ciated with 6-OHDA treatment was not induced transsynaptically.

The most likely explanation for our results is that 6-OHDA directly influenced HSV replication in ganglion cells. Alternative explanations, such as that the drug increased access of virus to the ganglion or interfered with immune defenses that limit intraganglionic viral spread, appear less likely in the light of the observations that drug-induced HSV replication was little influenced by antibody to HSV, and that viral replication in the TG and eye were not significantly altered by drug treatment.

In untreated animals, the outcome of HSV infection of individual ganglion cells is variable. It may be hypothesized that when virus reaches the ganglion cell, it can either actively replicate or become latent (*4, 7*). The factors influencing the outcome are poorly understood. Immunization appears to favor latent infection over active viral replication; whether this reflects a direct modulation of HSV replication by antiviral antibody (*8*) or is exerted by indirect means remains to be determined. In contrast, 6-OHDA exerts an effect opposite to that of immunization, markedly augmenting HSV replication and reducing latency. Furthermore, 6-OHDA overrides the protection conferred by passive immunization, inducing a greater than 1000-fold increase in viral titers while also reducing subsequent latent infection. The concomitant increase in acute HSV replication and decrease in latency following 6-OHDA treatment suggests that cell lysis accompanies augmented productive infection, thereby precluding a role for these infected cells as reservoirs for latent virus.

Further studies are needed to define how injury to terminal axons is communicated to the neuronal parakaryon (*9*), and what cellular metabolic changes underlie the ensuing increased permissiveness for HSV replication. Whether other viral infections may be similarly modified by intrinsic metabolic changes in neural cells or whether, indeed, infection of the central nervous system may be influenced in a manner similar to the SCG also remains to be established. In the case of HSV, it can be speculated that disturbances of peripheral sensory axon terminals might trigger recurrent epithelial eruptions (*5, 10*), while alterations of central nervous system neurons by toxic or physiologic influences, through mechanisms similar to those involved in the 6-OHDA effect on the SCG, might play a decisive role in the pathogenesis of the fulminant, sporadic encephalitis caused by this virus (*11*). If such intrinsic neural mechanisms prove to be important in determining the outcome of human infections, an avenue of therapeutics directed at countering such potentiating influences may warrant future exploration.

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References and Notes

1. R. T. Johnson and C. A. Mims, *N. Engl. J. Med.* **278**, 23 (1968); *ibid.*, p. 84; R. T. Johnson, *Adv. Neurol.* **6**, 27 (1974); V. ter Meulen and M. Katz, Eds., *Slow Viral Infections of the Central Nervous System* (Springer-Verlag, New York, 1977).
2. J. P. Tranzier and H. Thoenen, *Experientia* **24**, 155 (1968); R. M. Kostrzewa and D. M. Jacobowitz, *Pharmacol. Rev.* **26**, 199 (1974); G. Jonsson, T. Malmfors, C. Sachs, Ed., *6-Hydroxydopamine as a Denervation Tool in Catecholamine Research* (American Elsevier, New York, 1975).
3. R. W. Price, B. J. Katz, A. L. Notkins, *Nature (London)* **257**, 686 (1975); R. W. Price and J. Schmitz, *Infect. Immun.* **19**, 523 (1978).
4. R. W. Price and J. Schmitz, *Infect. Immun.* **23**, 373 (1979).
5. J. G. Stevens and M. L. Cook, *Science* **173**, 843 (1971); J. G. Stevens, *Curr. Top. Microbiol. Immunol.* **70**, 31 (1975); M. A. Walz, R. W. Price, A. L. Notkins, *Science* **184**, 1185 (1974).
6. R. W. Price, T. H. Joh, D. J. Reis, in *Catecholamines: Basic and Clinical Features*, J. Barchas and E. Usdin, Eds. (Pergamon, Elmsford, N.Y., in press).
7. A. Puga, J. D. Rosenthal, H. Oppenshaw, A. L. Notkins, *Virology* **89**, 102 (1978).
8. J. G. Stevens and M. L. Cook, *J. Immunol.* **113**, 1685 (1974).
9. A. R. Lieberman, *Int. Rev. Neurobiol.* **14**, 49 (1971).
10. T. J. Hill, W. A. Blyth, D. A. Harbour, *J. Gen. Virol.* **39**, 21 (1978).
11. J. R. Baringer, in *Handbook of Clinical Neurology* vol. 34, *Infections of the Nervous System*, P. J. Vinken and G. W. Bruyn, Eds. (North-Holland, New York, 1978), part 2, p. 145.
12. I thank R. Rubenstein, A. Kahn, and L. Fillippi for technical assistance. This work was supported by grants from the National Institutes of Health (NS 12396) and the National Foundation/March of Dimes (Basil O'Connor Starter Research Grant NF 5-123).

