

or negligible peak on the P-2 column at the position of the race 1 elicitor, and no specific elicitor activity was present in these fractions (Fig. 1). The column results disclosed the presence of several ultraviolet-absorbing peaks from races 1 and 3 that contained nonspecific elicitor activity, but only the one specific elicitor peak from race 1 was consistently observed with potassium phosphate elution.

Further attempts to purify the race 1 specific elicitor from P-2 column fractions have thus far been unsuccessful, in part due to a gradual loss of activity in the pooled fractions and to the apparent absence of charge for the specific elicitor. The peculiar behavior of the specific elicitor activity on the P-2 columns with or without salt suggests that the race 1 specific elicitor may be adsorbed on proteins and the polyacrylamide gel matrix.

A glucan elicitor of hydroxyphaseollin production in soybeans has been isolated from culture fluids of *P. megasperma* var. *sojae* by Ayers *et al.* (8). This metabolite is likely not the race 1 specific elicitor detected here since it has not been shown to have specific elicitor activity. Furthermore, I have not detected anthrone reactive material in the race 1 specific elicitor peak from Bio-Gel P-2 columns.

The detection of specific hydroxyphaseollin elicitors from race 1 but not race 3 cultures of *P. megasperma* var. *sojae* constitutes an additional independent proof that derepressed HP production is indeed the basis for resistance in soybeans to incompatible races of the fungus. Despite the failures thus far encountered in isolation and chemical characterization of the race 1 specific elicitor, the data presented here are consistent with the hypothesis that metabolites of pathogenic origin can indeed specifically predicate disease resistance or susceptibility through a differential effect on plant biosynthesis of phytoalexins such as HP. Since race 3 of *P. megasperma* var. *sojae* presumably evolved from race 1, and specific elicitor activity was not detected from race 3 crude culture fluids or P-2 column fractions, it is enticing to speculate that the genetic-chemical basis for race 3 evolution was loss of the ability to make the specific elicitor typical of race 1.

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References and Notes

1. Incompatible host-parasite combinations result in resistant plant reactions with no occurrence of disease; compatible combinations give susceptible plant reactions to the pathogen.
2. The responses of various resistance genotypes of soybeans to three known races of *P. megasperma* var. *sojae* are as follows (C = compatible response; I = incompatible):

Soybean cultivar	Race		
	1	2	3
Harsoy (H)	C	C	C
Harsoy 63 (H63)	I	I	C
D60-9647	I	C	I
Semmes	I	I	I
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5. Known mechanisms for the hypersensitive resistant reaction in higher plants are the permeability decompartmentalization mechanism and the production of antibiotic phytoalexins. Little is known about elicitors of the former mechanism, but many substances are recognized that elicit phytoalexin production in plants. Of these, the great majority are defined as nonspecific elicitors since they exhibit no known differential effects on various cultivars of a plant species; specific elicitors are metabolites, presumably of pathogenic origin, that elicit differential phytoalexin production on various host cultivars similar to the fungus race that produces them.
6. The medium consisted of the following, in the amounts specified per liter: sucrose, 15 g; asparagine, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 1 mg; $CaCl_2 \cdot 2H_2O$, 10 mg; thiamin-HCl, 1 mg; K_2HPO_4 , 1.04 g; KH_2PO_4 , 1.90 g; β -sitosterol, 20 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg; $CuSO_4 \cdot 5H_2O$, $NaMoO_4 \cdot 2H_2O$, and $MnCl_2 \cdot 2H_2O$, each 0.02 mg; and $CaCO_3$, 3 g.
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Halogenated Hydrocarbons in New Orleans Drinking Water and Blood Plasma

Abstract. Volatile organics from New Orleans drinking water and pooled plasma were collected on a solid phenyl ether polymer and analyzed by gas chromatographic and mass spectrometric techniques. Thirteen halogenated hydrocarbons were identified in the drinking water. Five halogenated compounds were found in the plasma. Tetrachloroethylene and carbon tetrachloride were found in both the plasma and the drinking water. Considerable variation in the relative concentrations of the halogenated hydrocarbons was noted from day to day in the drinking water.

