sible that NBD-PS and acetyl-PS either displace or otherwise perturb essential endogenous PS.

Three lines of evidence make it unlikely that NBD-PS acts to inhibit mast cell secretion by a more or less nonspecific cytotoxic effect. First, treatment of mast cells with NBD-PS at a concentration (25 μM) sufficient to inhibit secretion by 70 to 80 percent does not significantly decrease intracellular adenosine triphosphate (ATP) levels (*9*). Second, electron microscopy of mast cells treated with this concentration of NBD-PS indicates no evidence of cytotoxicity. Third, the inhibitory action of NBD-PS on secretion induced by A23187 is partially reversible by removing the medium containing NBD-PS and resuspending the cells in fresh balanced salt solution (BSS) (*10*). Significant NBD-PS dissociates from the cells under these conditions. Reversibility argues against displacement of endogenous PS as a mechanism of action of NBD-PS.

A possible trivial alternative explanation of the observed inhibition is complexing of A23187 by *N*-substituted PS aggregates in solution, as proposed for 48/80 and polymyxin B by Read *et al.* (*11*). Arguing against this hypothesis is the failure of PS itself to significantly inhibit secretion by A23187 at concentrations five times those required for half-maximal inhibition by the *N*-substituted PS derivatives.

Although the evidence that is available cannot establish the mode of action of the inhibitory *N*-substituted PS derivatives, it is evident that *N*-substitution of PS converts this phospholipid from a potent activator of a specific class of secretagogues to a potent inhibitor of two different secretory stimuli. *N*-Substituted derivatives of PS may prove useful for the investigation of stimulus-secretion coupling in the mast cell and perhaps in other cells as well (*12*).

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8. The DNS-PS and NBD-PS were originally synthesized for use as fluorescent probes to study the mode of the interaction of PS with mast cells. Since these *N*-substituted derivatives were found to be inactive, a requisite function of the unblocked amino group of PS in its action was indicated. As a further test of this hypothesis, we chose the acetyl substituent to block the amino group of PS with a minimal alteration in the structure of PS. The three *N*-substituted derivatives of PS were synthesized from PS isolated from bovine brain by the method of H. Sanders [*Biochim. Biophys. Acta* **144**, 485 (1967)]. The reaction conditions and the isolation of the products were virtually identical for the three derivatives. The PS (180 μmole) was reacted for 4 hours at 22°C under N_2 with 270 μmole of dimethylaminonaphthalene 5-sulfonyl chloride, ^{3}H-labeled acetic anhydride, or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole in a final volume of 4 ml chloroform-triethylamine (3:1 by volume). The reaction mixture was then made up to 20 ml with chloroform and extracted three times with 50-ml portions of methanol-H_2O (1:1 by volume, *p*H 4.0). The extracted chloroform phase was taken to dryness in vacuo; the residue was dissolved in 10 ml of chloroform and applied to a 50-g column of silicic acid. The column was eluted with five column volumes each of chloroform, chloroform-methanol (95:5 by volume), and finally chloroform-methanol (6:4 by volume). The last fraction contained the desired product, which was quantitatively separated from unreacted PS by chromatography on DEAE-cellulose (Whatman DE-52), using the solvent system chloroform-methanol-H_2O (2:3:1 by volume). The PS eluted from the column with this solvent containing 0.018*M* ammonium acetate. The *N*-substituted derivatives were eluted in 0.05 to 0.06*M* ammonium acetate. In some preparations a contaminant that comigrated on thin-layer plates with the corresponding *N*-substituted lyso-PS derivative remained after DEAE-cellulose chromatography. The purified *N*-substituted lyso-PS derivatives were found to be extremely cytolytic and were removed by preparative thin-layer chromatography on 1-mm silica gel G plates (Analtech) developed in diisobutylketone-CH_3COOH-H_2O (40:30:7 by volume). The final products migrated as single spots on silica gel H thin-layer plates developed in this solvent; R_F = 0.46.
9. Mast cells (10^6 per milliliter) in a final volume of 1 ml were treated with 25 μM NBD-PS for 10 minutes at 22°C, and intracellular ATP was assayed in parallel with untreated control cells [L. Ko and D. Lagunoff, *Exp. Cell Res.* **100**, 313 (1976)]. The ATP content of the cells (0.769 μg per 10^6 cells in untreated controls) was not decreased by treatment with 25 μM NBD-PS (0.858 μg per 10^6 cells) under these conditions.
10. Although 25 to 50 μM NBD-PS presented to the cells with A23187 results in 80 to 95 percent inhibition of secretion, secretion is inhibited by only 30 percent when the cells are washed and resuspended in fresh medium after exposure to NBD-PS but before challenge with the ionophore.
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Neurotoxic Fragrance Produces Ceroid and Myelin Disease

