benzylpenicillin and cephalosporin N, K_i/K_m was about 2.9. The quotient (Michaelis constant for cephalosporin N/Michaelis constant for benzylpenicillin) was therefore close to 1.0. A similar value for this quotient was deduced from the total rate of hydrolysis of mixtures of the two substrates.

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The Enzymic Cleavage of Aromatic Ethers

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A major route of the metabolism of many aromatic ethers involves cleavage to yield phenolic metabolites. For example, *p*-nitrophenyl ethers (Huggins, Jensen & Cleveland, 1948), phenacetin and *p*-phenetidine (Brodie & Axelrod, 1949; Smith & Williams, 1949), codeine (Adler & Latham, 1950) and a variety of nuclear-substituted anisoles (Bray, Craddock & Thorpe, 1955) are converted into their corresponding phenols in the body. Although much information is now available concerning the metabolism *in vivo* of aromatic ethers, little is known about the cellular mechanism involved in their metabolism.

A previous study has shown that codeine is converted into morphine by an enzyme system localized in the microsomes of the liver (Axelrod, 1955c). The present investigation is concerned with further studies on the intracellular processes involved in the enzymic cleavage of aromatic ethers. It will be shown that aromatic ethers are converted into phenols and aldehydes by enzyme systems, localized in microsomes of the liver, which require both

reduced triphosphopyridine nucleotide and oxygen. Evidence for the presence of more than one ether-cleaving enzyme will be presented. The relationship between the structures of ethers and their rate of enzymic cleavage will also be described.

MATERIALS AND METHODS

Materials. Triphosphopyridine nucleotide (TPN⁺) of 80% purity, diphosphopyridine nucleotide (DPN⁺) and glucose 6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. Reduced triphosphopyridine nucleotide (TPNH) was prepared by the procedure of Kaplan, Colowick & Neufeld (1952). Methyl and ethyl ethers were obtained from the Eastman Chemical Co.

Analytical methods. *p*-Acetamidophenol was hydrolysed to *p*-aminophenol by heating the biological material in *N*-HCl. *p*-Aminophenol was extracted into peroxide-free diethyl ether at pH 7, and then returned to 0.1 *N*-HCl. The compound was then determined by coupling with phenol in the presence of sodium hypobromite to form an indophenol dye (Brodie & Axelrod, 1948).

p-Ethoxy- and *p*-methoxy-acetanilide were hydrolysed to the corresponding amino compounds by heating the

biological material in *N*-HCl. *p*-Etoxy- and *p*-methoxy-aniline were extracted into benzene at pH 13, and then returned to 0.1 *N*-HCl. The compounds were determined by diazotization and coupling with α -naphthol (Brodie & Axelrod, 1949).

Formaldehyde was distilled from trichloroacetic acid extracts (Axelrod, 1955*b*) and determined by the chromotropic acid procedure of MacFayden (1945). Acetaldehyde was determined by the procedure of Stotz (1943).

Fractionation of tissue homogenates. The preparation of all tissue samples was carried out at 0–3°. Young adult male animals were stunned and exsanguinated. The liver was immediately removed and homogenized with 2 vol. of 0.05*M* potassium phosphate buffer, pH 7.3, with a Potter-Elvehjem type of homogenizer. The homogenate was centrifuged at 9000 *g* for 15 min. to remove unbroken cells, nuclei and mitochondria. The supernatant phase, consisting of the microsomes plus the soluble fraction, could be stored for at least 7 days at –10° without appreciable loss of activity.

Nuclei, mitochondria and microsomes were prepared by differentially centrifuging a 12% homogenate in isotonic sucrose (Schneider, 1948). The particulate fractions were washed twice with isotonic KCl and resuspended in 0.05*M* phosphate buffer to a concentration 3 times that of the original homogenate. The soluble fraction was prepared by centrifuging a 12% homogenate in isotonic KCl at 78000 *g* for 30 min. and dialysed against 0.01*M* phosphate buffer, pH 7.4, for 20 hr.

