were held at 25°C for an additional 30 minutes. Sphingomyelinase C was added, and the mixtures were placed in a 37°C bath for 30 minutes, and then in an ice bath for 10 minutes. After brief centrifugation, hemolysis was estimated from the quantity of hemoglobin in the supernatants. The amount of IgG that prevented Cor-PLD from inhibiting sphingomyelin-induced hemolysis was 28 μg, whereas 200 times as much IgG did not neutralize the inhibitory activity of Lox-PLD. The two enzymes are therefore immunologically distinguishable. Bacteriological examination of venom from live *L. reclusa* did not reveal the presence of corynebacteria.

Cor-PLD splits the sphingomyelin of the vascular endothelial membrane and this may account for increased vascular permeability concomitant with the development of lesions (*14*). Vascular damage is also considered to play an important role in the genesis of lesions by Lox-PLD because, presumably through splitting of sphingomyelin in platelet membranes, platelet aggregation accompanies thrombus formation. This effect is probably accompanied by release of inflammatory substances (*1, 2*). Further work is needed before a conclusion can be drawn as to the precise degree of similarity that may exist between the pathological changes produced by the two PLD's.

ALAN W. BERNHEIMER
Department of Microbiology, New York University School of Medicine, New York 10016
BENEDICT J. CAMPBELL
LAWRENCE J. FORRESTER
Departments of Biochemistry and Microbiology, University of Missouri, Columbia 65212

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Ethanol Neurotoxicity: Effects on Neurite Formation and Neurotrophic Factor Production in Vitro

Abstract. *The effects of ethanol on chick embryo sensory and spinal cord neurons growing on one of several biological substrates (poly-D-lysine, laminin, or neuron-produced neurite-promoting materials) were examined. Ethanol inhibited process formation by the neurons in a dose-dependent manner and inhibited the production of neurotrophic factors. Neuronal attachment to the substrates, survival of attached neurons, and receptor interactions of sensory neurons with nerve growth factor were not influenced by ethanol. It appears that ethanol alters certain metabolic characteristics of developing neurons.*

Prenatal exposure to ethanol is associated with characteristic effects in offspring (*1*), ranging from craniofacial dysmorphology and central nervous system abnormalities [the fetal alcohol syndrome (*2*)] to subtle neurological and behavioral disturbances. Neuropathological observations suggest that the effects of ethanol on the developing nervous system result from errors in neuronal migration (*3*). Migratory events are a very early component of neurogenesis and involve interactions of neurons with a matrix upon which they move and extend processes. These events depend on the ability of the neuron to interact with the extracellular matrix of growth and with diffusible trophic factors in its milieu. We measured matrix interaction and process formation in vitro in order to examine directly the effects of ethanol on embryonic neurons.

Dissociated neuronal cultures were prepared from 8-day chick embryo dorsal root ganglia (DRG) and spinal cord (*4*). Sensory neurons from DRG were plated in wells with nerve growth factor (NGF; 90 pg/ml) and ethanol (1, 10, 25,

