LXXIX. STUDIES IN THE SULPHUR METABOLISM OF THE DOG. II. THE CONSTANCY OF THE RELATIVE OUTPUT OF ETHEREAL SULPHATE AND OF NEUTRAL SULPHUR AFTER THE ORAL ADMINISTRATION OF THE HALOGEN SUBSTITUTED BENZENES.

By THOMAS SHIRLEY HELE.

From the Biochemical Department, University of Cambridge.

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BAUMANN and PREUSSE [1879] were the first to point out that bromobenzene was partly excreted by the dog as *p*-bromophenylmercapturic acid and partly as *p*-bromophenyl sulphate. It is known that *p*-bromophenol gives rise to *p*-bromophenyl sulphate, but as yet no mercapturic acid has been isolated from the urine of dogs taking this substance. It is largely a question of dosage. The phenol coefficient (*B. coli*) for *p*-bromophenol is 7.4 [Cooper and Woodhouse, 1923] and its toxicity to mice is about the same as that of phenol [Bechold and Ehrlich, 1906]. On the other hand bromobenzene can be administered safely to animals in comparatively large doses. To prepare mercapturic acids therefore the benzenes and not the phenols have always been administered to dogs. Observers generally do not make a distinction between the metabolism of bromobenzene and that of *p*-bromophenol. They assume that bromobenzene is first oxidised to *p*-bromophenol before it is converted to mercapturic acid. This is almost certainly true but the proof is incomplete. In other directions also our knowledge is very incomplete. The evidence for the synthesis of a mercapturic acid from any substance other than a monohalogen benzene or by any animal other than the dog is not definite, though there is presumptive evidence that the monohalogen naphthalenes also give rise to mercapturic acids in the dog [Baumann, 1883] and that the rabbit can synthesise a mercapturic acid from bromobenzene [Rhode, 1922].

It is this lack of knowledge that has tended to make writers draw a line of distinction between the metabolism of the monohalogen benzenes and the metabolism of all other benzene derivatives in the dog; and a line of distinction between the behaviour of the dog and the behaviour of all other species towards the monohalogen benzenes. Reference to their papers shows that such distinctions were not in the minds of Baumann and Preusse [1879]

or of Jaffe [1879], who shares with Baumann and Preusse the credit of the discovery of the mercapturic acids. Hopkins [1907] and Sherwin [1922] have both suggested that mercapturic acid is an intermediary stage in the formation of ethereal sulphate and that the addition of the halogen to the benzene ring prevents the further oxidation of the intermediary mercapturic acid in the dog but not in other species. It is to be noted however that the structure of the two types of compound is entirely different and that the change from one to the other is not a simple oxidation.

p-Bromophenylmercapturic acidPotassium *p*-bromophenyl sulphate

Such a theory is put forward to account for the occurrence of a remarkable synthesis, excited by a substance which the animal would never meet under natural conditions. It is surely much easier to assume that many substances of common nature are excreted as mercapturic acids, the isolation of which is not readily achieved, and that bromobenzene merely follows a general line of metabolism.

Muldoon, Shiple and Sherwin [1923, 1] have recently failed to find any animal, other than the dog, capable of synthesising mercapturic acid from bromobenzene. They failed also to demonstrate in the pig the direct synthesis of ethereal sulphate from sodium sulphate and either phenol, *p*-chlorophenol or bromobenzene. On the other hand the increase in ethereal sulphate after *p*-bromophenylmercapturic acid was not sufficient to prove it to be an intermediary product of metabolism. Presumably no toxic signs were observed after mercapturic acid, such as has been found by Hele and Callow in the dog [1924]. The full account of their work is awaited with interest.

It will be shown in this paper that chlorobenzene like guaiacol [Hele, 1924] may be excreted in combination with preformed sulphate added to the diet. This at once renders an intermediary stage unnecessary though it does not preclude the possible oxidation of a mercapturic acid to ethereal sulphate. In the next paper of this series there will be discussed the possibility of the excretion by the dog of mercapturic acids other than those already known [Hele and Callow, 1923].

Until accurate methods of estimating mercapturic acids are discovered it is necessary to rely on sulphur analyses of the urine for quantitative studies. This is unsatisfactory. Zeller and Straczewski [1914] and Kapfhammer [1921] obtained a rise in the neutral sulphur of the urine after giving bromobenzene by mouth to dogs on a protein-free diet and yet were unable to isolate a mercapturic acid. In the course however of the present investigations a quantitative relation was established between the output of ethereal sulphate and of neutral sulphur that would suggest that chlorobenzene given to a dog on

a standard mixed diet, containing protein, appears in the urine in only two sulphur combinations, as ethereal sulphate and as mercapturic acid. Whether this is true or not (and proof will only be forthcoming when accurate analyses of the several substances comprised in neutral sulphur are possible), it is certain that the dog reacts towards bromobenzene and chlorobenzene in a very constant fashion and that the quantitative effect on the sulphur partition in the urine can within limits be predicted for any given dose. For the sake of brevity it will be assumed in this paper that the increase in the output of neutral sulphur in the urine over the normal is due entirely to mercapturic acid but it must not be forgotten that the increase may be due to two or more sulphur-containing bodies.

EXPERIMENTAL.

The work described in this paper was commenced in the year 1914. It was found in the course of preparing mercapturic acid from the dog that the simultaneous administration of cystine seemed to increase the yield. In consequence quantitative experiments (Nos. 1, 2, 3) to show the direct union of bromobenzene and cystine, both given by the mouth, were carried out with apparently satisfactory results, though subsequently the experiments were proved fallacious. In 1920 this question was reopened (Nos. 4, 5, 6) but no proof of such direct union was obtained. On the other hand the constant quantitative character of the reaction of the dog to chlorobenzene became apparent and two experiments (Nos. 7, 8) were carried out to test this point more completely. Subsequently in 1921-22 four experiments (Nos. 9, 10, 11, 12) were carried out, which showed that chlorobenzene will react with sodium sulphate and with cystine without materially upsetting the quantitative relation between the amount of ethereal sulphate and the amount of mercapturic acid produced. The paper therefore falls into three parts: (1) early experiments, (2) experiments exhibiting a constant quantitative relation between ethereal sulphate and neutral sulphur, and (3) experiments exhibiting the union of administered sulphate and of administered cystine with the oxidation products of chlorobenzene.

Bitches, rendered easy of catheterisation by operation, were used for the experiments. The standard diets were composed of lean beef, suet, arrowroot biscuits and milk. The food was given at 9.30 a.m. and 5 p.m. The daily allowance was divided equally between the two meals except in the early part of Exp. 5. The monobromobenzene or monochlorobenzene was given in gelatin capsules, usually in a single dose with the morning meal. In the experiments in which these substances were given over several days the daily dose was divided between the morning and evening meals. The sodium sulphate and cystine were also given by the mouth and when given were administered at each meal. After the animal had been on a standard diet for four or five days the urine was collected every 24 hours, the animal being catheterised at least once a day just before the morning meal, when the

24 hours' urine was made up. Total nitrogen, total sulphur, total sulphate and inorganic sulphate were estimated in the urine and sometimes free and combined phenols, ammonia and nitrogen titratable in presence of formaldehyde. Ethereal sulphate was estimated by deducting inorganic sulphate from total sulphate, and neutral sulphur by deducting total sulphate sulphur from total sulphur. Total nitrogen, total sulphur and sometimes free phenols were estimated in the faeces.

For all the sulphur estimations Folin's methods [1905] were employed. For total sulphate and for inorganic sulphate these methods have become general. The methods are not unduly long and more accurate than any benzidine method. If duplicates did not agree within 1 %, further estimations were carried out. For total sulphur estimations Folin's technique by oxidation with sodium peroxide was preferred to any other method. The accuracy of the method has been well established by Folin. An agreement within 3 % between duplicates was regarded as sufficient save on important days. A spirit lamp was always used for the fusion of the peroxide. For filtration Gooch crucibles were used throughout and it was found that, if they were properly packed with asbestos, the filtration and washing of the barium sulphate precipitates did not take more than eight minutes for each estimation. About a litre of hot water for washing was used for each of the total sulphur estimations and half that quantity for each of the other estimations. The precipitates were washed until the washings were chloride-free. No essential departures were made from the methods as described by Folin.

For the estimation of phenols the method of Folin and Denis [1915, 1916] was used. Though open to certain objections that have been met in Tisdall's modification [1920], it was found to be more convenient than the latter method. As these estimations were not considered an essential part of the experiments, accuracy was allowed to give place to convenience.

For the estimation of total nitrogen and of total sulphur in the faeces, it was found better not to dry them. The faeces were rubbed up in a mortar with a quantity of water sufficient to make an emulsion as far as possible uniform. 25 to 50 cc. were taken for estimations of nitrogen or sulphur. For estimating sulphur Folin's technique was employed but with twice the quantity of sodium peroxide (20 g.).

The estimation of total sulphur in the food was not so easy. The food sample was weighed into the nickel crucible, containing 10 cc. of water and 3 g. sodium peroxide. The contents were evaporated to dryness on the water-bath and then heated for ten minutes. The crucible was allowed to cool, 1 cc. water poured in and then 17 g. sodium peroxide were sprinkled over the contents. The subsequent procedure was identical with that given in Folin's paper. The method was entirely satisfactory for meat and milk, but for the biscuit, containing large amounts of carbohydrate and but little sulphur, there was the almost certain risk of explosion. To prevent this it was found necessary to burn off most of the carbon in the preliminary heating before

fusion, the substance having been made strongly alkaline in the earlier stage. In this manner the method became moderately satisfactory but it is not suggested that the sulphur analyses for the biscuit are anything but approximate. Eight samples of each foodstuff were taken and estimations carried out in duplicate. The mean values obtained are shown in the following table. Figures in brackets are calculated from the tables of Attwater and Bryant [1899].

Table I.

	Total N %	Total S %	Calories K per 100 g.
Beef (lean shank)	3.41	0.226	132.6
Milk	0.51	0.044	(71.8)
Suet	(0.75)	(0.05)	(780.4)
Biscuit	1.10	0.127	605.7

In the tables of urine analysis total sulphur, total sulphate, ethereal sulphate and neutral sulphur are expressed in grams sulphur. The relation between total sulphur and total nitrogen is expressed as the ratio of the sulphur multiplied by 100 to nitrogen and not in the reverse manner, as the sulphur output is the more directly influenced by the compounds given. The relation between ethereal sulphate and neutral sulphur ($\frac{E}{E+NS}$) is expressed as the ratio multiplied by 100 of the excess ethereal sulphate to the sum of the excess ethereal sulphate and excess neutral sulphur. To give an example: for the 14 normal days of Exp. 4 the average output of ethereal sulphate is 0.007 g. S and of neutral sulphur 0.074 g. S. On August 12 the ethereal sulphate is 0.047 g. S and neutral sulphur 0.120 g. S. The excess figures are respectively 0.040 and 0.046 g. S. The sum of these is 0.086 g. S and the ratio $\frac{E}{E+NS}$ is 46.5. In effect the figure represents the percentage excreted as ethereal sulphate of the bromo- or chloro-benzene in combination with sulphur, as in each group one molecule of the benzene corresponds to one atom of sulphur.

