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.

PETER S. SPENCER
ARNOLD B. STERMAN
DIKRAN S. HOROUPIAN
MARION M. FOULDS

Program for Environmental Neurotoxicology and Teratology, Departments of Neuroscience and *Pathology (Neuropathology), Saul R. Korey Department of Neurology,* and *Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, New York 10461*

References and Notes

1. 1-[2-(3-Ethyl-5,6,7,8-tetrahydro- 5,5,8,8-tetramethyl)naphthalenyl]ethanone. Formula: $C_{18}H_{26}O$. Molecular weight: 258.4. Chemical Abstracts Service Registry Number: CAS 88-29-9.
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].
3. *Research Institute for Fragrance Materials report to U.S. F.D.A.* (Food and Drug Administration, Washington, D.C., November 1977). This report described clinical and neuropathological effects found in rats exposed percutaneously to AETT, or to products containing AETT. These effects were identical to those described here in rats treated orally with AETT. D. L. J. Opdyke, *Food Cosmetics Toxicol.*, in press; and P. S. Spencer, A. S. Sterman, D. Horoupian, M. Bischoff, G. Foster, *Neurotoxicology* **1**, 221 (1979).
4. The *Wall Street Journal* [(5 May 1978), p. 14] reported that use of AETT by one company continued until 1 March 1978.
5. *Environmental Defense Fund letters to FDA*, 4 and 22 May 1978; *FDA, letter to Environmental Defense Fund*, 21 August 1978.
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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10. These structures stained bright red with H & E. They were histochemically and structurally similar to "Lafora bodies of the protein type" first described by F. Seitelberger [*Fourth International Congress of Neuropathology* (Thieme, Stuttgart, 1961), vol. 1, pp. 3–13]. In AETT-treated rats, the structures were usually intracellular but, occasionally, they were found free in tissues or in close proximity to a degenerating neuron. Intracytoplasmic inclusions appeared as light blue-green lakes in epoxy sections stained with toluidine blue. Ultrastructurally, inclusions were composed of intracellular electron dense masses, with irregular borders and nodular surface protrusions. No limiting membrane was present. Electron lucent spaces containing cytoplasmic organelles punctuated the mass of dense material. These ultrastructural features are reminiscent of "Bunina bodies" described in anterior horn cells in motor neuron disease (*11*).
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19 September 1978

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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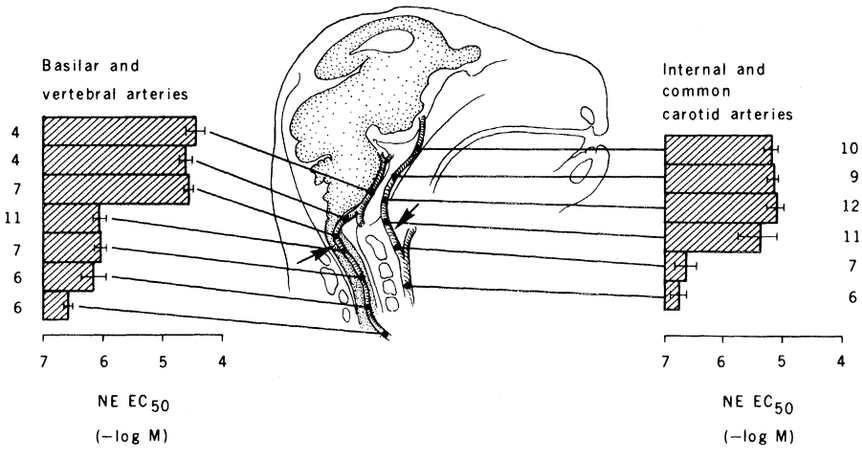

Fig. 1. Diagrammatic representation of rabbit vertebral internal and common carotid arteries superimposed on a sagittal section of the head. Bars show concentration of *l*-norepinephrine (*NE*) causing 50 percent maximum contraction (EC_{50}) of indicated arterial segment. Standard errors of the mean are indicated, and the number of observations in each case are shown at the bases of the bars.

fulness of such differences in regional vascular characteristics, we have defined the precise site of transition between systemic and cerebrovascular blood vessels.