Measurement of enzyme activity. When *p*-ethoxy- or *p*-methoxy-acetanilide was used as a substrate, enzyme activity was measured as follows. Tissue fractions obtained from 200 mg. of liver, 2 μ moles of substrate, 50 μ moles of nicotinamide, 25 μ moles of $MgCl_2$, 0.2 μ mole of TPN^+ , 0.5 ml. of 0.2*M* phosphate buffer, pH 7.4, and water to make a final vol. of 5 ml., were placed in a 20 ml. beaker. The mixture was incubated in a Dubnoff shaker for 1 hr. at 37° in air. At the end of the incubation period, a 4 ml. sample of the reaction mixture was transferred to a 15 ml. test tube containing 0.8 ml. of 12*N*-HCl and assayed for *p*-acetamidophenol. Enzyme activity was expressed as μ moles of *p*-acetamidophenol formed/hr. These values were corrected for a small blank formed when *p*-ethoxyacetanilide was incubated without tissue. The enzymic cleavage of other methoxy ethers was determined by estimating the amount of formaldehyde formed in the reaction. The formaldehyde generated was trapped with semicarbazide. The enzymic conversion of codeine into morphine was measured by a procedure described previously (Axelrod, 1955*c*).

RESULTS

Enzymic cleavage of *p*-ethoxyacetanilide to *p*-acetamidophenol

Tissue distribution of enzyme activity. *p*-Etoxy-acetanilide (2 μ moles) was incubated with 200 mg. of minced rabbit liver, kidney, diaphragm, spleen or brain under conditions described in the Methods section. The liver metabolized 0.8 μ mole of *p*-ethoxyacetanilide to *p*-acetamidophenol in 1 hr., while other tissues failed to metabolize the compound.

Requirements and properties of the enzyme system. Since the combined microsomal plus soluble fraction of rabbit liver could convert *p*-ethoxyacetanilide into *p*-acetamidophenol almost as effectively as the whole homogenate, the properties of the enzyme system were studied in the former fraction. Maximal enzyme activity was obtained in the presence of TPN^+ , $MgCl_2$, nicotinamide and oxygen (Table 1). DPN^+ could not replace TPN^+ . With phosphate buffers, the optimum pH for enzyme activity was at 7.4.

The rate of enzymic conversion of *p*-ethoxyacetanilide into *p*-acetamidophenol is shown in Fig. 1. The initial rate of metabolism was calculated to be about 0.07 μ mole of *p*-ethoxyacetanilide cleaved/min./g. of liver. No further metabolism of the compound occurred after 220 min. The relationship between substrate concentration and rate of metabolism is shown in Fig. 2. At substrate concentrations of 2.4 μ moles or above, the rate of metabolism followed zero-order reaction kinetics.

Table 1. Requirements of the enzyme system

Microsome plus dialysed soluble fraction, obtained from 200 mg. of rabbit liver, were incubated for 1 hr. at 37° with 2 μ moles of *p*-ethoxyacetanilide, 0.2 μ mole of TPN^+ , 50 μ moles of nicotinamide, 25 μ moles of $MgCl_2$, 0.5 ml. of 0.2*M* phosphate buffer, pH 7.4; final vol., 5 ml.

Conditions	<i>p</i> -Acetamidophenol formed (μ moles)
Complete system	0.84
TPN^+ omitted	0.22
$MgCl_2$ omitted	0.80
Nicotinamide omitted	0.50
Oxygen omitted	0.04
DPN^+ substituted for TPN^+	0.28

Fig. 1. Rate of enzymic cleavage of *p*-ethoxyacetanilide. Eight beakers, each containing microsome plus soluble fraction obtained from 200 mg. of rabbit liver, were incubated at 37° with 2 μ moles of *p*-ethoxyacetanilide and cofactors as described under Methods.

Intracellular localization of enzyme activity. Rabbit liver was separated into nuclear, mitochondrial, microsomal and soluble fractions by differential centrifuging and each fraction was examined for its ability to cleave *p*-ethoxyacetanilide. Negligible enzyme activity was observed in each cellular fraction alone, but when the microsomal and soluble fractions were combined the activity was almost as great as in the whole homogenate (Table 2). From these observations, it was concluded that factors present in both the microsomes and the soluble fraction were necessary for the conversion of *p*-ethoxyacetanilide into *p*-acetamidophenol.

Fig. 2. Relationship between substrate concentration and rate of enzymic cleavage of *p*-ethoxyacetanilide. Six beakers, each containing microsome plus soluble fraction obtained from 200 mg. of rabbit liver, were incubated for 1 hr. at 37° with varying amounts of *p*-ethoxyacetanilide and cofactors, as described under Methods.

