the initial enzyme activity to decay at 65 °C) for arylsulfatase B′ was about 14 min. Arylsulfatase B was more thermostable and arylsulfatase A more thermolabile than arylsulfatase B′ (table).

Both arylsulfatase B and B′ activities increase rapidly after 6 days of age and peak at approximately 18 days postnatal age (fig. 2), while developmental fluctuation of arylsulfatase A is less prominent. Similar developmental profiles were observed for SWR/J and A/J brain arylsulfatases. These trends suggest that arylsulfatases B and B′ may be subject to similar developmental regulation.

The immunotitration and neuraminidase experiments indicate that arylsulfatases B and B′ may be derived from the same primary gene product. SWR/J mice possess a more thermostable form of arylsulfatase B ($t_{1/2} \approx 75$ min) compared to C57BL/6J mice[13]. By contrast, SWR/J arylsulfatase B′ exhibited a half-denaturation time of 15 min which closely approximated that of C57BL/6J arylsulfatase B′. If arylsulfatases B and B′ are derived from the same primary gene product, then the posttranslational modification mechanism must sufficiently alter their structures to abolish their thermostability differences (with respect to arylsulfatase B′) or to enhance these differences (with respect to arylsulfatase B).

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Structural relationships between the endogenous volatile urinary metabolites of experimentally diabetic rats and certain neurotoxins[1]

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Summary. High resolution glass capillary gas chromatography and GC/MS were utilized to examine qualitative and quantitative variations from normal of urinary volatile metabolites of long-term alloxan and streptozotocin diabetic rats. Volatile metabolites were structurally compared with known neurotoxins to examine any possible relationship between these metabolites and the development of the diabetic polyneuropathy.

There is a growing body of evidence which implicates the axon as the initial site of damage in diabetic polyneuropathy in humans and experimental animals[3–8]. Strikingly similar axonal degeneration is seen in a number of other conditions. This includes many drug- and toxin-related polyneuropathies which exhibit a characteristic pattern of primary axonal nerve degeneration[9–12]. A recent report suggests the possibility that metabolic neuropathies such as diabetic polyneuropathy may arise from an abnormal in vivo production of neurotoxic metabolites[12].

Since previous reports[9–12] have implicated small organic molecules (e.g., n-hexane, 2,5-hexanedione, acrylamide and others) as potent axonal neurotoxic agents, we have investigated the possibility that such or chemically similar compounds are produced in vivo due to the diabetic condition. Urinary chromatographic profiles of volatile components were recorded for alloxan and streptozotocin diabetic rats. Volatile metabolites of physiological fluids suggest themselves for examination since these small neutral organic metabolites are chemically similar to known neurotoxic substances, and deviations in the concentration of some of these molecules from normal are seen in human diabetics[13,14] and alloxan diabetic rats shortly after the development of the hyperglycemic condition[15].

Based on the available evidence concerning the functional and structural changes in nerves that accompany the development of diabetic neuropathy in alloxan and streptozotocin diabetic rats[3,5–8], we have chosen experimentally diabetic animals of 2–12-month disease duration for this investigation. Comparisons with suitable control animals have enabled us to evaluate qualitative and quantitative variations in the volatile metabolites caused by the long-term diabetic condition. Metabolites exhibiting qualitative or large quantitative alterations from normal were structurally compared to known neurotoxins.

Materials and methods. Sprague-Dawley male rats (Harlan Industries, Indianapolis, Indiana) weighing between 150 and 170 g were utilized in these experiments. Streptozotocin diabetic rats received a single injection of 65 mg/kg citrated streptozotocin (a gift from the Upjohn Co., Kalamazoo, Michigan) in saline solution. Alloxan diabetic rats received a single injection, after a 24-h fast, of 40 mg/kg alloxan (alloxan monohydrate, Sigma Chemical Co., St. Louis, MO) in saline solution. All injections were i.v. using the tail vein of the rat. Solution concentrations were adjusted to keep injection volumes at approximately 1 ml. Control rats received a single injection of 1 ml saline solution.