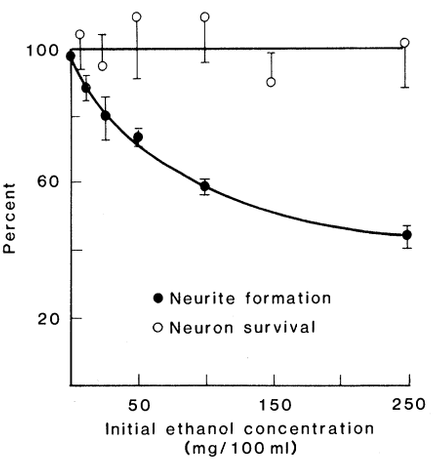

Fig. 1. Effect of ethanol on neuronal process formation and survival on poly-D-lysine. DRG neurons were obtained from 8-day chick embryos (*4*) and incubated in Ca^{2+}- and Mg^{2+}-free buffer with 0.01 percent trypsin and deoxyribonuclease (0.05 mg/ml; Worthington) for 10 minutes at 37°C. The reaction was stopped by removing the incubation solution and adding Ham's F12 medium containing 5 percent fetal calf serum (FCS). After trituration with a 5-ml pipette, the cell suspension was centrifuged (750*g* for 8 minutes at 4°C) through a concentrated FCS gradient, suspended in Ham's F12 with 5 percent FCS, plated on a Falcon culture dish, and incubated for 2 hours at 37°C in 5 percent CO_2. The nonadherent, neuron-enriched population was then seeded on 96-well Falcon microculture plates coated with poly-D-lysine (0.1 mg/ml for 24 hours) at a plating density of 10^4 cells per well in the presence of NGF (90 pg/ml) and ethanol. After 24 hours of incubation at 37°C in 5 percent CO_2, the wells were examined at ×200 and the number of cells with processes greater than 1.5 cell diameters were counted on a representative diameter of the culture well. Results for neurite formation are expressed as percentages (means ± standard deviations; $n = 3$) of the maximum response to NGF. When process formation was scored 10 μl of MTT (5 mg/ml) was added to 100 μl of medium in each well, and the wells were incubated for 4 hours at 37°C. Then 100 μl of 0.04*N* HCl in isopropanolol was added to all wells with thorough mixing. After a few minutes at room temperature the wells were read on a Titertek Multiskan MC Microelisa reader at a wavelength of 570 nm. Absorbance was directly proportional to cell number over the range 1×10^3 to 5×10^4 cells per well. Results for neuron survival are expressed as percentages (means ± standard deviations; $n = 4$) of control absorbance at 570 nm.

Table 1. Neurite formation and survival of DRG and spinal cord neurons in the presence of ethanol.

Substrate	Neuron type	TD_{50} of ethanol for neurite formation (milligrams per 100 ml)	Survival at TD_{50} (percentage of control)
Poly-D-lysine	DRG	171 ± 15	100
Laminin (5 μg/ml)*	DRG	164 ± 26	100
SAM†	DRG	175 ± 20	100
Poly-D-lysine	Cord‡	88 ± 9	100
Laminin (0.1 μg/ml)*	Cord	>250§	100
SAM†	Cord	115 ± 6	100

*Culture wells were coated with poly-D-lysine (0.1 mg/ml), washed, and overlaid with laminin for 24 hours. The wells were washed extensively before seeding of cells. †Chick embryo spinal cord cells were plated at high density (*6*). After 7 days the medium conditioned by the cells was removed, filtered through 0.2-μm Millipore filters, and added to poly-D-lysine–coated plates. After 24 hours the plates were washed and dissociated cells were added in fresh medium with ethanol. ‡Spinal cord cultures were prepared as described in the legend to Fig. 1 for DRG cells with the following exceptions: 0.1 percent trypsin for 30 minutes was used, NGF was not added, cultures were established for 24 hours in Dulbecco's minimum essential medium with 10 percent FCS and thereafter in defined (*6*) medium with 10^{-5} *M* cytosine arabinoside, and wells were examined for process formation after 5 days in culture. §Significantly different from corresponding values for poly-D-lysine and SAM ($P < 0.01$, analysis of variance).

50, 100, or 250 mg per 100 ml). After 24 hours at 37°C in a humidified atmosphere containing 5 percent CO_2, the culture wells were scored for process outgrowth. NGF produces dose-dependent neurite outgrowth from sensory neurons after their attachment to an appropriate substrate (*4*), and this response constitutes a biological assay for the protein. In these experiments the neurite outgrowth response to NGF at 90 pg/ml was designated as 100 percent; outgrowth in the presence of this concentration of NGF and ethanol was expressed as a percentage of the maximum response to NGF. Spinal cord neurons were grown for 5 days before the wells were scored for neurite-bearing cells. Sensory and spinal cord neuron survival was measured at the time the wells were scored for process formation by a colorimetric assay on the cleavage by active mitochondria of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (*5*).

To determine the concentrations of free ethanol and possible metabolites present at different times during the culture period, we added [^{14}C]ethanol (0.1 μCi/ml; specific activity, 25.7 mCi/mmol) to culture wells, removed portions of the medium, and counted the radioactivity in a Searle beta scintillation counter. The counts of ^{14}C were found to decrease logarithmically, with the percentage of the initial amount of ^{14}C remaining at 2, 4, 8, 12, and 16 hours being (means ± standard deviations) 75.0 ± 2.0, 57.6 ± 1.5, 30.4 ± 2.6, 24.0 ± 2.3, and 18.3 ± 0.5, respectively. Thus the neurons were exposed to the initial concentration of ethanol added for a brief time.

