hydrate with the same or closely related site structures (*15*). To define a minimum number of structural genes for $V_L$, Cohn and his colleagues considered that only amino acid interchanges outside "hypervariable" regions may be ascribed to different germ line genes, while changes within these regions may arise due to alteration of V genes during somatic cell division (*16*). Each of the three guinea pig antibodies to haptens has a distinctive pattern of residues at those variable positions outside the Hv regions, at N2, N16, and N79. Thus a minimum of three $V_H$ genes seems necessary for the three ligand-binding specificities. However, since each of these $V_H$ genes apparently results in a predominant product having a distinctive sequence in Hv1 and Hv2 as well, such coarse reproducibility in expression by different individuals would seem most simply accomplished by encoding the distinctive Hv1 and Hv2 regions in the same $V_H$ genes. The analysis of Hv3 from these antibodies, which seems to be less restricted in primary structure than Hv1 and Hv2, may uncover variability including useful markers for assessing whether alterations in $V_H$ genes may occur during the proliferation of plasma cell precursors. Meanwhile, the distinctive sequences of Hv1 and Hv2 are themselves proving useful markers for following the fate of clones of cells expressing the same or similar $V_H$ genes in animals caused to become tolerant or unresponsive, deviated with respect to isotype of antibody produced, or idiotype suppressed in order to probe the normal regulation of the immune response.

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6. Abbreviations for affinity labeling reagents signify: MNBDF, *m*-nitrobenzenediazonium tetrafluoroborate; BADL, $N^{\alpha}$-bromoacetyl-$N^{\epsilon}$-dinitrophenyl-lysine; BAAT, *N*-bromoacetyl-mono(*p*-azobenzene arsonic acid)-L-tyrosine; $V_H$ and $V_L$, variable regions of heavy and light chains, respectively; $C_H1$, $C_H2$, and $C_H3$, constant regions of heavy chain; CNBr fragment C-1-n spans N1 to N34 and encompasses the Hv1 region, C-1-$a_1$ spans N35 to N83 and encompasses the Hv2 region, and C-1-$a_2$ spans N84 to N120; anti-DNP, anti-ARS, anti-TMA, antibody to DNP, ARS, and TMA, respectively.
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12. Groups of 10 to 12 guinea pigs (strain 13) were immunized by footpad injection of 0.4 to 0.6 mg of DNP-, ARS- or TMA-hemocyanin (keyhole limpet) in complete Freund's adjuvant. Each preparation of antibodies—five anti-DNP ($K_a \approx 10^8$ liter/mole), three anti-ARS ($K_a \approx 5 \times 10^5$ liter/mole, and two anti-TMA preparations in all—was isolated from the pooled serum taken by cardiac puncture from a group of 10 to 12 animals between days 21 and 42 after the initial immunization injection. The CNBr fragments C-1-n and C-1-$a_1$ were isolated from digests of whole antibody molecules (*7*) and their primary structures were determined both by automatic sequential degradation (*7*, *8*) and by manual Edman degradation of small peptides isolated from enzymic digests of each fragment.
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3 April 1974

## N′-Nitrosonornicotine in Tobacco

Abstract. *N′-Nitrosonornicotine, a potential carcinogen, has been positively identified in unburned tobacco. The amount in commercial U.S. tobacco products is between 1.9 to 88.6 parts per million, one of the highest values of an environmental nitrosamine yet reported. The amount in food and drink rarely exceeds 0.1 part per million. This compound is the first example of a potential organic carcinogen isolated from tobacco.*

A number of *N*-nitroso compounds are carcinogenic or mutagenic (or both) in a wide variety of experimental animals (*1*). This biological activity has been demonstrated repeatedly and, although there is no direct evidence that these compounds are human carcinogens, they should be regarded as potential hazards. Because *N*-nitrosamines are easily formed by the reaction of secondary amines with nitrite and, to a lesser extent, by the reaction of tertiary amines with nitrite (*2*) and because various environmental sources contain both nitrite and the appropriate amine precursors, an intensive search has begun for *N*-nitrosamines in food, drink, and other materials ingested or inhaled by man (*3*). It is now the consensus of opinion that *N*-nitrosamines should be considered as potential health hazards at concentrations of part per billion (ppb, microgram per kilogram) (*4*).

