CLXXXVIII. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

IX. THE SYNTHESIS OF PHENYL SULPHATE AND INDOXYL SULPHATE.

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(Received September 1st, 1931.)

THE theory that the organic or ethereal sulphates arise from phenols by conjugation with sulphur compounds other than sulphate, oxidation occurring subsequently, rests entirely on negative evidence. In more recent years Rhode and Sherwin have supported this theory and Sherwin has gone further, suggesting that ethereal sulphates are formed only from sulphur-containing complexes of endogenous origin. Rhode [1922] in experiments on rabbits, and Sherwin in collaboration with Shiple and Muldoon [1924] in experiments on a pig failed to show any union between phenols and sodium sulphate administered concurrently. They made use of phenol itself and of certain halogenated phenols. The chief evidence for the older theory [Baumann, 1876], that ethereal sulphates arise by the combination of phenols with preformed sulphate, lies in the ability of the dog to bring about the union of guaiacol and the sulphate ion of sodium sulphate [Hele, 1924, 1]. In discussing these differences Pryde [1931] suggests that "guaiacol is a substance too far removed from the normal metabolic paths to warrant comparison with phenol in this respect." He makes no comment however on differences in animal species or differences in experimental technique.

The pig, unlike the dog, does not utilise sulphur compounds readily in detoxication processes and this may also be true of the rabbit [Coombs and Hele, 1926; Lawrie, 1931]. Under such circumstances to demonstrate the truth of Baumann's theory becomes a matter of extreme difficulty. No one has succeeded in proving it in either rabbit or pig, but failure to do so does not mean proof to the contrary. Apart however from the question of species Rhode and Sherwin with his co-workers, employing phenols of considerable toxicity and in relatively small doses, did not produce experimental conditions that could be successful. In my experiments the dog was used as the most suitable animal and guaiacol was chosen on account of its low toxicity, when given orally in the form of carbonate. The dog, as it were, could be saturated with guaiacol without any effect on its health. All sulphate arising from the tissues or the diet was used up in the detoxication process. The demand for

sulphate was greater than the supply, so that sodium sulphate, added to the diet, was immediately utilised by the animal.

In the present paper evidence is brought forward to show not only that the dog is capable of utilising sodium sulphate in the synthesis of phenyl sulphate and of indoxyl sulphate but also that, when the same syntheses are brought about at the expense of endogenous sulphur, the sulphur so utilised is in the form of the sulphate ion [Hele, 1927].

EXPERIMENTAL.

The experimental methods employed in this research have already been described [Hele, 1924, 2]. Two bitches were used, Patsy (6·8 kg.) and Pansy (9·0 kg.). As repeated doses of phenol could not be given safely, the "saturation" method, successfully utilised in the case of guaiacol, had to be discarded. The animals therefore were dosed daily with sodium sulphate and on certain days phenol or indole was administered orally in amounts sufficient to show an effect on the sulphur metabolism. This method of dosage had already successfully demonstrated the capacity of the dog to use sodium sulphate in the formation of chlorophenyl sulphate from chlorobenzene [Hele, 1924, 2]. In order to make the animal dependent for the formation of ethereal sulphate either on its endogenous sulphur or on the sodium sulphate, the protein of the diet and therefore the sulphate, arising therefrom, was reduced to the minimum. The quantity of meat retained in the diet was only just sufficient to place round the gelatin capsules, containing the phenol or indole on dose days. The meat was kept in position round the capsules by a few cotton stitches. Calories were provided in the form of margarine and sucrose. The actual amounts were determined experimentally by the quantities the bitches were prepared to swallow over long periods of time. The number of calories was inadequate and the bitches lost weight. It was found desirable to retain some milk in the diets.

Pansy's diet was as follows:

	Total N	Total S	Calories
	g.	g.	K
Meat: 10 g.	0·34	0·023	13
Sugar: 60 g.	—	—	246
Margarine: 50 g.	—	—	400
Milk: 50 cc.	0·26	0·023	36
Water: 100 cc.	—	—	—
Total	0·60	0·046	695

Patsy received only 40 g. of margarine but in other respects her diet was identical with that of Pansy.