Table 2. *Intracellular localization of the enzyme system*

Intracellular fractions were prepared by differential centrifuging. Samples of the various fractions, equivalent to those contained in 200 mg. of rabbit liver, were incubated for 1 hr. at 37° with 2 μmoles of *p*-ethoxyacetanilide and cofactors as described under Methods.

Intracellular fraction	<i>p</i> -Acetamidophenol formed (μmoles)
Whole homogenate	1.3
Nuclei*	0.1
Mitochondria	0.0
Microsomes	0.0
Soluble fraction	0.0
Nuclei* + soluble fraction	0.2
Mitochondria + soluble fraction	0.1
Microsomes + soluble fraction	1.1

* This fraction contained unbroken cells and erythrocytes as well as nuclei.

In previous studies on the metabolism of amphetamine *in vitro* (Axelrod, 1954a, 1955a), it was shown that the deamination of this compound was mediated by an enzyme system in the microsomes which required the presence of TPNH. The requirement of the microsomal plus soluble fraction for TPN⁺ suggested that the ether-cleaving enzyme may also require TPNH. The TPN⁺-dependent dehydrogenases in the soluble fraction could serve to generate the reduced cofactor. When a TPNH-generating enzyme system consisting of glucose 6-phosphate, glucose 6-phosphate dehydrogenase and TPN⁺ was added to the microsomes (Table 3) these cofactors were found to be about half as effective as the soluble fraction. Similar results were obtained when chemically prepared TPNH was substituted for the TPNH-generating enzyme system. These observations suggested that the cleavage of *p*-ethoxyacetanilide was catalysed by enzyme systems in the microsomes which require TPNH as well as other cofactors present in the soluble fraction. No further stimulation of enzyme activity in the microsomes was observed when adenosine triphosphate, ascorbic acid, fumarate or glutathione was added along with TPNH.

Identification of reaction products. *p*-Acetamidophenol, formed in the enzymic cleavage of *p*-ethoxyacetanilide, was identified as follows. Microsomes and soluble fraction, obtained from 30 g. of rabbit liver, were incubated with 150 μmoles of *p*-ethoxyacetanilide, 10 μmoles of TPN⁺, 250 μmoles of nicotinamide, 125 μmoles of MgCl₂ and 10 ml. of 0.2M phosphate buffer, pH 7.4; total vol., 200 ml. The enzyme reaction was stopped by the addition of 0.1 vol. of 40% trichloroacetic acid. After centrifuging, the supernatant solution was saturated with NaCl and adjusted to pH 7.0 with solid K₂HPO₄; the *p*-acetamidophenol was extracted into 5 vol. of diethyl ether. *p*-Acetamidophenol was then returned to a small volume of 0.5N-NaOH. Any

Table 3. *Requirement for TPNH*

Microsomes obtained from 200 mg. of rabbit liver, 2 μmoles of *p*-ethoxyacetanilide, 25 μmoles of MgCl₂, 50 μmoles of nicotinamide, 0.5 ml. of 0.2M phosphate buffer, pH 7.4, added cofactors and water to make a final vol. of 6 ml., were incubated at 37° for 1 hr.

Cofactors added	<i>p</i> -Acetamidophenol formed (μmoles)
TPN ⁺ , 2 μmoles	0.00
TPN ⁺ , 0.5 μmole; glucose 6-phosphate, 20 μmoles; and 1 mg. of glucose 6-phosphate dehydrogenase	0.40
TPNH, 2 μmoles*	0.44
TPN ⁺ , 0.1 μmole and soluble fraction from 200 mg. of rabbit liver	0.76

* This cofactor was added in five portions over a period of 50 min., since TPNH disappeared rapidly even in the absence of substrate.

Table 4. *Balance study on the enzymic cleavage of p-methoxyacetanilide and p-ethoxyacetanilide*

Microsomes and soluble fraction, obtained from 1 g. of rabbit liver, were incubated at 37° for 1 hr. with 6 μ moles of substrate, 1 μ mole of TPN⁺, 25 μ moles of MgCl₂, 50 μ moles of nicotinamide, 200 μ moles of neutralized semicarbazide hydrochloride, 2 ml. of 0.2M phosphate buffer, pH 7.4; final vol., 12 ml.