Tobacco smoke is carcinogenic in the experimental animal, but the overall biological activity can be explained only partially by the components isolated to date (*5*). Following the suggestion of Druckrey and Preussmann that nitrosamines may contribute, at least in part, to the observed carcinogenicity (*6*), several investigators have identified varying quantities of volatile nitrosamines in cigarette smoke (*3*, *7*). We have reported the identification of a nonvolatile nitrosamine, *N′*-nitrosonornicotine (NNN), in the unaged smoke of a popular American blended cigarette without a filter tip (85 mm) at a concentration of 137 ng per cigarette (*7*). This compound has also been reported in the smoke of cigarettes made from tobacco rich in nornicotine (*8*). *N′*-Nitrosonornicotine induces in mice multiple pulmonary adenomas with local invasion of the lung and the bronchi (*9*). Since our goal is to identify the source of potential hazardous substances in tobacco smoke and to devise methods to minimize their precursors, we have analyzed unburned tobacco for NNN.

We now report that various types of tobacco products contain NNN at 2 to 90 μg/g (dry weight of the tobacco) [2 to 90 parts per million (ppm)] (Table 1). This is to our knowledge the highest concentration of a positively identified *N*-nitrosamine yet reported in an environmental source. *N*-Nitrosamines in meat, fish, beverages, and related materials rarely exceed 0.1 ppm (*3*).

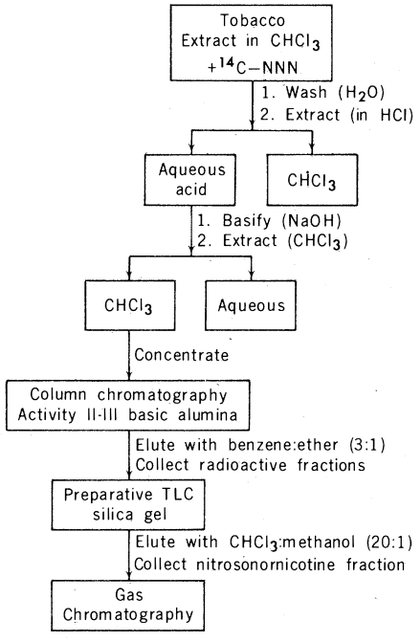


Fig. 1. Analytical procedure for NNN in tobacco.

Tobacco (50 to 60 g) from various commercially available products (1972 to 1973; stored in a cold room) was ground to a powder and extracted in a Soxhlet apparatus with $CHCl_3$ for 16 hours (*3*). *N'*-Nitroso[2'-$^{14}$C]nornicotine (0.56 $\mu$g; 14.1 mc/mmole) (*10*) was added, and the extract was analyzed (Fig. 1). The basic fractions were chromatographed on basic alumina (activity II-III; Woelm), and the radioactive fractions were collected, combined, and chromatographed by preparative thin-layer chromatography on silica gel. The band corresponding in $R_F$ to NNN was eluted and examined by gas chromatography (Fig. 2). The peak corresponding in retention time to NNN was collected and analyzed by mass spectrometry; it was identical with that of synthetic NNN. The other major components of the mixture shown in Fig. 2 have not been positively identified; however, none of these appear to be *N*-nitrosamines.

Since NNN could conceivably have been formed artifactually under our extraction conditions, possibly via aldehyde catalysis (*11*), we devised alternative conditions to rule out this possibility. Tobacco of cigarette B (50 g) was ground and extracted by stirring at room temperature in an aqueous ascorbic acid solution (4.0 m*M*) at *p*H 4.5 (*12*). Control experiments demonstrated that the maximum yield of NNN from $NaNO_2$ and nornicotine, or $NaNO_2$ and nicotine under these conditions, was below 0.4 percent. Since the average value of nitrite in tobacco is 0.004 percent (*13*), the maximum amount of NNN which could have been formed artifactually is 0.3 ppm. The extract was worked up in the usual way, and a value of 5.1 $\mu$g of NNN per gram of tobacco (dry weight) was found, in good agreement with the value obtained by extraction with $CHCl_3$.