Abstract. *Acetyl ethyl tetramethyl tetralin (AETT), a component of soaps, deodorants, and cosmetics, produces hyperirritability and limb weakness in rats repeatedly exposed to the compound. Brain, spinal cord, and peripheral nerves are discolored blue, show progressive neuronal ceroid degeneration, and develop spectacular myelin bubbling. These neurotoxic properties of AETT provide the basis for industry's decision to withdraw the compound from consumer products. In addition, AETT offers the experimentalist a new probe to explore the etiology and pathogeneses of human ceroid and myelin diseases.*

Acetyl ethyl tetramethyl tetralin (*1*) (Fig. 1) was widely used as a musk fragrance in soaps and cosmetics (*2*) until industry scientists discovered that repeated application of the compound to the skin of experimental animals produced a blue discoloration of internal organs and a vacuolar degeneration of the brain (*3*). By the early part of 1978, the fragrance industry had voluntarily withdrawn AETT from all cosmetic formulations (*4*). On this basis, the U.S. Food and Drug Administration (FDA) denied a request from the Environmental Defense Fund to ban the use of AETT (*5*). Recall of the existing inventory of contaminated products was considered unnecessary, so that some of these may remain available in the United States until existing stocks have been exhausted.

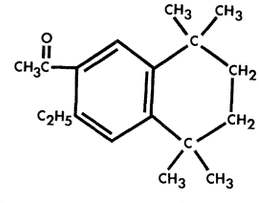

Fig. 1. Structure of acetyl ethyl tetramethyl tetralin.

We have studied the clinical and neuropathological changes in rats repeatedly exposed to AETT (*6*) and have found that this compound causes progressive ceroid-like pigmentation and widespread demyelination throughout the central and peripheral nervous systems. These degenerative changes are associated with hyperirritability, limb weakness, and ataxia.

Behavioral changes, developing as a function of dose and time, were noted within the first 2 to 3 weeks of treatment (*6*) in rats receiving 25 or 50 mg of AETT per kilogram of body weight per day. Animals became overtly aggressive and were easily startled. Rats receiving the

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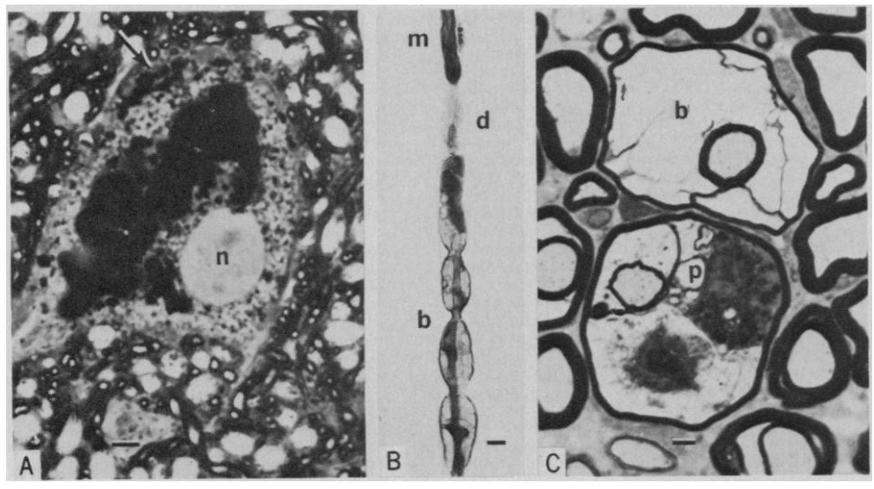

Fig. 2. (A) Neuron located in hypoglossal nucleus showing accumulation of dense lipopigments. Arrows indicate cytosomes that displayed the histochemical, fluorescence, and ultrastructural features of ceroid lipopigment (*8*). The larger mass of dense material is the second type of inclusion (*10*) (*n*, neuronal nucleus). Scale bar, 5 μm. (B) Single teased peripheral nerve fiber displaying intact myelin (*m*) adjacent to an internode with multiple myelin bubbles (*b*) and a short length of demyelinated axon (*d*). Scale bar, 10 μm. (C) Lumbar ventral root showing two cross-sectioned fibers displaying prominent myelin bubbling (*b*). One vacuolated fiber contains an intramyelinic phagocyte (*p*). Scale bar, 5 μm. Bright-field micrographs; (A) and (C) are taken from 1-μm epoxy sections stained with toluidine blue.

higher dose of AETT later developed intermittent abnormal arching of the back and progressive gait difficulties including hopping ambulation, limb weakness, and splaying. Although the severity of neurological dysfunction varied among individual animals, a stereotyped and stable picture of hyperirritability and weakness eventually developed in animals given the high dose. These signs slowly abated when the animals were placed on a normal diet.