Substrate	Substrate metabolized (μ moles)	<i>p</i> -Acetamidophenol formed (μ moles)	Formaldehyde formed (μ moles)	Acetaldehyde formed (μ moles)
<i>p</i> -Methoxyacetanilide	3.7	3.7	3.5	—
<i>p</i> -Methoxyacetanilide	4.2	3.9	3.8	—
<i>p</i> -Ethoxyacetanilide	3.2	3.3	—	3.2
<i>p</i> -Ethoxyacetanilide	2.7	2.2	—	2.3

residual *p*-ethoxyacetanilide was removed by shaking the alkaline extract with ether. The alkaline extract was then saturated with NaCl and adjusted to pH 7.0; the *p*-acetamidophenol was re-extracted into ether. The *p*-acetamidophenol in the ether extract was subjected to an eight-transfer Craig countercurrent distribution, with 1 vol. of M phosphate buffer, pH 6.0, and 2 vol. of ether as the solvent pair. The resulting distribution curve for the enzymically formed *p*-acetamidophenol was the same as that calculated for the authentic compound from the experimentally established partition ratio of the latter. The ultraviolet-absorption curve of the isolated *p*-acetamidophenol was also the same as that of the authentic compound. Further evidence for the identity of *p*-acetamidophenol was obtained by ascending paper chromatography with benzene-acetic acid-water (40:40:20, by vol.) as the solvent system (Bray, Clowes & Thorpe, 1952). The compound was detected by spraying with diazotized *p*-nitroaniline and Na₂CO₃ solution. Both the apparent and authentic *p*-acetamidophenol had the same *R_F* value (0.22).

A balance study of the reaction products formed in the enzymic cleavage of *p*-ethoxyacetanilide and *p*-methoxyacetanilide is shown in Table 4. Approximately 1 mole of *p*-acetamidophenol was formed for each mole of substrate metabolized. In addition, about 1 mole of the corresponding aldehyde was generated for each mole of *p*-acetamidophenol formed.

Enzyme activity in various species. The ability of the liver of a number of mammalian species to cleave *p*-ethoxyacetanilide was examined. All species showed considerable enzyme activity when a combined microsomal and soluble fraction of the liver was used. However, the whole liver homogenate of guinea pig, rat, mouse and dog showed little enzyme activity, and the whole rabbit-liver homogenate was as effective as the combined microsomal and soluble fraction (Table 5). These observations suggested the presence of inhibitors in the nuclei or the mitochondria, or both, of guinea pig, rat, mouse and dog liver. In order to investigate this possibility, the enzyme activity of guinea-pig microsomes plus soluble fraction was examined after the addition of nuclei or mitochondria. A marked depression in activity was observed in the presence of both (Table 6). The addition of rabbit

Table 5. *Species differences in the enzymic cleavage of p-ethoxyacetanilide*

Whole homogenate or microsomes plus soluble fraction, obtained from 200 mg. of liver, were incubated at 37° for 1 hr. with 2 μ moles of *p*-ethoxyacetanilide and cofactors as described under Methods.

	<i>p</i> -Acetamidophenol formed by	
	Whole homogenate (μ moles)	Microsomes plus soluble fraction (μ moles)
Rabbit	0.80	0.80
Guinea pig	0.18	0.84
Rat	0.10	0.36
Mouse	0.14	0.34
Dog	0.04	0.20

nuclei or mitochondria had no inhibitory action on the guinea-pig microsomal preparation, and guinea-pig nuclei or mitochondria depressed the activity in a rabbit-liver microsomal preparation. Similar inhibitory effects were observed with mouse, dog and rat nuclei or mitochondria. When the nuclei or mitochondria of the guinea pig were heated at 100° for 2 min. before addition to the combined microsomal and soluble fractions, no inhibition of activity was found. These observations suggested the presence in the nuclei and mitochondria of the guinea pig, mouse, rat and dog livers of heat-labile factors which inhibit the enzymic cleavage of *p*-ethoxyacetanilide.

Evidence for two ether-cleaving enzymes

Marked species differences were observed in the enzymic cleavage of *p*-ethoxyacetanilide and codeine by rabbit and guinea-pig microsomal preparations. Although both preparations metabolized *p*-ethoxyacetanilide equally well, the guinea-pig preparation converted codeine into morphine at about one-tenth of the rate of that from the rabbit liver (Table 7). These results suggested the presence of more than one ether-cleaving enzyme, and further evidence was obtained from inhibition studies. β -Diethylaminoethyl diphenylpropylacetate (SKF 525 A) (10⁻⁴ M) markedly inhibited the

Table 6. *Intracellular inhibitors in the enzymic cleavage of p-ethoxyacetanilide*

Intracellular fractions were prepared from liver by differential centrifuging. Various fractions, equivalent to those contained in 200 mg. of liver, were incubated at 37° for 1 hr. with 2 μ moles of *p*-ethoxyacetanilide and cofactors as described under Methods.

