9079

IV. p-Bromophenylmercapturic Acid and Ethereal Sulfates Synthesis in Dogs Maintained on Diets of Varying Sulfur Content.

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Hele[1] and White and Lewis[2] have suggested that when the dietary sulfur is too low to meet the needs for detoxication of bromobenzene to yield mercapturic acid, the dog is forced to employ to a larger extent other paths of detoxication of bromobenzene, namely, oxidation to bromophenol and conjugation with sulfuric acid. This conclusion was based on the observation that following the feeding of bromobenzene to dogs maintained on diets of various sulfur contents, the neutral sulfur of the urine was most pronounced on a high-sulfur diet, less so on a low-sulfur diet. On a low-sulfur diet, coincident with a lower output of the neutral sulfur, higher values for the ethereal sulfates in the urine were observed. In view of the limitations of the significance of the fluctuations of the neutral sulfur in the urine following the administration of bromobenzene,[3] it seemed of interest to check the suggestions of Hele[1] and White and Lewis,[2] mentioned above, using a recently developed direct method for the estimation of mercapturic acid in the urine.[4]

Each of 4 female dogs were kept in individual metabolism cages and fed each of the diets described in Table I. After the completion of the experiments on the casein diet, each dog was then fed the low-

TABLE I.
Composition of Diets.*

	Casein	Low-protein	Protein-free
Casein, gm.	43.7	22.0	
Sucrose, gm.	40.6	62.3	84.3
Vitavose, gm.	11.6	11.6	11.6
Salt mixture,† gm.	1.4	1.4	1.4
Bone ash, gm.	2.7	2.7	2.7
Butter fat, gm.	7.0	7.0	7.0
Lard, gm.	17.0	17.0	17.0
Nitrogen, %	4.92	2.57	0.20
Sulfur, %	0.250	0.124	0.030

*The diets were devised according to: Cowgill, G. R., Deuel, H. J., Jr., and Smith, H. H., *Am. J. Physiol.*, 1925, **73**, 106.

†As used by Karr, W. G., *J. Biol. Chem.*, 1920, **44**, 255.

1 Hele, T. S., *Biochem. J.*, 1924, **18**, 586; 1926, **20**, 606.

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

3 Stekol, J. A., *J. Biol. Chem.*, 1937, **117**, 147.

4 Stekol, J. A., *J. Biol. Chem.*, 1936, **113**, 279.

protein diet, and similar experiments carried out. After the low-protein diet, the dogs were fed the casein diet again until they recovered from the protein starvation while receiving the low-protein diet. Nitrogen and sulfur balance was taken as a criterion of such recovery. Then they were fed the protein-free diet. In all cases, our dogs received food yielding 60-65 calories per kilo of body weight, each dog receiving the same amount of food irrespective of the nature of the diet. The urine was collected every 24 hours by catheterization and the food was fed immediately after, at 9 a. m. One gm. bromobenzene was fed in a gelatine capsule daily for 4 consecutive days while feeding each of the 3 diets. The urine was analyzed by methods described previously[5] p-bromophenylmercapturic acid was determined by the recently described method,[4] until the urine collected for several days showed the absence of the acid. Water was allowed *ad libitum*. The ethereal sulfate sulfur values, as presented in Table II, indicate the rise in the output of ethereal sulfate sulfur on the days of administration of bromobenzene. The values were computed by deducting from the figures obtained on the day of feeding bromobenzene, the value for ethereal sulfates secured on the preceding days, usually the average of 3 to 4 days. The ethereal sulfate sulfur values were quite uniform.

TABLE II.
Excretion of Mercapturic Acid and Ethereal Sulfates on Diets of Varying Sulfur Content.