Free and total phenols are expressed as grams phenol. The method of Folin and Denis does not exclude hydroxy-acids, and there is little doubt that the high value for free phenols on the normal days is due to hydroxy-acids. After chlorobenzene therefore the rise in conjugated phenols appears to be out of proportion to the rise in free phenols. If these figures are treated in a manner similar to the treatment accorded to the sulphur figures, there is also found to be a constancy. The phenol ratio shown in the tables is the ratio multiplied by 100 of the excess free phenol over the normal to the excess total phenol over the normal.

In accordance with the observations of Folin and Denis [1916] no conjugated phenols were found in the faeces. There was no appreciable rise of free phenols in the faeces after the administration of chlorobenzene.

Ammonia (Folin) and "formal" nitrogen (Sørensen) were estimated in the urine on days on which the output of nitrogen was markedly high. This was

done to discover whether there was any increased output of amino-acid nitrogen. In no case however was there any such indication. The analyses have not been shown.

The mean normal daily output of each group of substances analysed is shown in the tables and the probable error for each mean, calculated by the method of mean squares. The probable error has also been determined for the ratios by compounding in a similar manner the errors on the figures used in the calculations. The analytical error (1 to 3 %) on each estimation has not however been taken into account. The probable error is large with the smaller doses of chlorobenzene and on the third and subsequent days after larger doses.

Some tables are given in full. Exp. 4 may be taken as a typical example. Others have been summarised to save space. In the summarised tables, days following the experimental days, during which the animal was returning to the normal, are not shown.

(1) EARLY EXPERIMENTS.

The chief difficulty experienced was the maintenance of the dogs in health. Confinement to the metabolism cages increased this difficulty but the main cause was the use of too large doses of bromobenzene, which caused loss of appetite.

Exp. 1. "Judy," May 24 to June 2, 1914. The dog was given a diet of meat and fat and received by mouth over a period of five days bromobenzene, 23 g., and cystine, 18.5 g.

Exp. 2. "Judy," June 17 to 30, 1914. The dog refused to take any fat and was given therefore only meat. Bromobenzene, 15 g., was given by mouth over a period of four days (June 19 to 22).

The urine analysis for the first six days is given in Table II.

Table II.

Date	Vol.	T. N	T. S	In. SO ₄	Eth. SO ₄	N. S	$\frac{E}{E+NS}$
1914	cc.	g. N	g. S	g. S	g. S	g. S	
June 17	210	3.08	0.164	0.079	0.015	0.070	—
" 18	160	3.31	0.174	0.101	0.013	0.060	—
" 19	280	4.12	0.313	0.083	0.083	0.147	(45.7)
" 20	310	6.06	0.432	0.000	0.123	0.309	(30.9)
" 21	260	6.35	0.403	0.000	0.073	0.330	(18.2)
" 22	410	6.64	0.392	0.000	0.089	0.303	(24.0)

The animal's weight fell from 8.17 to 6.60 kilo. in 14 days.

Nitrogen balance - 45.70 g.; sulphur balance - 2.345 g.

The output of neutral sulphur in the first experiment was very much greater than in the second and the output of nitrogen much less, suggesting that the cystine in the first experiment had united with some of the bromobenzene and prevented tissue breakdown. This was probably true but the results are vitiated by two considerations. The high nitrogen output in the second experiment is explained by the refusal of the dog to take the fat. The animal was being starved. The high value for neutral sulphur in the

first experiment is partly due to unoxidised products of bacterial decomposition of the cystine in the intestine. Exp. 2 does however offer confirmatory evidence to Exp. 10 and is therefore shown.

Exp. 3. "Doris," August 3 to 15, 1914. The diet was adequate but could not be maintained to the end of the experiment. It consisted of meat, biscuit and fat. Cystine, 2 g., was given daily from August 6 to 12 inclusive and bromobenzene, 5 g. daily, on August 7, 8, 9.

The urine analysis may be summarised as follows:

Table III.

Date 1914	Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S
Control period (average six days)	141	3.65	0.193	0.118	0.009	0.066
Probable error	± 45	0.73	0.035	0.033	0.003	0.010
August 7	156	3.98	0.479	0.000	0.147	0.332
" 8	270	4.52	0.645	0.000	0.194	0.451
" 9	300	4.69	0.591	0.000	0.190	0.401

The animal's weight fell from 5.63 to 4.90 kilo. in 13 days.
Nitrogen balance -4.57 g.; sulphur balance +1.706 g.

This experiment resembles and confirms Exp. 9. There is however no adequate means of checking the rise in neutral sulphur due to the effect of the cystine alone. It is clear that some of the cystine has been utilised to form ethereal sulphate. It is not however clear if any of it has been utilised to form mercapturic acid.

(2) EXPERIMENTS EXHIBITING A CONSTANT QUANTITATIVE RELATION BETWEEN ETHEREAL SULPHATE AND NEUTRAL SULPHUR.

In all these experiments and in those of the later group an adequate diet was maintained throughout. To keep the animals in a happy normal condition they were allowed more liberty. They were placed in the metabolism cages only at night. During the day they were chained to the laboratory bench and catheterised at 3, 4, or 5 hour intervals according to their habit. In this way long experiments could be continued without forcible feeding, which was always unsatisfactory. It was rare for an experiment to be discontinued suddenly on account of loss of urine or failure on the part of the dog to digest its food.

Exp. 4. "Phoena," August 8 to 31, 1920 (Table IV).

This experiment was devised to show the union between chlorobenzene and sodium sulphate both given by mouth, in the same way in which guaiacol may be shown to unite with sodium sulphate. Trial doses of chlorobenzene, 0.5 cc. or 0.55 g. and 3 cc. or 3.33 g., were given and it was then observed for the first time that the relative amounts of "extra" ethereal sulphate and of "extra" neutral sulphur were the same in the two cases within the error of the experiment. When however an attempt was made (August 23-26) to link up the chlorobenzene with sodium sulphate, the result was entirely negative. No additional ethereal sulphate was formed and the ratio $\frac{E}{E+NS}$ did not change.

Table IV. (Exp. 4.)

Diet				T. N g.	T. S g.	Calories K
Lean meat	75 g.	...		2.56	0.169	100
Suet	15 g.	...		0.11	0.007	117
Biscuit	60 g.	...		0.66	0.076	363
Milk	160 cc.	...		0.83	0.074	115
Total...				4.16	0.326	695

Sodium sulphate, cryst. : 3 g. daily by mouth from August 23 to 26.

Chlorobenzene by mouth at 9.30 a.m. : 0.55 g. on August 12 and 3.33 g. on August 16 and 24.

Date 1920	Urine										Faeces			Percent. of dose chloro- benzene excreted with S	
	Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	Phenols			T. N g. N	T. S g. S	Free phenols g. phenol			
							Free g. phenol	Conju- gated g. phenol	T. S T. N						
Aug. 8	273	3.60	0.183	0.102	0.006	0.075	0.062	0.006	5.08	Normal day	—	—	—	—	
" 9	260	3.48	0.180	0.108	0.006	0.066	0.054	0.012	5.17	"	0.45	0.093	0.060	—	
" 10	270	3.79	0.198	0.121	0.007	0.070	0.056	0.013	5.22	"	0.33	0.067	0.045	—	
" 11	268	3.80	0.206	0.119	0.009	0.078	0.056	0.011	5.42	"	—	—	—	—	
" 12	255	3.97	0.219	0.050	0.047	0.120	0.118	0.107	5.51	46.5 ± 4.0	40.9 ± 2.9	0.50	0.107	0.066	62.0
" 13	233	4.30	0.222	0.125	0.013	0.084	0.075	0.030	5.16	Normal day	—	—	—	—	
" 14	238	4.27	0.226	0.135	0.009	0.082	0.067	0.017	5.29	"	0.42	0.088	0.064	—	
" 15	243	3.99	0.218	0.131	0.008	0.079	0.060	0.015	5.46	"	—	—	—	—	
" 16	240	4.75	0.389	0.005	0.123	0.261	0.215	0.325	8.19	38.3 ± 1.0	34.1 ± 1.0	0.35	0.074	0.049	49.5
" 17	255	4.73	0.194	0.000	0.060	0.134	0.141	0.155	4.10	46.9 ± 3.0	38.2 ± 2.2	0.33	0.069	0.059	
" 18	275	3.94	0.134	0.000	0.023	0.111	0.075	0.058	3.40	30.2 ± 4.8	32.8 ± 7.4	0.30	0.065	0.047	
" 19	243	3.70	0.208	0.102	0.006	0.100	0.063	0.024	5.62	Normal day	—	—	—	—	
" 20	250	3.98	0.230	0.137	0.009	0.084	0.063	0.019	5.78	"	0.49	0.091	0.076	—	
" 21	265	4.10	0.213	0.137	0.005	0.071	0.052	0.005	5.19	"	0.38	0.083	0.054	—	
" 22	282	3.82	0.228	0.134	0.011	0.083	0.050	0.009	5.96	"	—	—	—	—	
" 23	275	4.52	0.495	0.411	0.006	0.078	0.054	0.008	10.96	—	—	0.42	0.074	0.056	—
" 24	368	4.96	0.606	0.222	0.131	0.253	0.233	0.299	12.22	40.9 ± 1.0	38.6 ± 1.0	0.16	0.049	0.023	55.2
" 25	335	4.41	0.442	0.212	0.073	0.157	0.136	0.129	10.02	44.3 ± 2.2	41.7 ± 2.3	0.37	0.108	0.061	
" 26	285	3.67	0.440	0.288	0.037	0.115	0.067	0.045	12.00	42.2 ± 4.5	30.4 ± 11.1	0.32	0.085	0.047	
" 27	330	3.64	0.182	0.093	0.011	0.078	0.053	0.025	5.00	Normal day	—	—	—	—	
" 28	200	3.30	0.165	0.094	0.006	0.065	0.041	0.015	5.00	"	0.29	0.058	0.040	—	
" 29	257	3.80	0.192	0.123	0.004	0.065	0.043	0.010	5.05	"	0.31	0.051	0.047	—	
" 30	350	3.89	0.198	0.124	0.007	0.067	0.045	0.015	5.09	"	0.29	0.059	0.046	—	
" 31	273	4.01	0.211	0.138	0.008	0.065	0.045	0.013	5.26	"	0.27	0.060	0.043	—	
Mean normal output (av. 14 days)	268	3.82	0.202	0.121	0.007	0.074	0.053	0.013	5.28	—	—	—	—	—	—
Probable error	± 35	0.24	0.019	0.015	0.002	0.007	0.007	0.005	0.27	—	—	—	—	—	—

The dog's weight fell from 7.26 to 7.12 kilo. in 24 days.