To study the effect of ethanol on neuronal process formation on immobilized substrate we used three growth substrates: poly-D-lysine, laminin, and neuron-produced substrate-attached material (SAM), factors released to the culture medium by neuron-enriched cultures that attach to the poly-D-lysine substrate and enhance neuronal performance (*6*). Ethanol caused dose-dependent inhibition of NGF-induced neurite outgrowth from sensory neurons on poly-D-lysine, with no effect on neuron survival at the concentrations used (Fig. 1). Dose-dependent inhibition of neurite outgrowth from sensory neurons also occurred on SAM and on laminin (5 μg/ml). The median toxic dose (TD_{50}) of ethanol for neurite formation did not differ significantly on the three substrates (Table 1). Similar observations were made for spinal cord neurons on poly-D-lysine and SAM, but laminin (0.1 μg/ml) afforded the cells significant protection. On all substrates, survival of sensory and spinal cord neurons was not affected at the TD_{50} of ethanol for neurite formation.

Dilutions of medium conditioned in the presence or absence of ethanol were used to study the effect of ethanol on the production of SAM by neurons. Medium conditioned in the presence of ethanol had less trophic activity than medium conditioned in its absence (Table 2). In the presence of ethanol the specific activity of neuron-produced SAM was 36 U per milligram of cell protein while in control cultures the specific activity was 200 U/mg. Binding of [^{125}I]NGF to its receptor on sensory neurons (*7*) was not affected by ethanol at the TD_{50} (215 pg of [^{125}I]NGF was bound per 10^6 cells with or without ethanol).

Table 2. Trophic activity of conditioned medium. Spinal cord cell–conditioned medium was prepared in the presence or absence of ethanol (initial concentration, 250 mg per 100 ml). Serial dilution of conditioned control medium and conditioned medium containing ethanol was carried out with fresh serum-free medium and the conditioned medium was added to poly-D-lysine–coated plates. After 24 hours the medium was removed and freshly dissociated cells were added. After another 24 hours triplicate wells were scored for process formation.

Addition	Trophic units*	Cell protein† (μg)	Specific activity (U/mg)
None	8	40	200
Ethanol	2	55	36

*The reciprocal of the dilution giving 50 percent of the maximum neurotrophic response. †As measured by the method of Lowry *et al.* (*14*).

At the molecular level, neuronal development in embryogenesis involves neuron surface receptors as recognition sites for diffusible factors and as sites for cell-to-cell and cell-to-substrate adhesion (*8, 9*). Dynamic alterations in these receptors or in the molecular species in the environment with which they interact probably account for the selectivity that must be invoked to explain axonal pathway guidance and exquisite connectivity in early neurogenesis (*10*). These interactions are occurring in a milieu in which many more neurons than will ultimately survive are generated and extend processes toward targets of innervation. Teratogenic influences during early neurogenesis might act (i) by influencing the survival of neurons extending processes toward targets; (ii) by preventing the interaction of neurons with neurite-promoting molecular species in the extracellular compartment; (iii) by inhibiting the attachment of neurons and their processes to immobilized templates; (iv) by altering neuronal metabolism to prevent process extension even though receptor interactions are appropriate; or (v) by disrupting cellular production of neurotrophic materials that neurons might require during critical periods of development before target connectivity is established. Our results suggest that the attachment of neurons to immobilized substrates, their survival, and receptor interactions with neurotrophic ligands in the extracellular environment are not influenced by ethanol. However, process formation and production of neurotrophic factors by the neurons are inhibited by ethanol at concentrations lower than those previously shown to have toxic effects in vivo (*11, 12*) and in vitro (*13*), suggesting that ethanol may alter specific metabolic characteristics of developing neurons.

KIMBERLY E. DOW
Department of Paediatrics (Neonatology), Queen's University, Kingston, Canada K7L 3N6
RICHARD J. RIOPELLE
Department of Medicine (Neurology), Queen's University

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Characterization of Envelope and Core Structural Gene Products of HTLV-III with Sera from AIDS Patients

Abstract. *The envelope (*env*) and structural (*gag*) gene products of human T-cell leukemia (lymphotropic) virus type III were identified by immunoaffinity chromatography, immunoprecipitation, and two-dimensional oligopeptide mapping methods. The* env *gene specifies a glycosylated polypeptide with a molecular weight of 160,000 (gp160) that is processed to gp120 and smaller gene products. The* gag *gene specifies two polypeptides of 70,000 and 55,000 molecular weight (p70 and p55), both of which contain p24, the major structural protein of the mature virion. The techniques in this study can be used to define the extent of variability of the* env *gene product among different virus isolates and may identify the nature and patterns of the humoral immune response that lead to an immunologically protected state.*