The sensitivity to *l*-norepinephrine (NE) of the vascular smooth muscle in sequential 4-mm ring segments from the entire length of the vertebral and internal carotid arteries of rabbits was determined according to standard pharmacological procedure (*2*). Briefly, vessels were mounted in a tissue bath in Krebs saline solution equilibrated with 95 percent O_2 and 5 percent CO_2 at 38°C and stretched with a load of 0.5 g. After 1 hour they were exposed to desmethylimpramine ($10^{-7}M$), and propranolol ($10^{-6}M$) to block neuronal uptake of NE and β-adrenoceptors, respectively. In the presence of these agents, NE was added cumulatively to provide data for a dose-response curve. The EC_{50}—the concentration of NE that caused a half-maximal contraction—was obtained for each segment. This value is independent of the initial resting tension applied to the preparation. In Fig. 1, the courses of the vertebral and internal carotid arteries are shown against a background of the sagittal section of the adult rabbit brain. The sensitivity of the smooth muscle in both vessels decreases abruptly as they course toward the brain. The NE EC_{50} for that part of the vertebral artery within the foramen of the transverse process of the atlas is the same as that of the more proximal parts of the vertebral artery and also that of the aorta and other large vessels (*5*). The sensitivity of the vertebral artery segment that runs in the groove on the posterior arch of the atlas and pierces the dura is low and indistinguishable from that of the basilar artery. The site of transition along the internal carotid artery occurs approximately where this vessel enters the carotid canal in the temporal bone. The vascular smooth muscle in the proximal part of the internal carotid artery is of similar sensitivity to that in the common carotid and proximal vertebral arteries. The NE EC_{50} of segments originating from that part of the internal carotid lying in the proximal part of the bony canal does not differ from more distal segments. Values obtained from segments of the internal carotid artery just before it enters into the canal show wide variation between animals presumably reflecting the individual differences in the precise site of the transition. Values for the internal carotid artery within the cavernous sinus are lower than those for the vertebral artery within the subarachnoid space and the basilar artery.

These data provide no evidence for a gradual transition of characteristics but rather, within the limits of our technique, an abrupt change. There is no parallel change in pattern or density of sympathetic adrenergic innervation determined by the glyoxylic-acid histofluorescence procedure, nor in the general histological structure of the blood vessel determined by light microscopy. In each instance the change occurs before but not at the site of entry of the vessels into the subarachnoid space (*6*).

The cerebral circulation has a complex embryological origin, the adult vertebral artery arising from two sources. The proximal or cervical portion arises from anastomoses between the seven dorsal cervical segmental artery branches of the two dorsal aortic roots (*7*), and the rostral part from the two bilateral longitudinal neural arteries that appear on both sides of the developing central nervous system. Vessels from both sources fuse during development, just cephalad to the first cervical segment. This site of fusion corresponds to the position of functional transition found in this study. The more rostral parts of the bilateral longitudinal neural arteries fuse to form the basilar artery and take part in the development of the circle of Willis.

The most proximal part of the internal carotid artery is derived from the third dorsal arch and the subsequent segment from the bilaterally represented embryonic dorsal aortas. These vessels in turn connect with the internal carotid system derived from the rhombencephalic equivalent of the bilateral longitudinal neural arteries—a system developing in situ and separate from those vessels derived from the dorsal aortas and its branches (*8*). It is this system that eventually forms the cerebral arteries and their branches and contributes, like the more caudal longitudinal neural artery system, to the circle of Willis. The precise site of the junction is unclear. It is undoubtedly distal to the level of the first arch, which is indicated in the embryo by the vestigal mandibular artery, the artery of the first arch. The petrosal part of the temporal bone develops from cartilage placed rostral to the skeletal structures derived from the first arch. It is upon entry into the petrous temporal bone that the changes in vascular smooth muscle characteristics in the internal carotid artery are found.

Differences in vascular characteristics that may be related to embryological patterns of origin have been described previously (*9*). Differences in the histamine-mediated response of intra- and extracranial vessels have also been documented, although the exact level of transition has not been defined (*10*). These observations taken together with the present results on the cerebral circulation of the rabbit suggest that the pattern of some regional vascular features can be related to the origin of the local mesodermal cells from which they are derived.

JOHN A. BEVAN

Department of Pharmacology, School of Medicine and Brain Research Institute, University of California, Los Angeles 90024

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4 December 1978

Gene Exchange and Natural Selection Cause *Bacillus subtilis* to Evolve in Soil Culture

Abstract. *Strains of* Bacillus subtilis *exchange linked blocks of genes when growing together in soil; such exchange leads to extensive reorganization of the genotypic structure of the population and to the appearance and eventual dominance of a single phenotype. This process illustrates how recombination and selection lead to adaptive changes in populations.*

The controversy over the escape of bacteria carrying cloned genes and the concern over the spread of antibiotic resistance determinants among bacterial pathogens have focused chiefly on elements of human danger, real or putative. Most experiments demonstrating gene exchange between bacterial strains in their native habitats have involved human commensals or pathogens carrying antibiotic resistance determinants. Among these studies are those of Anderson *et al.* on plasmid transfer by conjugation of *Escherichia coli* strains in the human intestine (*1*); those of Novick and Morse on plasmid transfer by transduction of *Staphylococcus aureus* in the mouse kidney (*2*); those of Ottolenghi and MacLeod (*3*) and of Conant and Sawyer on transformation of pneumococcal strains in mixed peritoneal infections in mice (*4*); and those of Conant and Sawyer on transformation of pneumococcal strains in mixed respiratory infections in mice (*4*). Much of the controversy surrounding recombinant DNA is due to lack of knowledge of the natural lives of bacteria.

