LXXXV. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

VI. THE METABOLISM OF THE PIG AND DOG COMPARED¹.

By HERBERT ISAAC COOMBS AND THOMAS SHIRLEY HELE.

From the Biochemical Laboratory, Cambridge.

(Received May 2nd, 1927.)

IN a former communication one of us [Hele, 1924, 1] was able to show that ethereal sulphates are formed by direct union of phenolic compounds with preformed sulphate. This work is directly opposed to the views held by Shiple, Muldoon and Sherwin [1924], who have published an account of work, which they interpret as evidence of the formation of ethereal sulphates from some product of endogenous sulphur metabolism, something of the type of a mercapturic acid being the intermediary. It seemed to us that perhaps this difference of opinion was caused by a difference in the species of animal employed, because, whereas dogs were used in this laboratory, a pig was used in the experiments of Shiple, Muldoon and Sherwin. We therefore decided to study the metabolism of the pig and to repeat as far as possible the experiments, which have been described in the former papers of this series and in which the dog was used as the experimental animal.

Early in our work it became obvious that there are great differences in the metabolism of the pig and of the dog. We found that the pig, unlike the dog, does not readily detoxicate phenolic substances by combination with compounds containing sulphur. The increase in the reducing power of the urine would suggest that the detoxicating agent is glycuronic acid. This then we believe to be the essential difference. Whereas in the dog ethereal sulphates and mercapturic acids are formed readily and glycuronic acids less readily, in the pig glycuronic acids are formed readily, ethereal sulphates less readily and mercapturic acids probably not at all. When our work was extended to the metabolism of the mercapturic acids, we also found a difference between the two species. No evidence could be obtained of a conversion of mercapturic acids to ethereal sulphates. In this respect the pig resembles the dog. On the other hand, with the doses used, mercapturic acids produced no sign of the haemoglobinuria which is so remarkable in the dog.

¹ The previous papers of this series are numbered 1—5 in the list of references.

EXPERIMENTAL.

Two experiments were carried out on different animals, pure bred female pigs, recently weaned, of the "Large White" breed. Young pigs had to be used, as there were no facilities in the laboratory for using adult pigs. An attempt was made to catheterise the pigs daily, a procedure so valuable in metabolism experiments on the dog. In the young pig this operation is difficult and was soon abandoned in favour of changing the pig from cage to cage every few hours, as each cage became fouled. The 24-hours' urine was made up each morning at 9 a.m. The volume was always large (1500 to 2000 cc. as compared with the 200 to 300 cc. of the dog). We found the pig's habits to be very regular and as any error in collection would be of urine passed many hours after food and therefore of low sulphur content, we thought failure to catheterise was not a serious matter. The analytical figures obtained justified this practice.

The diets used were as follows:

Exp. 1 (Fig. 1):

"Phyllis," born May 8th. Experiment July 16th to August 23rd, 1926. Weight at commencement of experiment, 14.5 kg.; at end, 18.5 kg.

9 a.m.	Sharps	175 g.	Water 1000 cc.
12 noon	Grey peas	200 g.	Fed dry
5 p.m.	Sharps	175 g.	Water 1000 cc.

The nitrogen content of the diet was 15.4 g.

Exp. 2 (Fig. 2):

"Rosamund," born July 23. Experiment October 29th to November 20th, 1926. Weight at commencement of experiment, 17.7 kg.; at end, 18.6 kg.

9 a.m.	Sharps	250 g.	Water 1500 cc., cabbage 100 g.
12 noon	Grey peas	200 g.	Fed dry
5 p.m.	Sharps	250 g.	Water 1500 cc., cabbage 100 g.

The nitrogen content of the diet was 19.6 g.

The "Sharps" were soaked for 24 hours in half the ration of water and the remainder of the water was added at the time of feeding. The sodium sulphate and guaiacol carbonate used in Exp. 1 were mixed with the morning and evening food. The remaining substances were administered in gelatin capsules. At first we had considerable difficulty in getting the pig to swallow the capsules but later, using the following technique, we were able to give the various doses easily and with certainty.

