suggesting that the cationic head of acetylcholine may bind to the active site by nonpolar interactions rather than by ionic or polar forces. If such is the case, there may not be any nucleophilic group capable of being alkylated at this position on the enzyme.

Present results do not exclude the possibility that alkylation of the enzyme by the mustard could be occurring at sites other than the acetylcholine binding site. Alkylation by the mustard at the active site or at other sites would tend to lower the velocity of its hydrolysis. This was observed both in the experiments designed to provide kinetic constants (Table I) and in a separate series where each substrate and equimolar concentrations of each substrate were assayed (Fig. 1).

In summary, results of the present study reveal that acetylcholine is hydrolyzed at a significantly faster rate than the mustard, even though their affinities for the enzyme appear to be comparable. In the light of previous results obtained in cholinergic receptor systems, which demonstrated alkylation of a portion of receptors (3), it is possible that the mustard is capable of covalent bond formation with, and subsequent inactivation of, acetylcholinesterase. Due to rapid turnover of enzyme–substrate complexes, conditions for alkylation may not be favorable. In any case, it appears likely that alkylation and enzyme inactivation are occurring during hydrolysis of acetylcholine mustard by acetylcholinesterase. Hydrolysis of mixtures of the substrates would be expected to yield mixed-type inhibition.

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Effects of Nitrogen Dioxide and 3-Methylcholanthrene on Pulmonary Enzymes

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Abstract □ Guinea pig lung phenol-*O*-methyltransferase, catechol-*O*-methyltransferase, and benzpyrene hydroxylase activities were examined after nitrogen dioxide and 3-methylcholanthrene treatment. While benzpyrene hydroxylase activity was enhanced by 3-methylcholanthrene, none of the pulmonary enzyme activities was altered after exposure to either 40 or 70 ppm of nitrogen dioxide for 2 hr.

Keyphrases □ Nitrogen dioxide—effects on guinea pig lung phenol-*O*-methyltransferase, catechol-*O*-methyltransferases, and benzpyrene hydroxylase □ 3-Methylcholanthrene—effects on guinea pig lung enzymes □ Enzymes, pulmonary—effects of nitrogen dioxide and 3-methylcholanthrene

A previous report (1) from this laboratory demonstrated that guinea pig lung contains appreciable phenol-*O*-methyltransferase and catechol-*O*-methyltransferases. Furthermore, catechol-*O*-methyltransferases were present both in the supernatant and the microsomal fractions. The presence of these enzymes also has been indicated in rat lungs (2, 3). In addition, benzpyrene hydroxylase activity has been reported in rat lung (4) and is inducible by a wide range of chemicals including cigarette smoke (5), 3-methylcholanthrene (4, 6), flavones (7), and *Cannabis* (6).

The question was raised as to the influence of air pollutants on the primary pulmonary enzyme identi-

Table I—Pulmonary Enzyme Activities after 3-Methylcholanthrene Administration

Lung Enzymes[a]	Untreated Animals	3-Methylcholanthrene-Treated Animals
Benzpyrene hydroxylase	0.22 ± 0.19 (6)[b]	0.43 ± 0.26 (6)[c]
Microsomal phenol-*O*-methyltransferase	6.4 ± 0.72 (3)	7.2 ± 0.92 (3)
Supernatant catechol-*O*-methyltransferase	22.7 ± 1.4 (3)	22.9 ± 0.55 (3)
Microsomal catechol-*O*-methyltransferase	3.0 ± 0.17 (3)	3.6 ± 0.76 (3)

[a] Transmethylation activities are expressed as nanomoles of O-methylated substrates per gram of tissue; hydroxylation activities are expressed as fluorescence units per gram of tissue. [b] Values are given as means ± *SE*, with the number of animals used in parentheses. [c] Values are significantly different ($p < 0.1$) from untreated animals.