Human T-cell leukemia (lymphotropic) virus type III (HTLV-III) is a human retrovirus closely related in a number of its properties to HTLV-I and HTLV-II (*1–8*). In addition, studies have suggested that this cytopathic virus is more closely related to visna virus, a pathogenic retrovirus in the subfamily Lentivirinae, than to any other retrovirus (*9*). HTLV-III is not endogenous to the human genome, and data have revealed that individual viral genomes show degrees of nucleic acid heterogeneity (*7, 8*). Virus isolation studies from patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC) and from individuals in several of the groups at high risk for the disease showed a high probability of virus presence, although HTLV-III appeared to be present in a minority of cells within an organ (*2, 8*). Antibody detection by an enzyme-linked immunosorbent assay or electrotransfer tests in individuals with AIDS or ARC and those in the high-risk groups have unambiguously defined the association of HTLV-III to AIDS (*4, 5, 10*).

Although the presence of antibody to HTLV-III in humans defines previous exposure to the virus, the presence of antibody to viral proteins does not impart immunity because advanced AIDS patients have readily detectable antibodies (*3–6, 11*). HTLV-III can be isolated from most antibody-positive subjects (*2*) and occasionally from normal-appearing, high-risk individuals who do not have detectable antibody (*12*). It therefore seems that a protected individual, that is, one with no residual virus and a strong neutralizing or other protective antibody response, has not been identified. In lieu of a simple quantal neutralization test for HTLV-III, we felt it would be advantageous to identify the HTLV-III viral envelope glycoprotein (gp) and antibodies to the glycoprotein in various virus-exposed groups because, for many viruses, the major envelope glycoprotein contains epitopes that elicit protective antibodies (*13*). The identification of the viral glycoprotein is a precondition for understanding the nature of the protective state and consequently the individual who could successfully cope with or overcome infection with HTLV-III.

A tentative description of one or more high molecular weight proteins precipitated by AIDS sera as the possible glycoprotein of HTLV-III has been made (*10*). We have identified the major viral glycoprotein and the major structural group-specific antigen together with their precursor polypeptides by a combination of immunoaffinity chromatography, radioimmunoprecipitation, and two-dimensional peptide mapping techniques. The identification of the envelope and the structural gene products was accomplished by immunoaffinity chromatography of cell culture fluids containing HTLV-III (Fig. 1). Lane 1 shows the immunoreactive polypeptides isolated from virus-containing cell culture fluids from H9 cells infected with

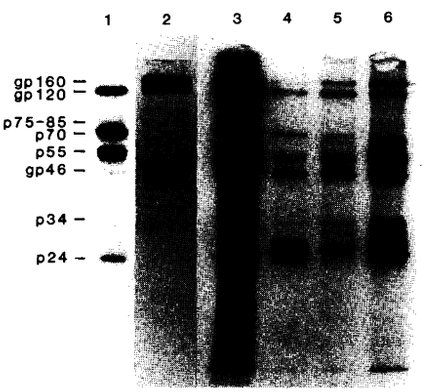

Fig. 1. Detection of HTLV-III–related antigens in extracellular extracts (lane 1) and detergent extracts (lanes 2 and 6) of HTLV-III–infected cells. Clarified cell culture fluids (lane 1) were chromatographed over AIDS IgG–Sepharose, eluted, labeled with ^{125}I by the chloramine-T method, concentrated by immunoprecipitation, and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*18, 21*). HTLV-III–infected H9 cells were labeled with [^{14}C]glucosamine (5 μCi/ml) for 16 hours; lysed with phosphate-buffered saline containing 1 percent Triton X-100, 0.5 percent sodium deoxycholate, and 0.1 percent sodium dodecyl sulfate; and immunoprecipitated with serum 149 after prior treatment with normal human serum (lane 2). HTLV-III–infected H9 cells were extracted with immunoaffinity buffer [1 percent Triton X-100, 1*M* KCl, and 0.01*M* tris (*p*H 8.5)] and chromatographed over AIDS IgG–Sepharose (lane 3), chimpanzee IgG–Sepharose (lane 4), AIDS IgG–Sepharose A3 (lane 5), and A4 (lane 6). The eluted material was labeled with ^{125}I as above and concentrated by immunoprecipitation with the respective homologous serum. The molecular sizes were estimated relative to the migration of the molecular weight standards myosin (200K), β-galactosidase (116K), phosphorylase B (93K), bovine albumin (68K), ovalbumin (46K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K), and lysozyme (14.4K). The molecular weights assigned to gp160 and gp120 are subject to some degree of error because of the nonlinear relation of the migration of molecular weight standards in the upper range of the gel. Also, the polypeptide conventionally designated as p24 is probably somewhat heavier.