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1. The predominant site for the hepatic reduction of aromatic and aliphatic ketones is the cytosol.

2. Compounds in which at least one aromatic ring is adjacent to the carbonyl group are reduced to aryl-alkyl or aryl-aryl carbinols in NADPH-requiring reactions, whereas either NADH or NADPH serves as cofactor in the analogous reaction with aliphatic ketones.

3. Acetophenone reductase activity is not enhanced in liver cytosol after treatment of rats with phenobarbital.

4. Ketone reductase activity was found in the cytosol fractions of some non-hepatic tissues, particularly in that of kidney.

Introduction

Over 80 years ago, Sundvik (1886) noted that when acetophenone was injected into dogs, conjugated glucuronic acid was excreted in the urine. Similar findings in rabbits have been reported for many different aliphatic ketones and alkylphenones (Neubauer, 1901; Thierfelder & Daiber, 1923). The urinary metabolite of acetophenone in the rabbit was shown to be the glucuronide of 1-phenylethanol (Smith, Smithies & Williams, 1954 a), and glucuronides of a number of alkylphenyl, alkyl-benzyl, and alkyl-phenethyl carbinols were identified in the urine after administration of the corresponding ketones (Smith, Smithies & Williams, 1954 b). Similarly, the glucuronide of benzhydrol was recovered from rabbit urine after feeding benzophenone (Robinson, 1958). Secondary alcohols have also been shown to be products of reduction *in vivo* of some aliphatic ketones; thus, the corresponding glucuronides were isolated from the urine after administration of dichloroacetone (Sundvik, 1886), methyl ethyl ketone (Saneyoshi, 1911), and 2-heptanone (Kamil, Smith & Williams, 1953). In the series of alicylic ketones, the glucuronides of the corresponding secondary alcohols are excreted by rabbits after administration of cyclohexanone (Elliott, Parke & Williams, 1959), various alkylcyclohexanones (Elliott, Tao & Williams, 1965; Cheo, Elliott & Tao, 1967), and the isomeric decalones (Elliott, Robertson & Williams, 1966). A number of terpenoid ketones are reduced in mammals to secondary alcohols that are excreted as glucuronides (Williams, 1940).

Relatively few studies of the reduction of xenobiotic ketones *in vitro* have been reported. The reduction of alicyclic ketones such as various cyclohexanone derivatives and decalones, but not that of noncyclic aliphatic ketones such as

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acetone and methyl ethyl ketone, is mediated by horse liver alcohol dehydrogenase (Winer, 1958; Merritt & Tomkins, 1959; Graves, Clark & Ringold, 1965); from studies of the reduction of optically active methylcyclohexanones, Elliott, Jacob & Tao (1969) have concluded that the responsible oxidoreductase in rabbits is quite similar to horse liver alcohol dehydrogenase.

The partial purification has been reported of an enzyme from dog erythrocytes and human liver cytosol that reduces α,β-unsaturated ketones, but not saturated ketones, in the presence of NADPH (Fraser, Peters & Hardinge, 1967). Intact rat erythrocytes, however, are capable of reducing both 4-phenyl-3-buten-2-one and 4-phenylbutan-2-one to 4-phenylbutan-2-ol (Fraser, Fancher & Strother, 1968). An NADPH-specific reductase for aromatic aldehydes and ketones was partially purified from the 30 000*g* supernatant fraction of rabbit kidney cortex homogenates (Culp & McMahon, 1968).

The present paper concerns the location and some of the characteristics of the enzymic reduction of ketones in mammals.

Experimental

Preparations

Male Holtzman rats (160–180 g) and male New Zealand White rabbits (1·5–2·0 kg) were used. When administered, phenobarbital was injected intraperitoneally, 75 mg/kg/day (rats) or 15 mg/kg/day (rabbits) for 3 days; animals were killed 18 h after the last injection. Livers were homogenized with 4 vol. of 0·1 M tris-chloride buffer, pH 7·5, in glass Potter–Elvehjem homogenizers (rat liver) or in a Waring blender (rabbit liver). The 9000*g* supernatant fractions of these homogenates were lyophilized after shell-freezing, stored at $-15°$, and reconstituted with water as needed. Microsomes and cytosol were prepared from fresh 9000*g* supernatant fractions or from reconstituted lyophilized preparations by centrifugation at 100 000*g* for one h. The microsomal pellets were resuspended in 0·1 M tris-chloride buffer, pH 7·5, and centrifuged again.