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Normal Levels of 5′-Nucleotidase Activity in Lymphocytes from Patients with X-Linked Agammaglobulinemia

Edwards *et al.* (*1*) reported a relative deficiency of 5′-nucleotidase (E.C. 3.1.3.5) in peripheral blood lymphocytes of patients with congenital agammaglobulinemia. However, the cell populations tested, which were mononuclear cell preparations obtained after Ficoll-Hypaque sedimentation, contained both lymphocytes and monocytes. We have found that patients with agammaglobulinemia have considerably increased numbers of monocytes. Human monocytes lack 5′-nucleotidase, acquiring this enzyme activity only after overnight culture (*2, 3*). It seems likely that a deficiency in 5′-nucleotidase in mononuclear cells from patients with congenital agammaglobulinemia could be accounted for by the relative increase in monocytes in the mononuclear cell preparations from these individuals.

To test this hypothesis, we examined cells from four patients with a primary diagnosis of congenital agammaglobulinemia (serum immunoglobulin G < 50 mg per 100 ml, immunoglobulins M and A undetectable). These patients also had affected maternal male relatives, permitting the diagnosis of X-linked agammaglobulinemia. Laboratory studies have demonstrated that these patients lack B lymphocytes. Peripheral blood was treated with 10 m*M* EDTA to prevent coagulation, and mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation. Ficoll-Hypaque

Table 1. Differential cell counts of peripheral blood. Figures are percentages of 200 cells counted, as determined by differential cell count of Wright's stained peripheral blood smear. Values for normal individuals are derived from differential counts of five healthy individuals.

Patient	Monocytes	Lymphocytes	Granulocytes
1	25	36	27
2	28	44	26
3	22	21	56
4	24	22	47
Normal subjects	1 to 6	27 to 45	47 to 59

Table 2. Specific activity of 5′-nucleotidase in monocytes or lymphocytes from individuals with agammaglobulinemia. The data are expressed as nanomoles per minute per milligram of protein. N.D., not determined.

Subject	Monocytes*	Lymphocytes
Patients with agammaglobulinemia		
1	< 0.190	0.745
2	< 0.060	1.827
3	< 0.313	1.326
4	< 0.057	0.358
Mean†	< 0.155 ± 0.122	1.064 ± 0.646
Normal subjects		
1	N.D.	0.246
2	N.D.	1.178
3	< 0.193	2.238
4	< 0.256	2.356
5	< 0.342	2.049
6	< 0.223	3.336
Mean†	< 0.254 ± 0.064	1.901 ± 1.064

*Specific activities for monocytes are maxium activities, which could have been present in the samples assayed, and reflect the sensitivity of the assay procedure. In most cases, samples of monocyte lysates were not distinguishable from control blanks. † Plus or minus standard deviation.

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separated cells from patient 1 showed a relative decrease in 5′-nucleotidase, compared to normal cells, when whole cells were tested at *p*H 8.5 as described by Edwards *et al.* (*1*). By this method, cells from the patient had 36 percent of the activity determined for cells from three normal individuals. Monocytes were separated by adherence to plastic culture dishes after a 45-minute incubation period in Dulbecco's modified Eagle's medium supplemented with 10 percent autologous serum. Previous studies have shown that nonadherent cells are more than 95 percent lymphocytes and adherent cells are more than 95 percent monocytes (*2, 3*). Cells were lysed in freshly prepared Triton X-100 (0.05 percent). Lysates were stored at −20°C until tested. 5′-Nucleotidase was assayed by the method of Avruch and Wallach, with ^{3}H-labeled adenosine monophosphate being used as substrate (*4*). The activity measured in these cells is sensitive to EDTA (10 m*M*) and is unchanged upon the addition of β-glycerophosphate (5 m*M*) or *p*-nitrophenylphosphate (5 m*M*) (*3*).

Differential cell counts documented an increased percentage of monocytes in the blood of these patients (Table 1). 5′-Nucleotidase activity in the monocytes from these four patients was not detectable (Table 2). Lymphocytes from each of the three patients had a normal amount of 5′-nucleotidase activity.

We conclude that the relative decrease in 5′-nucleotidase activity in mononuclear cell preparations is due to dilution of the 5′-nucleotidase-bearing lymphocytes by an increased proportion of 5′-nucleotidase-negative monocytes. The increase in peripheral blood monocytes in these patients is presumably secondary to their recurrent pyogenic infections.

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References and Notes

1. N. L. Edwards, D. B. Magilavy, J. T. Cassidy, I. H. Fox, *Science* **201**, 628 (1978).
2. W. D. Johnson, B. Mei, Z. A. Cohn, *J. Exp. Med.* **146**, 1613 (1977).
3. P. J. Edelson, unpublished data.
4. _____ and Z. A. Cohn, *J. Exp. Med.* **144**, 1581 (1976).
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The interesting observations presented by Edelson and Schwaber have little bearing on the data presented in our report (*1*).