Diet	Casein		Low-protein		Protein-free	
	N	S	N	S	N	S
Intake	gm. 4.92	gm. 0.250	gm. 2.57	gm. 0.124	gm. 0.20	gm. 0.030
	Urinary Output					
Day	Mercapturic acid mg.	Ethereal SO_4S mg.	Mercapturic acid mg.	Ethereal SO_4S mg.	Mercapturic acid mg.	Ethereal SO_4S mg.
1*	560	26	568	30	600	27
2*	732	27	575	33	613	40
3*	823	30	589	40	405	41
4*	921	29	643	43	376	50
5	175	2	286	10	286	21
6	125		100		143	
Total	3336	114	2752	159	2423	179
% Bromobenzene detoxicated	38.0	14.2	31.3	20.0	27.6	22.4
Total % Bromobenzene detoxicated	52.2		51.3		50.0	

*1 gm. bromobenzene in a gelatin capsule fed at 9 a.m.

[5] Stekol, J. A., *J. Biol. Chem.*, 1934, **107**, 225; 1934, **107**, 641; 1935, **109**, 147.

varying from 18 to 20 mg. per day, and remained practically unaffected by the change of diet.

For the sake of economy of space, typical results are presented in a summarized form in Table II. The data indicate that on the casein diet, the repeated administration of bromobenzene was followed by an apparently progressive increase in the synthesis of mercapturic acid. This apparent increase we attribute to the accumulation of mercapturic acid in the body with each successive dose of bromobenzene, due to a lag in the excretion of mercapturic acid.[3] No significant increase in the ethereal sulfate synthesis was noted on repeated feeding of bromobenzene on the casein diet. On the low-protein and the protein-free diet, the extent of synthesis of mercapturic acid was lower, on the protein-free diet being the lowest. The largest amount of ethereal sulfates was formed on the protein-free diet. Comparison of the per cent detoxication of bromobenzene on the 3 diets shows that the total amount of bromobenzene taken care of by our animals to yield ethereal sulfates and mercapturic acid, irrespective of the dietary sulfur, was about the same, 50-52% of the dose fed. The nature of the detoxication, however, was quantitatively different: on a low-protein or protein-free diet, the animal is apparently forced to employ the mechanism of ethereal sulfate formation to a greater extent than on a casein diet. The results are in accord with the observations of Hele[1] and White and Lewis[2] concerning the existence of 2 independent mechanisms of detoxication of bromobenzene in dogs. It should be, however, emphasized again that these observations by no means demonstrate the direct dependence of the extent of the synthesis of p-bromophenylmercapturic acid in dogs on the cystine or methionine content of the diet. Our data show that even though our diets varied in their sulfur content from 0.248 to 0.020%, *on the first day of maintenance on these diets our dogs yielded nearly the same amounts of mercapturic acid and ethereal sulfates.* It is improbable that cystine and methionine of the diet remained in circulation 24 hours after the last administration of food. The available data indicate that l-cystine and dl- or l-methionine, when fed to dogs in amounts as present in our casein diet, are readily oxidized to yield inorganic sulfates.[6] Had the extent of the synthesis of mercapturic acid been dependent directly on the dietary sulfur, no such constancy in the output of mercapturic acid on the 3 diets could be expected. We suggested previously that the immediate source of sulfur of the mer-

[6] Pirie, N. W., *Biochem. J.*, 1932, **26**, 2041; Stekol, J. A., and Schmidt, C. L. A., *Univ. California Pub. Physiol.*, 1933, **8**, 31; Virtue, R., and Lewis, H. B., *J. Biol. Chem.*, 1934, **104**, 59.