In recent years concern has been expressed about the quality of water available at the lower end of the Mississippi River. Several qualitative studies have identified halogenated hydrocarbons in local drinking water (1). Isolation procedures such as reverse osmosis, carbon adsorption, and solvent extraction have been commonly employed (2), in addition to total trapping techniques (3). Over 50 percent of the compounds isolated in the above studies were described as moderately toxic to very toxic, and two compounds as extremely toxic (1). Many studies have been conducted on the toxicity of halogenated hydrocarbons (4), and it is well known that such compounds as vinyl chloride (5) and chloromethyl ether (6) are carcinogenic whereas carbon tetrachloride and chloroform (7) are suspected carcinogens. Halogenated hydrocarbons will tend to accumulate in various tissues of animals and man (8). Instances of cancer, excluding skin cancer, in the New Orleans vicinity have been reported to be above the national

average (9). More recent studies on cancer mortality have supported these earlier findings (10). It is our purpose here to report a new and more rapid procedure to identify the major halogenated hydrocarbons in New Orleans drinking water and further to correlate, by the use of the same method, such observations with low-molecular-weight halogenated hydrocarbons in blood plasma from local residents.

Volatile organics were eluted from water and blood plasma samples by heating to 95°C under a stream of ultrapure helium. The helium stream was passed through a series of glass condensers to eliminate the bulk of water vapor. The volatile organics were trapped on poly(p-2,6-diphenylphenylene)oxide adsorbant having a mesh size of 35/60 (Applied Science Laboratories, Inc., State College, Pennsylvania) attached to the end of a condenser train. At the end of a 1-hour trapping period, the 1 g of polymer containing the adsorbed organics was transferred from the collection reservoir

to a silylated glass injection port liner (10 mm in outside diameter, 9.2 cm in length), and each end was plugged with silylated glass wool. The polymer, the glass wool, and liner were conditioned at 350°C for 1 hour in an ultrapure helium stream prior to use. Similar techniques in which a polyphenyl ether has been used as an adsorbant have been successful in studying volatile organics from biological samples (11).

The liners were immediately placed in the injection port (maintained at 200°C) of a gas chromatograph (Hewlett-Packard 7620A) which had been modified to accommodate the larger-diameter liners. The volatile components were transferred by means of a helium stream onto a column (1.5 m by 0.05 cm) coated with Emulphor ON-870 (Applied Science Laboratories, Inc.) held at Dry Ice-methanol temperatures. At the end of a 7-minute trapping period, the collected organics were swept onto a stainless steel capillary column (91.0

Table 1. Major halogenated hydrocarbons, isolated from New Orleans drinking water.

Compound	Chromatographic peak number in Fig. 1
1-Chloropropene	11
Chloroform	14
Carbon tetrachloride	19
Dichloroethane	20
Trichloroethylene	24
Dichloropropane	24
Dichloropropene	25
Bromodichloromethane	27
Dichloropropene	33
Dichloropropene	36
Tetrachloroethylene	40
Dibromochloromethane	45
1,3-Dichloropropene	60

m by 0.05 cm) coated with 10 percent GE SF-96 and 1 percent Igepal CO 880 (Applied Science Laboratories, Inc.) (12). During mass spectral analyses, the carrier gas effluent was passed through a heated transfer line (250°C) into a double-focusing mass spectrom-

eter (DuPont 21-491) by means of a jet-type separator. Spectra were obtained at 70 ev. All glassware was cleaned with chromic acid solution and baked out at 110°C for 1 hour just prior to use. Blanks were analyzed immediately before a given analysis to ensure that the helium carrier gas, glassware, vacutainers, and chromatographic system were free of any interfering compounds.

Drinking water was obtained directly from the tap at two different locations. Blood plasma was collected by means of vacutainer tubes (Becton, Dickinson, Rutherford, New Jersey,) each containing 10.5 mg of the disodium salt of ethylenediaminetetraacetic acid to prevent clotting. Two different groups of local residents, in good general health, ranging from 20 to 30 years of age were used. The first group included four males and four females, and the second group consisted of 13 males (13).

