gene within muscle cells (*24*). Muscle might also be a suitable tissue for the heterologous expression of a transgene that would modify disease states in which muscle is not primarily involved. The intracellular expression of genes encoding antigens may provide alternative approaches to vaccine development. The use of RNA and a tissue that can be repetitively accessed might be useful for a reversible type of gene transfer, administered much like conventional pharmaceutical treatments.

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27. Muscle extracts were prepared by excising the entire quadriceps, mincing the muscle into a 1.5-ml microtube containing 200 μl of a lysis solution [20 m*M* tris-HCl (*p*H 7.4), 2 m*M* $MgCl_2$, and 0.1% Triton X-100], and grinding the muscle with a plastic pestle (Kontes) for 1 min. To ensure complete disruption of the muscle cells, we then placed the muscle tissue under 600 psi of N_2 in a bomb (Parr) at 4°C for 30 min before releasing the pressure. Fibroblasts were processed similarly after they were removed from the plates by treatment with trypsin, taken up into media with serum, washed twice with phosphate-buffered saline, and then the final cell pellet was suspended into 200 μl of lysis solution. Portions (75 μl) of the muscle and fibroblast extracts were assayed for CAT activity by incubating the reaction mixtures for 2 hours (*28*). The autoradiograms were made by exposing the thin-layer chromatography (TLC) plates, after spraying them with NEN Enhance, to Kodak XAR5 film for 54 hours at −70°C. Percent conversions were determined by measuring the radioactivity in the scraped TLC spots. Percent conversions were converted to picograms of CAT with the use of a standard log-log curve established by measuring the percent conversion produced by purified CAT (Sigma) and the conversion factor of 100,000 units of protein per milligram (Sigma).
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Is Soot Composed Predominantly of Carbon Clusters?

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Soot generated from diesel fuel in a combustion tube is characterized by microanalysis, x-ray diffraction, chemical reactivity, and nuclear magnetic resonance to address the recent proposal of the significance of carbon clusters in soot. The data support a traditional model of soot as polynuclear aromatic compounds rather than as clusters of carbon atoms with minimal edge site density. The amounts of noncarbon atoms in the soot (hydrogen, oxygen, nitrogen, and sulfur) are commensurate with the edge density of the crystallites (2 by 2 nanometers) inferred from diffraction. The chemistry of soot, in being reduced by potassium metal and alkylated by alkyl iodides, is that known for aromatic compounds and not that anticipated for materials such as graphite, with a small fraction of carbon atoms on edges.

RECENTLY, IT WAS PROPOSED THAT the C_{60} carbon cluster might shed "a totally new and revealing light on several important aspects of carbon's chemical and physical properties that were quite unsuspected" [(*1*), p. 1139]. One of the outgrowths of this work was the prediction that "C_{60} should be a by-product of combustion and a key to the soot formation process" [(*1*), p. 1145]. Although there was no claim that the closed polyhedron C_{60} was a major component of soot, the idea was advanced that the known spherical morphology of soot could be interpreted as arising from open, spiraling, carbon clusters. Each spherical particle of soot would be a molecule.

There is no doubt that the proposal of carbon clusters is exciting and that the idea has captured the imagination of many. In the laser pyrolysis of carbonaceous substrates, however, two separate groups have failed to find C_{60} to be a dominant species (*2, 3*), and there is some disagreement over the interpretation of the experiment linking

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C_{60} to soot (*4, 5*). Herein, the plausibility of the "soot as cluster" model is examined through an analysis of the structure and chemistry of an actual sample of soot.

Evidence suggests that cluster, or polyhedral, carbon is involved in sooting flames of acetylene and benzene (*4, 5*). These results support a mechanism of formation of polyhedral carbon from small soot particles rather than a concept of small soot particles being polyhedral carbon (*5*). Polyhedral carbon ions are not observed in flames below the threshold of carbon to oxygen for soot formation (*5*). The entities that grow to become soot particles are large polynuclear aromatic compounds with masses greater than 350 atomic mass units (amu) (*5*). The formation of polyhedral carbon species occurs in parallel to soot particle formation without any observable influence on the soot particle growth (*5*).

To determine whether soot particles are open, spiraling, carbon clusters, as suggested by Kroto (*1*) and Zhang *et al.* (*6*), one can utilize many techniques, including microscopy, diffraction, and chemical approaches. The soot I discuss here was made in a turbulent diffusion flame by combination of preheated air (600°C) with preheated number two diesel fuel (350°C) in a combustion tube of inner diameter 9.8 cm (*7*). The properties of this soot are consistent with those found in the soot literature (*8*); soots produced under a wide range of synthetic conditions are known to contain significant quantities of hydrogen and oxygen, and they are known to have broad diffraction peaks associated with weakly ordered aromatic arrays (*8*).

