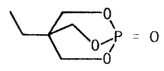
(Fig. 1). The spikes became more frequent and culminated in grand mal seizures. Analysis of a blood sample taken during a seizure showed 6 percent COHb (approximately the level expected in a heavy cigarette smoker). Chemical analysis (*6*) confirmed the presence of the bicyclic phosphate, 4-ethyl-l-phospha-2,6,7-trioxabicyclo[2.2.2.]octane-1-oxide (**1**) in the smoke.



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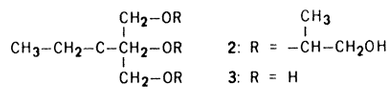
It is postulated by Voorhees *et al.* (*6*) that compound **1** is formed during the thermal decomposition of the urethane polymer by the production of the propoxylated trimethylolpropane adduct (**2**), which in turn decomposes to trimethylolpropane (**3**). Compound **3** is believed to further react with a reactive phosphorus species from the retardant additive to form compound **1**.



The chemical analyses were prompted by the similarities in the characteristics of intoxication of animals exposed to smoke from the fire-retarded foam and animals treated with intraperitoneal injections of the bicyclic phosphate compound **1** supplied by J. E. Casida (*7–9*). Homologous bicyclic phosphate compounds induce seizure activity in concentrations lower than 1 part per million (*7*). Compounds exhibiting such extreme toxicity could be present in biologically hazardous concentrations in a complex mixture (smoke) and be undetected by conventional analysis techniques such as gas chromatography and mass spectroscopy. This illustrates the necessity for a biological testing system to parallel chemical analytical methods during evaluation of a material's combustion products.

The sensitivity of a biological testing system should reflect the probable scenario of the fire hazard. The progressive loss of mental and behavioral functions followed by abnormalities in vital physiological functions leading to death is a typical sequence of impairment resulting from smoke intoxication. It is apparent from our observations that loss of behavior-dependent escape responses may occur well before lethal concentrations of "smoke" develop. The experimental approach of monitoring vital functions and behavior provides a biological testing system with an added level of sensitivity for the assessment of combustion products toxicity.

While our polyurethane foams are not commercial samples, the combination of ingredients is not uncommon in commercial formulations. This suggests that a major health hazard, independent of flame contact or CO intoxication, may be encountered by humans exposed to the combustion products of this class of materials during actual fires.

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Intestinal Metabolism of Phenacetin in the Rat: Effect of Charcoal-Broiled Beef and Rat Chow

Abstract. *The intestinal metabolism of phenacetin in vitro was increased 1100 percent in rats fed charcoal-broiled ground beef in a semisynthetic diet. The intestinal metabolism of phenacetin was increased 200 percent in rats fed a chow diet, as compared to rats fed the semisynthetic diet. The results obtained suggest a need for studies in man to determine whether charcoal-broiled meat and other dietary constituents can stimulate the intestinal metabolism of phenacetin or other drugs and thereby decrease their absorption and bioavailability.*

Recent studies showed that cigarette smoking enhances the metabolism, and lowers the plasma concentration, of orally administered phenacetin (*1*) in man without changing its plasma half-life (*2, 3*). These findings suggest that the enhanced metabolism of phenacetin in smokers may be occurring during the first pass through the liver, or in the gastrointestinal tract. Investigations in rats revealed the presence of an enzyme system in the wall of the small intestine which is capable of metabolizing phenacetin to *N*-acetyl-*p*-aminophenol (*3, 4*), and the results indicated that the activity of this enzyme system is increased in rats pretreated with cigarette smoke (*4, 5*); with 3,4-benzpyrene (*3, 5*), a constituent of cigarette smoke (*6*); or with 3-methylcholanthrene (*4*). Studies with everted sacs of rat small intestine, suspended in a phenacetin solution, were also made to determine whether changes in the intestinal metabolism of phenacetin may influence the amount of this drug that is absorbed unchanged into the bloodstream. These studies showed that an increase in phenacetin-metabolizing activity of the intestine decreased the amount of phenacetin and increased the amount of its metabolite, *N*-acetyl-*p*-aminophenol, that was transferred from the mucosal to the serosal side of the intestinal sac (*5*).

