LXXX. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

IV. THE MECHANISM OF MERCAPTURIC ACID FORMATION IN THE DOG.

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IN the preceding communication it was stated that Baumann discovered that administration of chloro-, bromo- or iodo-benzene to a dog on a mixed diet led to the excretion, in very good yield, of the corresponding halogen-phenyl-mercapturic acid in the urine.

I	II	III
Bromobenzene	*p*-Bromophenol	*p*-Bromophenylmercapturic acid
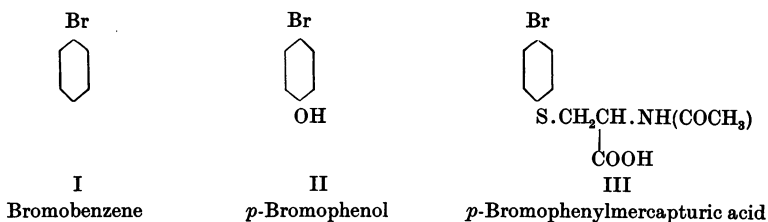
This excretion of mercapturic acid causes a marked rise in the output of neutral sulphur in the urine of a dog on a standard diet (cf. rise after iodo-benzene, Figs. 5 and 6). Conversely, in the absence of better methods of showing the presence of a mercapturic acid in the urine, it has often been assumed, in work on this subject, that a rise of neutral sulphur, after ingestion of a compound, indicates the presence and quantity of a mercapturic acid formed from that compound. While this assumption must be accepted with reserve, the assumption, which is made in this communication, that absence of a rise of neutral sulphur, after ingestion of a compound, indicates that a mercapturic acid has not been formed, is almost certainly true.

The only mercapturic acids which have ever been isolated are the three mentioned above, but the work of the preceding communication suggests that the *o*- and *m*-dichlorobenzenes, and benzene itself, also give rise to mercapturic acids. Baumann and Preusse [1881] were able to show that the mercapturic acids which they isolated were *para*-compounds and this was confirmed by Friedmann [1904].

In all the compounds which give rise to an increased neutral sulphur output in the urine—chloro-, bromo- and iodo-benzene and the *o*- and

m-dichlorobenzenes (benzene, having no halogen, being a special case)—it will be noticed that there is a position *para* to a halogen available for linkage with the cysteine. While working with the dichlorobenzenes it was intended to give the corresponding *para*-compound. In this case—if mercapturic acid were indeed limited to the *para*-position—no rise of neutral sulphur would be expected. Unfortunately, however, it was found that *p*-dichlorobenzene is rather insoluble, is not readily absorbed and is very irritant to the intestine [cf. Sollmann, 1920] so that no experiments could be carried out with it. To investigate the point, therefore, it was decided to substitute the less toxic *p*-chloroacetanilide and *p*-chloroanisole. As was expected, no rise of neutral sulphur was found after the ingestion of either of these two compounds (Figs. 1 and 3). To complete the work the corresponding *o*- and *m*-chloro-acetanilides and chloroanisoles[1] were also given (Figs. 2, 3 and 4), but it was found, with some surprise, that although a *para*-position to a halogen is always available, in no case was there a rise of neutral sulphur and therefore no mercapturic acid formation. It therefore seems that the mere introduction of an acetamino- or methoxy-group has profoundly altered the metabolism from that of chlorobenzene, and therefore the above compounds cannot be used to decide if mercapturic acid formation is limited to the *para*-position to the halogen or not. This change of metabolic process caused by slight changes in the compound used is being further studied.