The pig is held with the fore feet off the ground between the knees of an assistant, who is in a standing position. A piece of thin rope is tied round the upper jaw behind the snout and the snout is drawn upwards and backwards. Under these circumstances the pig keeps the mouth open. The operator then draws the tongue forward preferably with forceps and throws or blows through a tube the capsule to the back of the tongue. The tension on the rope is immediately released and the pig swallows the capsule. The assistant should place a stick between the teeth on one side of the pig's mouth to prevent it accidentally biting the operator. If available a second assistant can be employed in holding the pig's back legs off the ground.

All the chemicals that were administered were of the highest degree of purity. The guaiacol carbonate, phenol, bromobenzene and iodobenzene were obtained from Kahlbaum, the *p*-iodophenol was made from *p*-aminophenol by the method of Brazier and McCombie [1912] and the mercapturic acids were prepared from the urine of dogs by the usual method.

The analytical methods have all been previously described [Hele, 1924, 2] except those for the determination of iodine. For this the method described by Kendall [1920] proved very satisfactory. We preferred however to incinerate with sodium peroxide instead of with sodium hydroxide.

The formation of ethereal sulphate.

We tried to repeat the experiments described in the first paper of this series [Hele, 1924, 1], using the pig in place of the dog. Larger doses were used of sodium sulphate (4 g. daily) and of guaiacol carbonate (10 g. on each of two days), as the normal output of inorganic sulphate was larger in the pig (Exp. 1, Fig. 1, days 5 to 13). About 80 % of the sulphate was absorbed and excreted. The guaiacol carbonate, however, gave rise to only a minute amount of ethereal sulphate, the excess over the normal daily output of ethereal sulphate-sulphur on the two days being respectively 14 and 27 mg. S and on the day following 34 mg. S. Estimation of the phenolic substances in the urine by the colorimetric method [Folin and Denis, 1915, 1916] suggested that there were being excreted each day about 2 g. of the guaiacol, equivalent to about 500 mg. sulphur. As guaiacol is rather insoluble, we tried phenol (3 g., Fig. 1, day 11). The excess ethereal sulphate-sulphur on the phenol day was only 74 mg. In the dog this dose of phenol would have yielded an excess of at least 400 mg. The colorimetric method indicated an excretion of about 2 g. of the phenol in the urine, equivalent to 680 mg. S.

It therefore became obvious that the experiment, which proved so successful on the dog, could not be repeated on the pig, because so small an amount of ethereal sulphate was formed in the latter case. To prove the point at issue it is not sufficient merely to produce a rise in ethereal sulphate larger than the rise following the administration of the phenol alone, but it is necessary to produce such a rise that it is immediately clear that some of the administered sulphate has been utilised. It would have required at least 18 g. phenol, too large a dose for safety, to convert all the inorganic sulphate of food and tissue origin and some of the administered sulphate to ethereal sulphate. Quite apart from any direct action on sulphur metabolism such a dose, even if given successfully, would probably have caused an increase in tissue breakdown. The rise in the output of sulphate from this source would then have obscured the issue.

Subsequently we gave 10 g. guaiacol carbonate alone to the same pig (Fig. 1, day 25) and again obtained only a minute rise in the ethereal sulphate, a rise too small for any comparison with the previous guaiacol experiment. To the second pig (Fig. 2, day 18) we administered 4.4 g. *p*-iodophenol. We

Fig. 1. "Phyllis."

Days No. 5 to 13. 4 g. $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ daily.
 " No. 8 and 9. 10 g. guaiacol carbonate daily.
 " No. 11. 3 g. phenol.
 " No. 17. 4.08 g. ($2 \times \text{m.w.}/100 \text{ g.}$) iodobenzene.
 " No. 21. 3.14 g. ($2 \times \text{m.w.}/100 \text{ g.}$) bromobenzene.
 " No. 25. 10 g. guaiacol carbonate.
 " No. 32. 3.65 g. ($1 \times \text{m.w.}/100 \text{ g.}$) *p*-iodophenylmercapturic acid.

