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.

The immediately prepared organs were weighed and transfered to ice-cold medium composed of 0·25 M sucrose, 0·05 M nicotinamide and 0·01 M sodium phosphate buffer pH 7·0. Five % (w/v) homogenates were prepared in a Potter–Elvehjem homogenizer and squeezed by one layer of nylon bolting cloth (pores of 200 μ in diameter).

The incubation mixture (6 ml), slightly modified as compared with that of Cremer[1] consisted of: 200 mg of tissue, sodium phosphate buffer 0·016 M pH 7·0, $MgSO_4$ 0·018 M, NADP 0·00014 M, nicotinamide 0·033 M, and tetraethyllead 0·0007 M. It was poured into glass-stoppered 100 ml conical flask, and incubated at 37° for 30 min with shaking. The appropriate blanks (without tissue homogenate) were analyzed at the same time.

Triethyllead was determined using the method of Bolanowska.[5] The incubation media (6 ml) were diluted to 50 ml with 30% aqueous NaCl and extracted with benzene. Triethyllead was subsequently re-extracted from benzene using lead-free water and digested in concentrated sulfuric and nitric acids. Finally, lead was determined with dithizone according to Dutkiewicz *et al.*[6] The rate of conversion of tetraethyllead was expressed as μmoles of originated triethyllead per 100 mg of homogenate protein per hour.

The inorganic lead was isolated by precipitation as lead oxalate with subsequent digestion and determination with dithizone.[2] Protein was determined with Folin phenol reagent[7] using bovine serum albumine as standard.

TABLE 1. DEALKYLATION OF TETRAETHYLLEAD TO TRIETHYLLEAD *in vitro*

		μmoles Et_3Pb*/100 mg protein/hr			
		Liver		Kidney	Brain
Animal	Sex	Young†	Adult‡	Adult	Adult
Rat	Female	1·92 ± 0·74(8)	3·40 ± 0·35(5)	—	—
	Male	3·30 ± 1·45(8)	3·53 ± 0·76(9)	1·15 ± 0·31(5)	0·90 ± 0·52(5)
Rabbit	Male	—	3·78 ± 0·87(5)	0·94 ± 0·46(5)	0·67 ± 0·10(5)

* The values given are means ± S.D. with the number of animals used in parentheses.
† 1–2 months old.
‡ 6-month-old rats and 8-month-old rabbits.

Results and discussion

In both rats and rabbits the conversion of tetraethyllead into triethyllead occurred with the highest rate in the liver homogenates (Table 1). This conversion rate, found *in vitro* was 10-fold higher as compared with that reported by Cremer.[1] It seems probable that the difference in question was connected with some modifications in the way of homogenizing of the tissues as well as in the composition of the incubation medium. Moreover, due to the improved sensitivity of the discussed reaction, we were able to show that the conversion of tetraethyllead into triethyllead occurred not only in the liver, as suggested by Cremer,[1] but also in the homogenates of kidney and brain, both in rabbits and in rats. The conversion rate in these organs, however, was much lower as compared with the liver (Table 1). In the remaining tissues that were tested (blood, spleen, muscles) no evidence of this process could be stated.

The occurrence of metabolic conversion of tetraethyllead in the brain was unexpected since it is believed that the brain does not contain enzymatic systems metabolizing foreign compounds. The only reaction reported hitherto to occur in brain was the conversion of thiobarbiturates,[8] not mentioned, however, in the later review by Gillette.[9] Not having additional data to explain this fact the authors would like to emphasize, however, that the amounts of triethyllead found *in vitro* in the brain tissue homogenate greatly exceeded traces which would have been impossible to interpret with certainty and that the results do not represent merely an methodological artifact.

The influence of the sex of animals on the conversion rate of tetraethyllead was tested only in the liver homogenates. The results presented in Table 1 and Fig. 1 show that differences due to sex could be found only in young animals (1–2 months old), these, however, were statistically significant.

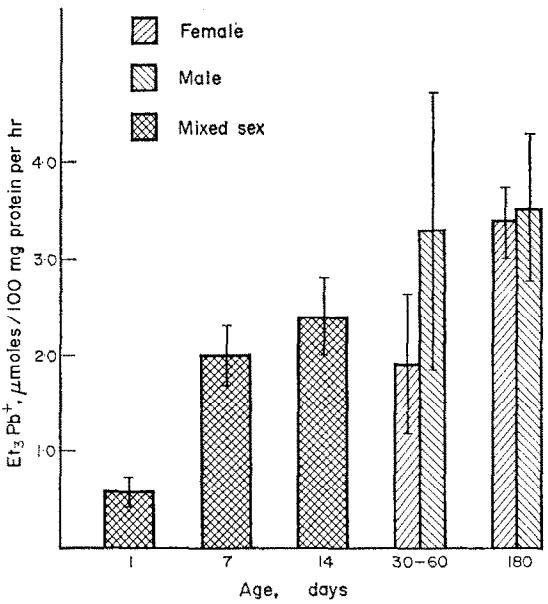

FIG. 1. Dealkylation *in vitro* of tetraethyllead to triethyllead in liver homogenates as dependent on age of rats. (Mean ± S.D.)

The influence of the age of rats on the rate of tetraethyllead dealkylation was tested again using the liver homogenates. From the data shown in Table 1 it follows that in the newborn rats the rate of dealkylation is much lower as compared with the adult animals and, that only after more than 1 week the ability to dealkylate tetraethyllead approaches the values found in adult animals. It seems also that in the females the mechanism in question is being developed more slowly than in males (Table 1 and Fig. 1).

In the present investigations done *in vitro* no ionic lead could be found in either of the samples examined. Our previous studies performed on rats *in vivo* had pointed to the existence of a parallel metabolic path leading from tetraethyllead to ionic lead in a way entirely independent from the dealkylation to triethyllead.[2] Whether or not this process may be reproduced also *in vitro* seems to be obscure at present.

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