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Mercapturic Acid Formation in Rabbits.

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The influence of diet on the detoxication of brombenzene in rabbits was studied by Abderhalden and Wertheimer[1], who found that an acid diet favors, while a basic diet inhibits the formation of mercapturic acid. This result was confirmed in our laboratory, using the same technique and method of determining mercapturic acid—isolation of the product by the Baumann-Schmitz method[3]. However, it was thought that the large urine volume might be responsible for the negative result obtained on the basic green-food diet, and preliminary experiments on this urine with various solvents indicated the presence of a small amount of mercapturic acid which could be extracted with chloroform.

Three rabbits were put on a diet of celery, lettuce and cabbage until the urines were definitely alkaline, and on 3 successive days each animal received 1.5 gm. brombenzene daily by subcutaneous injection. The combined urines for 6 days were divided in 2 portions, one of which was treated according to the Baumann-Schmitz procedure, and the other was extracted with chloroform. Not a trace of mercapturic acid was obtained by the former method, while the chloroform extract yielded 0.96 gm. of crystals, a yield corresponding to 3.5% of the brombenzene injected. The substance melted at 153-4°, gave a positive thiophenol reaction with concentrated sulfuric acid, and its barium salt analyzed 17.60% Ba, the calculated value for mercapturic acid being 17.81%. The experiment was repeated on another rabbit with a similar result—no mercapturic acid by the Baumann method, 2.7% yield by the chloroform extraction method.

When applied to the urine of a dog that had received 9.0 gm. of brombenzene over a period of 6 days, the chloroform extraction method proved to be more efficient than the Baumann-Schmitz and the Baumann-Preusse[2] methods, as shown by the following results:

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[1] Abderhalden, E., and Wertheimer, E., *Arch. ges. Physiol.*, 1925, **207**, 215; **209**, 611.

[2] Baumann, E., and Preusse, C., *Ber.*, 1895, **12**, 806.

[3] Baumann, E., and Schmitz, *Z. physiol. Chem.*, 1879, **20**, 586.

	Gm. Mercapturic Acid	% Yield
Baumann-Schmitz	2.764	15.3
Baumann-Preusse	2.508	14.0
Chloroform Extraction	3.372	18.5

The extraction method has not been perfected in detail, but may be described briefly. The urine is acidified with concentrated hydrochloric acid and let stand for 2 hours to liberate the mercapturic acid from the glycuronic acid complex. It is then extracted in a separatory funnel with 5 successive portions of chloroform, tracing the extraction by microscopic examination of a few drops of each extract. The combined extracts are evaporated, and the residue is taken up in very dilute ammonia, decolorized with carbon, and the filtrate acidified with hydrochloric acid. On standing overnight, an impure crystalline sediment is obtained. This is rubbed up with a few cc. of ether in a test tube, the ether is decanted from the crystals, and after another purification with dilute ammonia and carbon, almost colorless crystals are obtained. Modifications of this procedure are now being tested to eliminate certain undesirable features of the method.

Our results show that the Baumann-Schmitz method is unsuitable for application to alkaline rabbit urines. Also some doubt is thrown of the validity of interpreting any negative results with the above method as evidence that mercapturic acid is absent. Our findings on the synthesis of mercapturic acid in rabbits are significant in relation to a theory that the acid or basic character of the diet greatly modifies detoxication processes[1]. The oxidation of toluene and benzene and the conjugation of phenol, benzoic acid, and brombenzene have been cited in support of this theory. Braunstein and associates[4] showed that the oxidation of benzene and toluene, and the conjugation of phenol are independent of this dietary factor. Griffith[5] showed that the hippuric acid synthesis is not lessened on a basic diet as previously reported by Abderhalden[1]. Our findings seem to remove the last support of this theory, since the mercapturic acid synthesis is not inhibited by an alkaline diet.

[4] Braunstein, Parschin and Chalisowa, *Biochem. Z.*, 1931, **235**, 311.

[5] Griffith, W. H., *J. Biol. Chem.*, 1925, **64**, 401.