The anthropocentric emphasis, while important, has tended to obscure the fact that a quite general process of ecological and evolutionary adaptation is involved, its key feature being the generation by recombination of genetic variability in populations acted upon by selection. Hence, the question: If the parasexual processes of bacteria occur between organisms in their natural habitat, can they promote the acquisition of adaptive traits by bacterial populations? Our data show that for the soil bacterium *Bacillus subtilis*, the answer is yes. Genetic exchange is demonstrated by the transfer of linked blocks of chromosomal markers between strains growing together in soil culture; the ability of the exchange to promote adaptation is shown by the formation, spread, and eventual dominance in the cultures of a strain bearing markers from both parents. Here we present a complete analysis of the genetic results and consider them from an explicitly evolutionary point of view; the experimental details and supporting results have been described (*5*).

The strains used are called "168 R" and "SB-/R" and were constructed so that each carries a block of three linked genes and one outside marker unlinked to the block. Strain 168 R carries linked resistances to the antibiotics rifampicin (phenotypically, Rfm^r), erythromycin (Ery^r), and spectinomycin (Spc^r); the outside marker is lincomycin resistance (Lin^r). Strain SB-/R carries 3-aminotyrosine resistance (Amt^r) and histidine (His^-) and tryptophan (Trp^-) auxotrophic requirements as its linkage block and resistance to 4-azaleucine (Azl^r) as its outside marker. The two linkage blocks are opposite one another on the circular *B. subtilis* genetic map (*6*). This arrangement of markers permits the identification of triple transformants—the criterion for gene exchange—from both possible crosses by initially screening simultaneously for the outside marker and one marker in the linkage block of the recipient and one marker in the linkage block of the donor and by subsequent testing of the survivors for cotransfer of the other two markers in the donor linkage block. (We later discovered that Lin^r was linked to the Rfm^r Spc^r Ery^r block in soil transformations; however, the outcome of these experiments is not materially affected.)

Table 1. Occurrence of genetic exchange. All data are mean values of three mixed cultures.

Day	Triple transformants (%)	
	168 R × SB-/R*	SB-/R × 168 R†
1	0.02	0.02
2	0.005	0.02
3	0.003	0.005
4	0.0004	0.0003
6	0.000003	0.001
8	Not found	0.002

*Recipient × donor; triple transformants defined as Spc^r Lin^r Amt^r His^- Trp^-. †Recipient × donor; triple transformants defined as Amt^r Azl^r Spc^r Ery^r Rfm^r.

Spores of strains 168 R and SB-/R were separately treated with excess deoxyribonuclease and then heat-shocked (80°C; 15 minutes); 1×10^8 treated spores of each strain were then added to 50 g of sterile soil in a peat pot enclosed in a storage jar, along with 4 mg each of histidine and tryptophan; incubation was at 37°C. Samples were taken, suspended, diluted, and plated on selective minimal media or nonselective tryptose blood agar. Colonies arising on selective media were tested for cotransfer of linked markers (Table 1); colonies arising on nonselective media were tested for all markers (Table 2). These experiments take advantage of the facts that germinating spores both release transforming DNA (*7*) and become competent for transformation (*8*).

The rates of occurrence of triple transformants in mixed soil culture are shown in Table 1 for both possible crosses. These frequencies are at their greatest 10- to 100-fold lower than those obtained in laboratory transformation in liquid culture with saturating DNA, and they decrease with time. However, the frequencies are much larger than those that could result from three simultaneous mutations, about 10^{-18}. Higher frequencies, up to 17 percent, of triple transformants are observed when large amounts of phenol-extracted DNA (*9*) are added to strains growing alone in soil.

We have assumed that the demonstrated gene exchange in mixed soil culture is mediated by transformation since *B. subtilis* is not known to conjugate and since there are no known generalized transducing phages in these strains. However, we have not been able to block the exchange by the conventional laboratory methods of adding either deoxyribonuclease or competing DNA to the soil cultures.

The mixed cultures reached significantly higher stationary phase population sizes (2×10^8 to 4×10^8 colony-forming units per gram) than single-strain cultures either with or without

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