In transmission electron microscopy, the soot appears to be spherical, with diameters of order 20 nm (*9*). X-ray diffraction shows peaks associated with the stacking of aromatic layers at 351 pm [the (002) diffraction peak] and at 174 pm [the (004) diffraction peak], with linewidths that indicate correlation lengths of 2 nm (*7*). The correlation length is related to the reciprocal of the linewidth of the diffraction peak through the Scherrer equation. This suggests that approximately five to six aromatic thicknesses are involved in a coherent aromatic stack. Diffraction also shows peaks associated with order within a benzenoid network at 208 pm [the (100) diffraction peak] and at 120 pm [the (110) diffraction peak]. Linewidth analysis of each peak suggests a correlation length of 2 nm (*7*). A crystallite of this dimension would have 133 carbon atoms, by analogy to the two-dimensional unit cell of a single graphite sheet (*7*), and I shall consider this crystallite to be a soot "molecule." The presence of peaks at these *d* values (*d* is the spacing of atomic planes) has long been taken as evidence for benzenoid networks (*10, 11*), and, in fact, a C_{60} cluster does not show peaks at these *d* values (*7*). Although I will use the 133–carbon atom "molecule" for a calculation below, the reader should note that the soot is composed of aromatic entities of varying sizes, of which the size 2 nm by 2 nm is merely representative. Carbon-13 nuclear magnetic resonance (NMR) measurements support the interpretation that soot is aromatic in showing only a single peak at 129 δ [δ is parts per million downfield of tetramethylsilane (TMS)], a region associated with sp^2-hybridized carbon (*7*). Aromatic carbon bound to other aromatic carbon is found in this region, as is protonated aromatic carbon. Aromatic carbon associated with oxygen is generally downfield of 129 δ, and the spectrum shows evidence for a shoulder in the range 129 to 170 δ associated primarily with aromatic carbon singly bound to oxygen, as might be found in a phenolic or diaryl ether grouping.

Chemical arguments have been advanced to support the "soot as spiraling cluster" model. Initially, it was stated that carbon would continue to bond to carbon, thereby "satisfying its valence requirements without the aid of other atoms" (*6*). In reality, soot has other components in addition to carbon. Microanalysis of the soot sample I examined shows 92.06% C, 6.11% O, 1.11% H, 0.46% S, and 0.30% N. Normalizing to the 133 carbon atoms of our 2 nm by 2 nm "molecule" would give 26.33 atoms that are not carbon, of which hydrogen (19.10) and oxygen (6.63) are most abundant. In addition, the soot has free radicals, as shown by an electron paramagnetic resonance signal at a Landé *g* factor value of $g = 2.0028$ of derivative extremum linewidth 2.4 G and intensity 4×10^{18} spins per gram. As a simple estimate of edge site density, it should be noted that the acene class of molecules (for example, anthracene or tetracene) has one hydrogen atom for every 245 pm, so that the periphery of a 2 nm by 2 nm "molecule" would have 32.65 edge positions (= 8 nm/245 pm). Within the limits of error of this simple model, the number of noncarbon atoms is sufficient to "decorate" the available edge positions of the 2 nm by 2 nm "molecule."

Kroto allowed for the presence of some hydrogen in soot by invoking a "hydrofullerene" structure (*1*). Here, some carbon atoms are removed from the carbon polyhedron, and the edge of the newly created hole is decorated with hydrogen. The most obvious choice for such a hole (and indeed the one illustrated) is to leave the edges in coordination as found in the molecules phenanthrene, picene, and chrysene (that is, the phenacene edge). The interhydrogen distance for such coordination is short; for the molecule perylene it is 181 pm and for picene 188 pm (*12*). The respective second moments of the ^1H NMR are 10.2 G^2 and 8.1 G^2. With a Gaussian model line shape, these correspond to derivative extremum linewidths of, respectively, 6.4 and 5.7 G. The linewidth actually observed for the soot sample is 1.14 G, smaller by a factor of 5 to 5.6 than what we should observe for the phenacene coordination of the hydrofullerene model.