This figure may be compared directly with the figures in Papers III, IV and V of this series. The figure in Paper I is, however, drawn to a different scale.

used the iodine compound as the output of iodine in the urine afforded an indication of absorption and excretion. From the iodine figures it was clear that 76 % of the dose was excreted on the first day and 8 % on the second day. The ethereal sulphate analyses indicated an excretion of only 10 % of the dose in this form. The dogs "Vixen" and "Sophia" excreted 59 % and 53 % respectively of the corresponding chloro-compound in the form of ethereal sulphate [Coombs and Hele, 1926]. These results are summarised in Table I.

Table I. *The formation of ethereal sulphate.*

Percentage of dose of various compounds excreted as:

	Ethereal sulphate		Inorganic sulphate	
	Pig %	Dog %	Pig %	Dog %
Sodium sulphate alone	Nil	Nil	81	89
Guaiacol carbonate alone	2.5	12	Nil	Nil
Guaiacol carbonate and sodium sulphate together:				
Guaiacol carbonate	2.4	30	Nil	Nil
Sodium sulphate	Nil	66	69	22
Phenol alone	—	38	—	Nil
		57		
<i>p</i> -Chlorophenol alone	—	53	—	Nil
		59		
<i>p</i> -Iodophenol alone	10*	—	Nil	—
Phenol and sodium sulphate together:				
Phenol	8	—	Nil	—
Sodium sulphate	Nil	—	75	—

The numbers for the dog are taken from Papers I and IV of this series.

* The iodine excreted in the urine indicated that 84 % of the *p*-iodophenol was excreted.

It is clear from these experiments that ethereal sulphates are less readily formed in the pig than in the dog. We have no proof of the fate of the large balance of the absorbed phenols. The urine of course contained glycuronic acid and the increase in the reducing power of the urine suggested that the balance of the phenols was excreted combined with this substance.

In no instance was any rise in neutral sulphur observed in the urine after the administration of phenols; in this respect the pig resembles the dog.

The effect of the monohalogenbenzenes on metabolism.

We administered 4.08 g. iodobenzene to the first pig (Fig. 1, day 17) but did not obtain any rise in the neutral sulphur of the urine. On the dose-day there was a rise of 98 mg. S in the ethereal sulphate fraction and 18 mg. on the following day, representing in all only 18 % of the dose. In the dog the same dose would have yielded a rise in neutral sulphur and ethereal sulphate, corresponding respectively to 47 % and 25 % of the dose. Taking the two sulphur fractions together, 18 % in the pig should be compared with 72 % in the dog [Coombs and Hele, 1926]. On the dose-day 1125 mg. iodine (44 % of the dose) was excreted and on the following day 369 mg. (14.5 % of the dose), showing that there had been no failure in absorption. We repeated

Fig. 2. "Rosamund."

Day No. 5. 6.36 g. ($2 \times \text{m.w./100 g.}$) *p*-bromophenylmercapturic acid.
 „ No. 11. 6.28 g. ($4 \times \text{m.w./100 g.}$) bromobenzene.
 „ No. 18. 4.4 g. ($2 \times \text{m.w./100 g.}$) *p*-iodophenol.
 „ No. 22. 6.12 g. ($3 \times \text{m.w./100 g.}$) iodobenzene.

This figure may be compared directly with the figures in Papers III, IV and V of this series. The figure in Paper I is however drawn to a different scale.

the experiment with a larger dose (6.12 g.) on the second pig (Fig. 2, day 22) and obtained exactly similar results.

We administered 3.14 g. bromobenzene to the first pig (Fig. 1, day 21) and obtained a small rise in the output of neutral sulphur, equivalent to 6.7 % of the dose. This increase might be attributed to normal variations in metabolism. The rise in ethereal sulphate corresponded to 14 % of the dose. A larger dose (6.28 g.) was given to the second pig (Fig. 2, day 11). The rise in ethereal sulphate on the dose-day indicated an excretion of 10 % of the dose and on the following day 3 %. The effect on the neutral sulphur is not so easy of interpretation. The neutral sulphur had risen to 212 mg. on the previous day. The actual increase over this figure on the dose-day was only 59 mg. S (4.6 % of the dose). This was followed by a fall and a subsequent rise. We regarded these variations as extraneous in origin and without relation to the administration of the bromobenzene. In the dog the rise in neutral sulphur would have been about 600 mg. S. However, the fact that a small rise in neutral sulphur did occur in the pig after bromobenzene made us attempt the isolation of a mercapturic acid from bromobenzene and also from iodobenzene. Although the usual precautions were taken, we failed to isolate any. The results are summarised in Table II.