Figure 1 illustrates the gas chromatographic separation of the organic volatiles isolated from drinking water and pooled plasma from eight subjects. The volatile components isolated from plasma for each test group produced essentially identical chromatographic results. Mass spectral identifications of the major halogenated components in water are listed in Table 1. Spectral identifications were confirmed by comparison with published spectra of authentic samples (14). Considerable variation in the relative concentrations of the various halogenated compounds was observed from day to day. In some instances chloroform was the major component with concentrations in the low parts-per-billion range. Several of the compounds observed have not been shown to be present in earlier studies of local water (1). This could be in part due to a difference in isolation techniques or to normal variations in the organics in drinking water for the New Orleans area, or both (15). Analysis of one commercial source of artesian water (1 liter) revealed low quantities of halogenated compounds. The concentrations of organics observed in the artesian water were at least 10^3 times lower than those in drinking water.

Of particular interest is whether any of the halogenated compounds found in drinking water are also present in humans ingesting such water over long periods of time. Our preliminary studies reveal (Fig. 1) that both carbon tetrachloride (peak a) and tetrachloroethylene (peak b) are present in the

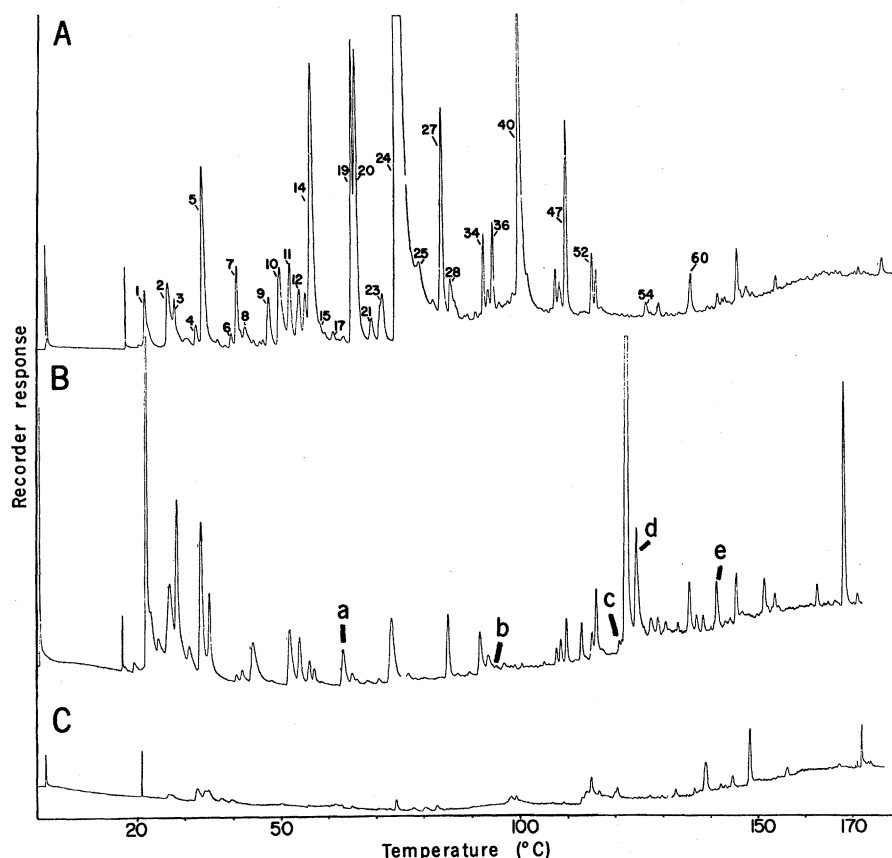


Fig. 1. Gas chromatographic separation of volatile organics from (A) 600 ml of New Orleans drinking water ($\times 40$). (B) 10 ml of pooled plasma from eight subjects ($\times 40$). (C) A glassware and chromatographic system blank obtained in the absence of plasma or water samples. Sample collection and injection techniques are discussed in the text. Numbers in (A) refer to identifications given in Table 1. In (B) the carbon tetrachloride and tetrachloroethylene are marked a and b, respectively, whereas c, d, and e refer to the isomers of dichlorobenzene. Temperature programming was employed after an initial hold of 10 minutes at 25°C. Programming from the initial temperature to 170°C was at 2°C per minute; the final temperature was maintained for 1 hour. The chromatograph was equipped with flame detectors maintained at 300°C. All analyses were at the same attenuation.

pooled plasma of the two test groups. As can be seen in Fig. 1, the concentration of carbon tetrachloride in plasma is substantially higher than in drinking water. In view of the lipophilic nature of carbon tetrachloride, our observations suggest that a bioaccumulation mechanism may be in operation, if drinking water is the only source of such materials. In addition, three isomers of dichlorobenzene (peaks c, d, and e of Fig. 1) were noted in the plasma but were not confirmed in the drinking water. Approximately 400 liters of New Orleans air were analyzed to determine if the atmosphere could be a major contributor of the halogenated compounds of plasma. No halogenated compounds were observed among the over 50 major organics evaluated by gas chromatography and mass spectrometry. The atmospheric samples were, however, rich in aromatic compounds.