Another striking effect of AETT was the progressive blue discoloration of organs (*7*). This became evident within 2 to 3 weeks of treatment and was manifest by darkening of the eyes and of the skin of the ears and feet. The brain, spinal cord, peripheral nerves, and other organs eventually developed a gray-green discoloration, the intensity of which varied with dose and duration of treatment. Segmental sensory ganglia were very densely stained; optic nerves and tracts were only faintly discolored.

Structural damage to the nervous system accompanied the development of behavioral changes and organ discoloration. Both gray and white matter were involved, as well as the peripheral nervous system. The earliest structural change consisted of a progressive and irreversible granular lipopigmentation of neuronal perikarya. Although almost all neurons were affected, lipopigmentation was especially prominent in the third laminar layer of the cortex, the pyramis of the entorhinal cortex—especially of the olfactory tubercles—the hypoglossal nuclei, the anterior horns of the spinal cord, and the dorsal root ganglia. Neuronal granules displayed the histochemical, fluorescence, and ultrastructural features of ceroid lipopigment (*8*) found in some types of human ceroid lipofuscinosis (*9*). Later in the disease process, neurons containing large numbers of ceroid-like granules developed massive intracellular inclusion bodies (Fig. 2A) before undergoing degeneration (*10*). Similar structures have been described in human ceroid lipofuscinosis and motor neuron disease (*11*). Since there are a limited number of experimental methods for producing these neuronal lipopigments, AETT may be useful for studying these neuronal degenerative disorders of man.

Abnormal dense cytosomes containing lamellar profiles were also seen in the paranuclear region of Schwann cells, oligodendrocytes, and astrocytes. In addition, Schwann cells accumulated a large number of π granules of Reich, as though AETT had accelerated the deposition of these age-related inclusion bodies (*12*).

Demyelination in the central and peripheral nervous system occurred after the appearance of cellular lipopigmentation. The demyelination was characterized by splitting of the myelin sheath at the intraperiod line, and edematous vacuolation and bubbling of individual myelin segments (*13*) (Fig. 2, B and C). Myelin bubbling first affected large-diameter nerve fibers, and was most prominent in ventral white matter of the medulla oblongata and spinal cord. A stereotyped and symmetrical pattern of white matter status spongiosus eventually developed. Scattered degenerating fibers were also found, especially in the corticospinal tracts. Shortly after the onset of myelin vacuolation of the central nervous system, an identical pattern of myelin bubbling affected large-diameter peripheral fibers in lumbar ventral roots. Dorsal roots and peripheral nerves were similarly affected later in the course of the disease. After the development of myelin bubbling, phagocytes stripped the edematous myelin sheaths leaving denuded and shrunken axon segments which subsequently became associated with short internodes of thin myelin (*13*). This typical pattern of remyelination occurred while animals were being treated with AETT and continued during the recovery period.

Myelin splitting and edematous bubbling are well known in toxic states, notably after exposure to hexachlorophene (*14*), triethyl tin (*15*), cuprizone (*16*), and isoniazid (*17*). Myelin phagocytosis has also been characterized in autoimmune, viral (*18*), and traumatic (*19*) demyelination, but has been infrequently observed in toxic myelinopathies (*20*). Unlike the pathologic picture in autoimmune demyelination, where there is usually a perivascular cellular infiltrate targeted against an apparently intact myelin sheath, invading phagocytes in AETT intoxication strip and remove overtly damaged myelin. The segmental pattern of myelin loss may be interpreted as indicating a Schwann cell locus of toxic damage (*20*), although remyelination during intoxication is more compatible with a primary toxic effect on the myelin sheath (*21*). These data indicate that AETT may be useful in resolving the etiology and pathogenesis of human (*22*) and animal (*23*) myelinopathies in which white matter disease is heralded by segmental (*13*) bubbling of the myelin sheath.

These results also have important implications in the field of public health. Acetyl ethyl tetramethyl tetralin joins hexachlorophene and zinc pyridinethione as the third experimentally proved neurotoxic agent applied to skin to be identified in the domestic environment. Hexachlorophene was included in many "over-the-counter" antiseptic and deodorant soaps for more than 20 years before the FDA decided to regulate its use. This action followed outbreaks of neurological disease in infants bathed in detergents containing hexachlorophene for the purpose of reducing staphylococcal infections (*14*). Zinc pyridinethione, a

compound that produces a primary axonal degeneration in animals (*24*) has been used as an effective antidandruff agent in shampoos for 10 to 15 years. AETT was in use for 22 years before toxicological testing (*3*) revealed its insidious neurotoxic properties. The fragrance industry subsequently brought its research findings to the attention of the FDA (*3*) and voluntarily withdrew the compound from fragrance formulations. There may be a need, however, to subject other ingredients (*25*) used in the preparation of cosmetic and cleansing products to rigorous scrutiny for neurotoxic properties.