Blood glucose values were measured with a Beckman Glucose Analyzer. Values were determined at 2-week inter-

Effects of alloxan and streptozotocin diabetes of long-term duration on the concentration of rat urinary volatile constituents

Compound	Peak No.	Months 2	5	8	12
4-Heptanone	4	60±6.7	29±1.4	50±7.5	24±3.5
2-Heptanone	5	19±3.6	53±5.2	54±3.3	38±3.6
5-Hepten-2-one	6	36±5.8	42±15.1	68±8.3	65±5.8
2-Heptenal	8	212±16.2	228±20.7	180±20.3	157±11.2
5-Methyl-5-octen-4-one	12	22±6.9	55±4.3	64±9.4	16±2.9
Pyrrole	14	212±14.5	321±17.8	245±23.0	140±5.3
N-Acetylpyrrole	17	337±21.8	458±31.8	219±15.3	145±9.8
3-Methyl-N-acetyl-pyrrole	18	158±12.3	626±55.1	343±28.5	162±11.2
Acetophenone	19	156±6.1	555±50.1	188±14.5	182±17.1
Sulfur compound (C_5H_8SO)	20	NS*	222±30.2	NS*	NS*
Indole	23	200±12.2	250±14.4	200±13.1	250±11.6

Other identified metabolites not quantitatively altered from normal

Compound	Peak No.		
2-Pentanone	1	4-Nonanone	10
2-Hexanone	2	Furfural	11
3-Penten-2-one	3	3-Octen-2-one	13
3-Ethyl-5-methyl-2-hexanone	7	2-Acetylfuran	15
6-Methyl-5-hepten-2-one	9	Benzaldehyde	16
		Benzothiazole	22

Concentrations reported as percent of control value ± SE. * NS, not statistically significant.

vals and just prior to urine collection. No insulin or other treatment was utilized to maintain the animals. Normal rat chow (Lab-blox, Wayne Feeds, Bloomington, IN) and water were supplied ad libitum throughout the duration of these experiments. Blood glucose values for the control animals ranged between 70 and 110 mg/100 ml, while those for diabetic animals were 350–618 mg/100 ml.

24-h urine samples were collected from suitable control animals and uncontrolled diabetic animals at 2, 5, 8, and 12 months after the initial induction of diabetes. Samples were collected in polycarbonate metabolism units (Maryland Plastics, Federalsburg, MD). Interim samples were kept frozen over dry ice during urine collection. The samples were then brought to room temperature and filtered. Since standardized urine volumes are necessary for the comparative headspace analysis procedure described below, all 24-h urine samples were diluted with highly purified water to a standard volume. Dilution water was passed through a Barnstead purification system and then through a high capacity reverse phase chromatographic column to remove organic impurities. The urine was then divided into suitable aliquots and refrozen at −20 °C until analysis.

Analysis of the volatile constituents of the urine samples was accomplished utilizing a headspace concentration method followed by glass capillary gas chromatography[16]. The procedures and automated chromatographic instrumentation utilized for the acquisition of these metabolic profiles have been previously described[16]. In the only modification of prior procedures, the h.w. thermostated sampling bottles were scaled down to accomodate a smaller sampling volume of 2 ml of urine. Reproducibly prepared glass capillary columns (60 mû × 0.25 mm inside diameter) coated with 0.2% UCON-50-HB-2000 (containing 0.015% BTPPC as surfactant) were employed for chromatographic analysis. All samples were run at a flow rate of 2 ml/min and temperature-programmed from 28 to 160 °C as indicated in the figure. Quantitative variations of volatile metabolites were calculated from the integrated peak areas of the chromatograms for the individual diabetic and control animals.

Identification of volatile constituents was accomplished using the same glass capillary gas chromatographic column coupled to a low resolution mass spectrometer. The glass capillary column was directly interfaced with the mass spectrometer ion source (maintained at 220 °C). Low-resolution electron impact ionization spectra were obtained at 70 eV with a Hewlett-Packard Model 5980A combined gas chromatograph/dodecapole mass spectrometer/computer system. All spectra were recorded at scan rates of 100 amu/sec. Chromatographic conditions were the same as above. Proposed structures were verified through comparison of mass spectra and retention times of authentic compounds.