Because of the possibility that various substances normally present in man's diet may influence the intestinal metabolism and bioavailability of drugs, we have initiated studies on the effects of dietary constituents on the metabolism of phenacetin by the rat intestine. We now report a stimulatory effect of charcoal-broiled ground beef and of a rat chow diet on the metabolism of phenacetin by the rat small intestine.

Male Long-Evans rats (Blue Spruce Farms, Altamont, N.Y.), weighing 170 ± 10 g, were maintained on a Wayne Lab-Blox rat feed (Allied Mills, Chicago) diet. After arrival in our laboratory, the rats were fed freely a nutritionally complete semisynthetic diet consisting of vitamin-free casein, 27 percent; starch, 59 percent; vegetable oil, 10 percent; salt mixture, 4 percent; and a complete vitamin supplement (normal protein test diet, Nutri-

tional Biochemicals, Cleveland) for 7 days. The rats were then fed one of the following diets without restriction for the next 7 days: (i) the semisynthetic diet, (ii) Purina rat chow (Ralston Purina Co., St. Louis), (iii) raw ground beef, (iv) ground beef cooked on aluminum foil, or (v) charcoal-broiled ground beef. Diets (iii), (iv), and (v) were mixed 3 : 1 by weight with the semisynthetic diet. The ground beef, in the form of patties measuring approximately 4 inches (1 inch = 2.54 cm) in diameter by 0.25 inch in thickness, was cooked on a grill over burning charcoal, either directly or protected by a layer of aluminum foil, at a distance of 2.5 inches, for 4 minutes per side. Ground beef cooked by these methods would, by human standards, be considered well-done but edible. The rats had free access to water, and animals in all of the diet groups gained weight at the same rate.

The rats were killed by cervical dislocation. The proximal 5-cm segment of small intestine was excised, washed free of contents with 20 to 30 ml of ice-cold 0.9 percent sodium chloride solution, split longitudinally, opened, and suspended in 35 ml of a solution of 5 μg of [^{14}C]phenacetin (ring-labeled, 8.6 μc/mg) per milliliter of Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing glucose (5 g/liter). The solution was continually gassed with a mixture of oxygen and carbon dioxide (95 : 5, by volume) and maintained at 37°C; the preparation was shaken gently. After 90 minutes of incubation, the segment of intestine was removed from the solution, washed with ice-cold 0.1*M* KH_2PO_4–K_2HPO_4 (*p*H 7.4) buffer, and then homogenized with six volumes of this buffer at 2°C. A 3.0-ml portion of the homogenate and a 1.5-ml portion of the incubation solution were analyzed for *N*-acetyl-*p*-aminophenol content by thin-layer chromatography, as previously described (*5*).

Phenacetin was metabolized to *N*-acetyl-*p*-aminophenol by the longitudinally split segments of rat small intestine (Tables 1 and 2), and the amount of intestinal metabolism was increased 200 percent in rats fed Purina rat chow, when compared to those fed the semisynthetic diet (Table 1). Intestine from rats fed charcoal-broiled ground beef that was combined with the semisynthetic diet metabolized phenacetin 1100 percent more rapidly during the 90-minute incubation than intestine from rats fed the semisynthetic diet alone (Table 2). Raw ground beef or ground beef that was cooked on aluminum foil had little or no stimulatory effect on intestinal phenacetin metabolism (Table 2). Studies with intestinal washings failed to reveal detectable phenacetin metabolism, indicating that bacterial contamination of the intestine did not account for the phenacetin metabolism observed. The oxidative metabolism of phenacetin and 3,4-benzpyrene are both catalyzed by an enzyme system in the wall of the small intestine (*3–5, 7*), and the metabolism of each compound is stimulated by polycyclic hydrocarbons (*3–5, 7, 8*) and cigarette smoke (*4, 5, 9*). Charcoal-broiled beef, which contains 3,4-benzpyrene and other polycyclic hydrocarbons (*10*), has been shown to stimulate the metabolism of 3,4-benzpyrene by rat liver and placenta (*11*). Several crude, commercial animal diets, and a number of vegetables, including Brussels sprouts, cabbage, turnips, broccoli, cauliflower, and spinach, enhance the metabolism of 3,4-benzpyrene by rat intestine (*12*). It would be of interest to determine whether these foods enhance the metabolism of phenacetin or other drugs in man.

