Metabolism of Naphthalene by Liver Slices

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The metabolism of naphthalene in animals was reviewed by Young (1950). Since that review was published experiments have shown that the oxidative pathways leading to 1-naphthol and to 1:2-dihydroxy-1:2-dihydronaphthalene (naphthalenediol), are probably separate and independent (cf. Boyland & Wiltshire, 1952). The conversion of naphthalene to naphthalene mercapturic acid (Bourne & Young, 1934) probably involves still another metabolic route. The present experiments indicate that isolated slices of rat liver are able to oxidize naphthalene to naphthalenediol.

EXPERIMENTAL

(a) Tissue slices not more than 0.5 mm. thick were weighed in a moist condition on a torsion balance, and 100 mg. quantities placed in Krebs-Ringer phosphate solution in Warburg vessels with 0.2 ml. 2N-KOH in the inner vessel. Naphthalene and other additions were made as shown in Table 1, so that the total volume was 3.4 ml. The vessels were equilibrated with O₂ and, at the end of the incubation (usually 2 hr.), the KOH was removed and *n*-butanol (5 ml.) was added to the incubated mixture. The flasks were shaken mechanically for 30 min. and the mixtures centrifuged. Portions (4 ml.) of the butanol

Table 1. *Formation of substance (probably 1:2-dihydroxy-1:2-dihydronaphthalene) which yields 1-naphthol in N-HCl in 15 min. at 100° by rat-liver slices*

(Reaction vessels contained 100 mg. wet tissue in 3.4 ml. Ringer-phosphate pH 7.4. Temperature 37°. Gas phase O₂.)

Rat no.	Naphthalene present (μg./ml.)	Condition of rat	Additions to system	Q _{O₂}	Apparent 1:2-dihydroxy-1:2-dihydronaphthalene (μg. 1-naphthol formed/g. tissue/hr.)
1	30	Normal	None	3.1	50
2	30	Fed on 40% protein diet	None	3.0	40
3	30	Normal	None	3.2	25
	30	Normal	0.25% Tetramethyluric acid	3.25	85
4	30	Fed on 40% protein diet	None	7.2	50
	30	Fed on 40% protein diet	0.25% Tetramethyluric acid	6.4	60
5	30	Fed on 40% protein diet	None	3.6	85
	30	Fed on 40% protein diet	0.05% Tetramethyluric acid	5.2	75
	30	Fed on 40% protein diet	0.1% Tetramethyluric acid	6.2	60
	30	Fed on 40% protein diet	0.1% Glucose	4.2	75
	30	Fed on 40% protein diet	0.1% Glucose + 0.05% tetramethyluric acid	3.5	75
	30	Fed on 40% protein diet	0.1% Glucose + 0.1% tetramethyluric acid	4.2	120
6	30	Fed on 20% protein diet	0.1% Glucose	5.2	40
	30	Fed on 20% protein diet	0.1% Glucose + 0.1% tetramethyluric acid	4.9	60
	30	Fed on 20% protein diet	0.1% Glucose + 0.1% nicotinamide	3.8	30
	30	Fed on 20% protein diet	0.1% Glucose + 0.2% nicotinamide	3.6	30
	30	Fed on 20% protein diet	0.1% Glucose + 0.1% tetramethyluric acid + 0.01% ascorbic acid	4.1	90
7	0	Normal	0.1% Tetramethyluric acid	4.8	0
	10	Normal	0.1% Tetramethyluric acid	3.5	0
	20	Normal	0.1% Tetramethyluric acid	4.4	12
	30	Normal	0.1% Tetramethyluric acid	5.5	35
	80	Normal	0.1% Tetramethyluric acid	4.5	80
	200	Normal	0.1% Tetramethyluric acid	3.9	110

extracts were removed and heated with 0.2 ml. 10N-HCl at 100° for 10 min. (This procedure was shown to convert synthetic DL-1:2-dihydroxy-1:2-dihydronaphthalene quantitatively into 1-naphthol.) The butanol was cooled and 1 ml. 0.01M diazotized sulphanilic acid added, followed by 1 ml. 4N-NaOH and 4 ml. ethanol. The red colour of the solution was measured in a photoelectric colorimeter using a green filter. Standard solutions of naphthalenediol and of 1-naphthol were treated in the same way for comparison.

