previous reports of neurotoxin molecular weights.[5–7] Clearly, botulinum toxin and botulinum neurotoxin, both kept for short times in pH 6·2, 0·9% NaCl, are two distinct molecular species.

The data presented here show that type A botulinum toxin does not bind to synaptosomal membranes. This finding is in accord with previous studies which found that the toxin does not bind to homogenates of whole brain,*,† but stands in contrast to studies which found that the toxin does bind to peripheral nerves.[4, 10] In light of our data, it may be questioned whether the observation that type A botulinum toxin does not interfere with calcium uptake by synaptosomes is germane to the pathophysiology of botulism. Unless data are presented to show CNS binding of the toxin, the fact that it does not impede a process can be given no interpretation. In any event, previous data have militated against a competitive antagonism of calcium as a mechanism of type A botulinum toxin action.[11]

The finding that the neurotoxic component of botulinum toxin does lose potency after incubation with synaptosomal membranes does not necessarily indicate that specific binding has occurred. Either nonspecific binding or inactivation due to biochemical reactions may be responsible. Additional studies are needed to resolve this matter.

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* L. L. Simpson, unpublished data.
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Stimulatory effect of charcoal-broiled ground beef on the hydroxylation of 3,4-benzpyrene by enzymes in rat liver and placenta*†

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YAMAGIWA and Ichikawa[1] demonstrated that coal tar possesses carcinogenic activity in laboratory animals. In 1933, a pure carcinogenic hydrocarbon was isolated from coal tar pitch and identified as 3,4-benzpyrene (BP).[2] This polycyclic, aromatic hydrocarbon is found in certain smoked and cooked

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foods,[3–6] polluted city air,[7,8] the soil,[8] tobacco smoke,[9–12] and in tars, mineral oil, mineral pitches and soots.[13,14]

BP is a stimulator of many drug metabolizing enzymes.[15,16] The administration of BP induces several-fold increases in BP-hydroxylase activity in liver, lung, gastrointestinal tract, skin and kidney. It has been demonstrated by Welch *et al.*[17] that compounds present in cigarette smoke can induce an enzyme in human tissues capable of metabolizing the carcinogen BP. In addition, orally administered BP can serve as a potent inducer of BP hydroxylase in rat placenta. Since Lijinsky and Shubik[18] extracted BP and many other polynuclear hydrocarbons from charcoal broiled steaks, the question arises whether hydrocarbons consumed while eating charcoal broiled foods can stimulate the drug metabolizing enzymes in the liver and the placenta of rats.

Female, pregnant and nonpregnant, Sprague–Dawley rats weighing 245–305 g were used in these experiments. Pregnant rats were received on the fifth day of gestation. Both the pregnant and non-pregnant rats were fed a basal diet for 7 days before being fed ground beef for 7 days. The basal diet was low in polycyclic hydrocarbon content and consisted either of evaporated milk mixed with equal parts of water or of pellets made from a synthetic diet containing 22% casein, 0·5% *l*-cysteine, 5% corn oil, 4·5% salt mixture, 68% glucose and high levels of all known vitamins.[16]

The ground beef fed to the experimental animals was subjected to four different methods of cooking, as follows:

1. Ground beef samples approximately 3 in. in diameter and 0·25 in. in thickness were exposed to burning charcoal at a distance of 2·5 in. for 15 min on each side.
2. Ground beef samples were treated the same way as described above, but were protected during the exposure to charcoal by a layer of aluminum foil.
3. Ground beef samples of the same size which had been exposed to the method of cooking known as "Char-Broiling", i.e. exposure to heated stones rather than charcoal, were obtained from local chain restaurants.
4. Ground beef samples of the same size which had been cooked by pan frying were obtained from local chain restaurants.

The rats given the commercially cooked ground beef were given the evaporated milk diet described above, while the animals receiving the laboratory-cooked ground beef were given the pellets basal diet. In all of the feeding experiments, adequate control animals were run with each experimental group of animals.

Rats were decapitated and their livers and placentae removed. A five per cent liver homogenate and 10 per cent placental homogenate were prepared in ice-cold 0·25 M sucrose solution. The assay procedure used to determine the amount of 8-hydroxy-3,4-benzpyrene (8-OH-BP) formed was described previously by Welch *et al.*[17,19] Authentic 8-OH-BP* controls in sodium hydroxide solution were run with each experiment and found to have spectra almost identical with the assayed BP metabolites formed by the placenta and liver homogenate samples. The amount of 8-OH-BP metabolized was calculated as the difference between the amounts extracted from the zero-time flasks and the incubated flasks. The rate of hydroxylation of BP was expressed as nanograms of 8-OH-BP formed per gram of tissue per hr (ng/g/hr).

There was no significant difference between the amount of 8-OH-BP formed in placental homogenates from rats which were fed pan fried ground beef and rats fed ground beef commercially prepared on a grill over heated stones (0·9 $\pm$ 0·2 and 1·4 $\pm$ 0·6 ng/g/hr respectively).

Data from animals fed ground beef which was cooked in our laboratory are shown in Tables 1 and 2. For both liver and placental homogenates there was a significant difference between the animals receiving ground beef which was protected from the charcoal flame by aluminum foil and those animals which were fed broiled ground beef unprotected ($P \leqslant 0{\cdot}05$). It would appear from these data that when the beef is protected from the charcoal flame by aluminum foil, or prepared commercially by broiling over heated stones, there is no induction of drug metabolizing enzymes when the beef is subsequently fed to rats; such induction does occur if the beef has been subjected to charcoal broiling without protection by aluminum foil.