Another aspect of mercapturic acid formation was also investigated. Baumann and Preusse [1879] were the first to observe that *p*-bromophenol was also excreted after the administration of bromobenzene. Subsequent workers in this field have assumed that the phenol (II) is an intermediary between the halogen-benzene (I) and the mercapturic acid (III). To confirm this supposition, dogs were fed on *p*-chlorophenol. It at once becomes obvious from a comparison of the curves for this compound with those for iodobenzene (Figs. 5 and 6) that these compounds are metabolised differently—whereas the chlorophenol is only excreted as a phenol linked with sulphate, the iodobenzene is excreted both as a phenol and also as a mercapturic acid. *p*-Chlorophenol cannot, therefore, be an intermediate substance and it seems probable that mercapturic acids are formed by direct condensation of the halogen-benzene with the cysteine. In the light of this new evidence, the arguments put forward in the second paper of this series [Hele, 1924] on the balance between the formation of ethereal sulphate and mercapturic acid require some modification. The determining factor in the relative amounts of ethereal sulphate and mercapturic acid formed from chlorobenzene appears to be the rate of oxidation of the benzene nucleus to

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[1] Observations of our own and other investigators have shown that the three halogens, chlorine, bromine and iodine can be interchanged in compounds of this type without any notable change in the metabolism of the compound, *e.g.* the animal deals with *o*-bromoanisole as with *o*-chloroanisole. As we had a sample of *o*-bromoanisole and not the chloro-compound the former was given. *p*-Bromoanisole was also used (Fig. 3).

the phenol compared with the rate of condensation of the same nucleus with the sulphydryl group of the cysteine.

After administration of o- and m-chlorophenols exactly the same effect was noticed as with the o- and m-chloroacetanilides and chloroanisoles. From the graphs (Figs. 3 and 4) it is seen that no mercapturic acid was formed although a position para to the halogen in each case is unsubstituted. Phenol itself (Fig. 6) also does not give rise to a mercapturic acid.

Experimental.

The experimental methods were essentially the same as those described in an earlier paper of this series. The same dogs were employed. "Sophia" and "Vixen" received the diets already described [Hele, 1924, pp. 595 and 597]. "Phoena" and "Patricia" were given the diets shown below:

			T. N. g.	T. S. g.	Calories K.
"Phoena" 7·0 kg.	Meat:	90 g.	3·07	0·202	120
	Sugar:	50 g.	—	—	191
	Margarine:	30 g.	0·21	0·015	234
	Milk:	160 cc.	0·83	0·074	115
		Total	4·11	0·291	660
"Patricia" 6·8 kg.	Meat:	100 g.	3·41	0·226	133
	Sugar:	50 g.	—	—	191
	Margarine:	20 g.	0·14	0·010	156
	Milk:	160 cc.	0·83	0·074	115
		Total	4·38	0·310	595

All the chemicals were from Kahlbaum except the acetanilides, which were made by standard methods and were easily obtained in a pure condition. The phenols were dried in a desiccator for several weeks over calcium chloride before weighing. All the substances were given by mouth in gelatin capsules. It was thought, however, that if the phenols were administered in the ordinary way, quick digestion might produce a high local concentration of the phenol and therefore injury to the stomach wall. To obviate this difficulty each capsule was wrapped in a long thin piece of meat (part of the diet) and the whole kept together by a few small stitches. No symptoms of any kind were noticed with the phenols or any other of the compounds given except p-chloro-acetanilide. This compound appeared to be much more toxic than the corresponding o- and m-isomerides (compare the dichlorobenzenes above). After receiving a dose of this compound the animal remained apparently unaffected till the evening with a normal rectal temperature (38·3°) but next morning the animal was found in a state of collapse and the temperature was depressed (37·1°). A slightly larger dose given to another dog caused the same symptoms and death through heart failure. With the smaller dose recovery was very rapid.

The results are shown graphically. The daily outputs of sulphur in the form of inorganic sulphate, neutral sulphur and ethereal sulphate and also of total nitrogen are given. In every experiment analyses were carried out on many days, before and after the period shown, to make sure that the animal

was in a state of equilibrium before the compounds were given, and also to see that the animal returned to normal after them. To save space these days have been omitted in the figures. Moreover, most of the experiments have been repeated and the results confirmed.

