoma of cattle and demonstrate that this radioimmunoassay can be used to detect animals that have been exposed to the virus.

The BLV was obtained from tissue culture fluids of chronically infected fetal lamb spleen cells (*12*), and was concentrated 1000-fold by centrifugation in a su-