New York Meeting

New York Academy of Medicine, October 16, 1935.

8274 C

Metabolism of Bromobenzene in Growing Dogs and Mice Maintained on Adequate Diets.*

JAKOB A. STEKOL.

From the Department of Chemistry, Fordham University, New York City.

Monohalogen benzenes, when fed, are excreted in the urine of adult dogs,[1] cats,[2] rats,[3] and rabbits,[4] partly as ethereal sulfates, partly as mercapturic acids. Hele,[5] in an attempt to compare the synthesis of mercapturic acid in dogs and pigs, has come to the conclusion that the pig does not synthesize mercapturic acid readily. Hele[5] used young growing pigs as experimental animals in comparing the metabolism of bromobenzene in pigs to that in the adult dog. Inasmuch as Abderhalden[6] believes that the synthesis of mercapturic acid is limited in the animal body by the need of that animal for sulfur for reactions more essential than the detoxication of bromobenzene, it seemed probable that the limitation of synthesis of mercapturic acid in the pig as found by Hele[5] was due to the fact that he employed young growing pigs instead of the adult animal. White and Jackson[7] have adduced evidence which seems to indicate that bromobenzene, when fed to growing rats, affects the utilization by

* I wish to express my gratitude to Dr. F. Brogan and to Mr. A. Vajda for the analysis of the mercapturic acid.

1 Baumann, E., and Preusse, C., *Ber. deutsch. chem. Ges.*, 1879, **12**, 806.

2 Gibson, R. B., *J. Biol. Chem.*, 1909, **6**, 16.

3 Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037.

4 Abderhalden, E., and Wertheimer, E., *Pflüger's Arch.*, 1925, **207**, 215; **209**, 611; Nishimura, K., *Acta schol. med. univ. imp. Kioto*, 1929-30, **12**, 73; Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037; Lough, S. A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **94**, 739.

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

6 Abderhalden, E., and Wertheimer, E., *Z. physiol. Chem.*, 1931, **198**, 8; **201**, 267.

7 White, A., and Jackson, R. W., *J. Biol. Chem.*, 1933, **100**, ciii.

the animals of cystine necessary for normal growth. These workers have not, however, demonstrated that mercapturic acid was actually synthesized by the growing rat.

As far as we know, the metabolism of bromobenzene in the growing dog or growing white mouse has not been studied previously. Pending the development of the method for the estimation of mercapturic acids in the urine, now in progress, we used the changes in the partition of urinary sulfur produced by bromobenzene feeding to animals, and the isolation of mercapturic acid from the urine as the criterion of the synthesis of mercapturic acid by the growing dog and mouse, while maintained on adequate diets.[8] It was anticipated that the metabolism of bromobenzene in the growing dog would be similar to that in the adult animal, inasmuch as the metabolism of naphthalene, which also yields mercapturic acid, was found by us to be the same in the adult and growing dog.[9]

Three female mongrel pups of 2 to 7 months of age and one Dalmatian male thoroughbred pup of 5 months were used. The general experimental procedure and the diet used were the same as used previously.[9] Fifteen 21-days-old white mice, born and raised in the laboratory were fed *ad libitum* a diet consisting of 70 parts of oats, 15 parts of milk powder and 15 parts of yeast powder (Harris) and placed in a group in a small false-bottom metabolism cage. The cage was placed over a large funnel which contained a small filter to separate the feces from the urine. 0.1 cc. of bromobenzene was injected into each mouse subcutaneously on 2 consecutive days and the urine collected. No attempt was made at this time to secure any analytical data similar to that obtained on the growing dog's urine, in view of the obvious impossibility to maintain mice in equilibrium. All animals received water *ad libitum*.

p-brom-phenylmercapturic acid was isolated by the method of McGuinn and Sherwin.[10] The isolations were made on the 24-hour sample of each pup separately and on the entire 2-day sample of urine collected from the fifteen mice.

Table I shows the results obtained on the pups. The data apparently indicate formation of an ethereal sulfate and a sulfur compound or compounds which are excreted in the neutral sulfur fraction of the urine. The raised excretion of neutral sulfur continued for 2-3 days after the feeding of bromobenzene. It was found that

[8] Stekol, J. A., *J. Biol. Chem.*, 1934, **107**, 641; 1935, **109**, 147.