As all the dogs were approximately the same weight it was found convenient to adopt a "standard dose" for the substances administered and most of the doses were either the molecular weight in grams divided by one hundred (M.W./100 g.) or a small multiple of this. In the earlier experiments this was not done but for convenience of comparison the doses used have been expressed in terms of molecular weight.

It is evident from the graphs that there is no rise of neutral sulphur after any of the compounds given except after iodobenzene, which was given as a standard of comparison. However, without exception, it will be noticed that there is a very big rise in the ethereal sulphate. This can also be seen from the table wherein the approximate rise of ethereal sulphate, caused by the administration of the substance, and the approximate percentage of the dose excreted as ethereal sulphate is given. This increased output is an indication that all the substances have been freely absorbed and excreted as phenolic compounds linked with sulphate.
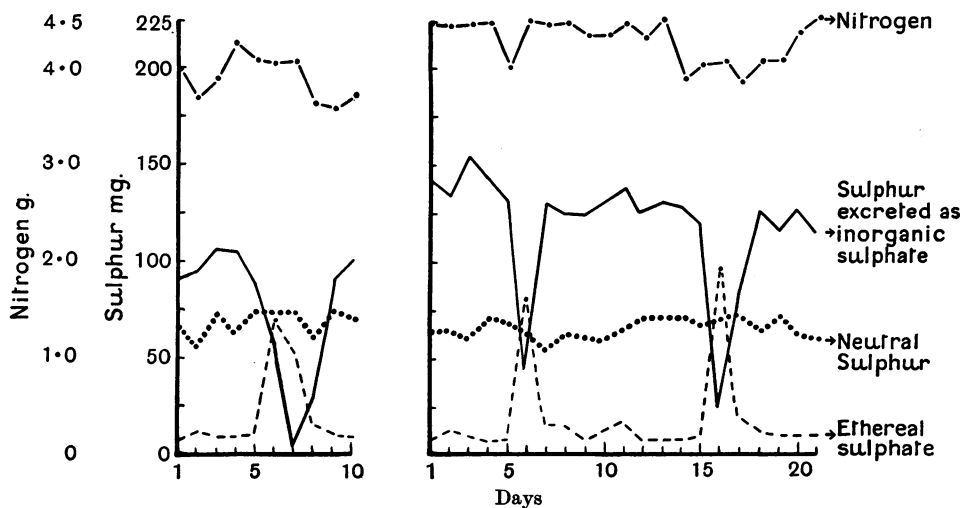

Fig. 1.

Fig. 1. "Phoena" Day no. 6 0·6 × M.W./100 g. p-Chloroacetanilide
Fig. 2. "Patricia" Day no. 6 0·6 × M.W./100 g. o-Chloroacetanilide
Day no. 16 0·6 × M.W./100 g. m-Chloroacetanilide

The curves also indicate that this increase of ethereal sulphate is at the expense—in part at least—of the inorganic sulphate. Shiple, Muldoon and Sherwin [1924], however, working on the pig, and Rhode [1923], working on the rabbit, could not satisfy themselves that there was any diminution of inorganic sulphate after the ingestion of phenols. The actual percentage, however, of the doses excreted as ethereal sulphate in the experiments
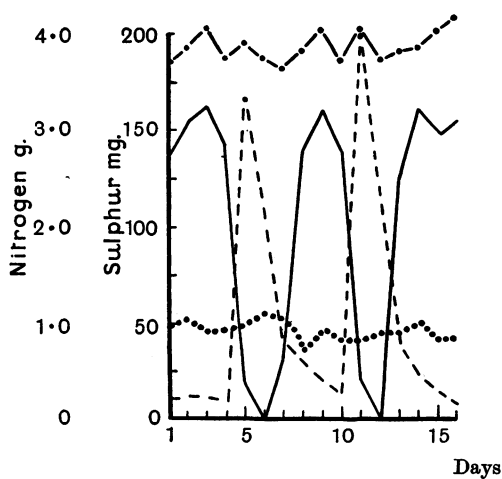
40—2

Fig. 3.