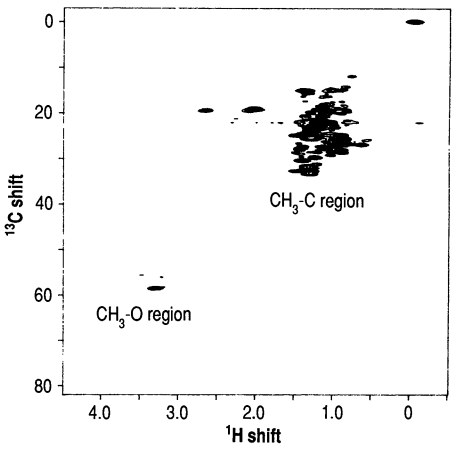

Fig. 1. HETCOR NMR spectrum of the filtrate from the reductive methylation (99.7% ^{13}C-enriched methyl iodide) of soot. The ^{13}C observation frequency is 75 MHz.

The idea has been advanced that carbon clusters can undergo "intercalation" chemistry somewhat analogous to the chemistry of graphite [(*1*), p. 1144]. A single C_{60} cluster is claimed to enclose as many as three potassium entities (*13*). The most potassium-rich intercalation compound, C_8K, is a hexagonal lattice of $a_0 = 0.4961$ nm, $c_0 = 2.176$ nm, with the shortest K–K contact 0.4961 nm and a C–K–C sandwich thickness of 0.544 nm (*14*). The maximum diameter of a C_{60} cluster is about 0.7 nm. Three potassium entities in C_{60} are significantly more crowded than in the intercalation compound C_8K.

However, the general idea that carbon clusters might have chemistry more like that of graphite than like that of small polynuclear aromatic compounds is probably correct. Aromatic molecules, but not graphite, are chemically reactive at molecular edges (*14*); one presumes that clusters, which exist because they minimize edges, would be like graphite. To determine whether the chemistry of soot was more like that of graphite or more like that of aromatic molecules, I performed two sets of experiments: (i) reaction with K^+ naphthalene$^-$ followed by quench with alkyl iodide [which should lead to alkylation only with molecules (*14*)] and

(ii) reaction with K^0 (which should lead to the intercalation of graphite, with concomitant structural change, but mainly edge chemistry with molecules). The chemistry of reductive alkylation, which works for aromatic entities with a large fraction of edge positions, is effective with soot, and the chemistry of intercalation, the chemistry of graphite, is not effective with soot.

The reaction of K^+ naphthalene$^-$ with the carbonaceous substrate coal, followed by alkylation with alkyl iodide, is well studied (*15, 16*). This reaction is predicated on the "molecular" identity of aromatic moieties in the carbonaceous substrate, and one does not observe alkylation products when working with graphite, or graphite-like, materials (*14, 17*). With coal chemistry, one finds that the alkyl group adds to both oxygen and carbon anions, to yield adducts that can be identified in NMR. For instance, methyl groups added to oxygen tend to appear in the 51 to 61 δ region in ^{13}C NMR and methyl groups added to carbon tend to appear in the region 10 to 50 δ (*12, 15, 16*).

Reacting soot with K^+ naphthalene$^-$ can lead to a net uptake of 15.9 mmol of K per gram of soot), which is 1 K^0 consumed for every 4.8 carbon atoms of the soot (*7*). Quenching this reduced soot with methyl iodide (99.7% ^{13}C-enriched) and examining the filtered solid and filtrate (fine frit; pore size, 4 to 5.5 μm) show that methyl groups are bound to the substrate in both phases. Figure 1 represents the heteronuclear correlated (HETCOR) NMR spectrum of the filtrate and shows evidence for both $C_{aliphatic}$-$^{13}CH_3$ and O-$^{13}CH_3$ groups. Figure 2 shows the NMR spectrum of the filtered solid [which constitutes more than 90% by weight of the recovered material (*7*)], taken with cross-polarization and magic angle spinning (denoted CP/MAS), and also shows evidence for $C_{aliphatic}$-$^{13}CH_3$ (region centered at 21 δ) and O-$^{13}CH_3$ groups (region centered at 56 δ). The HETCOR spectrum of the filtrate shows the complexity of the product slate, but C-$^{13}CH_3$ groups constitute the major part of the added methyl in both filtrate and filtered solid. Most importantly, Figs. 1 and 2 show that, like aromatic molecules and unlike graphite, the soot readily undergoes reductive alkylation reactions.