Balance Sheet (24 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	96.42	6.179		
" faeces	6.30	1.342		
Given by mouth, food	99.84	7.824		
" " sodium sulphate	—	1.192		
Balance			99.84	9.016
			-2.88	+1.495

Exp. 5. "Phoena," Sept. 7 to Oct. 3, 1920 (Table V).

It was felt that perhaps the failure in the previous experiment to show the direct union of sodium sulphate with chlorobenzene was due to the fact that the sodium sulphate was absorbed and excreted by the kidney before the chlorobenzene could be oxidised to chlorophenol and therefore could not upset the balance at the seat of synthesis. In the fifth experiment the sodium sulphate was given in small doses every hour throughout the day. The sulphate had however no effect on the ethereal sulphate or on the $\frac{E}{E+NS}$ ratio. Two attempts were made to show the direct union of cystine and chlorobenzene

Table V. (Exp. 5.)

Diet as in Exp. 4.

From Sept. 6 to 14, inclusive, the food was given thus:

Meat: 10 g. at 10.30 a.m., 25 g. at 12.30 p.m., 40 g. at 6.30 p.m.

Biscuit: 30 g. at 9.30 a.m. and at 6.30 p.m.

Suet: 10 g. at 12.30 p.m., 5 g. at 6.30 p.m.

Milk: 20 cc. every hour from 9.30 a.m. to 4.30 p.m.

Sodium sulphate, cryst.: 0.4 g. with each feed of milk, 16 cc. daily - 0.32 g. S on Sept. 9, 10, 11.

Cystine by mouth: 1.5 g. on Sept. 17, 25, 26, 27.

Chlorobenzene by mouth: 1.11 g. at 10.30 a.m. and 2.22 g. at 12.30 p.m. on Sept. 11; 3.33 g. at 9.30 a.m. on Sept. 17 and on Sept. 26.

Urine													Percent. of dose chloro- benzene excreted with S
Date 1920	Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	Phenols		T. S T. N	E E+NS	Phenol ratio		
							Free g. phenol	Conju- gated g. phenol					
Mean normal output (av. 13 days)	287	3.93	0.213	0.124	0.006	0.083	0.050	0.014	5.42	—	—	—	
Probable error	± 39	0.19	0.021	0.017	0.002	0.007	0.006	0.002	0.37	—	—	—	
Sept. 11	345	4.91	0.640	0.218	0.149	0.273	0.203	0.309	13.03	42.9±1.0	34.1±0.9	62.9	
" 12	273	4.42	0.450	0.198	0.066	0.186	0.174	0.149	10.18	36.8±1.7	47.9±1.3		
" 13	240	3.82	0.164	0.014	0.031	0.119	0.097	0.071	4.29	41.0±5.1	45.2±3.3		
" 17	372	4.07	0.535	0.080	0.136	0.319	0.258	0.301	13.14	—	42.0±0.7	50.4	
" 18	258	4.08	0.204	0.001	0.063	0.140	0.145	0.140	5.00	—	43.0±1.6		
" 26	278	4.74	0.630	0.176	0.130	0.324	0.258	0.293	13.29	—	42.7±0.7	68.6	
" 27	255	4.30	0.474	0.196	0.066	0.212	0.140	0.148	11.03	—	40.2±1.6		
" 28	255	4.06	0.245	0.086	0.028	0.131	0.078	0.058	6.04	—	38.8±5.2		

The dog's weight fell from 7.48 to 6.96 kilo. in 27 days.

Balance Sheet (27 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	108.85	7.838		
" faeces	7.89	1.804		
			116.74	9.642
Given by mouth, food	112.32	8.802		
" " S compounds	0.70	2.560		
			113.02	11.362
Balance			-3.72	+1.720

Table VI. (Exp. 6.)

Diet:—Meat, 300 g.; suet, 20 g.; biscuit, 15 g.; T. N, 10.54 g.; T. S, 0.707 g.; cal., 645 K.

From Nov. 10 diet irregular.

Chlorobenzene 3.33 g. given by mouth at 9.30 a.m. on Nov. 9.

Date 1920	Urine											Phenol ratio
	Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	Phenols			T. S T. N		
							Free g. phenol	Conju- gated g. phenol				
Mean normal output (av. 7 days)	294	8.21	0.449	0.284	0.012	0.153	0.074	0.007	5.47	—		
Probable error	± 15	0.44	0.034	0.019	0.004	0.030	0.005	0.002	0.30	—		
Nov. 9	305	8.85	0.524	0.042	0.136	0.346	0.256	0.295	5.92	38.7±0.8		
„ 10	300	6.35	0.313	0.046	0.061	0.206	0.144	0.129	4.93	36.4±1.7		

The dog's weight fell from 6.75 to 6.49 kilo. in 12 days.

Balance Sheet (12 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	85.15	4.709		
" faeces	3.62	0.714		
			88.77	5.423
Given by mouth, food			98.11	6.665
Balance			+9.34	+1.242

both given by mouth. These experiments were vitiated by the failure to appreciate the effect of the daily dose (1.5 g.) of cystine on the neutral sulphur output. In consequence the $\frac{E}{E+NS}$ ratio has not been calculated for these days. The phenol ratio is similar to that in Exp. 4.

Exp. 6. "Phoena," November 2 to 13, 1920 (Table VI).

In this experiment the dog was placed on a high protein diet. It was considered that perhaps protein might be more effective than cystine in the synthesis of mercapturic acid. The absorption of cystine from the intestine under these circumstances would not be so rapid and consequently there would be a greater likelihood of maintaining over a long period an increase in the concentration of cystine at the seat of synthesis. The dog refused to take so large an amount of meat before the experiment was completed. Apart from this failure however the variations in neutral sulphur output (from 0.150 to 0.196 g.) were very extreme, so that no average normal value could be determined. It is interesting to note that doubling the protein of the

Exp. 7. "Sophia" June 29 to July 17, 1921.

Table VII. (Exp. 7.)

		Diet		T. N		T. S		Calories					
				g.		g.		K					
		Lean meat	75 g.	...	2.56	0.169	100						
		Suet	10 g.	...	0.07	0.005	78						
		Biscuit	60 g.	...	0.66	0.076	363						
		Milk	150 cc.	...	0.78	0.069	108						
		Total...			4.07	0.309	649						
		Oral dose bromo- benzene g.	Urine.							Faeces		Percent. of dose bromo- benzene excreted with S	
			Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	T. S T. N	E E+NS	T. N g. N		T. S g. S
Date													
1921													
June	29	—	204	3.74	0.146	0.096	0.007	0.043	3.91	Normal day			—
"	30	—	204	3.72	0.123	0.084	0.004	0.035	3.31	"	0.83	0.128	—
July	1	—	206	3.26	0.127	0.085	0.003	0.039	3.90	"	—	—	—
"	2	—	188	3.58	0.150	0.106	0.005	0.039	4.19	"	—	—	—
"	3	0.5	210	3.90	0.167	0.040	0.035	0.092	4.28	38.1 ± 3.4	—	—	74.5
"	4	—	212	3.50	0.140	0.082	0.007	0.051	4.00	Normal day	—	—	—
"	5	—	246	3.67	0.179	0.126	0.009	0.044	4.88	"	0.71	0.107	—
"	6	—	228	3.87	0.179	0.130	0.008	0.041	4.63	"	—	—	—
"	7	3.0	290	4.85	0.351	0.008	0.114	0.229	7.24	37.0 ± 0.9	—	—	—
"	8	—	154	4.37	0.175	0.000	0.046	0.129	4.00	32.2 ± 1.9	—	—	75.2
"	9	—	124	3.86	0.110	0.015	0.032	0.063	2.85	—	0.77	0.129	—
"	10	—	148	3.92	0.193	0.130	0.005	0.058	4.92	Normal day	—	—	—
"	11	—	136	3.97	0.202	0.142	0.005	0.055	5.10	—	—	—	—
"	12	0.75	176	4.20	0.240	0.055	0.044	0.141	5.72	28.4 ± 1.7	—	—	87.6
"	13	—	150	3.88	0.171	0.091	0.011	0.069	4.41	—	—	—	—
"	14	—	132	3.47	0.151	0.102	0.008	0.041	4.35	Normal day	1.11	0.149	—
"	15	0.37	205	3.65	0.186	0.064	0.024	0.098	5.10	25.3 ± 3.0	—	—	93.4
"	16	—	210	3.38	0.160	0.108	0.008	0.044	4.73	Normal day	—	—	—
"	17	—	132	3.70	0.186	0.133	0.007	0.046	5.02	"	0.74	0.133	—
Mean normal output (av. 12 days)		}	187	3.65	0.161	0.110	0.006	0.045	4.41	—	—	—	—
Probable error			± 40	0.21	0.025	0.020	0.002	0.006	0.53	—	—	—	—

All doses of bromobenzene were given by mouth at 9.30 a.m.
The dog's weight fell from 5.91 to 5.82 kilo. in 19 days.

Balance Sheet (19 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	72.49	3.336		
" faeces	4.16	0.646		
Given by mouth, food			76.65	3.982
			77.33	6.061
Balance			+0.68	+2.079

food doubled the neutral sulphur output. There was no indication that there had been any greater production of mercapturic acid. The phenol output, though larger than in the last experiment, did not show large variations, so that an average normal output could be determined. The phenol ratio was similar to that in the previous experiments.

Exp. 8. "Phoena," August 11 to September 2, 1921.

Table VIII. (Exp. 8.)