The relatively large amounts of NNN in cigarette, cigar, and chewing tobacco could have important biological implications. Epidemiological studies have shown a correlation between tobacco chewing and the development of oral cancer; cigarette, pipe, and cigar smoking are also positively correlated with oral cavity cancer (*14*). Since NNN is extracted from tobacco by stirring with $H_2O$ at room temperature, one can assume that it would also be extracted in the mouth of a tobacco chewer. *N'*-Nitrosonornicotine is the first example of an *N*-nitrosamine in tobacco and of a potential organic carcinogen isolated from unburned tobacco (*3, 5*). Certain tobacco extracts have been shown to have weakly carcinogenic or tumor promoting activity (*5, 15*). However, the structures of components responsible for this activity have never been fully elucidated.

We have reported that both nornicotine and nicotine can serve equally as precursors for NNN in smoke. Nicotine, because of its higher concentration in tobacco, should play a more important role (*7*). However, transfer of NNN from tobacco must now also be considered as a possible contributing mechanism (maximum transfer rate = 6.3 percent for cigarette A). The transfer rate for nornicotine in cigarette A is 4.1 percent; for nicotine it is 12.8 percent (*7*).

Table 1. *N'*-Nitrosonornicotine (NNN) in commercial U.S. tobacco products.

| Product | NNN in dry tobacco* ($\mu$g/g) |
|---|---|
| Cigarette A | 2.2 |
| Cigarette B | 6.6 |
| Cigar A | 3.0 |
| Chewing tobacco A (scrap leaf) | 3.5 |
| Chewing tobacco B (scrap leaf) | 3.9 |
| Chewing tobacco C (fine cut) | 88.6† |
| Chewing tobacco D (plug) | 3.4 |
| Burley tobacco (air cured) | 4.2 |
| Burley tobacco (homogenized leaf cured) | 1.9 (*16*) |

* *N'*-Nitroso[2'-$^{14}$C]nornicotine was used as the internal standard for the quantitative analysis. † Determined by extraction with ascorbic acid solution (see text).

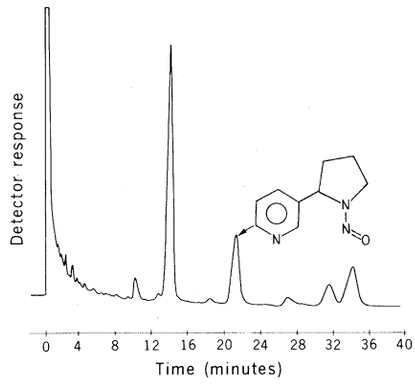


Fig. 2. Gas chromatogram of NNN fraction.

At what stage of tobacco growth or of processing most of the NNN formation occurs is not yet known. We hypothesize that reduction of nitrate to nitrite by bacteriological or enzymatic (or both) action during the curing of tobacco leads to formation of NNN from nicotine or nornicotine and possibly to the formation of other nonvolatile *N*-nitroso compounds from other tobacco alkaloids. The high concentration of NNN in one type of chewing tobacco may be a consequence of the additives used.

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## Monocularly Deprived Cats: Improvement of the Deprived Eye's Vision by Visual Decortication

Abstract. *Monocularly deprived cats were tested for visual perimetry before and after visual cortex lesions. Such a lesion greatly enhances the deprived eye's performance and impairs that of the nondeprived eye so that the pronounced preoperative interocular asymmetry is lost postoperatively. Apparently this destruction of abnormal corticotectal pathways allows the expression of previously suppressed, normal retinotectal pathways.*

A cat raised with the lids of one eye sutured together develops severe abnormalities in its geniculocortical system. In such a cat, stimulation of the deprived eye drives very few cortical neurons (*1, 2*) or lateral geniculate Y cells, although it does drive X cells in apparently normal numbers (*3*). Also, the geniculate cells innervated by the deprived eye are abnormally small (*4, 5*). These deficits are, however, limited to the binocular segment of the geniculocortical system (*5, 6*). In the monocular segment (*6*), the "deprived" geniculate neurons are of normal size (*5*), and the deprived eye drives the normal complement of both Y cells (*3*) and, apparently, cortical cells (*2*).

