XIV. STUDIES IN THE SULPHUR METABOLISM OF THE DOG. I. THE SYNTHESIS OF ETHEREAL SULPHATE.

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FROM the year 1876, when Baumann [1876] first isolated from horse's urine an ethereal sulphate in a pure state and proved it to be potassium phenyl sulphate, the manner in which the synthesis of ethereal sulphate is carried out in the animal body has been a matter of controversy. Baumann thought he had proved in the dog the direct union of phenol introduced subcutaneously with sodium sulphate given by the mouth. The experimental data given in his paper are however too meagre. It is not clear that the ethereal sulphate in the urine arose from the administered sulphate and not from catabolised protein. Sonnenburg [1878] found that sodium sulphate was beneficial in cases of phenol poisoning in man. Schmiedeberg [1881] repeated Baumann's experiments on the dog under slightly different conditions. Instead of administering phenol subcutaneously he gave benzene by the mouth with and without sodium sulphate. The data are again too meagre. The dose of benzene given was very variable and it is quite impossible to make any comparison between the two series of experiments. Marfori [1894] injected dogs with phenol and with phenol together with ammonium sulphate. The output of ethereal sulphate was considerably greater in the second series of experiments.

Tauber [1895] attacked the problem in a different way. He determined the minimum lethal dose of phenol for rabbits and for guinea-pigs and tried to find out if various sulphur-containing compounds afforded any protection against such a dose. The phenol was given subcutaneously and the sulphur compounds intravenously or subcutaneously. He found that sodium sulphate, sodium ethyl sulphate, sodium pyrosulphate, sodium dithionate, sodium bisulphite, sodium thiosulphate and taurine had no beneficial effect. Sodium sulphite had a small beneficial effect. The lethal dose of phenol alone was 0.55 g. per kilo. rabbit, with sodium sulphate 0.66 g. Pfeiffer [1890] had already shown that sulphite was readily oxidised to sulphate in the animal. Boix and Noé [1902] could not find that sodium thiosulphate given subcutaneously protected guinea-pigs in any degree from sodium phenate also injected subcutaneously.

Sato [1909] administered phenol by stomach tube to rabbits and gave also by the stomach various sulphur-containing substances. Isethionic acid did not increase the formation of ethereal sulphate; thio-urea gave a small increase, while cystine and "colloidal sulphur" gave a larger increase. He did not try the effect of sodium sulphate. Granström [1910] could not observe any increase of ethereal sulphate in man after the administration of sodium sulphate in phenol poisoning. Masuda [1910] found that thio-urea caused an increase in neutral sulphur and in ethereal sulphate as well as in inorganic sulphate apart from any phenol administered at the same time. He suggested that phenols are not the only causative factor in the formation of ethereal sulphate. Kenji Kojo [1911] found that thio-urea whether given by the mouth or subcutaneously caused a slight increase in the output of ethereal sulphate, even when phenols were not administered at the same time. He suggested that the thio-urea by oxidation and union with phenols, formed in the gut by bacteria, protected the phenols from further oxidation in the tissues and in consequence the output of ethereal sulphate was increased. Tauber [1895] had previously demonstrated that phenol is capable of oxidation by the tissues.

Rhode's experiments [1922] are more elaborate and greater detail is given. He determined the daily output of inorganic and ethereal sulphate by rabbits, receiving by mouth a daily dose of phenol. On certain days sulphur compounds were administered subcutaneously and the output of inorganic and of ethereal sulphate compared with that on the days on which phenol was given alone. He found that cystine and sodium sulphite increased the output of ethereal sulphate markedly, taurine slightly, sodium sulphate and sodium thiosulphate not at all. Sodium sulphate so administered was readily excreted unchanged without entering into combination with the phenol.

Hopkins [1907] and more recently Sherwin [1922] have suggested that a mercapturic acid, such as is excreted by the dog after a dose of bromobenzene, is an intermediary stage in the formation of ethereal sulphate from phenols and cystine. This last theory will be dealt with in the second and third papers of this series.

As all the sulphur compounds mentioned above, with the probable exception of the mercapturic acids, are oxidised in the animal body to sulphate, some more readily than others, the demonstration of the direct union of any phenol with preformed sulphate would explain any positive effect of these compounds in increasing the output of ethereal sulphate. In the present paper evidence is brought forward of the direct union in the dog of guaiacol and sodium sulphate to form ethereal sulphate.