Intracellular fraction	<i>p</i> -Acetamidophenol formed	
	Guinea-pig liver (μ moles)	Rabbit liver (μ moles)
Whole homogenate	0.16	1.30
Microsomes + soluble fraction	1.14	1.26
Microsomes + soluble fraction + nuclei	0.28	1.26
Microsomes + soluble fraction + heated nuclei*	1.16	—
Microsomes + soluble fraction + mitochondria	0.70	1.36
Microsomes + soluble fraction + heated mitochondria*	1.16	—
Rabbit microsomes + soluble fraction + guinea-pig nuclei	—	0.52
Guinea-pig microsomes + soluble fraction + rabbit nuclei	1.30	—

* Preheated for 2 min. in a boiling-water bath.

Table 7. *Comparison of ether-cleaving enzymes*

Intracellular fractions, obtained from 200 mg. of liver, were incubated at 37° for 1 hr. with 1 μ mole of codeine or *p*-ethoxyacetanilide and cofactors as described under Methods. SKF 525 A = β -diethylaminoethyl diphenylpropylacetate.

Fractions and additions	Morphine formed (μ mole)	<i>p</i> -Acetamidophenol formed (μ mole)
Rabbit microsomes + soluble fraction	0.50	0.80
Guinea-pig microsomes + soluble fraction	0.04	0.84
Rabbit microsomes + soluble fraction + 1 μ mole of SKF 525 A	0.10	0.74
Rabbit microsomes + soluble fraction + 1 μ mole of <i>p</i> -ethoxyacetanilide + 20 μ moles of codeine	—	0.80
Rabbit microsomes + soluble fraction + 1 μ mole of <i>p</i> -ethoxyacetanilide + 20 μ moles of <i>p</i> -methoxybenzylamine	—	0.08
Rabbit microsomes + 3 μ moles of TPNH	0.75	0.42

Table 8. *Substrate specificity*

Microsomes plus soluble fraction, obtained from 200 mg. of rabbit liver, were incubated at 37° for 1 hr. with 2 μ moles of substrate, 100 μ moles of neutralized semicarbazide hydrochloride, and cofactors as described under Methods. When methyl ethers were used as substrates, the reaction mixture was analysed for formaldehyde formed.

Substrate	Relative activity (%)
<i>p</i> -Ethoxyacetanilide	100
<i>p</i> -Methoxyacetanilide	100
<i>o</i> -Methoxyacetanilide	14
<i>p</i> -Methoxybenzoic acid	56
<i>m</i> -Methoxybenzoic acid	22
<i>o</i> -Methoxybenzoic acid	3
<i>p</i> -Methoxybenzonitrile	200
<i>p</i> -Methoxybenzaldehyde	155
<i>p</i> -Methoxybenzyl alcohol	130
<i>p</i> -Methoxybenzylamine	50
<i>p</i> -Methoxypropenylbenzene	33
<i>p</i> -Ethoxyaniline	11
<i>p</i> -Methoxybenzene	6
3-Methoxypropylamine	0
Methoxyacetic acid	0
1:2-Dimethoxyethane	0
Codeine	50
Papaverine	42
Quinine	22
Colchicine	17
Mescaline	13
Atebrin	0

cleavage of codeine by rabbit-liver microsomal preparations, but had only a slight effect on the metabolism of *p*-ethoxyacetanilide. Furthermore, the addition of a 20-fold excess of codeine had no effect on the metabolism of *p*-ethoxyacetanilide, whereas *p*-methoxybenzylamine markedly inhibited the cleavage. In addition, TPNH was more effective than the soluble fraction plus TPN⁺ in the conversion of codeine into morphine by rabbit-liver microsomes, and the reduced cofactor was only half as effective when *p*-ethoxyacetanilide was used as the substrate.