Table 1. Effect of a rat chow diet on the metabolism of phenacetin by intestine in vitro in the rat. Adult male Long-Evans rats were fed Purina rat chow or a nutritionally complete semisynthetic diet for 7 days. Phenacetin *O*-dealkylation by the proximal 5 cm of small intestine was determined. Each value represents the total amount of *N*-acetyl-*p*-aminophenol in tissue plus incubation solution and is the mean ± standard error for four rats. Results were analyzed statistically by Student's *t*-test.

Diet	*N*-Acetyl-*p*-aminophenol formed (micrograms per 5 cm of intestine per 90 minutes)
Semisynthetic	1.97 ± 0.25
Rat chow	5.99 ± 0.45
	$P < .001$

Table 2. Effect of charcoal-broiled ground beef on the metabolism of phenacetin by intestine in vitro in the rat. Adult male Long-Evans rats were fed for 7 days a nutritionally complete semisynthetic diet, or a 3 : 1 ratio of beef in semisynthetic diet. Phenacetin *O*-dealkylation by the proximal 5 cm of small intestine was determined. Each value represents the total amount of *N*-acetyl-*p*-aminophenol in tissue plus incubation solution and is the mean ± standard error for three rats. Results were analyzed statistically by Student's *t*-test.

Diet	*N*-Acetyl-*p*-aminophenol formed (micrograms per 5 cm of intestine per 90 minutes)
Semisynthetic	1.35 ± 0.17
Ground beef (raw)	2.77 ± 0.40*
Ground beef (cooked on foil)	1.97 ± 0.38†
Ground beef (charcoal-broiled)	15.88 ± 4.11‡

* Significantly different from control ($P < .05$).
† Not significantly different from control ($P > .05$). ‡ Significantly different from control ($P < .05$), from raw ground beef ($P < .05$), and from ground beef cooked on foil ($P < .05$).

Considerable variability exists in the responsiveness of man to a wide variety of drugs. This variability in drug action is caused, in part, by genetic differences in rates of drug metabolism in different individuals (*13*), as well as by the effects of environmental chemicals, such as pesticides and cigarette smoke, on the metabolism of drugs (*3, 14*). Some drugs, such as chlorpromazine (*15*), L-dopa (*16*), and phenacetin (*3–5*), are metabolized by enzymes in the gastrointestinal tract, and factors which influence the activity of these enzymes can influence the amount of unmetabolized drug that reaches the systemic bloodstream after an oral dose is given. Accordingly, administration of a decarboxylase inhibitor increases the blood level of L-dopa (*17*), and cigarette smoking lowers the blood levels of phenacetin (*2, 3*), at least in part, by altering the gastrointestinal metabolism of these drugs. The results reported here indicate that charcoal-broiled beef and a laboratory chow diet stimulate the intestinal metabolism of phenacetin in the rat. Additional studies are needed to determine whether various foods can influence the intestinal metabolism of phenacetin and other drugs in man and to determine the effects of changes in intestinal metabolism on the bioavailability of drugs.

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Ventral Medial Hypothalamus: Involvement in Hypoglycemic Convulsions

Abstract. *After the ventral medial hypothalamus of mice was lesioned with gold thioglucose, the dose of insulin required to produce convulsions in 50 percent of the animals was doubled compared to that in nonlesionad controls. No dose of insulin, up to 50 milliunits per gram, produced convulsions in more than 60 percent of the lesioned mice, even though blood glucose levels fell to approximately 24 milligram percent.*

The ventral medial hypothalamus (VMH) is thought to be a site of glucose receptors in the brain (*1*). Gold thioglucose (GTG) injected intraperitoneally into mice results in lesions of the VMH (*2*) and consequent hyperphagia leading to obesity. Other gold thio compounds such as gold sodium thiomalate (GTM) do not produce such lesions (*3*). The extent of hypothalamic lesions produced by a given dose of GTG appears to be directly related to the rate of glucose utilization at the time of injection (*4*). Mice that have been made diabetic before GTG injection do not develop hypothalamic lesions (*5*); however, intravenous (*6*) or intrahypothalamic (*7*) injections of insulin have been found to restore the sensitivity of the VMH to lesion by GTG. The mechanism by which the intrahypothalamic injection of insulin restores GTG sensitivity to diabetic mice is not fully understood. It suggests a local effect of insulin in the brain, a concept which is at odds with the generally accepted idea that glucose utilization by brain is not influenced by insulin (*8*).