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2. Including after-shave preparations, creams, lotions, antiperspirants, deodorants, and perfumes. Certain detergents also contained some AETT. In addition, AETT is included in a list of artificial flavoring substances which may be added temporarily to foodstuffs without hazard to public health [see Council of Europe, *Natural Flavouring Substances, Partial Agreement in the Social and Public Health Field* (Council of Europe, Strasbourg, 1974), List 2, No. 2220, p. 328].
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6. Young adult male Sprague-Dawley rats (200 to 250 g; $N = 16$) were placed on a diet containing AETT (25 or 50 mg per kilogram of body weight per day) dissolved in 67 percent ethanol for 12 to 20 weeks. Four control rats received a diet containing similar quantities of ethanol; these rats remained normal. All animals were weighed periodically and observed for signs of physical or neurological deterioration. One animal from each dose level was allowed to recover for 7 weeks after 20 weeks of treatment. The remaining rats were killed by intracardiac perfusion with 4 percent paraformaldehyde followed by 5 percent glutaraldehyde, each in a 0.1*M* phosphate buffer (*p*H 7.4). Brain, spinal cord, and peripheral nerves were removed; tissue slices were obtained from the sciatic nerve and its branches, lumbar dorsal root ganglia, corresponding dorsal and ventral roots, lumbar, thoracic, and cervical spinal cord, medulla oblongata, pons, cerebellum, inferior and superior colliculi, mammillary bodies, lateral geniculate nuclei, several levels of the optic nerve and tract, corpus callosum, hippocampus, lateral cerebral cortex, entorhinal cortex, and olfactory tubercles. Muscle from the ankle and calf was also sampled and found to be unremarkable when examined under the light microscope. Tissue sections were postfixed in phosphate-buffered 2 percent Dalton's chrome osmium tetroxide, dehydrated in increasing concentrations of ethanol, immersed in propylene oxide, embedded in epoxy resin, and processed for light and electron microscopy. Sections (1 μm) were stained with 1 percent toluidine blue; thin sections were stained with uranyl acetate followed by lead citrate. Histochemical studies were also performed on brain, spinal cord, peripheral nerves, and visceral organs. These tissues were removed from additional control and experimental animals perfused with 4 percent paraformaldehyde in 0.1*M* phosphate buffer. Paraffin sections were stained with hematoxylin and eosin (H & E), Luxol fast blue, periodic acid-Schiff (PAS), Ziehl-Neelsen, Schmorl's reagent, Prussian blue, and Sudan black B reagents. Nervous tissues displayed granular lipopigments (see text); other organs were unremarkable. Selected unstained paraffin sections of the brain were treated with 15 percent hydrogen peroxide and examined for autofluorescence.
7. Industry studies (*3*) identified an ortho diacetyl derivative and metabolite of AETT which is responsibe for the blue tissue discoloration. Tissues turn blue on contact with this derivative.
8. Granules stained light-brown with H & E, pinkish-red with Schmorl's reagent, and dark blue with toluidine blue. They were PAS-positive, strongly acid-fast, and displayed intense, primary yellow autofluorescence peaking at 460 nm. Ultrastructurally, the granules were angular in shape, delimited by a single membrane, and displayed a dense particulate matrix containing rare lamellar or curvilinear profiles.
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Sites of Transition Between Functional Systemic and Cerebral Arteries of Rabbits Occur at Embryological Junctional Sites

Abstract. *The vascular smooth muscle of cerebral blood vessels is relatively insensitive to sympathomimetic stimulation compared with muscle from systemic vessels. The transition in the vertebral artery occurs just rostral to the emergence of that artery from the foramen of the lateral process of the atlas and in the internal carotid artery just before it enters the carotid canal. These sites in the adult correspond to embryological junctions between segments of the vertebral and internal carotid arteries derived from the primitive dorsal aortas and their branches with vessels originating locally from the bilateral longitudinal neural arteries. Topographic patterns of vascular properties may in some cases be explained by the different sites of origin of their primordial mesodermal cells.*

The characteristics of the alpha-adrenergic receptor on the smooth muscle of cerebral blood vessels and the response it mediates differs in many ways from that of other major blood vessels of the same species (*1, 2*). In the rabbit, for example, the smooth muscle of the cerebral vasculature, in comparison with that of the aorta or pulmonary artery, is relatively insensitive to sympathomimetic amines (*3*); in addition, the relative potency of a series of such drugs differs from one site to the other, suggesting differences in the alpha-adrenergic receptor (*2, 4*). Because of the fundamental interest as well as the potential practical use-

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