[9] Stekol, J. A., *J. Biol. Chem.*, 1935, **110**, 463.

[10] McGuinn, A., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1115.

TABLE I.
Metabolism of Bromobenzene in Growing Dogs Maintained on Cowgill's Diet.

	Intake			Urinary Output						
	Age	N	S	Total N	Urea N	Total S	Total SO_4S	Inorganic SO_4S	Ethereal SO_4S	Neutral S
	days	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Pup 21 ♀	58	3.0	.155	2.42	1.72	.104	.055	.043	.012	.049
3.15 kg.	59	"	"	2.26	1.80	.095	.052	.040	.012	.043
	60	"	"	2.91	2.30	.160	.071	.025	.046	.089*
	61	"	"	3.29	2.60	.087	.017	.000	.017	.070
	62	"	"	2.52	1.95	.090	.049	.036	.013	.041
	63	"	"	2.37	1.93	.090	.046	.030	.016	.048
Pup 21 ♀	126	4.8	.248	3.29	2.39	.173	.121	.103	.018	.052
5.0 kg.	127	"	"	3.26	2.37	.144	.100	.083	.017	.044
	128	"	"	3.36	2.48	.195	.088	.044	.044	.107*
	129	"	"	3.54	2.87	.096	.019	.001	.018	.077
	130	"	"	3.44	2.50	.139	.085	.068	.017	.054
	131	"	"	3.32	2.53	.148	.102	.086	.016	.046
Pup 22 ♀	56	3.0	.155	2.02	1.45	.079	.042	.032	.010	.037
2.72 kg.	57	"	"	2.16	1.61	.091	.052	.041	.011	.039
	58	"	"	2.60	1.82	.151	.053	.023	.030	.098*
	59	"	"	2.81	2.17	.077	.016	.000	.016	.061
	60	"	"	2.10	1.46	.081	.040	.027	.013	.041
	61	"	"	2.00	1.40	.073	.041	.030	.011	.032
Pup 18 ♂	140	6.6	.341	4.56	3.88	.225	.149	.128	.021	.076
Dalmatian	141	"	"	4.35	3.53	.212	.140	.120	.020	.072
7.0 kg.	142	"	"	4.51	3.75	.233	.150	.129	.021	.083
	143	"	"	4.65	4.02	.285	.121	.080	.041	.164*
	144	"	"	5.06	4.26	.158	.037	.012	.025	.121
	145	"	"	4.50	3.74	.234	.128	.110	.018	.106
	146	"	"	4.81	3.78	.253	.144	.123	.021	.109
	147	"	"	4.89	3.70	.220	.134	.112	.022	.086
Pup 19 ♀	190	6.0	.310	4.43	3.00	.169	.129	.109	.020	.040
6.0 kg.	191	"	"	4.33	2.92	.160	.120	.100	.020	.040
	192	"	"	4.40	3.00	.180	.077	.046	.031	.103*
	193	"	"	4.70	3.40	.161	.105	.080	.025	.056
	194	"	"	4.40	3.10	.174	.117	.098	.019	.057
	195	"	"	4.31	3.00	.175	.120	.100	.020	.055

*1.0 gm p-Bromobenzene fed in gelatin capsule at 11 a.m.

as long as the neutral sulfur excretion continued above normal values, mercapturic acid was present in the urine. The raised output of neutral sulfur of the urine extending over a period of 2-3 days may indicate either slow synthesis of mercapturic acid in the growing dog or delayed excretion of the acid from the body or both. Analysis of the purified p-bromo-phenylmercapturic acid gave the following data: M.P. 152°C. (uncorr.); N, 4.21%; S, 10.39%. (Calculated for Br $C_{11}H_{12}O_3N$ S, N, 4.40; S, 10.08.) The average yield of purified p-brom-phenylmercapturic acid from each 1.0 gm. dose of bromobenzene fed to pups was 120-130 mg.

p-brom-phenylmercapturic acid isolated by a similar method from the urine of mice gave the following data: M.P. 151-152°C. (uncorr.) N, 4.27%; S, 9.97%.