Fig. 4.

Fig. 3. "Sophia" Day no. 5   2 × M.W./100 g.   o-Bromoanisole
             ,, no. 11   2 × M.W./100 g.   p-Bromoanisole
Fig. 4. "Sophia" Day no. 6   1 × M.W./100 g.   m-Chloroanisole
             ,, no. 12   2 × M.W./100 g.   m-Chloroanisole

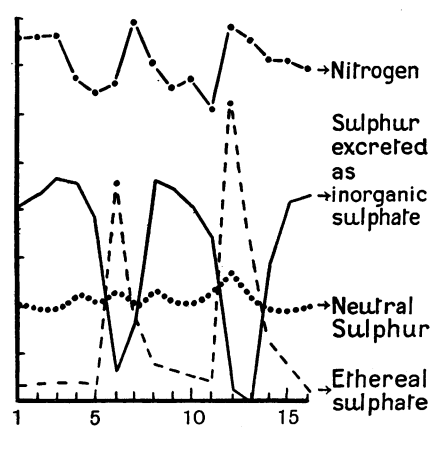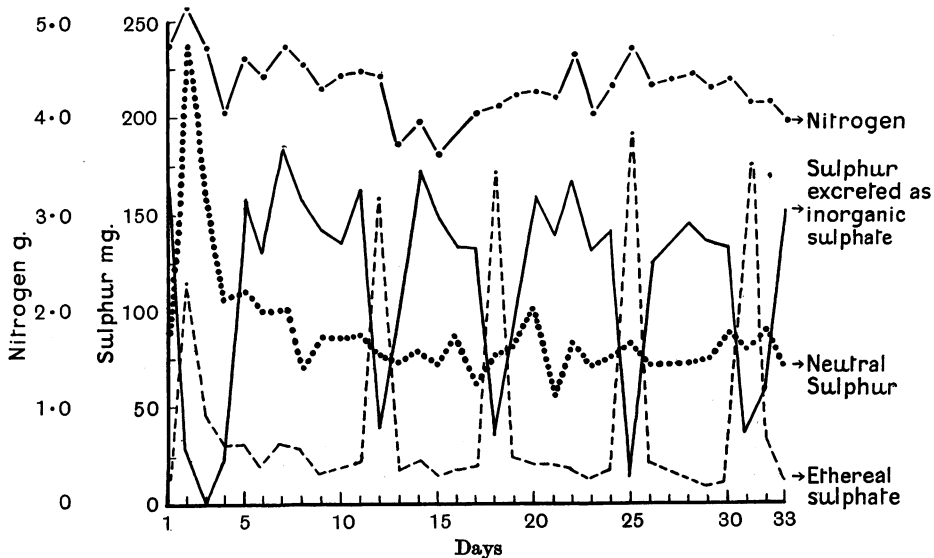Fig. 5.

Fig. 5. "Vixen" Day no. 2   1·8 × M.W./100 g.   Iodobenzene
            ,, no. 12   0·8 × M.W./100 g.   p-Chlorophenol
            ,, no. 18   1 × M.W./100 g.   m-Chlorophenol
            ,, no. 25   1 × M.W./100 g.   Phenol
            ,, no. 31   1 × M.W./100 g.   o-Chlorophenol

