Neurotoxicity of human eosinophils
(Gordon phenomenon/leukocytes/white matter/Parkinson's cells)

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ABSTRACT Eosinophils contain a substance that is neurotoxic when injected intracerebrally or intrathecally into laboratory animals—an effect known as the "Gordon phenomenon." We found neurotoxic activity in eosinophils from three patients with eosinophilic syndromes by injecting cell preparations into rabbits and guinea pigs. These animals developed a syndrome of muscular rigidity and ataxia, progressing to severe paralysis. No neurotoxic activity was found in preparations of polymorphonuclear or mononuclear leukocytes from normal donors. Examination of the brains of affected animals confirmed widespread loss of Purkinje cells, as described by earlier investigators. A new finding was severe spongy change occurring in the white matter of the cerebellum, brainstem, and spinal cord. Electron microscopic examination showed that vacuoles formed within the myelin sheaths of axons by separation of lamellae. Associated axonal degeneration was common and was also seen occasionally in peripheral nerves. Gray matter in the cerebral hemispheres and spinal cord was normal. This eosinophil-derived neurotoxin was partially purified by ultracentrifugation of sonicated eosinophils and fractionation of the supernate by gel filtration. Fractions with neurotoxic activity eluted at a position consistent with a molecular weight of approximately 15,000. The neurotoxic activity of this material withstood lyophilization and dialysis but was destroyed by heating to 90°C. Injection of eosinophil-derived neurotoxin into laboratory animals may provide a useful short-term experimental model for study of mechanisms of damage to myelinated nerve fibers. The clinical significance of the Gordon phenomenon has yet to be established.

Fifty years ago, while seeking an etiologic agent for Hodgkin disease, Gordon (1) found that intracerebral injection of lymph node homogenates from patients with Hodgkin disease into laboratory animals produced a remarkable syndrome of stiffness, ataxia, and paralysis. Other investigators soon confirmed his observations and promoted the use of "the Gordon phenomenon" as a diagnostic test for Hodgkin disease. Summarizing the total experience up to 1938, McNaught (2) reported a positive reaction in 69.3% of 179 cases of Hodgkin disease, whereas 98.1% of 214 lymph node preparations from normal individuals or patients with non-Hodgkin lymphadenopathy were negative. At first Gordon believed that he had successfully transferred a living infectious agent such as a virus from Hodgkin disease tissue to laboratory animals, but the relationship of the neurotoxic reaction to Hodgkin disease was soon shown to be nonspecific (2-5), depending primarily upon the presence of eosinophils (EOS) in the lymph nodes (2, 4).

This remarkable property of eosinophils has received only sporadic attention since the 1940s (6-8). Because patients with hypereosinophilic syndromes may exhibit varied neurologic abnormalities (9), an eosinophil-derived neurotoxin (EDN) may play a role in central nervous system disease in humans. We undertook the present study in an attempt to characterize further the substance in eosinophils that has neurotoxic properties and to observe its effect on central nervous system tissue.

MATERIALS AND METHODS

Preparation of Leukocytes. Human peritoneal eosinophils were obtained from the ascitic fluid of a patient who had eosinophilic gastroenteritis with peritonitis (patient 1) and from the blood of two patients with the hypereosinophilic syndrome (patients 2 and 3). Cell suspensions were partially purified by centrifugation after layering on Ficoll/Hypaque (10). After hypotonic lysis of erythrocytes and three washes in saline, the remaining leukocytes [patient 1, 0% polymorphonuclear leukocytes (PMN), 2% mononuclear leukocytes (MNC), 98% EOS; patient 2, 23% PMN, 17% MNC, 60% EOS; patient 3, 8% PMN, 2% MNC, 90% EOS] were suspended in isotonic saline at a concentration of approximately 3.5 × 10⁶ cells per ml and stored at -75°C. PMN and MNC were isolated from the blood of three normal volunteers (10) and stored in similar fashion. The final differential counts were 92-99% PMN with 0-5% MNC and 97-99% MNC with 1-3% PMN, respectively.

Preparation of EDN. EOS suspensions were thawed and sonicated until virtually all cells were disrupted. After centrifugation at 100,000 × g for 1 hr at 4°C, the supernatant fraction was filtered through a 0.22-µm Millipore filter and stored at -75°C. Portions (3 ml) of this material were fractionated by gel filtration on a 56 × 1.5 cm Sephadex G-50 (Pharmacia) column equilibrated with 0.015 M phosphate-buffered saline at pH 7.4. Fractions with EDN activity in rabbits were pooled. Portions of this pooled material were dialyzed against 2000 vol of isotonic saline overnight with two changes or heated to 56, 80, 90, or 100°C for 30 min. Ultrafiltrated supernatant fractions were prepared from sonicated PMN and MNC as described above for EOS. Protein content was determined by the method of Lowry et al. (11).