(b) Tissue slices (500 mg. wet wt.) were suspended in 10 ml. Krebs-Ringer phosphate solution at pH 7.4 and incubated at 37° in 50 ml. conical flasks. Naphthalene was added as a 10% solution in methyl cellosolve. The flasks were shaken slowly and gassed with a stream of O₂. At the end of the incubation period the slices were removed and the reaction mixture was transferred to 50 ml. centrifuge tubes, the flasks being rinsed into the centrifuge tubes with 20 ml. ethanol. The tubes were warmed to 60° and the coagulated protein centrifuged down.

Naphthol was estimated colorimetrically in 1 ml. portions of the supernatant fluid acidified with 0.1 ml. 10N-HCl before and after heating for 15 min. at 100°, by reaction with 2:6-dichloroquinone chloroimide as described by Gibbs (1927). The solution was neutralized by dropwise addition of saturated Na₂CO₃ and 1 ml. 0.1M-Na₂B₄O₇·10H₂O, and shaken with 10 ml. *n*-butanol and 0.2 ml. ethanolic 2:6-dichloroquinone chloroimide. After standing for 30 min. the upper layer was centrifuged and read against a blank in a Spekker colorimeter using filter OY2. The naphthol estimated in the unheated aliquot represents the free 1-naphthol while that in the heated solution would contain free 1-naphthol plus naphthol derived from 1-naphthylsulphuric acid and 1:2-dihydroxy-1:2-dihydronaphthalene.

In two experiments ethanol was removed from the supernatant, and the remaining aqueous solution neutralized to pH 7.4 and extracted twice with an equal volume of ether. Subsequent analysis of the aqueous and ethereal layers gave separate estimates of the 15 min. naphthol due to naphthylsulphuric acid and naphthalenediol.

(c) Rat-liver homogenates were prepared in a glass homogenizer cooled in ice. Rabbit-liver and kidney homogenates were made in a Waring Blendor in a cold room at +2°. The tissue was removed immediately after killing the animal, weighed and cooled on ice. It was then chopped with scissors and ground for 30 sec. in 2 vol. of ice-cold 0.15M-KCl solution. Other homogenates of rat-kidney and liver were made in glass homogenizers using water or 0.2% sodium citrate.

RESULTS

(1) Some of the experimental results obtained by the technique described in the experimental method under (a) are given in Table 1. Preliminary experiments showed that incubation of 0.01 mg. 1:2-dihydroxy-1:2-dihydronaphthalene with 100 mg. of liver slices or with 1 ml. of 1:10 liver homogenate for 1 or 2 hr. caused no decrease in the diol as estimated by the method. The results of the last experiment (rat 7) suggest that the amounts of diol formed vary with the initial amount of naphthalene present, although in this experiment never more than 4% of the naphthalene present was transformed into diol and the highest yield of diol

(120 µg./g. tissue/hr.) represents only 10% conversion of the naphthalene added to the medium. The comparison of the rate of reaction in rats fed on a normal (approx. 20% protein diet) and a 40% protein diet indicate that the maintenance of the animals on the higher protein diet for 3–5 days before using the liver did not affect the ability of the liver to oxidize naphthalene. The oxidation was not increased by addition of glucose, but was generally increased by addition of tetramethyluric acid (which forms a water-soluble complex with naphthalene).

(2) Data obtained by the second technique (b) are given in Table 2. These results show clearly that negligible amounts of free phenol are formed and that the apparent diol formation by rat-liver slices varied from 18 to 124 µg./g. wet wt. tissue/hr. These rates are of the same order as those obtained by technique (a). Under the experimental conditions 1 g. liver tissue in the presence of naphthalene can produce about 0.1 mg. of diol/hr. If the reaction proceeded at the same rate *in vivo*, a rat weighing 200 g. with 10 g. liver would produce 24 mg. diol/day. In experiments in which the excretion of diol in urine and faeces from rats dosed with naphthalene was measured (Boyland & Wiltshire, 1952) never more than 10 mg. were found in 24 hr. The rate found in the *in vitro* experiments would therefore be more than sufficient to account for the diol excreted.

There appeared to be very little difference in the reaction according to the sex of the animal from which the tissue was derived. The mean rates of diol formation were 76 and 89 µg./g. wet wt./hr. for tissue from male and female rats respectively.

Although prior treatment with naphthalene increases the resistance of rats to naphthalene as indicated by measurements of the lethal dose of naphthalene, such treatment did not appear to change the ability of the liver to metabolize naphthalene. The rates of diol formation were 18, 54, 54, 61, 78, 94 and 114 (mean 67) µg./g. tissue/hr. for liver from a series of rats which had had ten daily doses each of 50 mg. naphthalene compared with 83 µg./g. tissue/hr. in liver tissue from untreated rats.