Table II. *The effect of the monohalogenbenzenes on metabolism.*

Percentage of dose excreted as:

	Ethereal sulphate		Neutral sulphur		Halogen	
	Pig	Dog	Pig	Dog	Pig	Dog
	%	%	%	%	%	%
Iodobenzene	18	25	Nil	47	58.5	79
	19	30	Nil	47	51	68
Bromobenzene	14	28	6.7 (?)	47.2	—	—
	13	25	4.6 (?)	62.6	—	—
Chlorobenzene	—	24.9	—	34.8	—	57.9
	—	19.2	—	35.8	—	55.2

The numbers for the dog are taken from Papers II, III and IV of this series.

The effect of the mercapturic acids on metabolism.

To the first pig (Fig. 1, day 32) we administered *p*-iodophenylmercapturic acid. The rise in the neutral sulphur output on the first day corresponded to 83 % and the iodine excretion to 76 % of the dose. There was no appreciable rise in the ethereal sulphate. The excretion of the mercapturic acid was over within 24 hours. There was no haemoglobinuria.

As a control an equivalent dose of *p*-iodophenylmercapturic acid was given to a large female dog. The pig, weighing 18 kg. received 3.65 g., the dog, weighing 11 kg., received 2.23 g. The dog's urine was free from haemoglobin for the first 24 hours following the dose. For the next 23 hours the urine contained haemoglobin but on the third day the urine was clear. On the first day the iodine estimations in the urine showed an excretion of only 9 % of the dose, on the second day 12 %. The haemoglobinuria showed exactly

the same features as those observed following the administration of the bromo- and chloro-compounds [Callow and Hele, 1927]. It is interesting to compare the rapid excretion of mercapturic acid by the pig and the slow excretion by the dog.

To the second pig (Fig. 2, day 5) 6.36 g. *p*-bromophenylmercapturic acid, or twice the equivalent dose of the *p*-iodophenylmercapturic acid used for the first pig, were given. The rise in the neutral sulphur indicated that 80 % of the dose was excreted in the first 24 hours and 6 % on the following day. None was converted to ethereal sulphate. There was no haemoglobinuria. If the dosage, g. per kg. of body-weight, is compared, this dose is nearly three times the amount which produced haemoglobinuria in the dog [Callow and Hele, 1927]. More than half of the mercapturic acid administered was recovered unchanged from the urine. The results are summarised in Table III.

Table III. *The effect of the mercapturic acids on metabolism.*

Percentage of dose excreted as:

	Ethereal sulphate		Neutral sulphur		Halogen	
	Pig %	Dog %	Pig %	Dog %	Pig %	Dog %
<i>p</i> -Iodophenylmercapturic acid	Nil	Nil	83	(?)	76	21
<i>p</i> -Bromophenylmercapturic acid	Nil	Nil	86	48	—	—
<i>p</i> -Chlorophenylmercapturic acid	—	Nil	—	(?)	—	36

The numbers for the dog are taken from this paper and from Paper V of this series.

DISCUSSION.

From our results we feel justified in concluding that the metabolism of our pigs was very different from that of our dogs. The pig seems incapable of synthesising mercapturic acids. It is clear also that the pig does not synthesise ethereal sulphates with the same readiness as does the dog. In our experiments glycuronic acid was excreted and the increase in the reducing power of the urine, following the administration of phenolic compounds, suggested that the pigs detoxicated foreign substances of this sort mostly by the glycuronic acid synthesis, but there is no exact proof. It is to be noted that in our experiments with one exception (*p*-iodophenol, Fig. 2, day 18) the rise in ethereal sulphate was accompanied by a fall in inorganic sulphate. We know that the dog can synthesise ethereal sulphate directly from at least one phenol and sulphate [Hele, 1924, 1] and we have no reason to believe that the pig cannot do the same. However, we regard the conclusive proof of this in the pig as extremely difficult or impossible, because the pig does not synthesise ethereal sulphates readily.