When administered, the daily dose of sodium sulphate was 2 g. of the decahydrate (containing 200 mg. of sulphur), half given at 9.30 a.m., half at 5 p.m., either in the milk of the diet or subcutaneously, dissolved in 5 cc. of water, and the daily dose of phenol or indole was 1/100 of the g. molecular

Fig. 1. "Patsy."

Days 5-22 2 g. sodium sulphate, cryst. orally, daily.
Day 34 2 g. " " " "
" 38 2 g. " " " "
" 7 1 x M.W./100 g. phenol, orally.
" 11 1 x M.W./100 g. indole, "
Days 15-17 1 x M.W./100 g. " " daily.
Day 25 1 x M.W./100 g. " "
" 37 1 x M.W./100 g. " "

Line A. Daily sulphate sulphur on diet ... 32 mg.
" B. " " " on diet and sodium sulphate 220 mg.
" C. B less A ... 188 mg.

Patsy's weight fell from 6.8 to 6.0 kg. in 41 days.

weight (sufficient to combine with 320 mg. sulphur as sulphate), half given orally at 9.30 a.m., half at 5 p.m. The urine was collected from 9 a.m. to 9 a.m.

The first experiment carried out under these conditions was immediately successful. It is shown diagrammatically in Fig. 1. The excretion of total nitrogen and of neutral sulphur is shown in simple curve form. The excretion of sulphate is shown in column form in order to exhibit certain quantitative relations. The black portion of each column represents ethereal sulphate and the white portion inorganic sulphate. The average output of sulphate-sulphur on diet only was 32 mg. Line *A* represents this value. The average output of sulphate-sulphur on sodium sulphate days was 220 mg. (line *B*). On day 7 phenol was given orally and it is manifest that the major portion of the excreted sulphur was in the form of ethereal sulphate. In the formation of ethereal sulphate, it is best to assume that the sulphur first utilised is derived from all sources other than the administered sulphate. Line *C* has therefore been drawn at level 188 mg. S (220 mg. less 32 mg.). The black portion of the column is thus divided into three parts:

(1) a part above line *B* (43 mg. S), in excess of daily sulphate output and therefore derived from the tissues;

(2) a part between lines *B* and *C* (32 mg.), representing the daily output of sulphate derived from the diet and the tissues;

(3) a part below line *C* (133 mg. S), derived from the sodium sulphate.

At least 66 % of the administered sodium sulphate has been utilised.

On day 8 the output of sulphate is less. 53 mg. S from some source or other, normally excreted as sulphate, has been retained. The amount retained corresponds in some measure to the excess excreted on the dose day. Presumably the one is the consequence of the other.

A result, similar though not so good, follows on the administration of indole on the eleventh day.

By repeating the daily doses of indole over a period of three days (days 15-17) it was hoped that a greater drain on the endogenous sulphur would result. However there was actually some retention of sulphur on the second day of the series and the net excess sulphur excreted over the 3 days was only 39 mg., a figure very like those obtained for single day doses. It therefore appears that the amount of endogenous sulphur, immediately available for the formation of ethereal sulphate, is limited in amount. The retention of sulphur, following the dose days, was 61 mg.

It was next decided to attempt the replacement of the sulphur lost on dose days by the sulphate of the sodium sulphate. On day 25 indole was administered without sodium sulphate. There was an increased sulphate-sulphur excretion of 102 mg. To make good this loss sulphur was retained for the following 5 days, the total being 103 mg. This must have been derived partly from the food and partly perhaps from the tissues. On day 7 indole was again given alone and on the following day sodium sulphate. On the indole day 50 mg. excess sulphate-sulphur was excreted and on the sulphate

Black columns = Ethereal sulphate.
White columns = Inorganic sulphate.

Fig. 2. "Pansy."

Days 3-11	2 g. sodium sulphate, cryst. orally, daily.
Day 17	2 g. " " " " "
" 20	2 g. " " " " "
" 5	1 x M.W./100 g. phenol, orally.
" 8	1 x M.W./100 g. indole, "
" 14	1 x M.W./100 g. " "
" 16	1 x M.W./100 g. " "

Line A.	Daily sulphate-sulphur on diet	...	...	...	55 mg.
" B.	" " " " " " " " " " "	...	...	...	262 mg.
" C.	B less A...	...	...	...	207 mg.