capturic acid in all cases, irrespective of the dietary sulfur, is the tissue sulfur.[3] When l-cystine, cysteine, dl- or l-methionine were fed to dogs kept on the casein diet, no retention of the sulfur of these amino acids was observed[5]; nor was there any increase in the extent of the synthesis of p-bromophenylmercapturic acid as compared to that on the casein diet alone when l-cystine or dl-methionine were added to the casein diet.[3] When these amino acids were added to the low-protein diet, a retention of the sulfur of the amino acids was observed[5]; also an increased synthesis of p-bromophenylmercapturic acid as compared to that on the low-protein diet alone was noted.[3] It seems that when the dog is maintained on the casein diet, its sulfur stores, which are utilized in the detoxication of bromobenzene, cannot be further increased by the addition of l-cystine or dl-methionine to the diet, while on the low-protein diet supplemented by l-cystine or dl-methionine, a restoration of the tissue sulfur occurs and consequently an increased supply of the sulfur stores which are used in the detoxication of bromobenzene is available. As shown in Table II, on the first day of maintenance on the low-protein or the protein-free diets which were fed immediately after the casein diet, the stores of sulfur which the animal possessed while on the casein diet are still present and consequently the same amount of sulfur is available for the detoxication of bromobenzene on all 3 diets. On repeated administration of bromobenzene to dogs kept on a casein diet, these sulfur stores which are attacked by bromobenzene are restored at the expense of the dietary sulfur; while on the low-protein and the protein-free diets, less efficient recovery of the tissue and none at all respectively, takes place. Consequently, depletion of the sulfur stores of the animal follows, with subsequent increase in toxicity of bromobenzene with each new dose. Decreased synthesis of mercapturic acid is thus directly due to the depletion of the tissue sulfur available for the detoxication. It is of interest to mention in this connection that only l-cystine, cysteine and dl-methionine, but not taurine, were effective in augmenting the synthesis of p-bromophenylmercapturic acid in dogs.[2, 3] It will be remembered that only the first 3 amino acids are capable of replacing the tissue wear and promoting growth in animals kept on cystine-low diets. It seems that the ability of a sulfur-containing amino acid to restore the tissue sulfur of the animal is essential for its being an effective agent for augmenting the mercapturic acid synthesis in dogs.

Summary. 1. Dogs were fed diets of varying sulfur content and 1.0 gm. doses of bromobenzene were fed on 4 consecutive days on each of the diets. The synthesis of p-bromophenylmercapturic acid

and ethereal sulfates on these diets was estimated. 2. The extent of the synthesis of the mercapturic acid and of the ethereal sulfates is apparently a function of the nutritive state of the animal: when the animal was deprived of dietary sulfur, a decrease in the output of mercapturic acid and an increase in the output of ethereal sulfates was noted. 3. The results offer further support to the previous suggestion that the dietary sulfur is not the immediate source which is used for the detoxication of bromobenzene in dogs.

9080

Action of Parahydroxyphenylisopropylamine on Induced Cardiac Standstill.

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The pressor response of the experimental animal has been used almost exclusively in the investigation of the comparative pharmacological actions of the epinephrine-like compounds (the sympathomimetic amines).[1] In previous studies,[2] the cardiac standstill which may be induced in many individuals by pressure over the right carotid artery in the neck (carotid sinus reflex) was used as a basis for the investigation of the comparative activities of these substances on the human heart. In susceptible subjects a cardiac arrest of many seconds' duration can be induced consistently by compression of the carotid sinus. The standstill is the result of an active reflex stimulation of the vagus nerve so that the heart is temporarily deprived of its pacemaker, the sinus node.

Upon administration of drugs of the epinephrine series the cardiac standstill could be prevented due to the development of new centers of stimulus formation, usually in the ventricles. This effect on cardiac rhythmicity is definitely a sympathomimetic action as it could be produced by a number of sympathomimetic compounds and could not be affected by a variety of unrelated substances. The rate of the ectopic pacemaker was as an index of the intensity of the effect, and this served as a method for the comparative study of these substances using the human heart as the test object. As an illustration, after the intravenous administration of epinephrine 0.05 mg. the cardiac standstill was prevented by the development of a

1 Barger, G., and Dale, H. H., *J. Physiol.*, 1910, **41**, 19.

2 Nathanson, M. H., *Arch. Int. Med.*, 1934, **54**, 111.