Shiple, Muldoon and Sherwin [1924] express the view that there are two ways of detoxicating phenolic substances, the one by union with some intermediary sulphur compound of endogenous metabolism, which is oxidised to ethereal sulphate, and the other by utilising exogenous cystine to form a mercapturic acid or like substance, which may be either excreted unchanged or oxidised to ethereal sulphate wholly or in part. In the pig they obtained

a rise in neutral sulphur after the halogen-benzenes but were apparently unable to isolate a mercapturic acid from that animal [Muldoon, Shiple and Sherwin, 1923]. On the other hand, they believe that ethereal sulphates cannot be formed by the direct union of phenols with preformed sulphate, whether arising endogenously or exogenously. Their views are based on work on two animals, a dog of 15.5 kg. [Muldoon, Shiple and Sherwin, 1924] and a pig of 24 kg. [Shiple, Muldoon and Sherwin, 1924]. Their ideas are in accordance with theories put forward by Thomas and Straczewski, Zeller and Straczewski, Kapfhammer and Rhode, theories already discussed in this series [Hele, 1924, 2]. It is not clear however that the experimental evidence adduced by the American workers will bear none other than the interpretation put upon it by the authors.

In the work on the dog [Muldoon, Shiple and Sherwin, 1924, p. 679] the only suggestion of a controversial kind is the theory put forward that ethereal sulphate is solely formed from endogenous sulphur. The experimental basis for this theory is the fall in output of ethereal sulphate when cystine is given to a dog receiving a daily dose of bromobenzene. There does not seem, however, conclusive proof that such a fall occurs under these circumstances. The authors consider mercapturic acid to be derived from the union of bromobenzene with exogenous sulphur and ethereal sulphate from the union of bromobenzene with endogenous sulphur. Any increase therefore of exogenous sulphur would tend to upset the balance, increasing the mercapturic acid at the expense of the ethereal sulphate. They assume as others have done that the intermediary in either case is a phenol. We have however shown [Coombs and Hele, 1926] that the phenols are not intermediaries in the synthesis of mercapturic acids. When the series of reactions

is considered, it is clear that the decrease in ethereal sulphate, if it really does occur, must be due to an increased formation of mercapturic acid as the concentration of available cysteine is raised. There is no necessity to distinguish between endogenous and exogenous metabolism. In the second paper of this series [Hele, 1924, 2] stress was laid on the uniformity of the response of dogs to the halogen-benzenes, but some evidence, not very conclusive, was put forward in that paper to prove that the balance could be upset, if dosage with sulphate or with cystine was continued for more than one day.

The experimental work on the pig summarised in the first table of the American authors (p. 61) is not antagonistic to our experimental results and our views except in one particular. They found an increase in ethereal sulphate following the administration of *p*-bromophenylmercapturic acid. Perhaps it may be allowed that animals of the same species may vary in their power to carry out certain oxidations. We find it, however, very difficult from structural considerations to conceive of any oxidation of a mercapturic acid to ethereal sulphate without the fission of the molecule and the liberation of

a phenol, which might then be detoxicated by direct union with sulphate. This suggestion has already been mooted in the second paper of this series [Hele, 1924, 2]. An oxidation of a mercapturic acid to a sulphonic acid, the sulphur of which would still appear in the neutral sulphur fraction, is much more likely to occur [König, 1892]. In the rest of the table evidence is collected, which shows that the oral administration of cystine assists in the formation of ethereal sulphate from phenols, while sodium sulphate does not. We would expect cystine to be the more efficacious, because it is excreted more slowly, being in an unoxidised form, and because very likely it is oxidised at the seat of ethereal sulphate synthesis, thus producing sulphate where it is most required [Hele, 1924, 1]. For reasons already given we feel it would be very difficult to obtain conclusive proof of the formation of ethereal sulphate in the pig from preformed sulphate, even if the nitrogen-free diet, utilised by the American workers, were employed. Contrary to their findings we obtained generally a fall in inorganic sulphate when the ethereal sulphate rose. The pig behaves in this respect like the dog.