Pansy's weight fell from 9.0 to 8.3 kg. in 21 days.

Fig. 3. "Patsy."

Day 4 2 g. sodium sulphate, cryst. subcutaneously.

" 6 2 g. " " " "

" 9 2 g. " " " "

" 13 2 g. " " " "

" 22 2 g. " " " "

" 26 2 g. " " " "

" 8 1 × M.W./100 g. indole orally.

" 23 1 × M.W./100 g. phenol "

" 21 1 × M.W./100 g. " "

Line A. Daily sulphate-sulphur on diet ... 30 mg.

" B. " " " on diet and sodium sulphate 223 mg.

" C. B less A ... 193 mg.

Patsy's weight fell from 6.8 to 6.5 kg. in 28 days.

day 81 mg. sulphur were retained. Immediately afterwards the sulphate excretion returned to normal. Even if it is assumed that 32 mg. of the sulphur retained were derived from the normal daily output of sulphate, a balance of 49 mg. is left, which must have come from the administered sodium sulphate.

Similar experiments were carried out on "Pansy." Fig. 2 has been constructed on the same lines as Fig. 1. The results obtained by the administration of phenol (day 5) and of indole (day 8) are similar to those obtained in Exp. 1. In the latter case however there are certain discrepancies. The apparent retention of sulphur on day 9 is extremely great and on day 10 the excretion of sulphate is much in excess of the normal. The most likely explanation of these discrepancies is the delayed absorption and therefore the delayed excretion of the sulphate given on day 9. If the excess sulphate on day 10 is added to the sulphate on day 9, the figure for the retained sulphur on that day becomes 62 instead of 100 (see Table I). On days 14 and 16 indole was administered without sodium sulphate, again with the intention of draining the animal of all sulphur available for the synthesis of ethereal sulphate. The excess excretion on the two days was 184 mg. S. Sodium sulphate was administered on day 17. The sulphur retained on days 15 and 17 was 151 mg., of which at least 73 mg. must have been derived from the sodium sulphate.

In Exp. 3 (Fig. 3) the sodium sulphate was given subcutaneously, to eliminate the possibility of any of the sulphate failing to reach the tissues. Indole was given on day 8 and the excess sulphate-sulphur, excreted on this day, was 84 mg. Sodium sulphate was administered on the following day and the sulphur retained on that day was 78 mg. The corresponding figures for phenol (days 21, 22) were 65 and 81. On day 13 phenol was given with sodium sulphate and the excess sulphate-sulphur excreted on that day was 42 mg. The sulphur retained on the four following days was 77 mg. 37 % of the administered sodium sulphate was utilised in the formation of the phenol sulphate. It makes no difference therefore whether the sodium sulphate is given orally or subcutaneously.

The calculations are based on averages for normal diet and normal sulphate days and are therefore subject to error¹. The nitrogen output in each experiment and to a lesser degree the neutral sulphur output varied most markedly, but in most instances these variations do not seem related to variations in the sulphate output. However, the corresponding variations in the sulphate excretion, if they occur, would be small and would be masked by the much greater variations due to the administration of the sodium sulphate, of the phenol and of the indole. On the whole it is somewhat remarkable that the calculations bear out the main thesis of this paper so consistently as they do.

¹ For example: in Exp. 2 line C is absolutely too high; it is drawn at the level of 207 mg. S and actually only 200 mg. S were given as sodium sulphate on sulphate days.

DISCUSSION.

In calculating the figures, which appear in Table I, two assumptions have been made—that the administered sodium sulphate is the last source of sulphur to be used (1) in the formation of ethereal sulphate and (2) in making good the sulphur lost from the tissues on dose days. The figures given therefore in the Table under sodium sulphate are minimum values.

Table I.