Substrate specificity

The ability of the microsomal preparation to cleave a variety of methyl ethers was examined. From the results shown in Table 8 it appeared that aromatic ethers were extensively cleaved, and aliphatic ethers were not. The nature and position of the substituent on the aromatic nucleus exerted considerable influence on the rate of enzymic cleavage. The microsomal preparation also cleaved a number of methoxy-substituted drugs and naturally occurring compounds. In the absence of TPN⁺ and nicotinamide there was a marked reduction in metabolism of all of the substrates examined.

DISCUSSION

This investigation has shown that *p*-ethoxyacetanilide and other aromatic ethers are cleaved into their corresponding phenols and aldehydes by enzyme systems localized in the microsomes of the liver. The microsomal-enzyme preparation is unusual in that it requires both triphosphopyridine nucleotide and oxygen. The role of TPNH in an oxidative reaction is difficult to explain. This cofactor may serve to generate hydrogen peroxide through the transfer of its hydrogen, by means of an intermediate electron-transport system, to molecular oxygen. However, it was found that hydrogen peroxide generated from D-amino acid oxidase could not replace TPNH. The fact that TPNH could not completely replace the soluble fraction plus oxidized TPN⁺ suggests the need for additional cofactors.

A microsomal-enzyme system requiring TPNH and oxygen was first described for the deamination of amphetamine (Axelrod, 1954*a*, 1955*a*). It has been found subsequently that a wide variety of drugs and foreign organic compounds are metabolized by TPNH-dependent enzymes localized in the microsomes. Metabolic transformations catalysed by these enzyme systems include demethylation of sympathomimetic amines (Axelrod, 1954*b*, 1955*b*), aminopyrine and other alkylamines (La Du, Gaudette, Trousof & Brodie, 1955) and narcotic drugs (Axelrod, 1955*d*), hydroxylation of aromatic compounds (Mitoma & Udenfriend, 1955), side-chain oxidation of barbiturates (Cooper & Brodie, 1955), and the cleavage of ethers, described in this paper. In the case of ether cleavage, it appears that more than one enzyme system is involved.

It would seem unlikely that the microsomal-enzyme systems dependent on TPNH serve only to detoxify drugs and foreign organic compounds. Although the nature of their normal substrates remains to be elucidated, the enzymes are probably engaged in carrying out normal metabolic functions. The relative non-specificity of the substrates attacked by the microsomal enzymes would suggest that they could act as 'scavenger' enzymes and perform normal metabolic roles as well.

A definite relationship between the type of substitution on the nucleus of aromatic ethers and the rate of enzymic cleavage was found. The rate of metabolism of *p*-methoxyphenyl ethers decreased with the following sequence of substitutions: -CN, -CHO, -CH₂.OH, -NH.CO.CH₃, -CO₂H, -CH₂.NH₂, -CH:CH.CH₃, -NH₂, -H. No enzymic cleavage occurred with any of the aliphatic ethers examined. The position of substitution on the aromatic nucleus also had a decided influence on the rate of metabolism. For example, *para*-substituted ethers were cleaved more readily than the corresponding *meta* or *ortho* derivatives.

Heat-labile factors, which depressed the enzymic cleavage of aromatic ethers, were found to be present in liver nuclei and mitochondria of the guinea pig, rat, mouse and dog. On the other hand, these inhibitory factors were absent from rabbit-liver nuclei and mitochondria. The presence of TPNases may account for the inhibitory action of nuclei and mitochondria in some species. However, the addition of a 20-fold excess of TPN⁺ did not suppress the inhibitory effects of guinea-pig nuclei or mitochondria on the enzymic cleavage of ethers. The nature of the inhibitory factors is under further investigation.

SUMMARY

1. Enzyme systems are described which can cleave aromatic ethers to yield phenols and aldehydes.
2. The enzyme systems are located in the liver microsomes of a number of mammalian species and they require reduced triphosphopyridine nucleotide, oxygen and other cofactors.
3. Heat-labile factors which inhibit the enzymic cleavage of ethers have been shown to be present in liver nuclei and mitochondria of guinea pig, mouse, rat and dog, but not in the rabbit.
4. Evidence is given for the presence of more than one ether-cleaving enzyme.
5. Ethers substituted in the *para* position are cleaved more readily than the corresponding *meta* or *ortho*-substituted compounds.
6. The rate of metabolism decreases with the following sequence of substitution on the *p*-methoxyphenyl nucleus: -CN, -CHO, -CH₂.OH, -NH.CO.CH₃, -CO₂H, -CH₂.NH₂, -CH:CH.CH₃, -NH₂, -H.

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