Incubation conditions

When the reduction product was to be determined colorimetrically or identified by chromatography, incubation flasks contained 600 μmol of *tris*-chloride buffer, pH 7·5, 100 μmol of nicotinamide, enzyme preparation equivalent to 400 mg (wet weight) of liver, and substrate in 5 ml total volume. When the enzyme preparation was the 9000*g* supernatant fraction or the cytosol, 25 μmol of glucose-6-phosphate and 0·3 μmol of NADP were included. No glucose-6-phosphate or NADP were included when isolated microsomal fractions were used, but 1·5 μmol of NADPH was added at 0, 15, 30, and 45 min of incubation. After incubation for 1 h at 37° in an atmosphere of N_2 or air, the reactions were stopped as described below.

When reaction rates were assayed spectrophotometrically, mixtures of 300 μmol of *tris*-chloride buffer, pH 7·5, cytosol equivalent to 40 mg (wet weight) of liver, and substrate in a total volume of 2·9 ml (sample) or 3·0 ml (reference) were placed in spectrophotometer cuvettes of 1·0 cm optical path. The reaction was started by addition of 0·1 ml of 0·5% NADPH or NADH to the sample cuvette, and was followed at 340 nm with a Beckman model DB spectrophotometer with logarithmic recorder.

All substrates were added in aq. soln., except for benzophenone and deoxybenzoin, which were introduced in 0·1 ml of dimethylformamide.

When boiled enzyme was to be used in incubation, the 9000*g* supernatant fraction was heated in boiling water for 15 min and cooled to room temperature prior to making other additions.

Colorimetric determination of secondary alcohols

A modification of the method of Mantel & Anbar (1964) for isopropanol was used. Incubations (in 5 ml total volume) were run in 60 ml bottles and were stopped by the addition of 2 g of lithium sulphate and 5 ml of vanadium oxinate reagent in benzene. The bottles were closed with glass stoppers and shaken for 20 min. After separation, 4 ml of the benzene layer was transferred to a 12 ml glass-stoppered centrifuge tube. After addition of 4 ml of 1 M-NaOH, the tube was shaken for 2 min and centrifuged. A portion of the benzene layer was transferred to a spectrophotometer tube and the absorbance measured at 380 nm. Standard curves were constructed for isopropanol, 1-phenylethanol, benzhydrol, and 1,2-diphenylethanol; the absorption maximum occurred at 380 nm in each case.

Chromatographic identification of reduction products

For thin-layer chromatography, reactions were stopped by the addition of 0·5 ml of 4 M perchloric acid. After neutralization with KOH and centrifugation, the supernatant fluid was extracted with 5 ml ether (for extraction of 1-phenylethanol, 2·5 g of NaCl was added prior to extraction). The ether phase was evaporated under a stream of N_2 and the residue was taken up in a small volume of ether and chromatographed, together with reference alcohols, on Eastman Chromagram sheets No. 6060 (silica gel with fluorescent indicator) in benzene or benzene-ethanol (97 : 3 by vol.). After development and air-drying, spots were visualized under ultra-violet light.

For gas chromatography of 1,2-diphenylethanol, five reaction mixtures were combined, 12·5 g of NaCl was added, and the mixture was extracted with 25 ml of ether. After centrifugation at 2°, 5 μl of the ether phase was subjected to gas chromatography on 2% OV-1/ 3% OV-17 on Chromosorb W (AW-DMCS), 60/80 mesh, in a 4 ft glass U-column. Oven temperature was 160°, flash heater

Table 1. Ketones (R′R″CO) and alcohols (R′R″CHOH) employed

Ketone	Alcohol	R′	R″
Acetone*	Isopropanol*	CH_3	CH_3
2-Butanone*	2-Butanol*	CH_3	C_2H_5
Acetophenone*	1-Phenylethanol†	C_6H_5	CH_3
Benzophenone*	Benzhydrol‡	C_6H_5	C_6H_5
Deoxybenzoin‡	1,2-Diphenylethanol‡	C_6H_5	$C_6H_5CH_2$
Metyrapone§	Metyrapol	C_5H_4N	$(C_5H_4N)C(CH_3)_2$

* Fisher Scientific Co., Pittsburgh, Pa.
† Aldrich Chemical Co., Milwaukee, Wis.
‡ Eastman Organic Chemicals, Rochester, N.Y.
§ Ciba Pharmaceutical Co., Summit, N.J.