Recently, I have shown a correlation between these geniculocortical deficits and visual behavior in monocularly deprived cats. On a visual perimetry test, these cats behave with the deprived eye as if they see objects in the monocular segment of visual field, but are completely blind for the binocular segment (*7*). While this behavior could be predicted from the geniculocortical and corticotectal deficits, the retinotectal pathways of monocularly deprived cats seem to develop normally (*8, 9*). This is of considerable interest because normally reared cats without visual cortex can perform on this perimetry test by means of their retinotectal pathways (*10, 11*). Furthermore, such decorticate cats apparently see the entire ipsilateral hemifield with each eye (*11*).

Since the deprived eye has apparently normal retinotectal input (*8, 9*), it might be expected to view the entire 90° of ipsilateral hemifield and not just the peripheral crescent that represents the monocular segment. Wickelgren and Sterling (*8*) provided electrophysiological evidence that in monocularly deprived cats the deprived eye's retinotectal input is somehow suppressed by the abnormal corticotectal pathway, and that this suppression is abolished by decortication.

I now describe a behavioral analog for the Wickelgren and Sterling data. I tested monocularly deprived cats before and after visual cortical lesions and determined that these lesions significantly improve nonlearned visual behavior guided by the deprived eye.

Three cats were studied, and previous reports provide details on the surgical, behavioral, and histological techniques (*7, 11*). At 8 days of age, each cat had one eye closed by lid suture, the left eye for cat LMD7 and the right eye for cats RMD8 and RMD11. At 10 to 12 months of age all had their eyes opened prior to testing. I evaluated their binocular and monocular fields of view by means of a simple perimetry test (*7*). In brief, the cat fixated on one object while a second visual stimulus was introduced into a limited portion of the visual field. Every 15° sector of the horizontal extent of visual field was repeatedly tested and the cat's response to the second stimulus, orientation or lack of orientation to it, determined the extent of functional visual field. As a control, the level of these orientations for each sector was compared to a baseline of "spontaneous" orientations in the absence of a second stimulus—that is, the "blank responses" in (*7*). In addition, the cats were tested for their ability to follow moving targets and for visual placing responses (*7*).

After initial testing, each cat underwent decortication (*11*). Cat LMD7 had most of the occipitotemporal cortex bilaterally aspirated, and this included all of the visual recipient zones of both the lateral geniculate nucleus and also the pulvinar and lateral posterior thalamic complex (*11, 12*). In addition, this cat had a split of the commissure of the superior colliculus to permit visual functioning of the midbrain (*10, 11*). Cats RMD8 and RMD11 had smaller bilateral lesions involving mostly just the lateral geniculate cortical zone (*11, 12*) (that is, this included all of areas 17 and 18, and most of area 19). In these

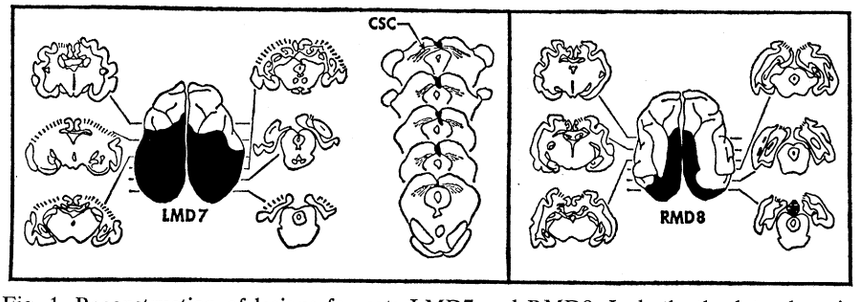


Fig. 1. Reconstruction of lesions for cats LMD7 and RMD8. In both, the lateral geniculate showed retrograde degeneration throughout its extent. The cortical lesion in LMD7 involved most of the occipitotemporal cortex, including all known projection zones of the lateral geniculate and pulvinar and lateral posterior thalamic complex (*12*). Also in this cat, the commissure of the superior colliculus (*CSC*) was completely transected, except for a few surviving fibers at the extreme anterior and posterior borders. For RMD8, dorsally the lesion involved all of the lateral gyrus, and medially it involved all cortex superior to the fundus of the splenial sulcus; thus all known lateral geniculate recipient zones were ablated, but the visual projections of the pulvinar and lateral posterior complex were largely spared (*12*).