CXVI. THE METABOLISM OF IODOBENZENE AND *p*-IODOPHENYLMERCAPTURIC ACID IN THE RAT AND THE RABBIT.

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THE present work was undertaken as a preliminary step in the investigation of the experimental haemoglobinuria described by Callow and Hele [1927]. These workers found that *p*-bromo- and *p*-chloro-phenylmercapturic acids, whether given by the mouth or subcutaneously, cause a transient haemoglobinuria in the dog. An attempt was made to find a small animal which would show a similar response to dosage with mercapturic acids. This attempt has not so far been successful; but in the course of the work certain interesting features of the sulphur metabolism of rats and rabbits were displayed. The possibility of mercapturic acid formation from iodobenzene and the effect of dosage with *p*-iodophenylmercapturic acid were studied in both these animals.

The ability of the rabbit to synthesise *p*-bromophenylmercapturic acid has been shown by Abderhalden and Wertheimer [1925], and some evidence in favour of the occurrence of this synthesis in the rabbit has been brought forward by Rhode [1922], and by Shiple, Muldoon and Sherwin [1924] in the case of the pig. The evidence suggesting a synthesis of mercapturic acid by the rabbit, brought forward by Rhode, was very inconclusive, being founded solely upon the diminished synthesis of ethereal sulphate from monohalogen benzene derivatives when cystine was given simultaneously.

Evidence in favour of the oxidation of mercapturic acid to ethereal sulphate has been adduced by Shiple, Muldoon and Sherwin [1924] for the case of the pig, and by Rose, Shiple and Sherwin [1924] for the case of the rabbit. On the other hand, previous work in this laboratory by Coombs and Hele [1927] indicated that this oxidation does not occur in the pig, and a criticism of the conclusions drawn by Sherwin and his co-workers is given in their paper.

No case of haemoglobinuria following the administration of a mercapturic acid has been described, other than the observations of Callow and Hele quoted above; nor has haemoglobinuria been obtained, either in the rat or the rabbit, in the course of the present work.

EXPERIMENTAL.

I. *Experiments upon the rat.*

An inbred, piebald strain was used. The degree of toxicity of iodobenzene and *p*-iodophenylmercapturic acid for the rat is indicated by the

following data. It was found that iodobenzene could be given to adult male rats, by subcutaneous injection, in doses of 1.5 mg. per g. of body weight, every three days, for 12–18 days, before any marked deterioration in the animal's general condition occurred. A single dose of this size was lethal within 48 hours when given intraperitoneally. *p*-Iodophenylmercapturic acid was given as the aqueous solution of its sodium salt, and a dose of 1 mg. per g. of body weight was lethal in over 50 % of cases. A dose of 0.6 mg. per g. of body weight was well tolerated when given subcutaneously. Mr Innes, of the Department of Animal Pathology, Cambridge, has kindly submitted the kidneys, livers and intestines of animals used in the above experiments to histological examination, and found no gross pathological changes.

In all experiments upon the rat, the following synthetic diet, made into a paste with distilled water, was fed *ad lib*.

"Light white casein"	...	...	23 %
Starch	...	...	40 %
Cane sugar	...	...	17 %
Salt mixture	...	...	5 %
Palm kernel oil	...	...	15 %

Cod-liver oil and marmite were added to the above diet in the proportions of 2.5 % and 7.5 % respectively. In each experiment two Hopkins metabolism cages were set up, each cage containing two adult male rats. Each morning, the urine which had been passed during the preceding 24 hours, from all four rats, was mixed with the rinsings from the collecting apparatus, made up to a suitable volume with distilled water and filtered before analysis.

Analytical Methods.

Total sulphur, total sulphate and inorganic sulphate were determined by the methods used in this laboratory for the analysis of dog's urine [Hele, 1924]. The ethereal sulphates and neutral sulphur were obtained by difference. Iodine was estimated by the method of Kendall [1920] with the exception that one incineration only was required in order to destroy completely the organic matter present in 10 cc. of urine. Iodine estimated in alkaline solutions of *p*-iodophenylmercapturic acid was found in approximately the proportion of 92 % of that present.

Exp. 1. In this experiment iodobenzene was twice given subcutaneously. The results of the sulphur analyses are recorded graphically in Fig. 1. The dosage, and a summary of the resultant changes in the sulphur partition are given in Table I A. Owing to the small quantity of urine available for analysis no satisfactory estimations of neutral sulphur and ethereal sulphate were obtained for the 4th day of this experiment.