EXPERIMENTAL.

A fox-terrier bitch, weighing 5 kilos. was used for the experiments. The posterior wall of the vagina was slit open in order to render catheterisation of the urethra easy. She was placed on a standard diet sufficient to maintain body weight. After a period of about 5 days the urine was collected daily

for analysis, the animal being catheterised at least once in every 24 hours. The output of total nitrogen (Kjeldahl), total sulphur, total sulphate and inorganic sulphate (Folin) in the urine was determined daily throughout the experimental periods. Total nitrogen and total sulphur estimations were also carried out on the food and faeces. The standard diet was made up as follows:

		Total nitrogen g.	Total sulphur g.	Calories K
Beef	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	107
Total		4.07	0.319	648

Exp. 1. This experiment lasted for 37 days, from July 1 to August 6, 1919. From July 7 to July 29 4 g. of guaiacol carbonate was administered daily by

Fig. 1.

the mouth. Guaiacol carbonate, though rather insoluble, was chosen because it is almost tasteless and because it is very little toxic. Probably not more than 1 g. was absorbed daily, the rest passing out unchanged in the faeces. The amount absorbed was very large in proportion to the daily output of sulphur in the urine, so that there was always more than sufficient guaiacol excreted to combine with all the sulphate produced in the course of metabolism. There was a very marked fall in the output of sulphur on the day following the last guaiacol day. This experimental observation will be considered in the second paper of this series.

There was administered by the mouth daily 2 g. sodium sulphate ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$) equivalent to 0.199 g. S, from July 5 to July 9; 0.8 g. cystine, equivalent to 0.213 g. S, from July 13 to July 15; and 0.652 g. sodium bisulphite (NaHSO_3), equivalent to 0.201 g. S, from July 20 to July 22. The daily output of total nitrogen, inorganic sulphate and ethereal sulphate (total sulphate less inorganic sulphate) is represented graphically above. Neutral

sulphur (total sulphur less total sulphate) has not been shown in the diagram as it was not considered desirable to complicate the figure. The variations in total nitrogen and in neutral sulphur bear little or no relation to the substances given.

EXPERIMENT 1.

Date	Urine							Faeces	
	Vol.	T.N.	T.S.	In. SO ₄	Eth. SO ₄	N.S.	T.S. × 100	T.N.	T.S.
1919	cc.	g. N	g. S	g. S	g. S	g. S	T.N.	g. N	g. S
July									
1	190	3.53	0.158	0.103	0.011	0.044	4.47	—	—
2	190	3.31	0.174	0.102	0.016	0.056	5.26	—	—
3	164	3.34	0.162	0.099	0.014	0.049	4.85	—	—
4	218	3.10	0.156	0.086	0.013	0.057	5.02	1.16	0.144
5	220	3.42	0.339	0.268	0.018	0.053	9.93	—	—
6	176	3.41	0.350	0.281	0.013	0.056	10.27	—	—
7	234	3.50	0.363	0.080	0.232	0.051	10.37	0.61	0.087
8	210	3.32	0.316	0.016	0.249	0.051	9.52	—	—
9	184	3.50	0.320	0.033	0.241	0.046	9.14	—	—
10	188	3.65	0.187	—	0.118	0.069	5.13	—	—
11	210	3.72	0.170	—	0.114	0.056	4.57	1.01	0.146
12	167	3.46	0.156	—	0.114	0.042	4.51	—	—
13	245	3.36	0.249	—	0.185	0.064	7.42	—	—
14	170	3.30	0.327	0.069	0.190	0.068	9.92	—	—
15	276	3.50	0.367	0.077	0.200	0.090	10.49	—	—
16	341	3.53	0.215	—	0.156	0.059	6.10	1.08	0.156
17	113	3.57	0.142	—	0.109	0.033	3.98	—	—
18	156	3.47	0.137	—	0.097	0.040	3.94	—	—
19	214	4.12	0.178	—	0.107	0.071	4.31	0.66	0.067
20	233	3.56	0.308	0.096	0.147	0.065	8.64	0.51	0.073
21	378	3.32	0.305	0.112	0.126	0.067	9.20	—	—
22	257	3.55	0.332	0.135	0.141	0.056	9.35	—	—
23	285	3.90	0.199	0.004	0.136	0.059	5.10	—	—
24	350	3.85	0.178	—	0.110	0.068	4.62	0.73	0.088
25	198	3.48	0.158	—	0.113	0.045	4.53	—	—
26	221	3.52	0.146	—	0.086	0.060	4.14	0.64	0.093
27	252	3.90	0.165	—	0.105	0.060	4.22	—	—
28	184	3.83	0.179	—	0.117	0.062	4.68	—	—
29	208	3.45	0.163	—	0.101	0.062	4.73	1.00	0.093
30	165	3.64	0.091	0.006	0.036	0.049	2.49	—	—
31	279	3.79	0.200	0.122	0.017	0.061	5.28	—	—
August									
1	310	3.79	0.182	0.113	0.019	0.050	4.80	—	—
2	302	3.77	0.174	0.110	0.014	0.050	4.63	1.13	0.162
3	204	3.82	0.166	0.107	0.015	0.044	4.34	—	—
4	224	3.66	0.152	0.101	0.011	0.040	4.16	—	—
5	285	3.79	0.157	0.102	0.014	0.041	4.13	0.80	0.109
6	295	4.04	0.199	0.144	0.010	0.045	4.92	—	—