Diet as in Exp. 4.															Percent. of dose chloro- benzene excreted with S
Date 1921	Oral dose chloro- benzene g.	Urine										Faeces			
		Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	Phenols		T. S T. N	E E + NS	Phenol ratio	T. N g. N	T. S g. S	
								Free g. phenol	Conju- gated g. phenol						
Aug. 11	—	164	3.90	0.197	0.133	0.010	0.054	0.045	0.014	5.05	Normal day		—	—	—
" 12	—	196	4.20	0.215	0.147	0.007	0.061	0.041	0.012	5.12	"		0.28	0.052	—
" 13	—	204	4.03	0.195	0.132	0.006	0.057	0.041	0.012	4.84	"		0.43	0.098	—
" 14	—	223	4.26	0.204	0.138	0.004	0.062	0.041	0.012	4.79	"		—	—	—
" 15	0.37	190	4.10	0.205	0.079	0.030	0.096	0.069	0.078	5.00	36.6 ± 4.2	27.9 ± 4.5	0.48	0.109	57.0
" 16	—	178	4.05	0.209	0.133	0.007	0.069	0.039	0.021	5.16	Normal day		—	—	—
" 17	0.28	184	3.89	0.209	0.080	0.028	0.101	0.053	0.071	5.38	31.7 ± 3.7	12.7 ± 7.0	0.54	0.120	79.6
" 18	—	166	3.91	0.203	0.127	0.012	0.064	0.047	0.025	5.20	Normal day		—	—	—
" 19	—	194	4.26	0.218	0.142	0.008	0.068	0.051	0.010	5.12	"		0.41	0.088	—
" 20	2.22	196	4.79	0.344	0.019	0.112	0.213	0.153	0.252	7.18	40.2 ± 1.0	31.4 ± 1.1	—	—	59.0
" 21	—	200	4.38	0.155	0.000	0.044	0.111	0.105	0.105	3.54	40.5 ± 3.0	40.2 ± 2.4	0.60	0.127	
" 22	—	170	4.22	0.159	0.070	0.020	0.069	0.069	0.044	3.77	"		—	—	—
" 23	—	174	4.46	0.195	0.127	0.009	0.059	0.046	0.031	4.37	Normal day		—	—	—
" 24	—	238	4.50	0.204	0.145	0.008	0.051	0.041	0.021	4.53	"		0.48	0.096	—
" 25	0.55	228	4.36	0.254	0.086	0.046	0.122	0.082	0.104	5.83	37.3 ± 2.5	29.6 ± 3.1	—	—	79.1
" 26	—	198	4.18	0.205	0.116	0.018	0.071	0.046	0.034	4.91	"		0.48	0.108	
" 27	—	162	4.39	0.216	0.147	0.010	0.059	0.036	0.022	4.92	Normal day		—	—	—
" 28	2.22	173	4.66	0.354	0.028	0.109	0.217	0.152	0.248	7.60	38.8 ± 1.0	31.5 ± 1.1	0.42	0.087	62.1
" 29	—	180	4.46	0.174	0.000	0.046	0.128	0.091	0.109	3.90	35.2 ± 2.3	33.1 ± 2.7	—	—	
" 30	—	140	3.96	0.145	0.054	0.020	0.071	0.056	0.052	3.66	"		0.52	0.113	—
" 31	—	162	4.13	0.191	0.122	0.013	0.056	0.052	0.033	4.63	Normal day		—	—	—
Sept. 1	—	162	4.18	0.198	0.143	0.007	0.048	0.052	0.013	4.74	"		—	—	—
" 2	—	132	4.27	0.209	0.152	0.008	0.049	0.050	0.018	4.90	"		0.52	0.117	—
Mean normal output (av. 13 days)		176	4.20	0.204	0.138	0.008	0.058	0.045	0.016	4.88	—	—	—	—	—
Probable error		± 15	0.18	0.008	0.008	0.002	0.006	0.005	0.005	0.25	—	—	—	—	—

All doses of chlorobenzene were given by mouth at 9.30 a.m. save on August 28, on which day 1.11 g. was given at 9.30 a.m. and 1.11 g. at 5.30 p.m.
The dog's weight fell from 8.10 to 7.94 kilo. in 23 days.

Balance Sheet (23 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	97.54	4.858		
" faeces	5.16	1.115		
Given by mouth, food			102.70	5.973
			95.68	7.498
Balance			-7.02	+1.525

Exps. 7 and 8 were carried out to determine whether the ratios were affected by the size of dose or not. Equivalent doses of bromobenzene and of chlorobenzene were given by mouth to two dogs on standard diets. 1 cc. or 1.5 g. bromobenzene is equivalent to 1 cc. or 1.11 g. chlorobenzene. The ratios are similar throughout, though the values for the $\frac{E}{E+NS}$ ratio are rather lower in Exp. 7 than in Exp. 8. This may be due to a difference in the dog or to a difference in the compound administered. The differences are not however great except for the small doses, where the experimental error is largest.

(3) EXPERIMENTS EXHIBITING THE UNION OF ADMINISTERED SULPHATE AND OF ADMINISTERED CYSTINE WITH THE OXIDATION PRODUCTS OF CHLORO-BENZENE.

In these experiments the same dog and the same standard diet were used throughout.

Exp. 9. "Vixen," November 19 to December 17, 1921.

Table IX. (Exp. 9.)

Diet				T. N			T. S			Calories				
				g.			g.			K				
Lean meat				80 g.	...	2.73	0.181			106				
Suet				20 g.	...	0.15	0.010			156				
Biscuit				60 g.	...	0.66	0.076			363				
Milk				160 cc.	...	0.83	0.074			115				
Total...				4.37			0.341			740				
Date	Chloro- benzene by mouth	Cystine by mouth	Vol. cc.	Urine								Faeces		Percent. of dose chloro- benzene excreted with S
				T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	T. S T. N	E E + NS	T. N g. N	T. S g. S		
Nov. 19	—	—	244	4.11	0.240	0.128	0.006	0.106	5.84	Normal day	—	—	—	
" 20	—	—	212	4.35	0.253	0.146	0.010	0.097	5.81	"	—	—	—	
" 21	—	—	188	3.92	0.223	0.135	0.009	0.079	5.68	"	—	—	—	
" 22	—	1	182	3.95	0.390	0.286	0.007	0.097	9.88	"	—	—	—	
" 23	—	1	186	4.33	0.482	0.344	0.006	0.132	11.13	—	—	—	—	
" 24	—	1	188	4.18	0.466	0.323	0.010	0.133	11.15	—	—	—	—	
" 25	2.22	1	224	5.10	0.534	0.029	0.155	0.350	10.47	40.9 ± 1.1	—	—	63.4	
" 26	2.22	1	220	5.10	0.534	0.029	0.155	0.350	10.47	39.7 ± 0.9	—	—		
" 27	2.22	1	190	4.98	0.528	0.024	0.155	0.349	10.60	39.8 ± 0.9	0.75	0.129		
" 28	2.22	1	252	5.12	0.532	0.002	0.157	0.375	10.38	37.5 ± 0.8	—	—		
" 29	2.22	1	202	4.96	0.533	0.000	0.160	0.373	10.74	38.2 ± 0.8	—	—		
" 30	—	1	181	4.60	0.276	0.069	0.049	0.158	6.00	58.0 ± 6.9	—	—	—	
Dec. 1	—	1	169	4.47	0.524	0.358	0.016	0.150	11.72	—	1.05	0.225	—	
" 2	—	—	196	3.74	0.434	0.298	0.019	0.117	11.60	—	—	—	—	
" 3	—	—	152	3.58	0.262	0.157	0.008	0.097	7.31	—	—	—	—	
" 4	—	—	266	4.57	0.277	0.168	0.009	0.100	6.06	Normal day	0.78	0.133	—	
" 5	—	—	218	4.61	0.294	0.194	0.009	0.091	6.38	"	—	—	—	
" 6	1.11	—	234	4.09	0.270	0.036	0.075	0.159	6.60	50.4 ± 3.2	—	—	55.6	
" 7	—	—	186	4.14	0.220	0.092	0.019	0.109	5.32	—	—	—		
" 8	—	—	216	3.90	0.269	0.169	0.011	0.089	6.90	Normal day	—	—	—	
" 9	—	—	178	4.36	0.265	0.163	0.007	0.095	6.06	"	0.95	0.149	—	
" 10	2.22	—	224	4.35	0.439	0.037	0.120	0.282	10.09	37.1 ± 1.1	—	—	58.6	
" 11	—	—	178	4.23	0.154	0.000	0.040	0.114	3.64	60.8 ± 9.6	0.81	0.128		
" 12	—	—	152	3.16	0.115	0.032	0.012	0.071	3.64	—	—	—	—	
" 13	—	—	211	3.61	0.216	0.141	0.010	0.065	5.98	(?) Normal day	—	—	—	
" 14	—	—	208	3.43	0.207	0.131	0.007	0.069	6.02	"	—	—	—	
" 15	—	—	202	3.63	0.229	0.148	0.010	0.071	6.30	"	0.62	0.099	—	
" 16	—	—	224	3.72	0.234	0.148	0.010	0.076	6.29	"	—	—	—	
" 17	—	—	266	3.67	0.231	0.155	0.006	0.070	6.29	"	—	—	—	
Mean normal output (av. 12 days)	—	—	219	3.99	0.245	0.152	0.009	0.084	6.13	—	—	—	—	
Probable error	—	—	± 26	0.45	0.025	0.018	0.002	0.013	0.31	—	—	—	—	
Mean normal output (av. 7 days, ex- cluding Dec. 13 to 17)	—	—	217	4.26	0.260	0.157	0.009	0.094	6.10	—	—	—	—	
Probable error	—	—	± 28	0.23	0.022	0.021	0.002	0.008	0.38	—	—	—	—	

The values for the ratio $\frac{E+NS}{E}$ are based on the mean normal output for 7 normal days, excluding December 13 to 17, and on the mean output of neutral sulphur derived from 1 g. cystine (Exp. 12).

The dog's weight fell from 7.50 to 7.43 kilo. in 29 days.

Balance Sheet (29 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	121.80	9.660		
" faeces	6.27	1.052		
Given by mouth, food	126.73	9.889	128.07	10.712
" " cystine	1.28	2.933		
Balance			-0.06	+2.110

2.22 g. chlorobenzene were given by mouth on five consecutive days and 1 g. cystine on eleven consecutive days. On later dates single doses of 1.11 g. and 2.22 g. chlorobenzene were given for comparison with the preceding series. It will be noted that the output of neutral sulphur on the last five days is distinctly low. In determining the fate of the cystine these days should probably be excluded in arriving at a mean normal output, which is to be deducted from the neutral sulphur output on the cystine days. The administration of cystine was not continued sufficiently long to obtain a true value for the cystine effect.

Exp. 10. "Vixen," January 24 to February 18, 1922.

Table X. (Exp. 10.)