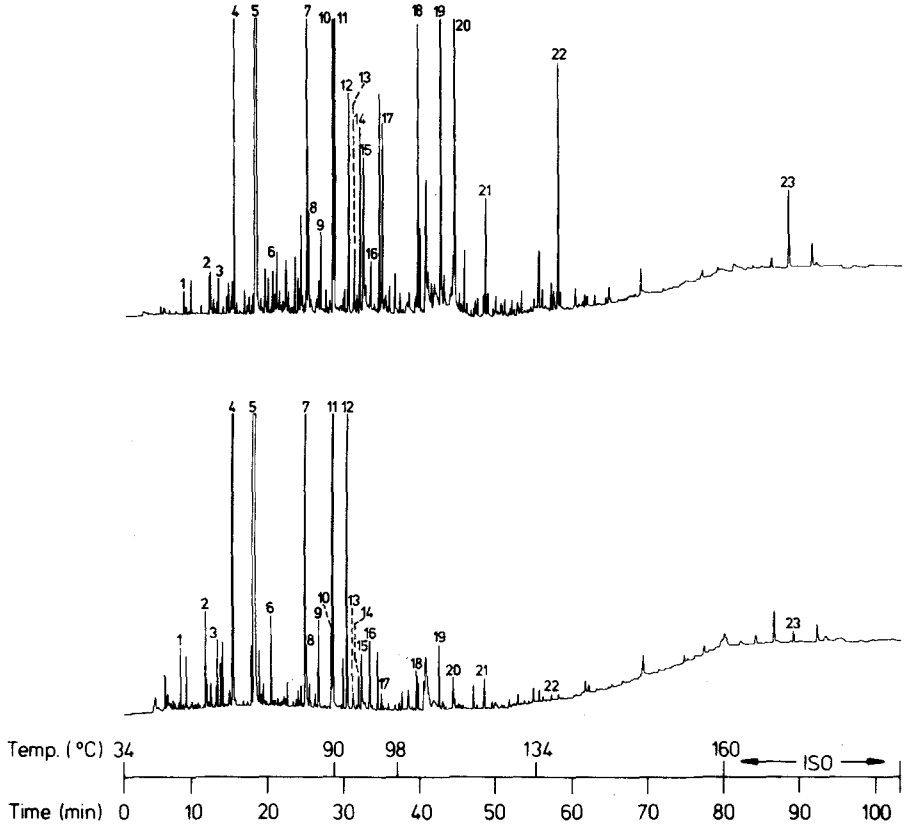
Results and discussion. Typical metabolic profiles for the urinary volatile constituents from diabetic and control animals are compared in the figure, which displays chromatograms representing a control animal (bottom) and an alloxan diabetic rat of 5-month disease duration (top).

The table lists the identified metabolites, along with the peak numbers to which they correspond, and the average quantitative variations from normal of urinary volatile metabolites in long term (2–12-month) experimentally diabetic rats. Quantitative data are displayed only for those compounds which were judged significantly altered from normal (Student's t-test, $p < 0.05$). The data were calculated as the percent of control average (i.e., 100 implies that the area of the average diabetic peak for a given metabolite equals that of the control). A total of 23 experimentally diabetic animals and 9 controls were examined in this way. The data presented were consistent from animal to animal over the disease duration examined. Although headspace analysis methods do not allow for direct absolute quantitation, the concentration of the individual urinary volatile metabolites lies in the range of 100–700 mg/24-h period.

We observed no consistent qualitative changes from normal in the urinary volatile chromatographic profiles of the experimentally diabetic animals examined, however, we identified a number of interesting metabolites and observed numerous quantitative alterations from normal.

The large number of saturated and unsaturated aliphatic ketones we identified are of interest since reports have indicated that those ketones or their metabolites which can undergo ω-1 oxidation to yield γ-diketones are neurotoxic[17–19]. 2-Hexanone, a known neurotoxic agent, has been identified in this study as a urinary metabolite of both long term experimentally diabetic rats and controls, however, no diketones or their intermediary metabolites were identified among the major chromatographic peaks.

In a previous study, we observed elevations in some urinary aliphatic ketones (including 2-hexanone and 3-ethyl-4-methyl-2-hexanone) soon after induction of diabetes in alloxan diabetic rats[15]. However, the data on the long-term experimentally diabetic rats we report here shows quite the opposite trend: the urinary aliphatic ketones are either



Urinary volatile metabolite chromatograms of a 5-month alloxan diabetic rat (top) and a suitable control animal (bottom). Identified chromatographic peaks are indicated in the table.

slightly below or significantly depressed from the levels in the age-matched control animals. Thus, if elevated levels of aliphatic ketones play a contributory role in the polyneuropathy observed in uncontrolled experimental diabetes in the rat, it would necessarily have to occur early in disease progression. The urinary aliphatic ketones identified here have been reported as arising from fat breakdown via decarboxylation of ketoacids (analogous to acetone formation)[13]. Since the uncontrolled long-term diabetic rats examined here had severely decreased weights and adipose tissue in comparison to control animals, it is not surprising that the urinary aliphatic ketone levels were decreased.

2-Heptenal, is greatly increased in the urine of the experimentally diabetic animals examined here. Although other carbonyl compounds of similar structure are neurotoxic, there is no current evidence in the literature implicating this compound or acetophenone which is also elevated in the urine of diabetic animals.

Finally, we observed characteristically elevated levels of pyrrole derivatives and indole in the urine of the experimentally diabetic rats. While little information exists concerning the metabolic origin of the pyrrole derivatives identified, indole is probably formed from tryptophan[20]. Since the uncontrolled diabetic rats used showed considerable weight loss throughout the experiments, it seems reasonable to conclude that enhanced protein catabolism accounts for the observed urinary elevation in pyrrole derivatives and indole. As with 2-heptenal, little information exists concerning the neurotoxicity of the pyrrole derivatives we identified.

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