Exp. 2. *p*-Iodophenylmercapturic acid was given once mixed with the food, when about one-third of its iodine was detected in the urine; and once by stomach tube. The results of the sulphur analyses are recorded graphically in

Fig. 2. The dosage, and a summary of the resultant changes in the sulphur partition are given in Table I B.

Table I.

Experiment and dose	Period in days	Neutral S daily av. mg. S	Ethereal SO ₄ daily av. mg. S	Total extra neutral S mg. S	Total extra ethereal SO ₄ mg. S	% dose excreted as sulphur compound	% dose excreted as iodine compound
A. 4 ♂ RATS. Figures calculated per rat							
Average figures for 9 normal days	1-6 } 10-13 }	6.8	0.9	—	—	—	—
On 7th day 0.18 g. C ₆ H ₅ I subcutaneously	7-9	—	—	12.4	3.5	56	102
On 14th day 0.34 g. C ₆ H ₅ I subcutaneously	14-19	—	—	38.9	4.0	80	102
B. 4 ♂ RATS. Figures calculated per rat							
Average figures for 12 normal days	1-8 } 13-16 }	7.2	1.0	—	—	—	—
On 9th day 0.75 g. <i>p</i> -iodophenylmercapturic acid mixed with food	9-12	—	—	26.5	nil	43	33
On 17th day 0.20 g. ditto by stomach tube	17-18	—	—	12.5	nil	71	32
C. RABBIT I. ♀							
Average figures for 28 normal days	1-10 } 13-18 } 25-32 } 35-38 }	180*	13	—	—	—	—
On 11th day 5.4 g. C ₆ H ₅ I by stomach tube	11-12	—	—	nil*	280	33	54
On 19th day ditto subcutaneously	19-24	—	—	92*	182	32*	98
On 33rd day 3.0 g. and on 34th day 2.0 g. <i>p</i> -iodophenylmercapturic acid by stomach tube	33-34	—	—	234*	nil	53*	64
D. RABBIT II. ♂							
Average figures for 12 normal days	1-12	8.2	3.0	—	—	—	—
On 13th and 14th days 2 g. <i>p</i> -iodophenylmercapturic acid by stomach tube	13-14	—	—	312	nil	89	73

* The significance of these figures is uncertain, owing to the irregularity of the neutral sulphur excretion.

Exp. 3. Isolation of p-iodophenylmercapturic acid from rat urine. Sixteen adult male rats, caged in Hopkins metabolism cages, were used in this

Fig. 1. Dosage. On 7th day, 0.18 g. iodobenzene per rat, subcutaneously.
 „ 14th „ 0.34 „ „ „ „

experiment. Urine was collected daily, and stored over chloroform. The full synthetic diet described above was fed throughout. Each rat received 0.36 g. of iodobenzene subcutaneously every three days, until a total of 34.6 g. had been given. Five litres of urine were obtained, and worked up in two batches of $2\frac{1}{2}$ litres each.

Fig. 2. Dosage. On 9th day, 0.75 g. *p*-iodophenylmercapturic acid per rat, mixed with food.
 „ 17th „ 0.20 „ „ „ „ given by stomach tube.

Treatment of first batch. This batch was separated into excess chloroform, precipitate, and filtrate. Analysis showed that nearly all the iodine was in the filtrate. An attempt was made to isolate *p*-iodophenylmercapturic acid from this fraction by Baumann's method, which had given good results with dog's urine in this laboratory. This method, however, failed to remove coloured impurities, and only a very small yield of pigmented material melting at 151° and decomposing at 187° was obtained. Attempts to decolorise this material

showed that the mercapturic acid was strongly adsorbed on to Merck's medicinal charcoal, and this property was utilised in the treatment of the second batch.

Treatment of second batch. The urine was filtered, and 600 cc. of the filtrate used in preliminary trials of the method. The remainder was acidified with hydrochloric acid and allowed to stand for eight days. The precipitate obtained was dissolved in dilute ammonia, and the solution shaken with Merck's medicinal charcoal until the filtrate gave no precipitate on acidification. The charcoal was then dried at 37° and extracted with successive 100 cc. portions of 97 % alcohol, until on evaporation of the alcohol only traces of solid residue were obtained. Approximately 20 g. of charcoal and 2 litres of alcohol were required.