The abundance of added methyl relative to initial aromatic carbon can give some idea of the significance of edge chemistry. If all measured carbon in the range 0 to 80 δ is added methyl groups of 99.7% ^{13}C enrichment and if the CP/MAS NMR experiment "sees" all the carbon atoms, the filtered solid product of the methylated soot contains 95.7% sp^2 carbon, 3.5% CH_3 on C, and 0.8% CH_3 on O (CH_3-C/CH_3-O = 4.4). One thus has 4.5 added methyl groups per 100 carbon atoms,

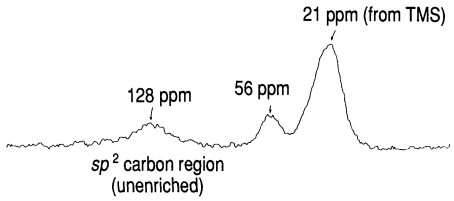

Fig. 2. Carbon-13 NMR spectrum of the solid product from the reductive methylation of soot. This is the filtered solid from the experiment discussed in Fig. 1. The ^{13}C observation frequency is 15 MHz; there is cross-polarization to protons, and there is magic angle spinning at 2.3 kHz. The cross-polarization experiment favors the detection of carbon atoms interacting with hydrogen atoms through dipolar coupling mechanisms.

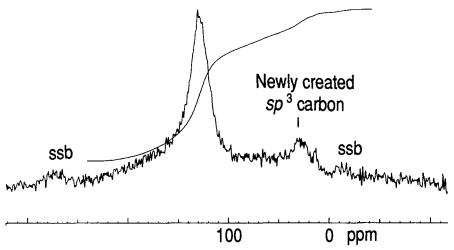

Fig. 3. Carbon-13 NMR spectrum of the solid product of adding water to a soot-potassium adduct, as discussed in (7). The ^{13}C observation frequency is 50 MHz; there is cross-polarization to protons, and there is magic angle spinning at 8 kHz. The peaks denoted "ssb" are spinning side bands from the aromatic peak, and the contour running across the spectrum is the integral of the spectrum. The initial soot has no measurable intensity in the sp^3-hybridized carbon region.

or 6 added methyl groups for the 2 nm by 2 nm soot "molecule" (*18*). This amount of addition is of the same order of magnitude as found for coals (*15*), which are generally considered to consist of relatively small aromatic molecules (*15*) rather than clusters. The aromatics of soot are, of course, larger than the two–, three–, and four–benzenoid ring molecules of bituminous coals (*15*) and are probably more similar to the aromatics of anthracite (*19*). Instrumental evidence for this is found in the failure to observe discrete infrared absorptions in the initial soot or in the methylated soot product with the use of either diffuse reflectance or photoacoustic approaches [techniques contrasted in (*20*)], which suggests that the soot is more highly absorbing than small aromatic molecules. Chemical evidence for this is found in the disparity between the level of reduction (4.8 carbon atoms per potassium consumed) and the level of alkylation (22 carbon atoms per added alkyl), arising from the reduction of alkyl iodide to alkyl radical and subsequent disproportionation and dimerization of alkyl radical; this disparity is also observed as the coal rank increases from bituminous to anthracite (*15*). However, the discrepancy between the chemistry of soot and that of graphite [8 to 24 carbon atoms per potassium (*14*); "infinite" carbon per added alkyl (*17*)] is apparent.

In terms of possible intercalation chemistry, direct reaction of the soot with K^0 at 110°C does not lead to any change of the (002) or (004) diffraction peaks of soot, a result totally unlike that manifested by graphite under the same conditions (*7*). Soot does not intercalate potassium and thus does not exhibit graphite chemistry even though it has a stacking order of aromatic planes. However, quenching this product with water creates sp^3-hybridized carbon atoms in the soot, as illustrated in Fig. 3. Even in a reaction that is favored by low edge site density, soot behaves like aromatic molecules in showing rehybridization, rather than intercalation, chemistry.

In summary, a variety of data on soot can be reconciled with a model of soot as aromatic molecules. Diffraction and ^{13}C NMR data for soot are as found for other aromatic mixtures and show no unexplained features. X-ray photoelectron spectroscopy of soot is also consistent with soot as conventional aromatics and heterocycles (*21*). The microanalysis of soot shows the presence of noncarbon atoms, commensurate with that expected from crystallite size measurements from diffraction. The chemistry of soot shows the significance of edge positions, as expected for aromatic compounds but not as expected for either graphite or clusters. Thus, although the proposal of "three-dimensional" aromatic carbon is truly exciting (*22*), it is possible that simple two-dimensional models can explain the structure and chemistry of soot.