Days
Fig. 6.
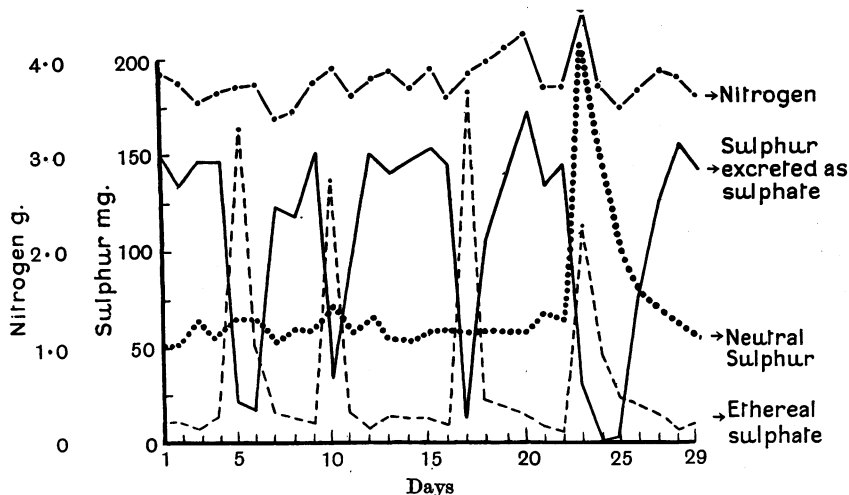
Fig. 6. "Sophia" Day no. 5   2 × M.W./100 g.   Anisole
      ,,   no. 10   1 × M.W./100 g.   Phenol
      ,,   no. 17   1 × M.W./100 g.   p-Chlorophenol
      ,,   no. 23   2 × M.W./100 g.   Iodobenzene

Dog	Substance	Dose M.W./100 g.	Numbers taken from figure	Total rise of ethereal sulphate as sulphur mg.	Percentage of dose excreted as ethereal sulphate
"Phoena"	p-Chloroacetanilide	0·6	1	101	52
"Patricia"	o-Chloroacetanilide	0·6	2	79	42
	m-Chloroacetanilide	0·6	2	100	52
"Sophia"	o-Bromoanisole	2	3	290	45
	p-Bromoanisole	2	3	328	51
"Sophia"	m-Chloroanisole	1	4	152	48
	m-Chloroanisole	2	4	250	39
"Vixen"	Iodobenzene*	1·8	5	173	30
	p-Chlorophenol	0·8	5	150	59
	m-Chlorophenol	1	5	170	53
	Phenol	1	5	182	57
	o-Chlorophenol	1	5	187	58
"Sophia"	Anisole	2	6	189	30
	Phenol	1	6	122	38
	p-Chlorophenol	1	6	171	53
	Iodobenzene*	2	6	160	25

* The rise of neutral sulphur after these doses was respectively 272 mg. and 299 mg. representing yields of 47 % of mercapturic acid in both cases.

described by these workers was small. Moreover, in the work of the former, the variations in the daily output of nitrogen were large and these would cause corresponding variations in the output of the inorganic sulphate, so that the point under discussion is somewhat obscured. The smaller rise of ethereal sulphate, obtained by the above authors, might be due to various causes—such as lack of absorption from the intestine, or greater destruction of the benzene nucleus by oxidation after absorption. Perhaps the difference

is entirely one of species—the pig and the rabbit detoxicating phenolic compounds more readily by means of glycuronic acid than by sulphate. With reference to this point the above authors have also assumed that a rise of neutral sulphur in the urine after administration of various compounds is due to the formation of a mercapturic acid. To our knowledge, however, nobody has yet isolated a mercapturic acid from any animal other than the dog, and it is conceivable that the rise obtained by them might be due to some other cause. We hope, shortly, to investigate this problem of mercapturic acid formation in animals other than the dog.

SUMMARY.

1. Attempts were made to decide if mercapturic acid formation is limited to the position *para* to the halogen of chlorobenzene.

2. Compounds with the *para*-position occupied were given. *p*-Dichlorobenzene was not suitable for administration but with *p*-chloroacetanilide and *p*-chloroanisole no mercapturic acid formation took place. These experiments were not decisive, however, because the corresponding *ortho*- and *meta*-compounds were also found to give no mercapturic acids, and therefore the introduction of an acetamino- or methoxy-group alters the metabolism from that of chlorobenzene.

3. *p*-Chlorophenol does not give rise to a mercapturic acid, and is therefore not an intermediary between chlorobenzene and its mercapturic acid.

4. *o*- and *m*-Chlorophenol and phenol itself also give no evidence of mercapturic acid formation.

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