The preliminary precipitation with hydrochloric acid and solution of this precipitate in ammonia removed 1.6 g. of the total iodine present in batch 2 of urine. Of this, 1.39 g. was absorbed on the charcoal. The extraction with alcohol removed 1.06 g. from the charcoal, and the final purified product contained 0.61 g. of iodine, thus giving a yield of mercapturic acid of 8.2 % from the iodobenzene injected.

Analysis of this product gave the following figures:

	N	S	I
Found	3.84	9.06	35.4
Theoretical for <i>p</i> -iodophenylmercapturic acid	3.84	8.77	34.8

The melting-point (152°) was unchanged on mixing with various proportions of pure *p*-iodophenylmercapturic acid, prepared in this laboratory from the urine of dogs which had been fed with iodobenzene.

II. *Experiments upon the rabbit.*

The rabbit was found to tolerate iodobenzene very readily. A dose as high as 5 g. given by subcutaneous injection to a rabbit weighing 3 kg. produced only a transient diminution of appetite. A similar slight reaction was observed in the case of the doe rabbit used in Exp. 4, after giving, by stomach tube, a dose of *p*-iodophenylmercapturic acid of 1.9 mg. per g. body weight. This rabbit was fed upon a diet of oats and cabbage. The buck rabbit used in Exp. 5, and fed upon a diet of sugar-beet, died 24 hours after receiving, by stomach tube, the second instalment of a dose of *p*-iodophenylmercapturic acid of 2.1 mg. per g. body weight.

The diet used in Exp. 4 consisted of 150 g. minced cabbage and 20 g. Sussex oats, given morning and evening. In Exp. 5, however, this diet was changed, in order to decrease the output of urinary sulphur. In this experiment the rabbit received 50 g. of sliced sugar-beet morning and evening.

In both experiments, the rabbit was kept in a cage with a wire floor, set in a large zinc funnel. Beneath the outlet of this funnel was placed a Hopkins separator of rather larger size than that used in rat work. Efficient separation

of urine and faeces was thus obtained. On every alternate morning the collecting apparatus was rinsed with distilled water, and the combined rinsings and urine were made up to a suitable volume, acidified with acetic acid to dissolve the copious phosphatic precipitate usually present, and filtered before analysis. In the case of the buck used in Exp. 5, catheterisation was carried out at the end of each two-day period of collection of urine. This procedure was not practicable in the case of the doe used in Exp. 4.

The same analytical methods were used as in the experiments upon the rat. Nitrogen was estimated by the Kjeldahl method.

Exp. 4. An adult doe rabbit, weighing approximately 2.5 kg. was used in this experiment. Iodobenzene was given once by stomach tube and once by subcutaneous injection. *p*-Iodophenylmercapturic acid was given once by stomach tube. The results of the sulphur analyses are shown graphically in Fig. 3. The dosage, and a summary of the resultant changes in the sulphur partition are given in Table I C.

Fig. 3. Rabbit 1.

Dosage. On 1st day of 6th period, 5.4 g. iodobenzene by stomach tube.
 " " 10th " " subcutaneously.
 " " 17th " 3.0 g. *p*-iodophenylmercapturic acid by stomach tube.
 " 2nd " 17th " 2.0 " " " "

Exp. 5. An adult buck rabbit, weighing approximately 2.0 kg. was used in this experiment. *p*-Iodophenylmercapturic acid was given by stomach tube.

Particulars of this experiment are given in Table I D.

Exp. 6. In order to determine whether the rabbit was capable of synthesising a mercapturic acid from iodobenzene, to an extent too small to be detected by estimations of the neutral sulphur, the following experiment was carried out.

As a preliminary test, a rabbit was injected subcutaneously with 3.6 g. of iodobenzene. The urine passed during the next six days was treated as in the case of the second batch of rat urine in Exp. 3. About 50 mg. of somewhat impure *p*-iodophenylmercapturic acid were obtained, having m.p. 148°–149°, and giving the following analytical figures: N, 3.64; S, 9.06; I, 34.8.