The dog received daily by mouth:

July	7 to 29, inclusive.	Guaiacol carbonate	4 g.
"	5 " 9	Sodium sulphate, cryst.	2 g.
"	13 " 15	Cystine	0.8 g.
"	20 " 22	Sodium bisulphite	0.652 g.

The weight of the dog was 5.53 kilos. on July 1 and 5.36 kilos. on August 6.

There is a marked rise in inorganic sulphate on the "sodium sulphate" days and an immediate fall on the addition of guaiacol to the diet, with a corresponding rise in ethereal sulphate. The effect of cystine and of bisulphite is less marked on the ethereal sulphate curve, though it is clear that part of the sulphur of these compounds appears in the ethereal sulphate fraction in

combination with the guaiacol. It is noticeable that on the days on which guaiacol is given alone the output of inorganic sulphate falls to zero and that incidentally the output of ethereal sulphate is very constant. In consequence of this fact the area marked *A* represents the administered sulphate in combination with the guaiacol, the area marked *D* the administered sulphate excreted unchanged, the area marked *B* the administered cystine sulphur in combination with the guaiacol, the area marked *E* the administered cystine sulphur excreted as inorganic sulphate, the area marked *C* the bisulphite sulphur in combination with the guaiacol and the area marked *F* the bisulphite sulphur excreted as inorganic sulphate.

If the actual figures from the tables of analyses (p. 113) are taken, the following results are obtained:

	Total ethereal sulphate g. S	Total neutral sulphur g. S	Daily average output of	
			Ethereal sulphate g. S	Neutral sulphur g. S
Control periods:				
First 6 days	0.085	0.315	0.014	0.052
Last 7 days	0.100	0.331	0.014	0.047
Both periods	0.185	0.646	0.014	0.050
Guaiacol days:				
July 10 to 12	0.346	0.167	0.115	0.056
" 17 " 19	0.313	0.144	0.104	0.048
" 24 " 29	0.632	0.357	0.105	0.059
All 12 days together	1.291	0.668	0.108	0.056

Sodium sulphate. The effect of the administered sodium sulphate is not prolonged beyond the last day on which it is given. From July 7 to July 9 the total output of ethereal sulphate is 0.722 g. S. If the average output of ethereal sulphate for three days on guaiacol alone (0.324 g. S) is deducted, there is left 0.398 g. S, the excess ethereal sulphate due to the effect of the added sodium sulphate. The output of inorganic sulphate for the same three days is 0.129 g. S. The output of neutral sulphur on these days is actually rather less than the normal average.

The results may be summarised as follows:

Sodium sulphate given from July 7 to 9 equivalent to	0.597 g. S
Amount excreted as ethereal sulphate	0.398 "
Amount excreted as inorganic sulphate	0.129 "
Amount unaccounted for	0.070 "

Therefore percentage of administered sulphate excreted in combination with guaiacol is 66.