Diet as in Exp. 9.													
Date 1922	Chloro- benzene by mouth g.	Urine								Faeces		Percent. of dose chloro- benzene excreted with S	
		Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	T. S T. N	E E+NS	T. N g. N	T. S g. S		
Jan. 24	—	194	3.50	0.203	0.104	0.012	0.087	5.80	Normal Day	—	—	—	
" 25	—	210	3.95	0.217	0.122	0.010	0.085	5.49	"	—	—	—	
" 26	—	186	4.02	0.225	0.141	0.008	0.076	5.60	"	—	—	—	
" 27	—	170	3.81	0.226	0.121	0.009	0.096	5.94	"	1.14	0.188	—	
" 28	—	203	3.92	0.221	0.124	0.008	0.089	5.64	"	—	—	—	
" 29	—	201	3.89	0.200	0.116	0.008	0.076	5.14	"	—	—	—	
" 30	—	203	3.94	0.210	0.110	0.009	0.091	5.32	"	—	—	—	
" 31	2.22	316	5.41	0.442	0.034	0.129	0.279	8.17	38.2±0.9	—	—	49.3	
Feb. 1	2.22	256	4.91	0.358	0.000	0.077	0.281	7.29	25.8±0.7	1.36	0.254		
" 2	2.22	220	5.21	0.329	0.000	0.067	0.262	6.31	24.7±0.8	—	—		
" 3	—	160	4.98	0.217	0.000	0.051	0.166	4.36	34.1±2.0	—	—		
" 4	—	130	4.75	0.108	0.000	0.028	0.080	2.27	—	—	—		
" 5	—	237	4.34	0.196	0.103	0.004	0.089	4.51	—	0.86	0.158	—	
" 6	—	300	4.26	0.282	0.154	0.008	0.120	6.62	(†) Normal day	—	—	—	
" 7	—	260	4.23	0.275	0.156	0.005	0.114	6.50	"	—	—	—	
" 8	—	296	4.75	0.274	0.140	0.012	0.122	5.77	"	—	—	—	
" 9	—	232	4.56	0.283	0.162	0.008	0.113	6.21	"	1.11	0.162	—	
" 10	—	156	4.18	0.250	0.146	0.008	0.096	5.98	Normal day	—	—	—	
" 11	2.22	302	5.66	0.473	0.041	0.149	0.283	8.36	41.4±0.9	—	—	77.2	
" 12	—	160	5.15	0.158	—	0.041	0.117	3.07	50.0±5.5	—	—		
" 13	—	243	5.02	0.327	0.179	0.014	0.134	6.50	—	—	—		
" 14	—	202	4.68	0.308	0.178	0.018	0.112	6.58	—	—	—		
" 15	—	194	4.45	0.260	0.166	0.010	0.084	5.84	Normal day	0.78	0.148		
" 16	—	193	4.17	0.260	0.177	0.009	0.074	6.24	"	—	—	—	
Mean normal output (av. 14 days)	}	—	214	4.12	0.242	0.139	0.009	0.094	5.86	—	—	—	—
Probable error		—	± 41	0.31	0.029	0.022	0.002	0.016	0.41	—	—	—	—
Mean normal output (av. 10 days, ex- cluding Feb. 6 to 9)	}	—	191	3.98	0.227	0.133	0.009	0.085	5.70	—	—	—	—
Probable error		—	± 16	0.28	0.022	0.023	0.001	0.007	0.31	—	—	—	—
Feb. 17	—	265	5.89	1.660	1.275	0.008	0.377	28.20	—	0.64	0.125	—	
" 18	—	250	4.98	0.663	0.382	0.013	0.268	13.32	—	0.52	0.371	—	

The values for the ratio $\frac{E}{E + NS}$ are based on the mean normal output for 10 normal days, excluding Feb. 6 to 9.

On Feb. 17 10 g. cystine were administered by mouth.

The dog's weight fell from 7.47 to 6.68 kilo. in 24 days.

Balance Sheet (Jan. 24 to Feb. 16, inclusive).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	107.74	6.302		
" faeces	5.25	0.910		
Given by mouth, food			112.99 104.88	7.212 8.184
Balance			-8.11	+0.972

2.22 g. chlorobenzene were given by mouth on three consecutive days. It was not possible to continue such dosage alone for a longer period without the grave risk of upsetting the dog. On a later date 2.22 g. chlorobenzene were given as a single dose for comparison with the previous experiment. The neutral sulphur output from February 6 to 9 inclusive is distinctly greater than during the fore-period. It is doubtful whether these days should be taken into account in determining the mean normal output.

Exp. 11. "Vixen," April 6 to 21, 1922.

Table XI. (Exp. 11.)

Date 1922	Diet as in Exp. 9.		Urine								Faeces	
	Chloro- benzene	Sodium sulphate	Vol. cc.	T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	T. S T. N	E E+NS	T. N g. N	T. S g. S
	by mouth g.	by mouth g.										
April 6	—	—	176	3.81	0.209	0.129	0.005	0.075	5.49	Normal day	—	—
" 7	—	—	216	4.03	0.253	0.151	0.007	0.095	6.28	"	—	—
" 8	—	—	236	3.66	0.237	0.137	0.006	0.094	6.47	"	—	—
" 9	—	—	250	4.27	0.243	0.155	0.008	0.085	5.80	"	—	—
" 10	—	2.6	275	4.22	0.499	0.397	0.008	0.094	11.82	Sulphate day	1.27	0.201
" 11	—	2.6	244	4.11	0.475	0.378	0.006	0.091	11.57	"	—	—
" 12	—	2.6	220	4.33	0.509	0.397	0.007	0.105	11.76	"	—	—
" 13	2.22	2.6	320	5.48	0.676	0.230	0.165	0.281	12.34	45.3±1.2	—	—
" 14	2.22	2.6	194	4.85	0.615	0.206	0.150	0.259	12.68	45.8±1.3	0.95	0.182
" 15	2.22	2.6	334	4.98	0.558	0.163	0.142	0.253	11.21	45.3±1.4	—	—
" 16	2.22	2.6	236	4.59	0.529	0.139	0.137	0.253	11.53	44.5±1.4	—	—
" 17	2.22	2.6	324	7.32	0.596	0.177	0.125	0.294	8.14	36.6±1.0	—	—
" 18	—	2.6	372	4.65	0.504	0.205	0.074	0.225	10.83	33.2±1.5	0.83	0.125
" 19	—	2.6	250	4.00	0.441	0.257	0.039	0.145	11.00	36.8±3.9	—	—
" 20	—	2.6	174	3.67	0.405	0.313	0.010	0.082	11.05	—	—	—
" 21	—	—	215	4.02	0.252	0.165	0.009	0.078	6.26	Normal day	0.88	0.135
Mean normal output (av. 5 days)	—	—	218	3.96	0.240	0.147	0.007	0.090 0.009	6.06	—	—	—
Probable error	—	—	± 24	0.21	0.016	0.013	0.001		0.36	—	—	—
Mean output 3 sul- phate days	—	—	246	4.22	0.494	0.391	0.007		11.72	—	—	—
Probable error	—	—	± 23	0.09	0.014	0.009	0.001		0.10	—	—	—

The dog's weight fell from 7.14 to 6.80 kilo. in 16 days.

The percentage of dose chlorobenzene excreted in combination with sulphur is 59.4.

Balance Sheet (16 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	71.99	7.006	75.92	7.649
" faeces	3.93	0.643		
Given by mouth, food	69.92	5.456	69.92	8.297
" " sodium sulphate	—	2.841		
Balance			-6.00	+0.648

2.22 g. chlorobenzene were given by mouth on five consecutive days and 2.6 g. sodium sulphate (Na₂SO₄, 10H₂O) on eleven consecutive days. Unfortunately the experiment had to be discontinued on the 16th day, because the glass catheter was accidentally broken in the dog's bladder.

THE FATE OF CYSTINE.

Exp. 12. "Vixen," January 30 to February 13, 1923 (Table XII).

1 g. cystine was administered by mouth on seven consecutive days.

In Exp. 12 the administration of 1 g. cystine (equivalent to 0.266 g. S) is followed by the additional elimination of 0.035 g. of neutral sulphur or 13 % of the dose, of 0.196 g. S as sulphate or 74 % of the dose. This leaves 0.035 g. S or 13 % of the dose unaccounted for.

Table XII. (Exp. 12.)

Diet as in Table IX. Cystine 1 g. daily by mouth February 3 to 9.

Date	Vol. cc.	Urine						Faeces	
		T. N g. N	T. S g. S	In. SO ₄ g. S	Eth. SO ₄ g. S	N. S g. S	T. S T. N	T. N g. N	T. S g. S
1923									
Jan. 30	292	5.02	0.273	0.180	0.011	0.082	5.43	—	—
" 31	189	4.66	0.288	0.192	0.011	0.085	6.18	—	—
Feb. 1	242	4.92	0.289	0.202	0.009	0.078	5.37	—	—
" 2	213	4.75	0.278	0.192	0.010	0.076	5.35	—	—
" 3	230	4.95	0.496	0.337	0.007	0.102	10.03	0.61	0.114
" 4	238	4.36	0.484	0.364	0.008	0.112	11.10	—	—
" 5	260	4.90	0.530	0.392	0.011	0.127	10.82	0.52	0.115
" 6	200	4.83	0.514	0.382	0.010	0.122	10.64	—	—
" 7	330	5.03	0.519	0.390	0.011	0.118	10.31	—	—
" 8	220	4.83	0.526	0.393	0.010	0.123	10.90	—	—
" 9	241	5.01	0.523	0.395	0.007	0.121	10.44	—	—
" 10	230	4.94	0.300	0.207	0.011	0.082	6.07	0.84	0.185
" 11	248	4.84	0.305	0.194	0.011	0.100	6.30	—	—
" 12	213	4.83	0.268	0.179	0.006	0.083	5.54	—	—
" 13	281	4.55	0.256	0.164	0.012	0.080	5.62	—	—
Mean normal output (av. 8 days)	240	4.81	0.282	0.189	0.010	0.083	5.86	—	—
Probable error	± 32	0.14	0.015	0.013	0.002	0.007	0.29	—	—
Mean output 7 cystine days	246	4.84	0.513	0.386	0.009	0.118	10.61	—	—
Probable error	± 38	0.21	0.016	0.010	0.002	0.007	0.34	—	—

The dog's weight fell from 8.14 to 7.94 kilo. in 15 days.

Balance Sheet (15 days).