Table II—Pulmonary Enzyme Activities after Nitrogen Dioxide Exposure

Test Group	Benzpyrene Hydroxylase[a]	Microsomal Phenol-*O*-methyltransferase[b]	Supernatant Catechol-*O*-methyltransferase[b]	Microsomal Catechol-*O*-methyltransferase[b]
Control	0.27 ± 0.18 (7)[c]	5.0 ± 0.6 (2)	22.4 ± 0.9 (2)	1.7 ± 0.2
versus				
40 ppm nitrogen dioxide	0.21 ± 0.13 (8)	4.8 ± 1.0 (4)	20.2 ± 0.48 (4)	1.7 ± 0.18 (4)
Control	0.33 ± 0.05 (2)	6.0 ± 0.5 (2)	29.2 ± 1.1 (2)	2.6 ± 0.4 (2)
versus				
70 ppm nitrogen dioxide	0.32 ± 0.028 (4)	6.4 ± 0.92 (4)	29.2 ± 0.99 (4)	2.8 ± 0.46 (4)

[a] Hydroxylation activities are expressed as fluorescence units per gram of tissue. [b] Transmethylation activities are expressed as nanomoles of O-methylated product per gram of tissue. [c] Values are given as means ± *SE*, with the number of animals used in parentheses.

fied to date. Thus, the effects on benzpyrene hydroxylase, phenol-*O*-methyltransferase, and catechol-*O*-methyltransferases were studied in guinea pigs after treatment with nitrogen dioxide by inhalation. Also included in this report, as a control, are the effects of 3-methylcholanthrene subcutaneous injection upon the same lung enzyme systems in guinea pigs.

EXPERIMENTAL

Guinea pigs, 300–500 g, of either sex were used. 3-Methylcholanthrene, dissolved in arachis oil, was administered at a dose of 20 mg/kg ip. Control animals were injected with arachis oil only. Exposures of animals to nitrogen dioxide at 40- and 70-ppm levels for 2 hr were carried out in a dynamic exposure chamber as described previously (8). Chamber concentrations were controlled by flowmeters which were checked periodically during the exposure.

At 24 hr after 3-methylcholanthrene or nitrogen dioxide treatment, the animals were sacrificed. The lungs were removed, rinsed, blotted, and weighed. Preparation of subcellular fractions and assays of *O*-methyltransferase enzyme activities were carried out as previously described (1). The substrates employed were phenol for phenol-*O*-methyltransferase and isoproterenol for catechol-*O*-methyltransferase activities. Benzpyrene was the substrate for the determination of hydroxylase activity using the procedure of Wattenberg *et al.* (9).

The Student *t* test and the U test (10) were used for statistical comparisons. Results confined to the same day and with the same animal shipment were used in these statistical analyses.

RESULTS AND DISCUSSION

3-Methylcholanthrene administration increased guinea pig lung benzpyrene hydroxylase activity about twofold. However, neither microsomal phenol-*O*-methyltransferase nor microsomal and supernatant catechol-*O*-methyltransferase activities were affected (Table I). During these experiments, the basal levels of benzpyrene hydroxylase varied considerably with different shipments of guinea pigs from the supplier. These results are consistent with the findings (4, 6) that pulmonary benzpyrene hydroxylase activity varies with rats and is inducible by 3-methylcholanthrene.

The LD_{50} of nitrogen dioxide exposure for 2–4 hr to guinea pigs was reported to be about 75 ppm (11). The concentrations of 40 and 70 ppm of nitrogen dioxide used in this study were chosen to study the effects of maximally tolerated nitrogen dioxide levels. Lung weights and enzyme activities were not significantly different from experiments carried out on the same day and with the same animal shipment. Table II shows lung enzyme activities from several experiments after nitrogen dioxide treatments. There is no evidence from these studies that nitrogen dioxide affects pulmonary phenol-*O*-methyltransferase, catechol-*O*-methyltransferase, and benzpyrene hydroxylase activities after short-term exposure to nitrogen dioxide even at levels as high as 70 ppm[1].

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[1] M. S. Palmer, R. W. Exley, and D. L. Coffin [*Arch. Environ. Health*, **25**, 439(1972)] reported a similar lack of effect with nitrogen dioxide on benzpyrene hydroxylase activity from rat lung.