LXXXIV. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

V. TOXIC ACTION OF MERCAPTURIC ACIDS.

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So far as has been ascertained there are only two records in the literature of the administration of mercapturic acids to dogs. Baumann and Preusse [1881] administered by mouth 8 g. of the ammonium salt of *p*-bromophenylmercapturic acid. No ill effects were observed; no glycuronic acid was excreted and only traces of mercapturic acid. No sulphur analyses apparently were done. Marriott and Wolf [1907] gave 1 g. of the bromo-acid by mouth. No ill effects were recorded but in the record the sulphur analyses of the urine do not show clearly whether any mercapturic acid was excreted. Possibly absorption was incomplete in both cases.

In the year 1922 during the course of experiments recorded in Part III of this series [Callow and Hele, 1926] it was found quite unexpectedly that an oral dose of 1 g. of *p*-bromophenylmercapturic acid produced a transient haemoglobinuria in the dog "Vixen." This result has been confirmed in several other dogs.

EXPERIMENTAL.

The methods employed in this research were identical with those already described [Callow and Hele, 1926]. Five dogs were used. "Vixen" and "Patricia" received the diets mentioned previously. "Diana" was not placed on a standard diet. "Phoena" and "Pansy" were given the following diets:

"Phoena" (6.97 kg.):

		T.N. g.	T.S. g.	Calories K
Lean meat	80 g.	2.73	0.181	106
Margarin	40 g.	—	—	312
Arrowroot biscuit	20 g.	0.22	0.025	121
Milk	160 cc.	0.83	0.074	115
Total		3.78	0.280	654

"Pansy" (8.62 kg.):

		T.N. g.	T.S. g.	Calories K
Lean meat	80 g.	2.73	0.181	106
Margarin	20 g.	—	—	156
Sucrose	50 g.	—	—	205
Milk	160 cc.	0.83	0.074	115
Total		3.56	0.255	582

Exp. 1. "Vixen." At 11 a.m. 1 g. *p*-bromophenylmercapturic acid was administered by mouth. At 5 p.m. on the same day the urine voided was almost black in colour and contained much haemoglobin. The microscope showed the sediment to consist of red-cell debris. There was evidence neither of haematuria nor of a primary nephritis. At 5 p.m. on the following day the urine was free from haemoglobin. Traces of albumin however persisted for another two days.

Exp. 2. "Patricia." 0.5 g. *p*-bromophenylmercapturic acid was administered by mouth. No ill effects were noted. The dose was too small to make any appreciable alteration in the output of neutral sulphur.

Exp. 3. "Phoena." At 11 a.m. 2.5 g. *p*-chlorophenylmercapturic acid were administered by mouth. At 9 a.m. on the following day 15 cc. of deep red urine were removed from the bladder, the previous urine being clear. The red urine contained much free haemoglobin, much cell debris and a few blood cells. At 12.45 p.m. there was in the urine only a trace of haemoglobin with a few blood cells. Until 9 p.m. there persisted a trace of albumin but no further trace of haemoglobin. The dog vomited the evening meal on the first day and afterwards refused all food except the meat and milk. In consequence the outputs of nitrogen and of neutral sulphur, which rose considerably, had no experimental value. There was however no increase in the outputs of ethereal sulphate, free or conjugated phenols, indicating that there had been no conversion of mercapturic acid to ethereal sulphate. The "organic" chlorine excreted in the first 24 hours after the dose corresponded to only 6 % of the dose, but on the following day during the recovery period the organic chlorine indicated an excretion of 30 % of the dose. Although chlorides were freely excreted during the haemoglobin period yet there was a marked retention of nitrogen. Afterwards the chloride excretion fell and the nitrogen rose. The excretion of sulphate was similar to that of the nitrogen. Fig. 1 shows graphically the excretives in the urine during the experimental period. Fig. 2 shows the concentration of chloride (expressed as chlorine), total nitrogen and sulphate in the urine and also the variations in its volume, associated with variations in the water intake.

Exp. 4. "Diana." 0.5 g. *p*-chlorophenylmercapturic acid was given subcutaneously as the ammonium salt. No ill effects were noted.