Injection of Leukocyte Extracts into Animals. Young New Zealand White rabbits weighing 1.2-2.0 kg were sedated with fentanyl and droperidol (Innovar, McNeil Laboratories, Irvine, CA). For intracerebral injections, a 20-gauge needle was passed directly through the skull into the right occipital lobe and 0.5 ml of the test material was injected. The early mortality of this procedure was approximately 10%; rabbits that died within 12 hr of injection were excluded. For intrathecal injection, rabbits were sedated and a small needle was passed into the cisterna magna. When correct position had been established by withdrawal of a small quantity of cerebrospinal fluid (CSF), 0.1-0.5 ml of the test material was injected. The early mortality of intrathecal injection was less than 5%; animals that died within 12 hr were excluded. Adult Hartley guinea pigs were sedated

Abbreviations: EOS, eosinophilic leukocyte(s); EDN, EOS-derived neurotoxin; CSF, cerebrospinal fluid; MNC, mononuclear leukocyte(s); PMN, polymorphonuclear leukocyte(s).

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with Innovar, a burr-hole was made in the skull, and 0.2 ml of the test material was injected into the right occipital lobe. The skin was closed with a silk suture.

Histologic and Electron Microscopic Examination. The brain and cervical spinal cord of rabbits were fixed by intracardiac perfusion with the modified paraformaldehyde/glutaraldehyde fixative described by Karnovsky (12); immediately after perfusion, the brain and spinal cord were removed and kept overnight in the same fixative. Portions of the cerebrum, brainstem, cerebellum, and spinal cord were prepared for light and electron microscopy by standard techniques (13–15).

RESULTS

Neurologic Manifestations. The development of neurologic abnormalities in rabbits injected with EDN followed a predictable course. The first sign was a characteristic stiffness, most pronounced in the forelimbs. Rabbits that were picked up and gently dropped to the floor kept the forepart of their body raised high on stiff front legs (Fig. 1b). At this stage, mild ataxia was often present. Next, incoordination developed and ataxia became more severe, so that the animals had difficulty in remaining upright. Stiffness persisted. The third and final phase of the illness was characterized by progression to severe weakness and muscle wasting (Fig. 1c). Because the forelimbs retained more strength than the hind limbs, most animals were able to keep head and shoulders upright even when incoordination and weakness prevented them from standing (Fig. 1c).

Some animals demonstrated nystagmus and jerky, repetitive head movements. There was no evidence of abnormalities of higher levels of neurologic function; most animals remained alert and would eat and drink normally when food and water were placed within reach. Urinary or fecal incontinence was not observed. A few severely paralyzed animals had to be sacrificed because they were unable to lift their heads to reach food and water; a small number died.

The latent period between injection of EDN and onset of neurologic abnormalities ranged from 2 to 20 days. When high concentrations of EDN were injected, the latent period was short and neurologic abnormalities were severe. If left undisturbed, most animals with mild or moderately severe manifestations recovered, usually with some permanent residual tremor and ataxia.

All seven guinea pigs that were injected intracerebrally with 0.2 ml of undiluted EDN from patient 1 or 3 developed gross ataxia, progressing to severe paralysis within 3 or 4 days. These animals lay on their sides and showed repetitive spasmatic extension of stiffened legs. Three of the seven guinea pigs died within 5 days; the remaining animals were sacrificed because they could not reach water. Three control animals injected with saline showed no neurologic abnormalities.

Pathologic Changes. The only macroscopic abnormality found in the meninges, brains, and spinal cords of affected rabbits was a small focus of necrosis at the site of injection in those animals that were injected intracerebrally.

Disappearance of Purkinje cells from the cerebellum was a constant microscopic finding in affected animals (Fig. 2). When concentrated EDN was injected, almost all the Purkinje cells disappeared within 3 days, often leaving empty spaces where cell bodies had been. Some of these spaces contained a shrunken cell or pyknotic eosinophilic material. When lower concentrations of EDN were injected, Purkinje cell disappearance was gradual and patchy. In one representative experiment, no abnormalities were noted in rabbits killed during the first 8 days, but by day 12 a significant number of Purkinje cells had disappeared, in a patchy distribution. Many of the remaining Purkinje cells were shrunken and pyknotic. This was associated with a moderate proliferation of the adjacent Bergmann astrocytes. There was no predilection for the more superficial portions of cerebellum but, when EDN was injected into the right occipital cortex, disappearance of Purkinje cells was most pronounced on the right side of the cerebellum, adjacent to the injection site. The degree of pathologic change generally correlated with the severity of neurologic abnormalities present before the animals were sacrificed.