Severe hypoglycemia following insulin administration causes convulsions. Since it has been postulated that the VMH may sense glucose levels (*9*), we investigated a possible role of the VMH in initiating the behavioral response (convulsion) to hypoglycemia.

Young adult female CBA/J mice (Jackson Laboratories) weighing 18 to 22 g were divided randomly into three groups and housed in groups of ten. The mice were fasted for 24 hours before being given a single intraperitoneal injection of either GTG (0.4 mg per gram of body weight), GTM (0.4 mg/g), or saline (*10*). The fast was continued for an additional 24 hours following injection and then the mice were allowed free access to food. No mice died during the 30 days following injection and 76 percent of the mice injected with GTG attained a weight 2 standard deviations greater than the mean weight of the control groups during the 73-day period following injection. A typical lesion produced by this dose of GTG under the conditions described is shown in Fig. 1 (*11*).

Weights were recorded periodically, and 73 days after treatment a series of insulin injections was begun. At that time the average fasting weight was 21 percent greater for GTG-treated mice than for control groups treated with either GTM or saline ($P < .01$; Student's t-test). The mice were fasted for 18 hours before each late morning insulin injection (*12*). After each injection the mice were observed for 70 minutes in their home cages with no food available. A convulsion was scored

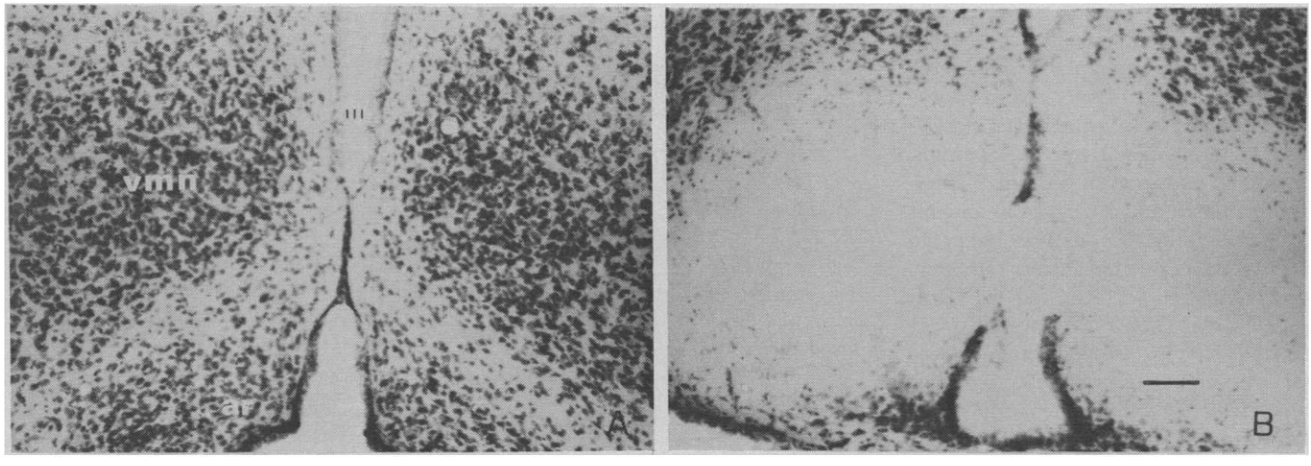

Fig. 1. Transverse sections 40 μm thick through the hypothalamus of CBA mice: (A) control and (B) GTG treated. The structures identified are: *vmn*, ventromedial nucleus; *ar*, arcuate nucleus; and *III*, third ventricle. Scale bar, 100 μm; magnification, $\times 78$.