Our original description of 5′-nucleotidase deficiency in congenital agammaglobulinemia was performed on Ficoll-Hypaque separated mononuclear cells with the enzyme activity measured on intact cells. This method was similar to the assay used to demonstrate 5′-nucleotidase deficiency in acquired hypogammaglobulinemia (*2*). We have measured 5′-nucleotidase on intact peripheral mononuclear cells before and after monocyte depletion by either the carbonyl iron or adherence techniques. The difference between 5′-nucleotidase activity in lymphocytes from normal subjects and agammaglobulinemic patients persists in the monocyte-depleted preparation (Fig. 1), an observation now reported by other investigators (*3*).

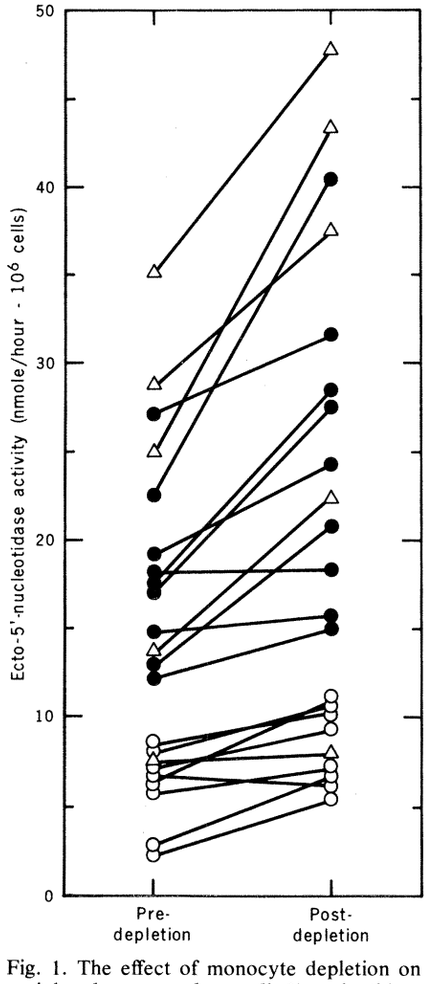

Fig. 1. The effect of monocyte depletion on peripheral mononuclear cell 5′-nucleotidase activity was investigated in normal subjects (●), patients with congenital agammaglobulinemia (○), and patients with common variable immunodeficiency (△). The Ficoll-Hypaque separated cells prior to monocyte depletion included 21.6 ± 6.5 percent monocytes (detected by a nonspecific esterase stain and latex phagocytosis) in normal subjects and 29.0 ± 10.6 percent in patients with congenital agammaglobulinemia. Samples after depletion included only 6.8 ± 2.1 percent monocytes in normal subjects and 9.0 ± 2.8 percent monocytes in patients with congenital agammaglobulinemia.

It is important to point out that intact cells are used in the enzyme analysis for our studies. Our own data showed that intact mononuclear cells from subjects with congenital agammaglobulinemia had a mean 60 percent decrease in ecto-5′-nucleotidase activity compared to the normal value. When lysed cells were used, only a 24 percent decrease was demonstrated [table 2 in (*1*)]. The mean 44 percent decrease in 5′-nucleotidase activity demonstrated by Edelson and Schwaber in lysed monocyte-depleted cells is even greater than the decreased value published in our study. A major difference in their observations from our data in cell lysates is their unusually large variability in the 5′-nucleotidase values. However, the practice of using cell lysates to investigate the activity of an "ecto-enzyme" such as ecto-5′-nucleotidase is questionable. Disruption of plasma membrane integrity exposes the ecto-enzyme to cytoplasmic contents, which may alter enzyme activity unless they are removed by dialysis. Cell lysis also results in release of cytoplasmic enzymes that may utilize the adenosine monophosphate substrate.

Thus Edelson and Schwaber's contention, that our report of a deficiency of 5′-nucleotidase results from a dilutional artifact of monocyte contamination, is not supported by our observations or the observations of others (*3*). Furthermore, the E-rosette forming cells from subjects with congenital agammaglobulinemia, which are not contaminated by monocytes, have the same relative deficiency of 5′-nucleotidase as do their mixed peripheral mononuclear cells or monocyte-depleted mononuclear cells (*1, 3, 4*).

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References

1. N. L. Edwards, D. B. Magilavy, J. T. Cassidy, I. H. Fox, *Science* **201**, 628 (1978).
2. S. M. Johnson, G. L. Asherson, R. E. W. Watts, M. E. North, J. Allsop, A. D. B. Webster, *Lancet* **1977-I**, 168 (1977).
3. G. R. Boss, L. F. Thompson, I. V. Jansen, J. E. Seegmiller, R. D. O'Connor, R. N. Hamburger, T. A. Waldmann, H. L. Spiegelberg, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 496 (1979).
4. N. L. Edwards, E. W. Gelfand, L. Burk, H-M. Dosch, I. H. Fox, *Proc. Natl. Acad. Sci. U.S.A.*, in press.

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