That the product produced from naphthalene by liver slices is 1:2-dihydroxy-1:2-dihydronaphthalene is made probable by the fact that in two experiments in which it was tested the substance yielding phenol on heating with acid was ether-soluble. Of the phenol precursor in the reaction mixtures from rats 8 and 9 (Table 2), 97 and 106% was apparently soluble in ether. The only known naphthalene metabolites which yield naphthol readily on heating with acid are 1-naphthylsulphuric acid and 1:2-dihydroxy-1:2-dihydronaphthalene; of these only the latter is extracted from neutral aqueous solution by ether. It will be noticed that the liver of rats 3–8 (Table 2) contained appreciable amounts of diol

Table 2. *Formation of a substance (probably 1:2-dihydroxy-1:2-dihydronaphthalene) which yields 1-naphthol in N-HCl in 15 min. at 100° by tissue slices*

(Reaction vessels contained 500 mg. wet tissue, 5 mg. naphthalene in 0.05 ml. ethylene glycol monomethyl ether. All vessels contained 10 ml. Ringer-phosphate solution pH 7.4. Temperature 37°. Gas phase O₂.)

Animal	Sex	Tissue	Incubation time (min.)	Naphthol found (μg.)						Apparent 1:2-dihydroxy- 1:2-dihydro- naphthalene (μg. 1-naphthol formed/g. tissue/hr.)
				Tissue only		Tissue + naphthalene		Formed		
				Free	15 min.	Free	15 min.	Free	15 min.	
Rat 1	M.	Liver	150	10	0	10	90	0	90	72
Rat	M.	Kidney	150	0	0	0	36	0	36	29
Rat	M.	Spleen	150	0	0	0	30	0	30	24
Rat 3	M.	Liver	215	—	84	—	234	—	150	84
Rat 4	F.	Liver	180	—	54	—	78	—	24	16
Rat 5	F.	Liver	105	—	90	—	186	—	96	110
Rat 6	F.	Liver	120	—	78	—	126	—	48	48
Rat 7	F.	Liver	120	—	84	—	138	—	54	54
Rat 8	F.	Liver	120	—	78	—	180	—	102	102
Rat 9	F.	Liver	120	—	10	—	74	—	64	64
Rat 10	F.	Liver	120	—	10	—	116	—	106	106
Rat 11	F.	Liver	120	—	18	—	60	—	42	42
Rat 12	M.	Liver	120	0	0	6	80	6	80	80
Rat 13	M.	Liver	120	6	0	12	72	6	72	72
Rat 14	M.	Liver	120	0	0	6	90	6	90	90
Rabbit	M.	Liver	120	—	12	—	126	—	114	114
Rabbit	M.	Kidney	120	—	24	—	48	—	24	24

when incubated without naphthalene. These rats had been previously injected with naphthalene.

(3) Experiments with homogenates prepared as described under (c), incubated aerobically with either 100 μg. naphthalene dissolved in water in a total volume of 4 ml., or 5 mg. naphthalene dissolved in 0.05 ml. methylcellosolve added to a total volume of 10 ml., for times varying from 70 to 215 min., gave no indication of metabolism of naphthalene.

DISCUSSION

The experiments show that isolated slices of rat or rabbit-liver can oxidize naphthalene to 1:2-dihydroxy-1:2-dihydronaphthalene although not to 1-naphthol. Although the rate of the change is slow, the oxygen used being equivalent to a Q_{O_2} value of 0.1 (μl. O₂/mg. dry wt. liver/hr.), it is sufficient to account for the metabolic conversion of naphthalene to the diol in rats treated with naphthalene. The apparent absence of 1-naphthol as a reaction product seems remarkable in view of the fact that after naphthalene injection into rats or rabbits, more 1-naphthol is excreted than naphthalenediol. It is possible that in the incubated liver 1-naphthol is formed and immediately conjugated with glucuronic acid. The conversion of naphthalene to 1-naphthol may occur in some tissue

other than liver but the few experiments carried out with spleen and kidney gave no indication that the oxidation to naphthol occurred in those tissues.

Although it had been suggested that the metabolic conversion of hydrocarbons to phenols might proceed through the stage of the corresponding diol, no conversion of a diol to a phenol has been seen in isolated liver preparations. The site of oxidation of naphthalene to naphthol and the enzymes involved in this oxidation and the oxidation to the diol still require investigation.

SUMMARY

1. On aerobic incubation with liver slices naphthalene is apparently slowly converted to 1:2-dihydroxy-1:2-dihydronaphthalene but not to 1-naphthol.

2. The rate of the reaction *in vitro* is sufficient to account for the metabolic production of the diol in rats.

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