Neither creatinine, uric acid nor allantoin output in the urine of pups were affected by the bromobenzene feeding. Naphthoresorcinol test for glycuronates was positive on the urine of all pups which were fed bromobenzene. No toxic effects were noticeable in the growing dogs after the administration of 1.0 gm. bromobenzene and the animals appeared quite normal. The mice, however, showed unmistakable signs of intoxication such as depression, loss of vitality and even complete prostration, resulting, in over 50% of the cases, in death.

The data presented indicate that the growing dog and mouse, under the conditions employed, are able to synthesize p-brom-phenyl mercapturic acid from the administered bromobenzene. The partition of various nitrogenous and sulfur fractions of the urine of growing dogs on the day of administration of bromobenzene is essentially similar to the partition of the same constituents in the urine of adult dogs which were fed bromobenzene while maintained on adequate diets.[11] The need of the growing dog for sulfur of the food did not prevent the animal from synthesizing the mercapturic acid from bromobenzene. We thought it possible that the synthesis of mercapturic acid by the growing dog took place because of the presence of cystine and methionine in the diet in excess to the needs of the animal for growth and maintenance. We found, however, in experiments which will be published shortly, that neither the adult dog nor the growing dog, while maintained on low-sulfur diets, protein-free diets, or after a fast of 4-5 weeks' duration was prevented from synthesizing mercapturic acid from bromobenzene. While it is probable that the inability to synthesize mercapturic acid by the growing pig is a species characteristic, experiments of Shiple, Muldoon and Sherwin[12] indicate the synthesis of mercapturic acid by the adult pig, if we accept the rise in the neutral sulfur of the urine on the day of feeding bromobenzene as a criterion of the synthesis of mercapturic acid, as has been done by Hele[5] and White and Lewis.[11] Since it has been shown by McGuinn and Sherwin[10] that the conventional methods for the isolation of mercapturic acid from the urine are not reliable, the failure of Hele[5] to isolate mercapturic acid from the urine of the pig may possibly be due to the

[11] White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

[12] Shiple, G., Muldoon, J., and Sherwin, C. P., *J. Biol. Chem.*, 1924, **60**, 59.

unreliability of the Baumann and Preusse[1] method and not to the absence of mercapturic acid in the urine.

Summary. 1. Bromobenzene was fed to mongrel pups, to a Dalmatian Coach pup and injected into growing mice. 2. p-brom-phenylmercapturic acid was isolated from the urine of all pups studied and from the urine of growing mice.

7275 C

Synthesis of p-Brom-phenylmercapturic Acid by the Fasting Growing Dog.

JAKOB A. STEKOL.

Department of Chemistry, Fordham University, New York City.

The literature on the subject of mercapturic acid synthesis in animals under various dietary regimes has been reviewed by White and Lewis.[1] One group of workers is of the opinion that exogenous cystine sulfur alone determines the synthesis of mercapturic acid,[2] while others maintain that the body tissues are capable of supplying cystine to meet the needs for detoxication of administered brom-benzene.[3] McGuinn and Sherwin,[4] contrary to Abderhalden,[3] have demonstrated that acidity or alkalinity of the diet has no effect on the synthesis of phenyl-mercapturic acid in rabbits. By chloroform extraction method, McGuinn and Sherwin[4] were able to isolate mercapturic acid from the urine of rabbits fed brom-benzene while maintained on either acid or alkaline diets.

Thomas[2] was unable to detect mercapturic acid in the urine of fasting dogs. Abderhalden,[3] however, isolated mercapturic acid from fasting dogs' urine, but he believes that the cystine requirements for more essential functions of the body limit the degree of mercapturic acid synthesis.

In all of the above mentioned experiments on fasting dogs, including that of Nishimura's on fasting rabbits,[3] the period of fasting was rather short. The experiments did not exclude the possi-

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

2 Thomas, K., and Straczewski, H., *Arch. Anat. u. Physiol., Physiol. Abt.*, 1919, 249; Kapfhammer, J., *Z. physiol. Chem.*, 1921, **116**, 302; Muldoon, J. A., Shiple, G. J., and Sherwin, C. P., *J. Biol.*, 1924, **59**, 675.

3 Abderhalden, E., and Wertheimer, E., *Z. physiol. Chem.*, 1931, **198**, 18; **201**, 267; Nishimura, K., *Acta Schol. med. univ. imp. Kioto*, 1929-30, **12**, 73.

4 McGuinn, A., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1115.