We also found striking histologic abnormalities in sections of white matter from the cerebellum (Fig. 2b), pons, and spinal cord (Fig. 3). Diffuse spongy change was present, apparently resulting from ballooning and disruption of myelin sheaths. Frequently, the axons contained within affected myelin sheaths had disappeared. White matter vacuolation was seen initially in the folia of the cerebellar hemispheres, usually several days after loss of the Purkinje cells. These changes were not more severe near the fourth ventricle or the subarachnoid space—that is, in areas nearest to the CSF. In the brainstem and the spinal cord, on the other hand, the spongy change was more severe in the subpial region. There was no predilection for individual fiber tracts. The white matter of the cerebellar hemispheres was usually normal. In some severely affected animals, vacuolation

![Image](https://example.com/image1.png)

**Fig. 1.** (a) Normal rabbit. (b) Rabbit showing early signs of the reaction, with characteristic stiff-legged stance. (c) Rabbit unable to stand due to severe incoordination, weakness, and wasting, most severe in the hind limbs. Note that the animal appears to be normally alert.
was observed in the optic tracts, and occasionally pyknotic neurons were observed in the hippocampus and cerebral cortex.

Electron microscopic examination confirmed that the spongiform change in white matter was due to large numbers of vacuoles of varying shapes and sizes (Fig. 4). Many were empty; others contained strands of flocculent material. Most of these vacuoles were bound by one or more layers of myelin, at least along a portion of their circumference. Vacuoles often showed septae, suggesting that the larger vacuoles may have resulted from fusion of several smaller spaces, each arising within a single myelin sheath. Frequently, the associated axon could not be identified (Fig. 4b). Some vacuoles appeared to be bound by a single-layered membrane (Fig. 4c).

Other electron microscopic findings in abnormal areas included normal-appearing axons separated from their myelin sheaths by multiple vacuoles, occasional naked axons completely devoid of myelin, and degenerating axons containing dense bodies and lipid droplets. Remnants of myelin sheaths could often be recognized around these processes. Abundant macrophages filled with lipid droplets and myelin fragments occupied areas of spongiform change. There was no other evidence of inflammation. The gray matter of the cord, including the anterior horn cells, was normal, as were the meninges and blood vessels. Peripheral nerves usually appeared normal, but occasional widely scattered foci of axonal degeneration with associated digestion chambers were seen in sections of peripheral nerves from the hind limbs. Sections of skeletal muscle were normal.

Cell counts and glucose and protein concentrations were determined in the CSF obtained by cisternal puncture from seven normal and nine severely affected rabbits. The values were normal even in rabbits with advanced paralysis, and cultures of CSF for bacteria all were sterile. Mean serum creatine kinase, aldolase, lactic dehydrogenase, and alkaline phosphatase levels were normal in five severely affected animals. Therefore, there was no biochemical evidence of striated muscle cell disease, even though the hind-limb muscles of affected animals were severely wasted.

Because hypoxia can cause loss of Purkinje cells, we measured the blood oxygen saturation in five normal and five severely affected rabbits by applying a neonatal probe connected to an oximeter to the ears of the animals. Mean oxygen saturation was not significantly different in the two groups.

Partial Purification of EDN. All rabbits injected intracerebrally or intracisternally with EOS suspensions from the three patients developed the typical neurotoxic reaction, whereas controls injected with water or saline all remained normal (Table 1). Sonic rupture of the cells did not alter activity of the preparation. Injection of 0.5 ml of a 1:10 dilution of a sonicated cell suspension produced the neurotoxic reaction in all of five rabbits, but a 1:100 dilution was inactive. Thus, the number of EOS required to produce the Gordon phenomenon in each rabbit lay between $2 \times 10^6$ and $2 \times 10^8$ cells.

Ultracentrifugation of the sonicated EOS suspension at 100,000 $\times g$ for 1 hr yielded a clear supernatant fraction with
undiminished neurotoxic activity (Table 1). This preparation contained 460 μg of protein per ml; injection of a dilution that contained 23 μg of protein produced the Gordon phenomenon in all of 13 rabbits; injection of 4.6 μg produced the syndrome in 3 of 7 animals. Lower concentrations had no effect.