	T. N g.	T. S g.	T. N g.	T. S g.
Excreted in urine	72.42	5.849		
" faeces	1.97	0.414		
Given by mouth, food	65.55	5.115	74.39	6.263
" " cystine	0.82	1.867		
Balance			-8.02	+0.719

In Exp. 9 the mean normal output of neutral sulphur over the control periods, excluding the last five days is 0.094 g. S. If to this figure is added the neutral sulphur output due to 1 g. cystine (Exp. 12) the value obtained is 0.129 g. S, a value almost identical with the output of neutral sulphur on the two days previous to the administration of the chlorobenzene. It appears therefore fair to apply the conclusions arrived at in Exp. 12 to Exp. 9.

It is safer to assume that all the sulphate on the chlorobenzene days is derived from the administered cystine. If it is assumed that part of this fraction is derived from protein, the figure for the utilisation of the cystine in forming mercapturic acid must be correspondingly increased. The output of sulphate on the five chlorobenzene days is given in Table XIII.

Table XIII.

(Derived from Table IX.)

Date	In. SO ₄ g. S	Eth. SO ₄ g. S	Total SO ₄ g. S	Difference from 0.196 g. S, figure for SO ₄ derived from cystine given alone g. S
1921				
Nov. 25	0.107	0.139	0.246	—
" 26	0.029	0.155	0.184	0.012
" 27	0.024	0.155	0.179	0.017
" 28	0.002	0.157	0.159	0.037
" 29	0.000	0.160	0.160	0.036

On the first day the output of sulphate is much greater than the average rise in sulphate after 1 g. cystine, given alone. In this experiment as in all other experiments it is clear that at first there is a certain amount of sulphate in the cell available for the detoxication of the chlorobenzene. On the following days the sulphate figure gradually falls, until on the last two days there is a difference of 0.036 g. S, a figure indicating that 14 % of the dose has been excreted as mercapturic acid. This is the minimum figure. If all twelve normal days are taken for the calculation of the mean normal output of neutral sulphur, this figure becomes 0.046 g. S or 17 % of the dose. It is also seen that all the sulphate appears ultimately as ethereal sulphate. It may be stated therefore that on the last two days 14 to 17 % of the cystine given appears as mercapturic acid, 57 to 60 % as ethereal sulphate. The higher sulphate output on the other days may quite well be due to traces of sulphate in the tissues derived from other sources.

On November 30, the day following the last chlorobenzene day, the output of sulphur is remarkable, in that it is reduced to the very small figure 0.276 g. S. This would suggest at once that there had been some error in the administration of cystine or in the absorption of cystine from the gut. No such error is known to have occurred. The figure is not an impossible one. It has been observed that the average output of sulphur after 1 g. cystine is 0.231 g. S. If this value is deducted from the sulphur output on the day in question, there still remains a balance for the sulphur derived from the protein. There is always a marked retention of sulphur after the administration of chlorobenzene, a fact well exemplified in Exp. 10, where there is no question of error. If it is assumed that there has been no error, this day affords clear proof of the direct conversion of cystine to mercapturic acid or other neutral sulphur compound. Experimental confirmation however is obviously required before the analyses for this day can be accepted.

THE FATE OF SODIUM SULPHATE.

This question is not so difficult as the last, as the administered sulphate can only appear as inorganic or as ethereal sulphate.

In Exp. 11 the average output of total sulphate on the three sulphate days is 0.398 g. S and on the five normal days is 0.154 g. S. This leaves a balance due to 2.6 g. sodium sulphate of 0.244 g. S.

The outputs of inorganic sulphate and of ethereal sulphate on the chlorobenzene days are given in Table XIV.

Table XIV.
(Derived from Table XI.)

Date	Eth. SO ₄	In. SO ₄	Difference from	% dose sodium
1922	g. S	g. S	0.244 g. S	sulphate excreted
			g. S	as ethereal SO ₄
April 13	0.165	0.230	0.014	5
" 14	0.150	0.206	0.038	15
" 15	0.142	0.163	0.081	31
" 16	0.137	0.139	0.105	40
" 17	0.125	0.177	0.067	26

In making calculations it is safer to assume that all the inorganic sulphate excreted after the chlorobenzene is derived from the administered sulphate and the ethereal sulphate is mostly formed from protein. On this assumption the percentages shown in the last column of the table are reached. There is no evidence that on the first day any of the administered compound has been utilised in detoxicating the chlorobenzene. On the remaining days from 15 to 40 % of the dose appears as ethereal sulphate.

The calculations may be made in a different manner. If the actual excess outputs of ethereal sulphate and of neutral sulphur over the experimental days are compared, Table XV is obtained.

Table XV.

Day	Cystine experiment		Control experiment		Sulphate experiment	
	Eth. SO ₄	N. S	Eth. SO ₄	N. S	Eth. SO ₄	N. S
	g. S	g. S	g. S	g. S	g. S	g. S
1	0.130	0.188	0.120	0.194	0.158	0.191
2	0.146	0.221	0.068	0.196	0.143	0.169
3	0.146	0.220	0.058	0.177	0.135	0.163
4	0.148	0.246	—	—	0.130	0.163
5	0.151	0.244	—	—	0.118	0.204

In constructing this table the figures taken for the mean normal output of neutral sulphur are such as would reduce the effect of the cystine in forming mercapturic acid to the lowest possible value. Any other combination would increase the value of the cystine effect.

There is but little difference between the figures for the first day of each experiment. Apparently sufficient sulphate was present in the tissues to obscure the issue. This bears out the failure to show any direct synthesis in the earlier experiments (Nos. 4, 5). On the remaining days however the effect of these added substances is quite well marked. There is much more ethereal sulphate in the cystine and sulphate experiments as compared with the output in the control experiment. In addition to this there is a distinct increase in the output of neutral sulphur in the cystine experiment. If the first day of each series is neglected and the mean values for the remaining days are taken, Table XVI is obtained.

Table XVI.

	Cystine experiment	Control experiment	Sulphate experiment
	g. S	g. S	g. S
Excess Eth. SO ₄	0.148	0.063	0.131
Excess Neut. S	0.233	0.186	0.175
Sum	0.381	0.249	0.306
E			
E + NS	38.8	25.3	42.8
% chlorobenzene excreted with S	60.2	39.4	48.4
Increase of Eth. SO ₄ over control	0.085	—	0.068
Expressed as % of dose S given	32.0	—	26.1
Increase of Neut. S over control	0.047	—	—
Expressed as % of dose S given	17.7	—	—

It must be noted in the above Table XVI that the figures are based on the averages of four days (two days in the case of the control experiment) and therefore differ from those given in the tables of the actual experiments.

These figures are similar to those obtained by the first method of calculation, except for the amount of ethereal sulphate, derived from the cystine. In the first method it is assumed that all the ethereal sulphate is derived from the cystine, in the second it is assumed that some is derived from protein. The first method of calculation is probably the more correct. In the control experiment more protein was being catabolised, as evidenced by the greater output of nitrogen. The cystine and to a lesser degree the sulphate partly prevented this tissue waste.

However these results may be interpreted, one thing is clearly defined, the comparative constancy in the relative outputs of ethereal sulphate and neutral sulphur. The mean value for the $\frac{E}{E+NS}$ ratio in the cystine experiment is 38.8, for the sulphate experiment is 42.8. If anything, the output of ethereal sulphate (relative to that of neutral sulphur) is increased by the addition of sulphate to the diet and the output of neutral sulphur (relative to that of ethereal sulphate) is increased by the addition of cystine to the diet, though with cystine there is an absolute increase of both. There has been some mass-action effect on the velocities of the two synthetic reactions but the effect is small in comparison with the doses given.

It is interesting to note that in the control experiment there is a marked falling off in the output of ethereal sulphate on the second and third days, though the output of neutral sulphur does not change. Exp. 2 shows the same phenomenon. This would seem to indicate that the production of sulphate from protein is slower than the production of sulphate from cystine, so that the ratio becomes altered. The alteration in the ratio could also be attributed to the increase in tissue breakdown (shown by the T. S/T. N ratio and the output of nitrogen) and the production of a neutral sulphur complex, not mercapturic acid.

DISCUSSION.

Sulphur and nitrogen balance.

It will be observed on looking through the tables that the nitrogen balance in the majority of the experiments is not very far out but that in general there is a marked positive balance for sulphur. This cannot be a question of lag in the sulphur excretion or entirely due to sulphur retention by the dog as the retention bears no relation to the state of nutrition of the animal. Wolf and Österberg [1911] and Lewis [1916] have discussed the question of sulphur retention in relation to periods of protein feeding after starvation, but although in some of the earlier experiments the state of nutrition of the animal improved during the course of the experiment, there was no alteration in the diet and the improvement was slight. The positive sulphur balance is much more

likely to be due to some definite experimental error or other cause. The difficulty of obtaining satisfactory sulphur analyses for the biscuit has already been discussed but the error is more likely to be in the direction of using too low a value for the sulphur of the biscuit than too high. There is a considerable loss of sulphur by the hair. The dogs were losing hair throughout the experiments and this loss was not estimated, as the relation between the loss of hair and the growth of hair was not known. Rough calculations of the quantity of hair on each dog gave the following figures: Phoena, 100 g.; Diana, 80 g.; Vixen and Sophia 75 g. Hair contains about 5 % of sulphur, so that there was 3.5 to 5 g. S in the hair of each dog. Assuming growth to be regular (which no doubt is not true) and the whole coat to be changed every twelve months, the daily consumption of sulphur in forming hair would be 10 to 15 milligrams, an appreciable percentage of the sulphur unaccounted for. There is also a loss of sulphur in estimating the sulphur of the faeces. Faeces are rich in sulphur and are not always easily collected from the metabolism cages. The loss of sulphur on the total would be relatively greater than that of the nitrogen. There must also be some loss of sulphur from the dogs in volatile compounds, though not necessarily of any considerable weight. Volatile compounds were particularly noticeable in the dog used in Exp. 10 after the dose of 10 g. cystine.

The output of neutral sulphur in relation to the diet.

The rise in the neutral sulphur of the urine (Exp. 6) on increasing the protein of the diet is a very marked feature. The dog was probably receiving more protein than it could digest, as it lost appetite. The large increase in the output of the neutral sulphur was almost certainly associated with some increased bacterial activity in the intestine, as the ethereal sulphate was somewhat raised.

The elimination of sulphur after cystine.

