

## Benzo[a]pyrene in Gasoline Partially Persists in Automobile Exhaust

**Abstract.** On average 36 percent of the benzo[a]pyrene in an automobile's exhaust gas comes from the benzo[a]pyrene originally in the gasoline. Between 0.1 and 0.2 percent of the benzo[a]pyrene in the gasoline survives the combustion process and is recovered from the exhaust; 5 percent accumulates in the crankcase oil. Some of the benzo[a]pyrene in the gasoline is converted into other polynuclear aromatic hydrocarbons and other more polar compounds. For our experiments we used commercial gasoline containing benzo[a]pyrene at 1.0 part per million to which was added benzo[a]pyrene-8,9-<sup>14</sup>C at 1.1 parts per million as a radioactive tracer.

Polynuclear aromatic hydrocarbons such as benzo[a]pyrene (BaP) have been found in the exhaust gas from gasoline engines (1-3). We find these compounds in commercial gasolines also; for example, the BaP content of the gasoline used by us ranged from 1 to 2 parts per million (ppm). Since, in average city driving, some gasoline goes through the engine unburned (4), some of the BaP found in exhaust may derive directly from that in the gasoline.

In order to determine the contribution by BaP in gasoline to the BaP emitted in exhaust, an engine was operated on gasoline to which radioactive benzo[a]pyrene-8,9-<sup>14</sup>C was added at 1.1 ppm as tracer. The gasoline, when purchased, contained nonradioactive BaP at 1.0 ppm, so that the total content was 2.1 ppm. The engine was relatively new; carburetion was within specifications and oil consumption was normal; it was operated on a cycle simulating city driving, and "tar" in the exhaust was recovered in a manner described (1, 3). The tar was analyzed for BaP and BaP-<sup>14</sup>C (5). The results of duplicate experiments are summarized in Table 1.

Although the percentages of BaP surviving combustion differed in the two runs, the percentages in exhaust gas, attributable to unchanged BaP from the gasoline, was approximately 36 percent in both runs; the percentage in the gasoline that survived the combustion process, to be recovered from the exhaust, was lower by at least an

Table 1. Survival in exhaust gas of benzo[a]pyrene; two runs. One liter of gasoline produced 8.7 m<sup>3</sup> of exhaust gas (wet, 24°C, 750 mm-Hg).

Benzo[a]pyrene		
In exhaust (μg/m <sup>3</sup> )	From fuel: surviving combustion (%)	In exhaust: from fuel (%)
1.2	0.21	34
0.6	0.10	38

order of magnitude than the average percentage of unburned gasoline in exhaust gas, as has been reported (4).

In addition to unchanged BaP-<sup>14</sup>C, the tar contained other radioactive polynuclear aromatic hydrocarbons such as pyrene-<sup>14</sup>C and chrysene-<sup>14</sup>C. These compounds may have resulted from the splitting of one or more benzo groups from benzo[a]pyrene-8,9-<sup>14</sup>C, followed by molecular rearrangement, or by buildup from smaller fragments (6).

Although only 0.1 to 0.2 percent of the BaP in the gasoline was emitted in the exhaust, slightly more than 5 percent of the total amount in the gasoline accumulated in the crankcase oil, which also contained lesser amounts of <sup>14</sup>C in compounds ranging in polarity from alkylbenzenes (alumina column,

eluted with cyclohexane) to oxygenated compounds (eluted with isopropanol).

The 36-percent contribution to the BaP in exhaust by BaP in the gasoline may be true only for the specific conditions of our experiment. Experiments with other concentrations of total BaP in gasoline, as well as other conditions of operation of the engine, are required for adequate understanding of the persistence of the polycyclics in gasoline in the total emission of these compounds from engines.

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## Host Resistance Reflected in Differential Nematode Population Structures

**Abstract.** Relative efficiency of host plants to support reproduction of the garlic race of *Ditylenchus dipsaci* can be partially explained by differential population structures. If axenic cultures of callus tissue from onion, white clover, red clover, and alfalfa are arranged in order of decreasing host suitability, the nematode populations are simultaneously arranged in order of increasing maleness.

The stem and bulb nematode, *Ditylenchus dipsaci*, consists of a number of physiological races; typically each race is restricted to a limited range of susceptible hosts. However, for each race there is a spectrum of less suitable hosts which show an increasing degree of resistance (1).

We used techniques of axenic culture of callus tissues to explore preferentially the basis of this resistance because the undifferentiated proliferation of cells would reduce the contribution of morphological factors. Sufficient host tissues, all grown on the same medium, were present so as not to limit the growth and development of nematode populations. Callus tissues from onion;

red clover, var. Kenland and var. Penscott; white clover, var. Ladino; and alfalfa, var. Caliverde and var. DuPuit were subcultured and inoculated with axenic *D. dipsaci* from three sources. These were mixed stages from onion plants, mixed stages from garlic plants, and fourth-stage larvae (preadults) from dried garlic scales. After 6 weeks at 27.5°C, the cultures were harvested, and the nematodes were recovered by means of the Baermann funnel technique. On all tissues, nematodes had penetrated, fed, developed, and moulted; and all stages appeared normally vigorous. Cultures of each tissue were replicated 20 times.