Similar calculations may be made for the cystine and bisulphite days, though in these instances the effect may be observed over an extra day, *i.e.* the excretion of the sulphate derived from these substances is not so rapid as in the former case.

Cystine days. July 13 to 15.

Cystine given	equivalent to	0.639 g. S
Total ethereal sulphate	"	0.731 "
Less the average output on guaiacol alone	"	0.432 "
Cystine in combination as ethereal sulphate	"	0.299 "
Cystine as inorganic sulphate	"	0.146 "
Cystine unaccounted for	"	0.145 "

Percentage of administered cystine excreted in combination as ethereal sulphate is 47.

The average output of neutral sulphur on the cystine days is 0.070 g. S, a figure rather above the normal average. This may be due to the excretion of the products of bacterial action on the cystine in the intestine.

Bisulphite days. July 20 to 22.

Bisulphite given	equivalent to 0.603 g. S
Total ethereal sulphate	" 0.550 "
Less the average output on guaiacol alone	" 0.432 "
Bisulphite in combination as ethereal sulphate	" 0.118 "
Bisulphite as inorganic sulphate	" 0.347 "
Bisulphite unaccounted for	" 0.138 "

Percentage of administered bisulphite excreted in combination as ethereal sulphate is 19.

The average output of neutral sulphur on the bisulphite days is 0.062 g. S, not much greater than the normal average.

Exp. 2. This experiment lasted from November 6 to December 12, 1919, with an interval of two days due to the animal missing one meal on November 24. The analyses on the faeces and of total sulphur on the urine were omitted. In other respects the experimental conditions were the same as in Exp. 1. Guaiacol carbonate was administered by mouth from November 12 to December 8, sodium sulphate on November 18, 19, 22, 23, 30, and sodium bisulphite on November 14, 15 and December 2. The dose of sodium sulphate given on November 18 and on November 19 was only one-fifth of the dose given on the other days. By calculation from the analyses the following results are obtained:

Fore period, Nov. 6 to 11.			Average daily output of ethereal sulphate				0.010 g. S	
After period, Dec. 10 to 12.			" "				0.013 "	
Mean			...				0.011 "	
Guaiacol days, Nov. 12, 13, 16, 17, 20, 21, 26 to 29; Dec. 4 to 8.								
Average daily output of ethereal sulphate							0.137 "	
Date	g. S in compound given	g. S Eth. Sulph. excreted	Less av. output on guaiacol days	g. S Eth. Sulph. due to S compound	g. S Inorg. Sulph. excreted from S compound	g. S in compound given, unaccounted for	Percent. used of S in compound	
Sodium bisulphite:								
Nov. 14, 15	0.402	0.349	0.274	0.075	0.304	0.023	18	
Dec. 2, 3	0.201	0.388	0.274	0.114	0.058	0.029	56	
Sodium sulphate:								
Nov. 18, 19	0.080	0.352	0.274	0.078	0.025	—	about 69	
Nov. 22, 23	0.398	0.273	0.274	Nil	0.436	—	Nil	
Nov. 30, Dec. 1	0.199	0.382	0.274	0.108	0.092	—	54	

On November 18, 19 and on November 22, 23, the amount of sulphur apparently derived from the dose of sodium sulphate was in excess of the dose given. Such discrepancies must occur, when the figures are based on a deduction of an average value. The experiment on November 22, 23 with sodium sulphate was entirely negative. There was no evidence that sodium metabisulphite was less or more effective than sodium sulphate. For the variations in results some other cause must be sought.

Exp. 3. This experiment lasted from October 9 to 27, 1921. The urine was collected in 48 hour periods. In other respects the conditions were the same as in Exp. 1. Guaiacol carbonate 4 g. daily was administered by mouth from October 13 to 27. Sodium sulphate 2 g. daily was given by mouth from October 17 to 23.

EXPERIMENT 2.