The increased output of sulphur following the administration of cystine was not observed for longer than 48 hours. This was the maximum period of lag in sulphur excretion. This lag has been considered in arriving at the mean output on normal days. As recorded by Goldmann in dogs [1885], Wohlgemuth in rabbits [1903], Blum in dogs [1904] and confirmed by Schmidt and Clark in dogs [1922] and by Sherwin and Rose in rabbits [1923] there may be a considerable rise of neutral sulphur after cystine, presumably in part from products of bacterial activity in the gut, though Sherwin and Rose attribute some at any rate of the "extra" neutral sulphur to incomplete oxidation of the cystine after absorption and state that apparently slow absorption facilitates oxidation.

On no occasion was the dose of cystine fully oxidised, though Rothera in man [1905] and Lewis and Root in rabbits [1922] were able to demonstrate the complete oxidation of this substance. Schmidt and Clark [1922] found

cystine in the urine of a dog which had received 7.5 g. by mouth. They attributed this to unusually rapid absorption. Perhaps the animal was an intermittent cystinuric [Garrod, 1923]. No cystine could be demonstrated by Gaskell's method [1907] in the urine of the dog used in Exp. 10 after a dose of 10 g. cystine, though the rise in neutral sulphur corresponded to 1.77 g. In this instance there was marked bacterial activity in the intestine, demonstrated by the evolution of much hydrogen sulphide from the anal canal.

The effect of chloro- and bromo-benzene on metabolism.

The effect of these substances on sulphur metabolism is best seen in the $\frac{T. S}{T. N}$ and $\frac{E}{E + NS}$ ratios. These and the phenol ratio are shown in Table XVII.

Table XVII.

Exp.	Dog	Dose g.	Day after dose	$\frac{T. S}{T. N}$	$\frac{E}{E + NS}$	Phenol ratio
Chlorobenzene:						
4	Phoena	Control period		5.28 ± 0.27		
		0.55	1	5.51	46.5 ± 4.0	40.9 ± 2.9
		3.33	1	8.19	38.3 ± 1.0	34.1 ± 1.0
		—	2	4.10	46.9 ± 3.0	38.2 ± 2.2
		—	3	3.40	30.2 ± 4.8	32.8 ± 7.4
6	Phoena	Control period		5.47 ± 0.30		
		3.33	1	5.92	—	38.7 ± 0.8
		—	2	4.93	—	36.4 ± 1.7
8	Phoena	Control period		4.88 ± 0.25		
		0.37	1	5.00	36.6 ± 4.2	27.9 ± 4.5
		0.28	1	5.38	31.7 ± 3.7	12.7 ± 7.0
		2.22	1	7.18	40.2 ± 1.0	31.4 ± 1.1
		—	2	3.54	40.5 ± 3.0	40.2 ± 2.4
		0.55	1	5.83	37.3 ± 2.5	29.6 ± 3.1
		2.22	1	7.60	38.8 ± 1.0	31.5 ± 1.1
		—	2	3.90	35.2 ± 2.3	33.1 ± 2.7
9	Vixen	Control period		6.10 ± 0.38		
		1.11	1	6.60	50.4 ± 3.2	—
		2.22	1	10.09	37.1 ± 1.1	—
		—	2	3.64	60.8 ± 9.6	—
10	Vixen	Control period		5.70 ± 0.31		
		2.22	1	8.36	41.4 ± 0.9	—
		—	2	3.07	50.0 ± 5.5	—
Repeated doses of chlorobenzene:						
10	Vixen	Control period		5.70 ± 0.31		
		2.22	1	8.17	38.2 ± 0.9	—
		2.22	1	7.29	25.8 ± 0.7	—
		2.22	1	6.31	24.7 ± 0.8	—
		—	2	4.36	34.1 ± 2.0	—
		—	3	2.27	—	—
Chlorobenzene with sodium sulphate:						
4	Phoena	Control period		—		
		3.33	1	12.22	40.9 ± 1.0	38.6 ± 1.0
		—	2	10.02	44.3 ± 2.2	41.7 ± 2.3
		—	3	12.00	42.2 ± 4.5	30.4 ± 11.1
5	Phoena	Control period		—		
		3.33	1	13.03	42.9 ± 1.0	34.1 ± 0.9
		—	2	10.18	36.8 ± 1.7	47.9 ± 1.3
		—	3	4.29	41.0 ± 5.1	45.2 ± 3.3

Table XVII (*continued*).

Exp.	Dog	Dose g.	Day after dose	T. S T. N	$\frac{E}{E+NS}$	Phenol ratio
Repeated doses of chlorobenzene with sodium sulphate:						
11	Vixen	Control period		11.72 $\pm$ 0.10		
		2.22	1	12.34	45.3 $\pm$ 1.2	—
		2.22	1	12.68	45.8 $\pm$ 1.3	—
		2.22	1	11.21	45.3 $\pm$ 1.4	—
		2.22	1	11.53	44.5 $\pm$ 1.4	—
		2.22	1	8.14	36.6 $\pm$ 1.0	—
		—	2	10.83	33.2 $\pm$ 1.5	—
		—	3	11.00	36.8 $\pm$ 3.9	—
Chlorobenzene with cystine:						
5	Phoena	Control period		—		
		3.33	1	13.14	—	42.0 $\pm$ 0.7
		—	2	5.00	—	43.0 $\pm$ 1.6
		3.33	1	13.29	—	42.7 $\pm$ 0.7
		—	2	11.03	—	40.2 $\pm$ 1.6
		—	3	6.04	—	38.8 $\pm$ 5.2
Repeated doses of chlorobenzene with cystine:						
9	Vixen	Control period		(11.14)		
		2.22	1	11.40	40.9 $\pm$ 1.1	—
		2.22	1	10.47	39.7 $\pm$ 0.9	—
		2.22	1	10.60	39.8 $\pm$ 0.9	—
		2.22	1	10.38	37.5 $\pm$ 0.8	—
		2.22	1	10.74	38.2 $\pm$ 0.8	—
		—	2	6.00	58.0 $\pm$ 6.9	—
Bromobenzene:						
7	Sophia	Control period		4.41 $\pm$ 0.53		
		0.5	1	4.28	38.1 $\pm$ 3.4	—
		3.0	1	7.24	37.0 $\pm$ 0.9	—
		—	2	4.00	32.2 $\pm$ 1.9	—
		0.75	1	5.72	28.4 $\pm$ 1.7	—
		0.37	1	5.10	25.3 $\pm$ 3.0	—
Repeated doses of bromobenzene:						
2	Judy	Control period		(5.28)		
		3.0	1	7.60	(45.7)	—
		5.0	1	7.14	(30.9)	—
		5.0	1	6.34	(18.2)	—
		2.0	1	5.90	(24.0)	—

The probable error could not be calculated for the figures in brackets, as the experiment was not continued long enough to determine a proper average for the mean normal output of the various sulphur fractions of the urine.

The analytical error has not been taken into account in calculating the probable error, so that the latter is somewhat greater. It must however be remembered that a constant analytical error would have no effect on the $\frac{E}{E+NS}$ ratio. It is obvious that to obtain a true value for this ratio a very large number of observations ought to be made. This is especially true for the smaller doses of bromo- or chloro-benzene.

The figures are however sufficiently striking to warrant attention being drawn to them. Where the variation is greatest, the probable error is usually greatest. The phenol ratio also shows some constancy except in Exp. 6. In this experiment the estimations of phenol were not carried out on the fresh urine and perhaps this series ought to be omitted. It is unfortunate that the

estimations of the phenols were not done throughout. They were discontinued because the results did not appear to have any meaning. The significance of the figures was only discovered at a very much later date.

The constancy of the $\frac{E}{E+NS}$ ratio is evidence for the balance between reactions taking place in the cell. The rate of production of ethereal sulphate to that of mercapturic acid bears the ratio of roughly 4 to 6. The assumption is made that the elimination of the two final products takes place with equal ease. In the first paper of this series [Hele, 1924], in order to account for the conflicting evidence of other workers on the synthesis of ethereal sulphate, the assumption was made that the rates of elimination of sulphate, sulphite and cystine by the kidney are different. This hardly amounts to an assumption as there is indirect evidence to substantiate it. On the other hand the assumption that mercapturic acid and ethereal sulphate are eliminated with equal ease has no experimental justification. If however the permeability of the kidney to these substances is markedly different, one would expect the $\frac{E}{E+NS}$ ratio to alter until the accumulation of end-products has established an equilibrium by the adjustment of the velocities of the syntheses to the rates of elimination. How rapidly such an adjustment would take place is not clear. The equilibrium should be attained more rapidly with large doses than with small. The work already done shows no marked difference between small and large doses. Perhaps by shortening the time interval differences would appear. Whatever causes however contribute to the results, the fact remains that under the experimental conditions a constant mixture is excreted by the kidney.

The phenol is roughly again four parts of free to six parts of total phenol. Here the story is much more complex. Unfortunately Folin's tungstate reagent is a group reagent, reacting with different degrees of intensity with different phenols (as well as with other substances) and it is not known how many phenols may arise from chlorobenzene. It is possible however that the products of oxidation may be distributed between the several fractions according to some definite proportion, so as to yield a constant relationship between free and conjugated phenols. Conjugated phenols include the sulphates and the glucuronates and there may be others.

The several reactions may be summarised thus:

The numbers in brackets indicate the molecular proportion of the several fractions excreted, that would satisfy the numerical relations given above. It is not known how much, if any, glucuronic acid is excreted in direct combination with phenols after chlorobenzene. Estimations of glucuronic acid would not be sufficient to determine this point, as mercapturic acid itself is excreted in combination with glucuronic acid [Baumann, 1883]. Isolation of

the products will alone give the correct answer. If such glycuronic acids are absent or present in very small amounts, the proportions in the above scheme must be altered. This question will be discussed further in the third paper of this series. It is interesting to note that on such a scheme the theoretical yield of mercapturic acid is only 37 %. This is in general agreement with the increased output of neutral sulphur after chlorobenzene and helps to explain the small yield of mercapturic acid (equivalent to 20–30 % of the dose of chlorobenzene) obtained from urine even under the most favourable conditions. The correspondence may however be accidental.