The usual ratio of adult males to

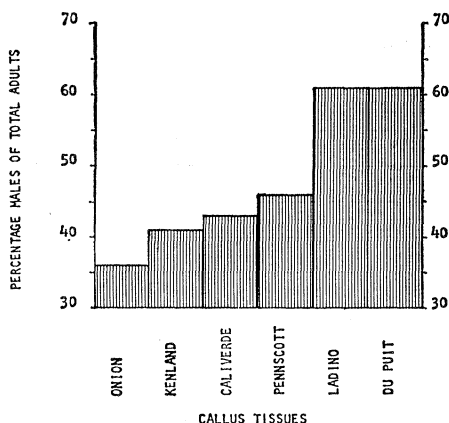


Fig. 1. Plant callus tissue varieties, arranged in order of increasing percentages of males of the total number of adult nematodes found after the tissues were cultured for 6 weeks at 27.5°C.

adult females in the garlic race of *D. dipsaci* is 1:2. (This holds for adults from onion or garlic plants or preadults from dried garlic scales.) In the cultures of callus tissue the overall ratio of males to females is standard (1:2), but the ratio of adult males to adult females differs markedly among cultures (Fig. 1).

In these host-parasite systems, resistance appeared to be expressed in the relative unbalancing of the sex ratio in favor of maleness, though there was no indication of sex reversal or the formation of intersexes.

The apparent trend to adult maleness was a result of the differential development between male and female stages, depending upon host suitability. Males were able to develop normally to adulthood, whereas the females were less able to do so. However, preadult females were normally active and feeding. The tendency of diverse nematode populations to increased maleness under environmental stress (2, 3) may be partially explicable in terms of arrested female development.

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## Histochemical Fluorescence after Application of Neurochemicals to Caudate Nucleus and Septal Area in vivo

**Abstract.** The movement of carbachol, norepinephrine, and dopamine from cannula sites in caudate nucleus and septal area of freely moving rats was traced by means of biogenic amine fluorescence. Fluorescent patterns seen after application of carbachol and norepinephrine to brain tissue did not appear to differ from controls. Three types of movement from the cannula site after administration of dopamine were observed. There was a spherical distribution approximately 2 millimeters in diameter. Fluorescence also followed axonal pathways in the orthodromic direction, suggesting that dopamine may have been transported by "axonal streaming" or by some other unknown mechanism in periaxonal spaces. Because fluorescence was present in both the ependymal lining and the choroid plexus, it was inferred that the cerebral ventricles were also involved in the movement of chemical. Any attempt to ascribe anatomical localization to behavioral effects resulting from chemical stimulation of the brain should take into account the widespread movement of chemicals after their local application to brain tissue.

In the study of the effects of neurochemicals applied to specific anatomical sites on behavior, it has been presumed (1) that the chemicals act at the site of stimulation, diffusing less than a millimeter from the cannula tip. Routtenberg (2) questioned this assumption on the basis of certain paradoxical results. Studies in which the movement of dyes have been traced (3) are only approximations since there is no way to be certain that the neurochemical would not behave somewhat differently from the dye.

We have studied the movement of chemicals from the cannula tip using the histochemical technique (4) for demonstration of biogenic amines. Although this technique demonstrates the presence of norepinephrine, dopamine, and serotonin, it will not show the diffusional movements and distribution of carbachol. However, since carbachol might cause release of biogenic amines (5), we observed the pattern of fluorescence after its application as well as after that of norepinephrine and dopamine.

Using stereotaxic methods, we implanted a stainless steel cannula into each of 25 female adult albino rats anesthetized with barbiturate (6). Ten additional rats served as operated and unoperated controls. Cannulas (7) were placed in either the caudate nucleus or the septal area. After a 2-day recovery period, approximately 10 µg of chemical were applied to each animal; fourteen animals received carbamylcholine chloride (carbachol), four received DL-norepinephrine hydrochloride, and six received dopamine hydrochloride. The rat was decapitated after 10 minutes, and the brain was rapidly removed (1 to 2 minutes) and im-

mediately frozen in liquid Freon 22, chilled first with liquid nitrogen to -150°C. An additional animal, receiving dopamine, was decapitated 1 minute after application. The tissue was then processed for the histochemical demonstration of biogenic amines (4).

The clearest demonstration of fluorescence was obtained with dopamine. With this chemical, three major types of movement from the cannula tip were seen. The first, seen in two cases, was spherical diffusion, which appeared as a circular pattern of fluorescence surrounding the chemical probe. Near the site of the cannula tip was a bright yellow fluorescence which became yellow-green and then green with increasing distance from the probe. This bright yellow fluorescence is believed to be related to the presence of high concentrations of the catecholamines (8). The spherical diffusion from the probe site was about 1 to 2 mm, variations likely depending upon the amount of dopamine applied.

A second type of transport from the cannula was associated with the axon (Fig. 1). This was seen in five of the seven cases. Dopamine applied to the caudate nucleus moved into the corpus callosum, traveling several millimeters to the contralateral side (Fig. 1A). A similar axon-associated movement was also demonstrated in the anterior commissure. In cases where dopamine entered this latter system, one could trace fluorescence on successive sections to the side contralateral to chemical application. Dopamine applied to the caudate nucleus also entered into the internal capsule and moved in the orthodromic direction (Fig. 1, B-D). A section slightly anterior to the probe site (Fig. 1B) showed no fluorescence in the in-