In the second table (p. 62) of the American work on the pig there are several findings contradictory to our theories. Bromobenzene caused a rise in neutral sulphur, and added cystine caused a further rise with a decrease in the output of ethereal sulphate. The effects of *p*-chlorophenol and cystine were similar. The fall in ethereal sulphate, which is not very great, may have been accidental and part of the neutral sulphur may be attributed to failure in oxidation of the cystine apart from any effect of the derivatives of benzene. This is certainly true in the *p*-chlorophenol—cystine experiments. The rise in neutral sulphur is about 400 mg. S. The dose (0.6 g.) of *p*-chlorophenol given could not theoretically have given rise to more than 150 mg. S. In this table there is also further evidence of the oxidation of *p*-bromophenylmercapturic acid.

In the American work on the pig there are only two findings which are seriously antagonistic to our theories; the rise in neutral sulphur after bromobenzene and the similar rise after *p*-chlorophenol. We believe that phenols bear no relation to the formation of mercapturic acids [Coombs and Hele, 1926] and even if these findings of the American workers are incontestable, it does not follow that such a rise in neutral sulphur indicates the formation of a mercapturic acid. It might be due to a sulphonic acid.

There are two defects in the work of the American authors, which are not unimportant. The variations in the output of nitrogen are considerable. These variations cannot always be attributed to the nature of the substances administered. Such variations indicate variations also in the metabolism of tissue sulphur, variations which would affect the neutral sulphur of the urine. It is true that these variations are most marked in the first "pig" table, where they have but little bearing on the authors' argument and it is probable that such variations are bound to occur when a nitrogen-free diet is employed, or at any rate are more fully exposed to view when the nitrogen excretion is

low. The second experiment described in this paper is open to the same criticism in a lesser degree. We have however taken into consideration these variations in nitrogen output and we do not think that our conclusions are inadmissible.

The second defect in the American work is avoidable. We have always found it necessary to allow a considerable interval of time to elapse between each stage of an experiment, in order to make certain that the metabolism has again reached the normal. It is true that in our first "pig" experiment we administered phenol almost immediately after guaiacol carbonate. In this particular experiment however we were testing merely the general effect of phenolic substances. The administration of guaiacol and phenol constituted one experiment. We have made an approximate division in the output of ethereal sulphate between these substances in the table as an indication of a general thesis. The American workers, however, were carrying out many different tests, and in our opinion they did not leave sufficient intervals of time between each stage of their enquiry. It is not always clear to what cause to attribute a particular effect.

In our experiments we have found that mercapturic acids produce haemoglobinuria in the dog, an animal capable of synthesising these compounds. On the other hand, we have found that the pig cannot synthesise mercapturic acids and that mercapturic acids in much larger doses per kg. of body-weight have no effect on the pig. The American workers also do not describe any toxic effects following the administration of mercapturic acids to the pig and the same seems true for the rabbit [Rose, Shiple and Sherwin, 1924]. Perhaps, too, the rabbit is incapable of synthesising mercapturic acids [Muldoon, Shiple and Sherwin, 1923]. This difference indeed seems remarkable, although it is possible that mercapturic acids in massive doses might cause haemoglobinuria even in the pig. The rapid excretion of mercapturic acids by the pig may be attributed to the fact that the kidney of the pig is not in any way blocked by red cell debris, the result of haemolysis.

SUMMARY.

1. The pig, unlike the dog, does not readily synthesise ethereal sulphate. For this reason it would be extremely difficult, if not impossible, to obtain conclusive evidence of the direct formation of ethereal sulphate from phenolic compounds and preformed sulphate. On the other hand, there is no evidence that the pig does not carry out this direct synthesis in the same manner as the dog.

2. The pig unlike the dog is probably unable to synthesise mercapturic acids.

3. The pig like the dog cannot convert mercapturic acids to ethereal sulphates.

4. Mercapturic acids in doses three times the toxic dose for the dog per kg. of body-weight do not produce haemoglobinuria in the pig.

The authors desire to thank the Government Grant Committee of the Royal Society for grants in aid of this research and one of them (H. I. C.) the Medical Research Council for a grant.

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