There is accumulative evidence that the liver is the chief seat of the sulphate and glycuronic acid syntheses. The subject has been discussed in the first paper of this series [Hele, 1924]. There is also evidence that the liver is the organ mainly responsible for the mercapturic acid synthesis. Zeller and Straczewski [1914] could not obtain more than 0.2 g. mercapturic acid from the urine of a dog receiving bromobenzene (28 g.) on a protein-free diet, though the rise in neutral sulphur indicated the presence of 6.9 g. of the acid. In another experiment they failed to demonstrate the presence of any mercapturic acid. They attributed the rise in neutral sulphur to compounds other than mercapturic acid and suggested that in the breakdown of tissue protein by bromobenzene on a protein-free diet no cystine is liberated and it is only when protein is included in the diet that mercapturic acid is formed. Thomas and Straczewski [1919] added *p*-bromophenylmercapturic acid to normal urine and were able to demonstrate its presence, when in concentration of only 0.2 g. in 500 cc. They administered bromobenzene *sub cutem* to dogs on a protein-rich diet. To one dog they gave 22.5 g. and to another 15.5 g. bromobenzene and only obtained traces of mercapturic acid from the urine. Kapfhammer [1921] administered in eight days 12 g. bromobenzene by mouth to a dog on a protein-free diet and failed to obtain any mercapturic acid from the urine. He then administered in four days 7 g. bromobenzene to the same dog on a protein-free diet and at the same time injected 29 g. of cystine *sub cutem*. He obtained 2.86 g. of impure mercapturic acid. These authors conclude that mercapturic acid is synthesised in the liver and from food protein only. Under these circumstances therefore one would expect to be able to alter the ratios of the syntheses in the liver by the simultaneous administration of cystine or of sodium sulphate with the bromo- or chlorobenzene by the mouth. Rhode [1922] has shown that the administration of cystine subcutaneously cuts down the output of ethereal sulphate, produced by bromobenzene given by the mouth. Such a method of administration apparently increases the velocity of the synthesis of mercapturic acid over that of ethereal sulphate. Unfortunately he carried out no estimations of neutral sulphur, no doubt because the cystine apart from the bromobenzene would have affected his results. Muldoon, Shiple and Sherwin [1923, 1, 2] administered bromobenzene with sulphate and with cystine by mouth to a dog. The sulphate was not converted to ethereal

sulphate though the cystine was in part converted to (?) mercapturic acid. The administration however both of the sulphate and of the cystine was only continued for two days. This may account for the failure in the utilisation of the sulphate. A full account of their work has not yet appeared.

In the experiments described in this paper the simultaneous oral administration of sulphate or of cystine had practically no effect on the relative output of ethereal sulphate and of neutral sulphur, though the total effect was considerable. Sodium sulphate was used in the synthesis of ethereal sulphate and thus there was more protein available for the formation of mercapturic acid. Cystine appeared largely as ethereal sulphate, though a small percentage appeared as mercapturic acid. The reactions may therefore be written:

Under these experimental conditions oxidation of the cystine precedes the other reactions or the rate of oxidation is influenced by the other reactions.

The constancy of the $\frac{E}{E+NS}$ ratio presents one difficulty to the theory of Thomas, Zeller and Straczewski. It is noticeable in the table of ratios, that the bromobenzene or chlorobenzene produces an immediate rise in the $\frac{T.S}{T.N}$ ratio with afterwards a compensatory fall. Larger doses produce a change of nearly 100 %. It is difficult to conceive that this output of sulphur only comes from food protein, as there is no known mechanism of storing amino-acids in quantity save as protoplasm. The more natural interpretation is that the toxic substances act directly on the cell with the liberation of sulphur complexes and that the later output of nitrogen represents the adjustment necessary to restore the normal cell-content of amino-acids. If this process involved the production of another sulphur compound of bromo- or chlorobenzene, an alteration in the $\frac{E}{E+NS}$ ratio would be expected, just as there is an alteration in the $\frac{T.S}{T.N}$ ratio. Yet no such alteration takes place, when the dose of bromo- or chlorobenzene is not repeated.

It is true that on repeating the doses of these substances there is a marked fall in the $\frac{E}{E+NS}$ ratio. This fall is always associated with the onset of signs of poisoning in the dog (fever, lassitude, loss of appetite and vomiting). If the sulphur analyses given in the paper by Marriott and Wolf [1907] on bromobenzene poisoning are examined, the same fact is made manifest. The control periods are hardly long enough to yield a true mean normal output, but so far as the analyses can show, the $\frac{E}{E+NS}$ ratio lies between 37.5 and 44.8, except in one experiment. In this experiment the dog was starved and given bromobenzene repeatedly. The ratio fell from 35.1 to 5 and then the dog died.

This fall would be readily explicable on the theory of Thomas, Zeller and

Straczewski but could be explained equally well on other lines. It has been noted that on the first day of such continued administration of chlorobenzene, cystine or sulphate added to the diet has no apparent effect. There is presumably enough sulphate in the cell to yield all the ethereal sulphate formed. On the second and later days, when sulphate or cystine is not given, the sulphate may be deficient in the cell and the oxidation of the protein cystine may not proceed so rapidly as its removal by the chlorobenzene to form mercapturic acid resulting in an alteration of the ratio. All the experiments in this paper bear out such a theory. However until a mercapturic acid has been isolated in sufficient quantity from the urine of dogs on a protein-free diet, it is clear that the theory of Thomas, Zeller and Straczewski must be accepted and another explanation sought for the constancy of the $\frac{E}{E+NS}$ ratio.

There is also a marked fall in the $\frac{T.S}{T.N}$ ratio after the prolonged administration of guaiacol [Hele, 1924]. This would suggest that even guaiacol has some slight toxic action on sulphur metabolism, too small to cause an appreciable rise of neutral sulphur during its administration, the effect being accumulative and only manifest after the dosage has been stopped.

It is frequently stated that species other than the dog are unable to synthesise mercapturic acid. Certainly it does not follow that the requisite enzymes are present in all species or, if present, are present in the same concentrations, but, apart from this, account is not always taken of the dosage given and the difficulty of separating mercapturic acid in low concentrations. For instance Mester [1890] administered doses of 4 g. and 6 g. bromobenzene respectively to two men and although he obtained a small rise in neutral sulphur, he was unable to demonstrate the presence of a mercapturic acid. It is true that in the experiments recorded in this paper on the dog, small doses yielded a value for the $\frac{E}{E+NS}$ ratio similar to that yielded by large doses. The percentage yield of mercapturic acid was presumably the same in the two cases, but, at the concentration in the urine after small doses, it would be difficult to obtain any definite evidence of the presence of a mercapturic acid. A 6 g. dose for a man would yield no higher concentration of mercapturic acid in the urine than a 0.5 g. dose in the dog. Mester's work is evidence as much for the synthesis of a mercapturic acid by man as the reverse.

SUMMARY.

1. Chlorobenzene administered by the mouth has an approximately constant quantitative effect on the sulphur metabolism of the dog. The "extra" ethereal sulphate bears a constant proportion (about 2 to 3) to the "extra" neutral sulphur. In this paper the relation is expressed as a ratio $\frac{\text{"Extra" Eth. SO}_4 \times 100}{\text{"Extra" Eth. SO}_4 + \text{"Extra" Neut. S}}$ or $\frac{E}{E+NS}$.

2. This ratio does not vary within the experimental error with the dose or with the dog.

3. Sulphur is eliminated very much more rapidly than the nitrogen after chlorobenzene. The ratio $\frac{\text{Total sulphur} \times 100}{\text{Total nitrogen}}$ or $\frac{T. S}{T. N}$ may be increased nearly 100 % with a compensatory fall afterwards. The increased elimination of sulphur must be derived in part from the tissues. The alteration in the $\frac{T. S}{T. N}$ ratio is not reflected in the $\frac{E}{E + NS}$ ratio.

4. The simultaneous oral administration of cystine or of sodium sulphate does not appreciably alter the $\frac{E}{E + NS}$ ratio, though when the dose of chlorobenzene is repeated both compounds take part in the syntheses. (After the first chlorobenzene day cystine 14 to 17 % as (?) mercapturic acid and 57 to 60 % as ethereal sulphate: sodium sulphate 26 to 40 % as ethereal sulphate.)

5. The $\frac{E}{E + NS}$ ratio may be altered by repeated doses of chlorobenzene, if no cystine or sulphate is given at the same time. A low ratio is associated with the onset of toxic signs in the dog.

6. It is suggested that the constancy of the $\frac{E}{E + NS}$ ratio is proof under the experimental conditions of a balance between three series of reactions in the cell, the synthesis of mercapturic acid, the synthesis of ethereal sulphate and the oxidation of cystine to sulphate, the velocities of the several reactions being dependent on the concentrations of the catalysts and the effective masses of the reacting compounds at the seat of the syntheses. An explanation is advanced for the fall in the value of the ratio on repeating the dose of chlorobenzene.

7. The constancy of the $\frac{E}{E + NS}$ ratio seems to be antagonistic to the theory of Thomas, Zeller and Straczewski, that there are two neutral sulphur compounds of halogen substituted benzenes (bromobenzene), mercapturic acid formed in the liver from food protein and an unknown compound formed from tissue protein. If these compounds were formed at the same velocity, the $\frac{E}{E + NS}$ ratio would not then alter, whatever alterations the $\frac{T. S}{T. N}$ ratio underwent. There seems no reason however why the velocities should be identical. On the other hand the fall in the $\frac{E}{E + NS}$ ratio on repeating the dose of chlorobenzene would be readily explained on the theory of Thomas, Zeller and Straczewski.

8. As chlorobenzene as well as guaiacol unites directly with preformed sulphate there is no necessity to regard mercapturic acid as an intermediary stage in the formation of ethereal sulphate.

9. The ratio of "extra" free phenol to "extra" total phenol ($\frac{\text{Free phenol} \times 100}{\text{Total phenol}}$ or the Phenol Ratio) also appears to be constant. The figures are however less satisfactory.

10. There is no increase of free phenols in the faeces after chlorobenzene. In accordance with the observations of Folin and Denis no conjugated phenols occur in the faeces.

11. The action of bromobenzene appears to be similar to that of chlorobenzene.

12. In each experiment there was a marked positive balance for sulphur, even when the nitrogen intake and output corresponded closely. It is suggested however that this positive balance may be due to experimental error, loss of sulphur in the faeces, loss of sulphur in volatile compounds and to the growth of hair.

13. The oral administration of cystine always produced a small rise in the output of neutral sulphur. It is not clear whether this was due to products of intestinal decomposition or to failure in oxidation after absorption.

14. A rise in the protein intake may give a considerable rise in the output of neutral sulphur, possibly intestinal in origin.

15. Guaiacol appears to have some slight toxic action on sulphur metabolism, as, although there is no apparent rise in neutral sulphur during its administration, there is a marked fall in the $\frac{T.S}{T.N}$ ratio when the administration is stopped.

16. Too little account has often been taken of the dosage, when bromobenzene has been administered to individuals of different species. Thus Mester's work is as much in favour of the occurrence of the mercapturic acid synthesis in man as the reverse, though it is generally stated on the evidence of his work that man does not synthesise mercapturic acid.

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