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Thymic Epithelium Tolerizes for Histocompatibility Antigens

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The role of thymic epithelium in the establishment of tissue tolerance was analyzed with a murine chimeric system. All T cells differentiated from birth onward in a thymus comprising allogeneic epithelium and syngeneic hematopoietic cells. Embryonic thymic rudiments that contained no hematopoietic cells from C3H (H-2^k) donors were grafted to newborn athymic (nude) BALB/c (H-2^d) mice. Chimeras that had normal T cell numbers and function rejected third-party skin grafts, but permanently accepted grafts syngeneic to the thymic epithelium. In vitro functional assays did not always correlate with the state of tolerance in vivo. Thus, pure thymic epithelium induces tolerance to histocompatibility antigens.

THE THYMUS HAS A KEY ROLE IN THE development of the immune system. Intrathymic differentiation and selection of T lymphocytes seem to result in the establishment of self major histocompatibility complex (MHC) restriction and in tolerance to self antigens. Two types of thymic stroma cells, endodermally derived epithelial cells and accessory cells of hematopoietic origin, are implicated in these processes and are attributed different functions. Tolerance and clonal deletion (negative selection) of autoreactive lymphocytes are thought to be mediated by the hematopoietically derived dendritic cells of the medulla, whereas selection leading to MHC-restriction (positive selection) may be mediated by contact of the differentiating lymphocytes with epithelial cells (EC) (*1*).

Previous studies aimed at inducing allotolerance were carried out in the adult mouse by grafting fetal thymuses depleted of hemapoietic cells (HC) either by deoxyguanosine treatment or by culture at low temperature; the results were controversial. Deoxyguanosine-treated thymuses, although expressing donor MHC antigens, were not rejected when implanted into normal or nude mice, and yet did not induce allotolerance [as tested in mixed lymphocyte reaction (MLR) and cytolytic activities (*2*)]. However, T cells from deoxyguanosine-treated thymus grafts showed H-2–restricted specificity for antigen (*1*). It has also been found that thymic grafts depleted of HC can tolerize precursors of cytolytic T cells, but only for the minor histocompatibility antigens carried by the thymic epithelial stroma (*3*). In contrast, thymuses cultured at low temperature before grafting into an athymic nude mouse induced tolerance in both intrathymic and spleen cells, as determined by MLR tests (*4*). In none of these experiments was tolerance assessed through grafts of tissues other than the thymus itself, particularly since deoxyguanosine-treated thymus is not rejected even by immunocompetent allogeneic hosts (*2*).

In the experimental protocols above, it was impossible to ascertain that all the HC-derived cells (lymphocytes and medullary dendritic cells) of the thymus were completely eliminated. Moreover, T cells developing in those chimeras arose into the immunologically mature, though deficient, environment of an adult animal. We, therefore, investigated the roles of the endodermal epithelium and the HC-derived dendritic cells in tolerance induction by grafting the early embryonic thymic epitheliomesenchymal rudiment before its invasion by HC. If such an early allogeneic thymic anlage is introduced into a nude mouse at birth, all the thymic-dependent T cells that develop in the animal would have differentiated by contact with the MHC alloantigens of the donor thymus at nearly physiologic developmental stages. Their ability to recognize these alloantigens as self could then be tested in vivo with skin grafts or with in vitro assays.

We studied thymus development in birds by the construction of quail and chick chimeras. A chick that receives a xenogeneic (quail) graft of either a limb or a bursa of Fabricius at embryonic day 4 (E4) acutely rejects it after birth, when its immune system is mature (*5*). This could be prevented, however, if the epithelial thymic rudiment from the donor was also implanted; thus, thymic EC can present self antigens to the differentiating T cells in a tolerogenic manner. In the mouse thymus, development proceeds from the endoderm of the third branchial pouch, which starts to grow and is colonized by HC from E11 onward. At E10 the presumptive thymic rudiment is devoid of HC and is unable to develop into a lymphoid thymus if cultured in the absence of a source of HC (*6*). Neither the lymphoid nor the macrophage or dendritic cells labeled with appropriate Thy-1 and MHC class II markers, respectively, were ever found to be of donor type (*7*). We have, therefore, investigated whether the thymic epithelial component has the same effect on the induction of tolerance to tissue grafts in the mouse as it has in birds (*8*). Fully allogeneic thymic epithelium reconstituted the T cell compartment of athymic nude mice and induced tolerance to skin grafts of the donor strain. Therefore, thymic EC in the absence of MHC-matched thymic HC ensured the state of tolerance.

Between 1 and 13 days after birth we grafted 10 to 15 third branchial arch regions

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