Date	Urine				Date	Urine			
	Vol.	T.N.	In. SO ₄	Eth. SO ₄		Vol.	T.N.	In. SO ₄	Eth. SO ₄
1919	cc.	g. N	g. S	g. S	1919	cc.	g. N	g. S	g. S
Nov.					Nov.				
6	198	3.64	0.133	0.009	25	—	—	—	—
7	200	3.26	0.126	0.011	26	221	4.30	—	0.130
8	300	3.15	0.111	0.016	27	202	3.75	—	0.141
9	157	3.22	0.117	0.013	28	222	3.76	—	0.135
10	200	3.38	0.119	0.006	29	233	3.91	—	0.145
11	315	3.67	0.137	0.005	30	277	3.88	0.092	0.205
12	227	3.67	0.022	0.129	Dec.				
13	220	3.63	—	0.156	1	232	3.57	—	0.177
14	199	3.39	0.141	0.172	2	222	3.54	0.058	0.235
15	200	3.46	0.153	0.177	3	253	3.81	—	0.153
16	222	3.42	0.010	0.140	4	221	3.53	—	0.134
17	228	3.20	—	0.149	5	270	3.06	—	0.112
18	224	3.54	—	0.179	6	218	3.02	—	0.130
19	222	3.82	0.014	0.173	7	273	3.56	—	0.118
20	222	3.67	0.011	0.142	8	231	3.66	—	0.137
21	232	3.81	—	0.155	9	196	3.66	0.031	0.050
22	233	3.97	0.208	0.148	10	217	3.65	0.121	0.015
23	253	4.06	0.228	0.125	11	261	3.51	0.119	0.011
24	—	—	—	—	12	210	3.62	0.117	0.013

The dog received daily by mouth:

Nov. 12 to Dec. 8 inclusive.	Guaiacol carbonate	4 g.
" 14 " Nov. 15	Sodium bisulphite	0.652 g.
" 18 " " 19	Sodium sulphate, cryst.	0.4 g.
" 22 " " 23	" "	2 g.
" 30	" "	2 g.
Dec. 2	Sodium bisulphite	0.652 g.

The weight of the dog was 4.99 kilos. on Nov. 6 and 5.10 kilos. on Dec. 12.

EXPERIMENT 3.

Date	Urine							Faeces	
	Vol.	T.N.	T.S.	In. SO ₄	Eth. SO ₄	N.S.	T.S. × 100	T.N.	T.S.
1921	cc.	g. N	g. S	g. S	g. S	g. S	T.N.	g. N	g. S
October									
9-11	334	7.24	0.339	0.215	0.015	0.109	4.68	—	—
11-13	363	7.66	0.373	0.242	0.020	0.111	4.87	0.63	0.132
13-15	400	7.83	0.396	—	0.300	0.096	5.06	0.83	0.155
15-17	389	7.67	0.368	—	0.272	0.096	4.80	—	—
17-19	332	7.68	0.676	0.185	0.379	0.112	8.80	1.07	0.155
19-21	370	7.37	0.700	0.274	0.330	0.096	9.50	—	—
21-23	372	7.27	0.692	0.201	0.391	0.100	9.52	—	—
23-25	333	7.94	0.366	—	0.272	0.094	4.61	0.93	0.145
25-27	264	7.50	0.324	—	0.226	0.098	4.32	0.82	0.139
27-29	300	7.57	0.275	0.082	0.096	0.097	3.63	—	—
29-31	278	7.50	0.289	0.153	0.029	0.107	3.85	0.92	0.129

The dog received daily by mouth:

Oct. 13 to 27, inclusive.	Guaiacol carbonate	4 g.
" 17 " 23	Sodium sulphate, cryst.	2 g.

The weight of the dog was 6.12 kilos. on Oct. 9 and 6.04 kilos. on Oct. 31.

The following results are obtained from the analyses:

The mean output of ethereal sulphate on the first 4 days	...	...	...	...	0.009 g. S
The mean output of ethereal sulphate on 8 guaiacol days	...	...	...	...	0.134 „
The total dose of sodium sulphate given over 6 days	...	...	...	...	1.194 „
The total output of ethereal sulphate on these days	...	...	...	...	1.000 „
The ethereal sulphate calculated from the output on the guaiacol days to be deducted	...	...	...	...	0.804 „
The inorganic sulphate excreted over sulphate period	...	...	...	...	0.660 „
Sulphate unaccounted for	...	...	...	...	0.338 „
The percentage of administered sulphate used	...	...	...	...	16

The results of all the experiments may be summarised thus:

Compound given by mouth	Experiment	Total dose g. S	Percentage used
Sodium sulphate	1	0.597	66
	2	0.080	about 69
	2	0.398	Nil
	2	0.199	54
	3	1.194	16
Cystine	1	0.639	47
Sodium bisulphite	1	0.603	19
	2	0.402	18
	2	0.201	56

DISCUSSION.