285°, and hydrogen flame ionization detector 210°. Helium carrier gas flow rate was 100 ml/min. Under these conditions, the retention time of deoxybenzoin was 13·8 min.

Chemicals

Alcohols and ketones used and their sources are shown in Table 1.

Results

When incubated under N_2 with the 9000*g* supernatant fraction of rabbit liver in the presence of an NADPH-generating system, several ketones were reduced to secondary alcohols (Table 2). Acetone underwent reduction to a small extent; at 0·1 M concentration about one % was reduced, while with 1 M acetone, 0·35%

Table 2. Reduction of ketones in rabbit liver 9000*g* supernatant fraction*

Ketone	Concentration	System	Alcohol formed (μmol/h)
Acetone	0·1 M	Complete	5·2
	1·0 M	Complete	17·4
	1·0 M	No NADP or G-6-P	4·6
	1·0 M	Boiled enzyme	0
Acetophenone	8 mM	Complete	5·0
	8 mM	No NADP or G-6-P	0·7
	8 mM	Boiled enzyme	0
Benzophenone	8 mM	Complete	7·6
Deoxybenzoin	8 mM	Complete	1·0

* Reconstituted from lyophilized preparation.

reacted in one hour. When the NADPH-generating system was omitted, the extent of reduction fell to about one-quarter of that which occurred in its presence. The remaining activity probably depends upon residual pyridine nucleotide in the preparation. When the liver fraction had been boiled, no reaction occurred. The aromatic ketones were in general more extensively reduced. In the case of benzophenone, about 20% of the ketone was reduced in one hour. Acetophenone was reduced to a slightly lower extent, and at the same concentration about 3% of the deoxybenzoin was converted to a secondary alcohol. Again, omission of the NADPH-generating system caused a loss of most of the activity, and no reduction was mediated when the liver fraction had been inactivated by heating.

When acetophenone was incubated with this system in air, reduction proceeded to about 75% of that which occurred under nitrogen.

The secondary-alcohol products of reduction of the aromatic ketones were identified by chromatography (Table 3). The metabolite from acetophenone had the same R_F value as 1-phenylethanol in two different solvent systems. Similarly, the reduction product of benzophenone was chromatographically identical to

benzhydrol. Finally, the alcohol formed from deoxybenzoin had identical gas chromatographic properties to 1,2-diphenylethanol.

Table 4 shows that very little reduction of acetophenone or of acetone took place in rabbit liver microsomes, and that most of the reducing activity of the post-mitochondrial supernatant fraction was resident in the 100 000*g* supernatant fraction. All incubations shown in Table 4 were carried out under N_2.

Table 3. Chromatographic identification of reduction products

Substrate	Chromatographic system	Metabolite	Reference compound
	t.l.c.	R_F value	
Acetophenone	benzene	0·26	0·27*
	benzene-ethanol (97 : 3, v/v)	0·72	0·71*
Benzophenone	benzene	0·41	0·41†
	g.l.c.	Retention time (min)	
Deoxybenzoin	OV-1/OV-17	11·1	11·0‡

* 1-Phenylethanol.
† Benzhydrol.
‡ 1,2-Diphenylethanol.

Table 4. Ketone reduction in rabbit liver microsomes and cytosol*

Ketone	Cell fraction	Alcohol formed (μmol/h)
Acetophenone	Microsomes	0·7
	Cytosol	7·3
Acetone	Microsomes	0·6
	Cytosol	10·6

* Fractions were prepared by centrifugation of reconstituted lyophilized 9000*g* supernatant fraction.

In Table 5 are presented the results of spectrophotometric assays of the oxidation of reduced pyridine nucleotides by rabbit liver cytosol in the presence of various aromatic and aliphatic ketones. With acetophenone, both NADPH and NADH were oxidized; however, NADPH was oxidized over ten times faster than

was NADH. In other preparations, the ratio of reaction rates of the two nucleotides was not as great, but acetophenone always caused a much greater rate of oxidation of NADPH than it did of NADH. The same was true of metyrapone. When it came to the aliphatic ketones, however, the situation was different. In the presence of either acetone or 2-butanone, liver cytosol oxidized NADPH and NADH at about the same rates.

