In this series of experiments 46 operations were performed on 40 dogs. Under intraperitoneal nembutal anesthesia and intratracheal oxygen insufflation the chest was opened in the 5th interspace. The lung was manually compressed and held out of the way. The pericardium was opened and approximately one dram of the powder was lightly dropped and sprinkled onto the surface of the epicardium. The pericardium was then closed with a continuous suture. The lung was reëxpanded and the chest closed by percostal sutures and approximation of the soft tissues of the chest wall.

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p-Bromophenol as the Intermediate in Synthesis of p-Bromophenylmercapturic Acid from Bromobenzene in the Rat.

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White and Lewis[1] in agreement with Coombs and Hele[2] demonstrated that the suggestion that in dogs "the oxidation to the phenol is a preliminary step in the synthesis of p-bromophenylmercapturic acid after the administration of bromobenzene appears improbable."[1] These workers reported that p-bromophenol, when fed to dogs, was in part excreted as an ethereal sulphate. No appreciable effect on the excretion of the neutral sulfur in the urine was noted. This observation indicated that apparently no p-bromophenylmercapturic acid was formed from p-bromophenol.

It appeared desirable to investigate the question of the synthesis of p-bromophenylmercapturic acid from p-bromophenol in the rat, using the direct method for the estimation of mercapturic acids in the urine. Aside from the fact that such an investigation had not previously been made on the rat, we felt that apparently the question of the formation of p-bromophenol from bromobenzene as a preliminary step in the synthesis of p-bromophenylmercapturic was not as yet settled. Thus Bodansky[3] in discussing the mechanism of the synthesis of the mercapturic acid from bromobenzene, states that "the reaction involves first oxidation to p-bromophenol and its subsequent conjugation with cysteine. The resulting p-bromophenyl-

1 White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

2 Coombs, H. I., and Hele, T. S., *Biochem. J.*, 1927, **21**, 611.

3 Bodansky, M., *Introduction to Physiological Chemistry*, 4th edition, p. 393, 1938, John Wiley, New York.

TABLE I.

Effect of p-Bromophenol and Bromobenzene on Sulfur Partition and Excretion of p-Bromophenylmercapturic Acid in Urine of Rats.*

Rat	Period	Total S mg	Inorganic SO_4S mg	Ethereal SO_4S mg	Neutral S mg	Mercapturic acid mg	Supplement with diet
1	1	24.50	16.11	3.47	4.92		None
	2	23.29	9.23	9.65	4.41	0	210 mg p-bromophenol
	3	93.04	75.56	12.48	5.00	0	" " " and 420 mg Na_2SO_4
	4	31.34	19.62	6.22	5.50		None
	5	36.47	6.26	5.20	25.01	197.3	210 mg bromobenzene
2	1	26.34	16.57	4.00	5.77		None
	2	25.60	10.21	8.77	6.42	0	210 mg p-bromophenol
	3	76.72	57.86	11.08	7.78	0	" " " and 420 mg Na_2SO_4
	4	29.16	17.20	3.97	7.99		None
	5	39.57	11.95	4.28	23.34	152.8	210 mg bromobenzene
3	1	31.84	13.71	4.88	16.25		None
	2	29.15	8.72	8.44	12.99	0	210 mg p-bromophenol
	3	103.84	78.02	12.30	13.52	0	" " " and 420 mg Na_2SO_4
	4	32.32	16.54	5.82	9.96		None
	5	43.51	12.38	4.34	26.79	160.0	210 mg bromobenzene

*The results shown above are representative of experiments obtained with 8 rats. Each period in the table represents 6 days during which each rat ingested 42 g of diet C-10 with or without the supplements. The food was fed daily in 7 g portions and the urine was collected, under toluene, every 3 days. The pooled 6-day sample of urine was made up with water to a suitable volume and analyzed.

cysteine then undergoes acetylation." A similar statement is also made by Schmidt.[4]

We administered p-bromophenol to adult white rats which were fed constant amounts of a diet of uniform composition. The urine was collected and analyzed for total sulfur, inorganic and ethereal sulfates (Folin method) and for p-bromophenylmercapturic acid.[5] The urinary values were then compared with those obtained on the same rats which ingested the same amounts of the diet free from p-bromophenol. The diet C-10 had the following composition: casein 10, cane sugar 15, corn starch 46, yeast powder 5, Osborne-Mendel salt mixture[6] 4, cod liver oil 5, and Crisco 15. The supplements of p-bromophenol and Na_2SO_4 were fed mixed with the diet. The amounts of the supplements and the diet fed, the general procedure of collection of urine are indicated in Table I.

The results shown in Table I indicate that p-bromophenol, when fed to rats, causes an increased excretion of ethereal sulfates in the urine. The formation of the ethereal sulfates from p-bromophenol takes place apparently at the expense of the inorganic sulfates. No effect on the excretion of the neutral sulfur fraction of the urine is produced by the administration of p-bromophenol, nor any appreciable amount of p-bromophenylmercapturic acid is formed from this substance. On the other hand, comparable amounts of bromobenzene, when fed to the same rats under identical conditions, raised the output of the neutral sulfur in the urine which could be accounted for by the excretion of p-bromophenylmercapturic acid. The increase in the excretion of the ethereal sulfates sulfur during the p-bromophenol administration indicates that only 10 to 15% of the administered p-bromophenol is excreted as the ethereal sulfate. The major portion of the ingested p-bromophenol might, perhaps, be accounted for as a glucuronate. This possibility is being investigated.

The results are in complete accord with those of Coombs and Hele[2] and of White and Lewis,[1] and emphasize anew the improbability of the theory that p-bromophenol is the intermediate substance in the synthesis of p-bromophenylmercapturic acid from bromobenzene in dogs or rats.

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[4] Schmidt, C. L. A., *The Chemistry of the Amino Acids and Proteins*, p. 236, 1938, Thomas, Springfield, Ill.

[5] Stekol, J. A., *J. Biol. Chem.*, 1936, **113**, 279.

[6] Osborne, T., and Mendel, L. B., *J. Biol. Chem.*, 1917, **37**, 572.