At this point, one can only speculate about the origin of the organics in the drinking water and their relation to halogenated hydrocarbons in plasma. However, in view of the lipophilic nature of halogenated hydrocarbons and their occurrence in drinking water, it is not surprising that they might be found accumulating in blood or other body tissues.

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Retrograde Amnesia: Temporal Gradient in Very Long Term Memory following Electroconvulsive Therapy

Abstract. *A newly designed remote memory test has been used to assess the temporal dimension of prolonged retrograde amnesia. Patients given a course of electroconvulsive treatments for relief of depressive illness exhibited a temporal gradient of retrograde amnesia after five treatments. Memories acquired up to about 3 years before treatment were impaired, but memories acquired 4 to 17 years before treatment were not affected. The results suggest that the neural substrate of memory gradually changes with the passage of time after learning and that resistance to amnesic treatment can continue to develop for years.*

Retrograde amnesia is the loss of memory for events that occurred before some precipitating incident such as head trauma, drug injection, or electroconvulsive stimulation. Typically, as the interval between learning and amnesic treatment increases, the resulting amnesia is diminished. This phenomenon has usually been taken to mean that the neural substrate of memory changes or consolidates with the passage of time (1). It is not yet clear how long these changes can continue after learning. A large experimental literature based primarily on animal studies has suggested that the gradual increase in resistance to amnesic treatment may be complete within hours or days after learning (1). Yet in man the amnesic syndrome can affect memories acquired many years before the onset of amnesia (2-4), and sometimes memories acquired in the remote past appear to be less affected than those acquired more recently (2).

In such cases of prolonged retrograde amnesia, however, it is difficult to determine whether the amnesia is temporally graded or whether all memories are affected about equally. Sampling artifacts could easily lead to clinical impressions of a temporal gradient of retrograde amnesia (3, 5).

Thus, when an interview covers a period of many months or years, questions about the remote past tend to sample a greater time interval and to be more general than questions about the recent past. In addition, memories sampled from different time periods are likely to be of different strengths. That is, questions about the remote past are directed at events that have proved resistant to forgetting, whereas questions about the recent past are directed at events that may be rapidly forgotten. Recently developed questionnaires about events and persons formerly in the news have been usefully applied to some aspects of the amnesic syndrome (3, 4), but these methods are also limited by the possible operation of sampling bias in the selection of questions.

A new remote memory test is available which may overcome these limitations by permitting equivalent sampling of events from different time periods (6). In this test the subject is asked to recognize the names of television programs that were broadcast nationally between 6 and 11 p.m. for a single season between 1957 and 1972. Program names were selected by a method designed to minimize sampling bias. In addition, popular ex-