The reduction of acetophenone in rabbit liver cytosol was found to be a reversible reaction. Table 6 shows experiments with a preparation in which the oxidation of NADH proceeded at 40% of the initial rate of that of NADPH in the

Table 5. Oxidation of pyridine nucleotides in rabbit liver cytosol in the presence of ketones*

Ketone	Concentration of ketone	Nucleotide	Initial rate of oxidation (nmol/min/100 mg liver)
Acetophenone	8 mM	NADPH	278
		NADH	25
Metyrapone	13 mM	NADPH	350
		NADH	28
Acetone	1 M	NADPH	283
		NADH	296
2-Butanone	50 mM	NADPH	157
		NADH	173
None	—	NADPH	8
		NADH	< 5

* Cytosol was prepared by centrifugation of reconstituted lyophilized 9000*g* supernatant fraction.

presence of this ketone. When the substrate was 1-phenylethanol, NADP and NAD were reduced, the former at a more rapid initial rate than the latter.

It was found that the ketone reductase activity of rat liver cytosol was not inducible with phenobarbital. In the presence of 8·3 mM acetophenone, the mean initial oxidation of NADPH ± standard error was 84 ± 6 nmol/min/100 mg liver in cytosol from three untreated rats, while in that from 5 rats treated with phenobarbital (75 mg/kg/day for 3 days), the initial rate was 83 ± 5 nmol/min/100 mg. In these experiments, the cytosol fractions were freshly prepared after homogenization of the livers.

Table 7 shows the initial rates of NADPH oxidation in the presence of acetophenone in cytosol fractions from various rabbit tissues. Of the cytosol fractions

studied, that of liver showed the highest rate of acetophenone reduction, but the activity of kidney cytosol was also quite high. The soluble supernatant fractions of heart and lung had low activities, and no reduction of acetophenone could be demonstrated in that of brain.

Table 6. Reversibility of acetophenone reduction in rabbit liver cytosol*

Substrate (8 mM)	Nucleotide (0·2 mM)	Initial rate of oxidation or reduction (nmol/min/100 mg liver)
Acetophenone	NADPH	320
	NADH	128
1-Phenylethanol	NADP	320
	NAD	152

* Cytosol was prepared by centrifugation of reconstituted lyophilized 9000*g* supernatant fraction.

Table 7. Acetophenone reduction in various rabbit tissue cytosols*

Organ	Initial rate of NADPH oxidation (nmol/min/100 mg tissue)
Liver	138
Kidney	96
Heart	30
Lung	30
Brain	0

* Cytosol fractions were obtained by centrifugation of freshly-prepared 9000*g* supernatant fractions.

Discussion

The data reported here demonstrate that a number of ketones may be reduced in the liver as well as in other tissues. The largest part of this reduction takes place in the cytosol. There would appear to be at least two mechanisms of ketone reduction in the liver cytosol. One, which is specific for NADPH, is responsible for the reduction of ketones in which the carbonyl group is adjacent to an aromatic ring. Ketones in which the carbonyl function is attached to two aliphatic groups are equally well reduced in the presence of either NADPH or NADH in liver cytosol. Whether the same enzyme mediates the NADPH-dependent reduction of aromatic and aliphatic ketones is not known.

A few reports in the literature have been concerned with the reduction of various ketones in the cytosol of mammalian tissues. Culp & McMahon (1968)

described the partial purification of an enzyme from kidney 30 000*g* supernatant fraction which catalyzed the reduction of a group of aromatic aldehydes and ketones. The starting material for this purification could contain part of the microsomal fraction. The α,β-unsaturated ketone reductase of Fraser, *et al.* (1967) was purified from the 105 000*g* supernatant fraction of human liver. Both of these enzymes were shown to be specific for NADPH. Maylin & Anders (1969) have implicated the reduction reaction of acetophenone in the liver cytosol in the control of stereospecificity of ethylbenzene hydroxylation.

The fact that the reduction of the aromatic aldehydes in cytosol is specific for NADPH rather than for NADH indicates that the normal liver alcohol dehydrogenase is not involved. For a number of cycloalkanones, however, reduction in liver appears to be mediated by enzymes identical with or similar to alcohol dehydrogenase (Winer, 1958; Merritt & Tomkins, 1959; Graves, *et al.*, 1965; Elliott, *et al.*, 1969).

In these experiments, the oxidation of NADPH proceeded rapidly in the presence of metyrapone, whereas under similar conditions NADH was oxidized quite slowly. Kahl (1970) has reported the reduction of metyrapone to metyrapol in rat liver cytosol in the presence of a mixture of NADPH and NADH; with this substrate, however, he found the greatest hepatic reduction to occur in the microsomal fraction.

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