It would appear from the above experiments that guaiacol and therefore presumably other phenols will unite in the animal body with preformed sulphate, provided the right conditions are present. Even in the above series there is one complete failure to bring about the synthesis. What are the necessary conditions? It must be remembered that phenols are also excreted in combination with glycuronic acid. Tollens [1910] found that in man the greater portion of the carboic acid excreted is in combination with glycuronic acid, a lesser portion in combination with sulphuric acid, while the greater portion of indole is excreted in combination with sulphuric acid and a lesser portion in combination with glycuronic acid. Further, Knapp [1911] found that in man after the administration of guaiacol carbonate rather more guaiacol is excreted combined with sulphuric acid than with glycuronic acid. Such a difference in behaviour between phenols might account for the failure of Rhode and of others to demonstrate a synthesis between carboic acid and preformed sulphate.

In any case it is clear that any one phenol is dealt with in many ways, some part is excreted uncombined, some part is oxidised completely, some part is conjugated with glycuronic acid and some part with sulphuric acid. The mixture, which reaches the blood stream and is excreted by the kidney, will depend upon the relative velocities of the several reactions. It is also certain that the permeability of the kidney to the various reacting substances and end-products is very different. To upset the balance it is necessary to increase the effective concentration of one of the reacting substances at the seat of synthesis. In the case under consideration it is the effective concentration of sulphuric acid. Further, it is necessary to consider the organ or organs, in which the syntheses take place. One is inclined to believe that all tissues contain in some degree the enzymes that catalyse the reactions in question. There is, however, strong evidence that the liver is the chief seat of the

syntheses of ethereal sulphate and of glycuronic acid. Baumann [1876] obtained phenol from liver and from blood by distillation, after hydrolysis with hydrochloric acid, of alcoholic extracts, which before hydrolysis yielded no phenol on distillation. The liver contained 19 times more phenol-yielding substance than blood. Christiani and Baumann [1878] found that ethereal sulphates were not produced in the kidney to any appreciable extent. Kochs [1879, 1880] found that liver mush and muscle mush would synthesise phenyl sulphate from phenol and sodium sulphate. Embden and Glaessner [1902] perfused organs with phenol. They obtained considerable amounts of ethereal sulphate from the liver, small amounts from the kidney and lung. The experiments with muscle and intestine were negative. Satta [1908] extracted organs for ethereal sulphate after the administration of phenol. He obtained the highest yield from the liver, lesser amounts from the kidney, stomach, and blood and practically none from muscle. Pelkan and Whipple [1922] have found that extensive liver injury in dogs lessens phenol conjugation and extreme injury to the liver will reduce phenol conjugation to zero. They conclude that phenol conjugation is a function of liver parenchyma cells and of no other body cells.

It is now possible to discuss the reason why some have failed to demonstrate any direct union between phenols and sulphate. If the liver is the chief source of ethereal sulphate, sodium sulphate injected subcutaneously or intravenously is likely to be excreted by the kidney without affecting in any marked degree the concentration of sulphate in the liver cells, whereas substances like cystine and sulphite, which are not readily excreted by the kidney, may reach the liver and be oxidised actually at the seat of the ethereal sulphate synthesis. Further, phenol itself is so toxic that relatively only small doses can be used with impunity and in consequence any change in the rate of conjugation would be very difficult of detection. There will also always be sufficient sulphate present, derived from protein, to meet the immediate requirements of the tissues. Rhode's observations may be explained on these lines. One could hardly expect such experiments as those of Tauber, Boix and Noé, to yield positive results. Phenol in such doses injected subcutaneously would have a fatal result, long before any synthesis could take place. In the experiments described above the large doses of the relatively non-toxic phenol, guaiacol, given orally and the simultaneous oral administration of sodium sulphate constitute conditions under which the direct synthesis of ethereal sulphate is most likely to be demonstrated.

SUMMARY.

1. Sodium sulphate, sodium bisulphite and cystine are partly excreted as ethereal sulphate by the dog, when administered by the mouth together with guaiacol carbonate.
2. Under these conditions sodium sulphate is utilised as readily as cystine or as sodium bisulphite.

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