The supernatant fraction obtained by ultracentrifugation of sonicated EOS from patient 1 was further purified by gel filtration of Sephadex G-50, and fractions were tested by intrathecal injection of 0.1–0.5 ml into rabbits. Activity was limited to fractions 11 and 12 (Fig. 5), which were pooled. This pooled material contained 71 μg of protein per ml; intracerebral injection of 0.5 ml of a 1:10 dilution (3.6 μg of protein) produced the neurotoxic reaction in four of seven rabbits (Table 1).

**Distribution of EDN among EOS, PMN, and MNC.** The supernatant fractions obtained by ultracentrifugation of sonicated PMN and MNC from three normal donors were injected intrathecally into 24 rabbits. None of these animals developed neurologic abnormalities, but 11 of 13 injected with similarly prepared EOS fractions developed the typical manifestations of the Gordon phenomenon (Table 1).

**Properties of EDN.** Neurotoxicity of the partially purified material was unaffected by heating to 56°C for 30 min (all of 7 rabbits tested developed signs), partly inactivated by heating to 80°C (4 affected of 7), and destroyed by heating to 90°C or by boiling (0 affected of 13). EDN remained active after lyophilization and reconstitution, and it was not altered by seven cycles of freezing and thawing. Dialysis did not inactivate the neurotoxin, but rabbits injected with dialyzed EDN showed a longer latent period to onset of symptoms and less severe neurologic abnormalities.

**DISCUSSION**

These experiments confirm that intracerebral or intrathecal injection of human EOS causes severe damage to the central nervous system of rabbits and guinea pigs. The loss of Purkinje cells, which was described as the hallmark of this reaction by previous investigators (1–3, 5, 6, 8), was striking. We have demonstrated another prominent pathologic feature: spongiform degeneration of cerebellar, brainstem, and spinal cord white matter. Similar changes have been described in a heterogeneous group of human (16–18) and experimental (19–21)

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**Table 1. Incidence of neurotoxic reaction in rabbits injected with various preparations from human leukocytes**

<table>
<thead>
<tr>
<th>Material injected</th>
<th>Source</th>
<th>No. rabbits affected/total</th>
<th>Intra-cerebral</th>
<th>Intra-thecal</th>
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<tr>
<td>EOS</td>
<td>Patient 1</td>
<td>4/4</td>
<td>4/4</td>
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</tr>
<tr>
<td></td>
<td>Patient 2</td>
<td>—</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient 3</td>
<td>—</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>Sonicated EOS suspension</td>
<td>Patient 1</td>
<td>5/5</td>
<td>—</td>
<td>—</td>
</tr>
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<td>1:10</td>
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<td>1:100</td>
<td></td>
<td>0/5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ultracentrifuged EOS supernate</td>
<td>Patient 1</td>
<td>6/6</td>
<td>11/13</td>
<td></td>
</tr>
<tr>
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<td>13/13</td>
<td>—</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>3/7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1:100</td>
<td></td>
<td>0/4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Active fraction from Sephadex G-50</td>
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<td>12/12</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>4/7</td>
<td>—</td>
<td>—</td>
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<tr>
<td>1:50</td>
<td></td>
<td>0/4</td>
<td>—</td>
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<tr>
<td>Ultracentrifuged PMN supernate</td>
<td>Normal donor 1</td>
<td>—</td>
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<td></td>
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<tr>
<td></td>
<td>Normal donor 2</td>
<td>—</td>
<td>0/4</td>
<td></td>
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<tr>
<td></td>
<td>Normal donor 3</td>
<td>—</td>
<td>0/4</td>
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<tr>
<td>Ultracentrifuged MNC supernate</td>
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<td></td>
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<tr>
<td></td>
<td>Normal donor 3</td>
<td>—</td>
<td>0/4</td>
<td></td>
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<tr>
<td>Sterile water</td>
<td></td>
<td>0/7</td>
<td>—</td>
<td>0/6</td>
</tr>
<tr>
<td>Sterile saline</td>
<td></td>
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* 0.5 ml.
† 0.1–0.2 ml.

**Fig. 4.** (a) Two adjacent myelinated axons (Ax) showing large intramyelinc vacuoles (V). (x12,500.) (b) Two large vacuoles (V) within intact myelin sheaths, which have lost their axons. Process at left contains an intramyelinc vacuole (V;) as well. (x8650.) (c) Portion of an intramyelinc vacuole (V) showing that it is formed by separation of the outer myelin lamella (arrowhead). (x25,000.)