Day	Substance	Percentage phenol or indole excreted on dose day as ethereal sulphate	Ethereal sulphate excreted on dose day				Percentage sodium sulphate utilised	Sulphur retained after dose day mg. S	Minimum amount of sodium sulphate retained mg. S
			From excess sulphur mg. S	From sulphur of diet mg. S	From sodium sulphate mg. S	Total mg. S			
<i>Exp. 1:</i>									
7	Phenol	62*	43	32	133	208	66	53	21
11	Indole	34*	44	32	43	119	22	85	53
15-17	Indole	40*	39	96	278	413	46	61	29
25	Indole	36*	102	24	—	126	—	103	—
37	Indole	23	50	32	—	82	—	81	49
<i>Exp. 2:</i>									
5	Phenol	63	45	55	112	212	56	43	—
8	Indole	50*	71	55	49	175	25	100	45
14	Indole	41*	109	34	—	143	—	23	—
16	Indole	37*	75	55	—	130	—	128	73
<i>Exp. 3:</i>									
8	Indole	30	84	20	—	104	—	78	48
13	Phenol	43	42	30	73	145	37	77	—
21	Phenol	27	65	30	—	95	—	81	51

* Some in addition was also excreted on the day following the dose day.

It is clear from the Table that 23 to 62 % of the phenol or indole administered has been excreted on the dose day in the form of ethereal sulphate and at least 22 to 66 % of the sodium sulphate given has been utilised in the synthesis of the ethereal sulphate. One cannot expect all the administered sulphate to be utilised. Some will always escape through the kidney, before the synthesis can take place. Also not all the phenol or indole is available for the sulphate synthesis; part is excreted with glycuronic acid and part is probably oxidised. The utilisation of the sodium sulphate has been as full as can be expected. The mode of formation of ethereal sulphate from phenol or indole is the same as that from guaiacol.

In the Table are also shown the supposed sources of origin of sulphur, taking part in the ethereal sulphate synthesis. In column 4 are given the amounts of excess sulphur excreted on dose days. Such sulphur would commonly be called endogenous. Some persons might demur to attaching such a label to small amounts of excess sulphur, amounts that might be regarded as exogenous products delayed in excretion. When however the excess figures become large, it would be difficult to avoid the term endogenous. In the Table the corresponding figures for retained sulphur are also given and in the last column from these have been deducted the normal sulphate output

on diet days. The balance must represent the minimum amounts of sodium sulphate retained to make good the loss of sulphur from the tissues.

The most likely explanation of these experimental facts is the simplest. There is no reason to suppose that there are any differences in this particular respect in the oxidation products of endogenous and exogenous sulphur. It is unnecessary to distinguish between the two lines of metabolism. It is enough to say that sulphate arises by oxidation of sulphur compounds in organs. Before the sulphate is finally excreted by the kidneys, it accumulates in the body fluids. In a small dog this accumulation amounts to about 50 to 100 mg. S.

In the detoxication of phenols the first sulphur to be used is probably the sulphate ion in the cell fluids. As the immediate supply of sulphate is used up, it is replaced by oxidation products of metabolism. When the body fluids are depleted of sulphate by the administration of phenols, sulphate accumulates slowly over a series of days as it arises in the course of metabolism or as it can be replaced by sulphate in the diet, until the concentration is such that excretion again becomes normal. It seems likely that the sulphate excreted in excess on dose days and the sulphate retained afterwards are present in the tissues in the ionised condition and not in combination and undissociated, but of this there is no proof. To some the retention of sulphate by the animal may seem remarkable but it must be remembered that the concentrations involved are no greater than the concentration of sulphate (2 to 3.5 mg. S per 100 cc.) in normal dogs' blood [Denis, 1921]. If the sulphate retained were distributed uniformly (a more than improbable assumption) throughout the body fluids, the concentration would be about 1 to 2 mg. S per 100 cc.

SUMMARY.

1. The dog can utilise sodium sulphate, given orally or subcutaneously, to form phenyl sulphate and indoxyl sulphate from phenol and indole respectively.
2. When ethereal sulphate is formed by the dog from phenol or indole at the expense of endogenous sulphur, this sulphur can be replaced at least in part by the sulphate ion.
3. It is suggested that in every case ethereal sulphate, whether arising endogenously or exogenously, is formed by the union of the sulphate ion with phenolic compounds.

The expenses of this research were largely defrayed out of grants from the Royal Society.

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