Six adult doe rabbits were then placed upon a diet of carrot and oats, fed *ad lib.*, and housed in metabolism cages. Each rabbit received 3.6 g. iodobenzene subcutaneously and five days later a further injection of 3.6 g. The urine was collected daily for ten days after the first injection and preserved over chloroform. This urine was rendered distinctly alkaline to litmus with ammonia, and the phosphate precipitate filtered off. Its volume was then 5600 cc., and it contained about 10 g. of iodine. Treatment of this material according to the method described in Exp. 3 yielded 0.64 g. of impure *p*-iodophenylmercapturic acid, having m.p. 149°–150°. After purification by recrystallisation of the ammonium salt, the melting-point was 150°, and analysis gave the following figures: N, 3.70; S, 8.88; I, 34.75.

DISCUSSION OF RESULTS.

I. *Experiments upon the rat.* Fig. 1 shows that the chief change brought about in the sulphur partition by a dose of iodobenzene is an increase in the neutral sulphur and total sulphur fractions. The ethereal sulphate shows only a slight increase. A large increase in the excretion of neutral sulphur, such as that occurring after the second dose of iodobenzene, may have been due, in part at least, to toxic effects of the dose. This possibility is shown more strikingly in the experiments with mercapturic acid (Fig. 2). Dosage with *p*-iodophenylmercapturic acid leads to an increase in the neutral sulphur excretion greater than that which would correspond to the mercapturic acid excreted, as judged by the iodine analyses. This excess of neutral sulphur appeared to be directly related to the severity of the signs of poisoning shown by the rats. In no case did dosage with *p*-iodophenylmercapturic acid cause haematuria or haemoglobinuria in the rat. The indication of mercapturic acid synthesis given by Exp. 1 was followed up in Exp. 3 by isolation of *p*-iodophenylmercapturic acid from rat urine. Thus the metabolism of iodobenzene in the rat is similar to its metabolism in the dog; but the rat differs from the dog in that it shows no haemoglobinuria after dosage with mercapturic acid.

II. *Experiments upon the rabbit.* Administration of iodobenzene to the rabbit, either orally or subcutaneously, causes a large increase in the ethereal sulphate output and a corresponding drop in the inorganic sulphate. No indication of mercapturic acid formation is given by the neutral sulphur figures (Fig. 3), although the conversion of a very small fraction of injected iodobenzene into *p*-iodophenylmercapturic acid is conclusively demonstrated in Exp. 6. The large fall in the excretion of all sulphur fractions, except ethereal sulphate, on the 10th day of Exp. 4 is accounted for by the fact that the animal refused its food for one day after this injection.

The effect of giving *p*-iodophenylmercapturic acid to rabbits is shown in Figs. 3 and Table I C and D. Both experiments show clearly that no appreciable oxidation of this substance to ethereal sulphate has occurred. This result contrasts with the findings of Sherwin and his co-workers, who stated that the rabbit oxidises *p*-bromophenylmercapturic acid to ethereal sulphate to the

extent of 23 % to 30 % [Rose, Shiple and Sherwin, 1924]; and that the pig is capable of oxidising phenyl-, *p*-chlorophenyl- and *p*-bromophenyl-mercapturic acids to ethereal sulphate to the extent of 38 %, 43 % and 23 % respectively. Previous work in this laboratory [Coombs and Hele, 1927], however, indicated that neither *p*-bromophenyl- nor *p*-iodophenyl-mercapturic acid could be oxidised to ethereal sulphate by the pig. The rise in the neutral sulphur excretion after giving *p*-iodophenylmercapturic acid is not well shown in Fig. 3 (Exp. 4) on account of the large and irregular quantities of neutral sulphur excreted throughout this experiment. In Exp. 5, a diet was chosen which gave a very low sulphur excretion, and the rise in neutral sulphur is very evident. In this experiment the rise in neutral sulphur is in excess of that which would correspond with the iodine excretion. This condition is doubtless due to the severe poisoning which occurred in the case of this animal. No haematuria or haemoglobinuria was observed in these experiments upon the rabbit.

SUMMARY.

(1) It has been shown that the rat is able to synthesise *p*-iodophenylmercapturic acid from iodobenzene; is unable to oxidise that mercapturic acid to ethereal sulphate; and does not develop haemoglobinuria after the administration of that mercapturic acid.

(2) The rabbit is unable to oxidise *p*-iodophenylmercapturic acid to ethereal sulphate. It does not develop haemoglobinuria after the administration of that mercapturic acid. It is able to convert a very small fraction of injected iodobenzene into *p*-iodophenylmercapturic acid.

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