**Fig. 5.** Gel filtration on Sephadex G-50 of ultracentrifuged supernate from sonicated eosinophils. Absorption at 280 nm and fractions containing neurotoxic activity are shown; molecular weight markers are blue dextran (2,000,000), α-chymotrypsinogen A (25,000), and cytochrome c (12,500).
conditions. In the experimental lesions, vacuolation has been attributed to an unusual form of cerebral edema causing accumulation of fluid within the myelin sheath by separation along the interperiod lines. These changes involved almost all the white matter of the brain in experimental triethyltin edema (19) and hexachlorophene encephalopathy (22). Although cerebral white matter was also affected in ducklings with isoniazid intoxication (20), vacuolation was much more extensive in the cerebellum, brainstem, and spinal cord (21). No axonal degeneration or microglial reaction was found in triethyltin poisoning, but these changes were prominent after isoniazid (21) and hexachlorophene (22) intoxication, as in our animals. Purkinje cells were spared in isoniazid intoxication (20).

The mechanism by which EDN induces white matter vacuolation is unknown. The greater severity of these changes on the side of the intracerebral injection and in the white matter closest to the CSF pathways in the pons and spinal cord suggests a direct toxic effect. Inhibition of Na⁺,K⁺-ATPase activity has been suggested as the cause of intramyelinic fluid accumulation in Canavan disease (16) and in isoniazid and hexachlorophene intoxication (21, 22).

The pathologic changes caused by EDN are reproducible and can be induced in only 2–3 days if a sufficient dose is injected. These features suggest that the lesions caused by EDN may provide a useful laboratory model for determining mechanisms of white matter damage as well as adding to our understanding of the properties of EOS.

The neurotoxic reaction described here appears to be identical in all respects to that described in earlier studies, except for the striking white matter abnormalities. Among previous studies, only Swartz et al. (7) mentioned "focal degeneration" of the cerebrum, cerebellum, and brainstem without further elaboration, and Flor (23) reported "edema" of the subpial white matter in some animals. An artifactual abnormality of white matter in our study is unlikely for the following reasons: (i) vacuolization was confined to certain areas of the brain and spinal cord, sparing the cerebral hemispheres; (ii) the presence of many macrophages in sections of abnormal white matter indicated that changes had occurred during life; (iii) when rabbits injected with EDN on the same day were sacrificed serially, white matter lesions were absent initially and developed progressively, correlating with the progress of the clinical illness; (iv) sections from the central nervous system of two control rabbits injected intrathecally with isotonic saline did not show any abnormalities. Moreover, the absence of neurologic abnormalities in rabbits injected with preparations from normal human PMN and MNC indicates that these changes do not represent a nonspecific reaction to leukocytes.

The neurologic manifestations developed by animals with the Gordon phenomenon are entirely consistent with a major abnormality of white matter such as we report here. Previous descriptions have uniformly recorded ataxia, suggesting a cerebellar lesion. However, these descriptions also emphasized the presence of muscular rigidity, spasticity, paralysis, and muscle wasting, all more severe in the hind limbs (1, 3, 5, 7). These signs are not compatible with an isolated cerebellar lesion, but they are consistent with the presence of abnormalities in the corticospinal tracts as well as the cerebellum.

The ability of neutrophils to damage tissue is established; EOS may similarly mediate tissue damage. For example, the pathologic changes of Loffler's eosinophilic endomyocarditis may be found in various conditions associated with hypereosinophilia (24). The Gordon phenomenon represents EOS-mediated tissue damage in another site—the central nervous system. In humans there is no single neurologic syndrome commonly associated with eosinophilia. Widely varying neurologic abnormalities are common in patients with the hypereosinophilic syndrome (9) or eosinophilic leukemia (25), including weakness, hemiplegia, incoordination, and many others. Terplan et al. (26) observed eosinophilic meningoencephalitis and cerebellar abnormalities in a child who died with trichinosis. Snead and Kalavsky (27) described a child suffering from a neurologic disorder resembling multiple sclerosis whose CSF contained 30% EOS. Patients with eosinophilic meningitis due to Angiostrongylus cantonensis may have cranial nerve palsies, paresthesias, and paralyses, but cerebellar signs have not been reported (28). One of our patients with the hypereosinophilic syndrome (patient 3) had paresthesias, weakness, incoordination, and intention tremor in the upper limbs. Although it is tempting to speculate that some of these signs might represent manifestations of the Gordon phenomenon, its clinical significance for man remains to be established.

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23. Flor, W. J. (1972) Dissertation (Stanford University, Stanford, CA).