A week later 1 g. *p*-bromophenylmercapturic acid was given subcutaneously as the ammonium salt at 10 a.m. At 10 p.m. there was a trace of haemoglobin in the urine. The urine voided during the night was very dark coloured and the haemoglobin persisted until 2.30 p.m. on the following day. After 4.30 p.m. the urine contained no albumin. During the haemoglobin period the sediment contained much cell debris with a very occasional blood cell or epithelial cell. There were no casts until the recovery period and then only a few epithelial and blood casts.

Exp. 5. "Pansy." This experiment is shown in Fig. 3. At 10 a.m. 1.23 g. *p*-bromophenylmercapturic acid was administered by the mouth. The urine

Fig. 1.
Exp. 3.

Fig. 2.
Exp. 3.

Fig. 3.
Exp. 5.

Figs. 1 and 2. "Phoena." Day No. 5. 2.5 g. *p*-chlorophenylmercapturic acid.
Fig. 3. "Pansy." „ No. 9. 1.23 g. *p*-bromophenylmercapturic acid.

remained clear until 5 p.m., when the dog was placed in the metabolism cage. The urine passed during the night was mostly clear, though there was a little blood at the bottom of the collecting vessel. At 9 a.m. on the following day 17 cc. of bloody urine were removed from the bladder. This urine contained much cell debris, only a few red cells and no casts. For the quantitative work this urine was discarded. For the next 24 hours the urine was cloudy, containing traces of blood and some albumin. On the third day the urine was clear and free from albumin. The diet was maintained throughout, so that variations in the quantities of the urinary products could not be ascribed to errors of diet. During the "albumin" period estimations in duplicate were carried out on the untreated urine, on the urine rendered protein-free by trichloroacetic acid and on the urine rendered protein-free by "dialysed iron." The agreement between these was very close, showing that the quantity of albumin present was too small to affect the neutral sulphur figure appreciably. The rise in neutral sulphur corresponded to an excretion of 32 % of the dose in the first 24 hours and 16 % in the second. There was no retention of nitrogen as in Exp. 3, showing that the permeability of the kidney was hardly affected. There was no alteration in the outputs of ethereal sulphate or conjugated phenols, indicating that there had been no conversion of mercapturic acid to ethereal sulphate. No explanation is offered for the rise in the output of nitrogen on the tenth day and of inorganic sulphate on the eleventh day. The rise in free phenols was slow and continuous and bore no relation to the dose-day.

DISCUSSION.

From the above experiments it is clear that the administration of *p*-bromo- or chloro-phenylmercapturic acid may produce a transient haemoglobinuria in the dog. There is a minimum dose necessary to bring about this effect. If the haemoglobinuria is considerable and the kidney tubules are largely blocked with cell debris, there is a retention of nitrogen and the excretion of the mercapturic acid is delayed. These experiments would suggest that this effect of mercapturic acid might be used as a method of producing experimentally a variable degree of "blocked kidney," of the kind observed in blackwater fever. The cause of the haemolysis which precedes the excretion of the haemoglobin is not clear. The effect is observed, whether the mercapturic acid is administered orally or hypodermically. Preliminary experiments on the direct action of mercapturic acids on the blood cells of the dog *in vitro* suggest that mercapturic acids have no direct haemolysing action. The action of mercapturic acids is remarkable, when the comparatively non-toxic character of bromo- and chloro-benzene is considered. These dogs are capable of dealing with 5 g. of bromobenzene in a single dose without haemolysis being produced, that is ten times the dose of bromobenzene equivalent to the toxic dose of the corresponding mercapturic acid. Of course the concentration of mercapturic acid in the effective organ may never be so high

at any given moment after bromobenzene as after the administration of the mercapturic acid. Until, however, something is known about the mechanism of the production of the haemolysis, it would be wrong to regard the formation of mercapturic acid in the dog as a protective synthesis.

It must be noted that in these experiments there is no indication of any conversion of mercapturic acid to ethereal sulphate. Any such conversion would be seen in the outputs of ethereal sulphate and of conjugated phenols.

SUMMARY.

1. *p*-Bromo- and *p*-chloro-phenylmercapturic acids, whether given by the mouth or subcutaneously, cause a transient haemoglobinuria in the dog. There is a minimum dose required to produce this effect.

2. There is no evidence of any conversion of mercapturic acids to ethereal sulphates in the dog.

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