The data obtained by Lijinsky and Shubik,[18] quantitating the amount of polycyclic hydrocarbons in steaks which were charcoal broiled, suggest that the increase in benzpyrene hydroxylase activity may be related to the hydrocarbons deposited on the ground beef. These authors indicate that the most important source of the polycyclic hydrocarbons is the melted fat which drips on the hot coals and is thus subjected to pyrolysis. The hydrocarbons in the smoke are then deposited on the meat as the smoke rises. It is possible that broiling over heated stones, a commercial process used in many chain restaurants, does not provide a catalytic surface on which pyrolysis can take place.

Our data show that the pyrolysis products deposited on ground beef cooked over an open charcoal flame induce an enzyme in rats capable of metabolizing the hydrocarbon BP. On the other hand, the

* A sample of 8-OH-BP was given to us by Drs. R. M. Welch and A. H. Conney of the Wellcome Research Laboratories, Burroughs Wellcome & Co. (U.S.A.) Inc., Tuckahoe, N.Y.

TABLE 1. EFFECT OF FEEDING WITH CHARCOAL-BROILED GROUND BEEF ON THE FORMATION OF 8-HYDROXY-3,4-BENZPYRENE BY RAT PLACENTA

Exp.	No. of animals	Ground beef*	8-OH-BP formed (ng/g/hr ± S.E.)	P†
I	3	C	2·5 ± 1·0	<0·05
	3	T	15·0 ± 3·8	
II	3	C	7·5 ± 0·0	<0·05
	3	T	22·0 ± 4·4	
III	3	C	3·45 ± 0·6	<0·05
	3	T	9·15 ± 2·1	
IV	3	C	4·65 ± 0·4	<0·01
	3	T	26·25 ± 0·5	

* C = Ground beef cooked over charcoal and protected from flame with aluminum foil. T = Ground beef cooked over charcoal unprotected from flame.
† Student's *t*-test for ungrouped data.

TABLE 2. EFFECT OF FEEDING WITH CHARCOAL-BROILED GROUND BEEF ON THE FORMATION OF 8-HYDROXY-3,4-BENZPYRENE BY RAT LIVER

Exp.	No. of animals	Ground beef*	8-OH-BP (ng/g/hr ± S.E.)	P†
I	6	C	470·0 ± 85·3	<0·01
	6	T	767·5 ± 86·1	
II	6	C	352·4 ± 58·0	<0·05
	6	T	977·5 ± 272·3	
III	6	C	103·8 ± 13·1	<0·05
	6	T	163·0 ± 20·8	
IV	5	C	180·3 ± 11·2	<0·01
	5	T	286·8 ± 22·2	
V	3	C	463·0 ± 66·1	<0·01
	3	T	1160·0 ± 100·4	

* C = Ground beef cooked over charcoal and protected from flame with aluminum foil. T = Ground beef cooked over charcoal unprotected from flame.
† Student's *t*-test for ungrouped data.

pyrolysis products deposited on the commercially prepared ground beef cooked over heated stones did not produce this effect. This induction of BP hydroxylase by the pyrolysis products found on the charcoal-broiled ground beef occurs within 7 days.

If these results can be extrapolated to man, it is possible that, since the products of BP metabolism are less carcinogenic than the parent compound,[13] persons with elevated levels of BP hydroxylase in liver may be less susceptible to the carcinogenic action of polycyclic hydrocarbons ingested from the meat cooked directly over the charcoal flame, whereas those persons with low levels may be more susceptible. Similarly, fetuses whose maternal placenta have high BP-hydroxylase activity may be less affected by environmental carcinogens than those whose maternal placentae have low BP hydroxylase activity. Our observations can be compared to studies done by Welch *et al.*[17,19] These authors reported that smoking an average of 19 cigarettes per day during pregnancy significantly increased the

BP-hydroxylase activity in human placenta. However, it is not known whether or not the level of these hydrocarbons in cigarette smoke is great enough to result in deleterious effects in man.

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Dealkylation of tetraethyllead in the homogenates of rat and rabbit tissues

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TETRAETHYLLEAD (Et_4Pb) is dealkylated *in vivo* and the known metabolites are triethyllead (Et_3Pb^+) and inorganic lead in rat,[1,2] rabbit[3] and man.[4] Triethyllead is stable in the rat[2] and in man,[4] whereas in the rabbit[3] dealkylation proceeds progressively to give inorganic lead. Toxic symptoms following administration of tetraethyllead result from *in vivo* formation of triethyllead.[1]

Tetraethyllead is converted to triethyllead in rat liver microsomes *in vitro*.[1] The reaction requires NADPH and oxygen; it is inhibited by anaerobic conditions or SKF 525-A (diethylamino-ethanol ester of diphenylpropylacetic acid).

In this work the ability of some tissues of rat and rabbit to convert Et_4Pb to Et_3Pb^+ was investigated and the influence of age and sex of rats on this conversion has been studied.

Material and methods

Experiments were carried out on Wistar rats and Chinchilla rabbits of known age. The animals were bred in standard laboratory conditions and fed with commercial chow "Bacutil".

Animals were killed by decapitation and, in the adult animals, organs of each individual were analyzed separately. In the case of newborn, 7- and 14-day-old rats, the livers from whole litters were tested jointly.