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Sulphur Metabolism.

III. The Effect of Flowers of Sulphur on the Growth of Young Rats fed an otherwise Well-balanced Ration.

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According to the work of several investigators the oral administration of flowers of sulphur offers interesting possibilities of success in checking intestinal putrefaction in chicks in confinement (Phillips, Carr and Kennard, 1921) and worm infestation in sheep (Steyn, 1932). Montgomery (1932) and Burman (1932) also found that the ingestion of an adequate amount of flowers of sulphur served as a good prophylactic in malaria.

Unfortunately the administration of sulphur cannot be left in inexperienced hands and the promiscuous feeding of sulphur should be warned against, in view of the fact that the dosing of high levels of sulphur was found to be definitely toxic. Steyn (1931), for instance, observed that the dosing of 45 gm. of flowers of sulphur "per week per sheep over a period of thirty-five days given in daily doses of 15 gm. cause symptoms of poisoning and high mortality." Lawson, Redfield and Boyd (1934), who experimented with rabbits and guinea pigs, found that by giving doses of from 0·5 to 2·0 gm. of either colloidal or flowers of sulphur per kgm. of body weight directly into the stomach through a small stomach tube all the animals died within twenty-four hours. The stomach walls were necrotic, the kidneys intensely congested and the livers slightly so. The spleens showed little change as did the hearts and lungs. Lewis and Lewis (1927) observed that sulphur when added to the milk powder-starch diet of Sherman and Merrill (1925) was definitely

toxic and caused the death within periods of from 3 to 21 days of fourteen out of twenty-two animals whose diet contained it to the amount of 1 per cent. With a few exceptions, the livers of the rats which died showed a marked peripheral zonal necrosis.

However, the latter authors also observed that the feeding of 1 per cent. of sulphur with the Osborne-Mendel low protein diet was less toxic than when added to the same extent to the Sherman-Merrill diet. This observation seems to show that the toxicity of sulphur can be controlled to a certain extent by the composition of the food given. If that is true, it will be of great practical importance in regions where sulphur is administered as a prophylactic to disease in man and animals.

As will be pointed out later on in this article both the Sherman-Merrill and Osborne-Mendel diets are deficient in certain food essentials whereas the mixed diets of man and animals, under normal conditions, usually contain enough of the various essential constituents. It was thought, therefore, of interest to study the effect of elementary sulphur on the growth of young animals given diets, which were as complete as possible, from a nutritional standpoint.

Experimental.

Albino rats of about three to four weeks old and of the London strain of the Wistar Institute stock were used in these experiments. In the first experiment (preliminary) three groups of five rats each were used. Each group of animals was housed in a big cage with a raised screen bottom. The animals were fed *ad libitum* and the total food consumption for each group recorded. The basal ration used in this experiment was a modification of the Osborne-Mendel low protein diet given in Table I. One group received the basal ration and the other two the basal ration supplemented with 0·8 per cent. of flowers of sulphur. The sulpur was added at the expense of the dextrinized starch. The animals were weighed once a week throughout the experimental period and on the last day of the experiment. Fresh distilled water was at all times available to the animals except to those of one of the sulphur groups which received fresh orange juice *ad libitum* instead of water. The orange juice was substituted for water because it was thought that its basic elements might play a rôle in the detoxication of hydrogen sulphide. As a matter of fact the author (1936) found that H_2S is oxidized (detoxicated) better in an alkaline than in an acid or neutral medium. If this is also true for the oxidation of H_2S in living organisms, the observations of Saywell and Lane (1933) and Schuck (1934) that the hydrogen ion concentration of the urine of men and women subjects can be decreased and the alkaline reserve of their bodies increased by taking 1,000 c.c. of orange juice daily might still prove itself of value in the detoxication of sulphur. The results are given in Table II.

TABLE I.

Composition of Basal Rations in Percentage by Weight.

	Modified Osborne-Mendel Diet.	Stock Diet.
Merk's casein	10	—
Crude casein	—	5
Modified Steenbock salts 40*	4·5	—
Dextrinized starch	54·5	—
Powdered sucrose	5	—
Merk's prepared lard	19	—
Cod liver-oil	3	—
Agar	2	—
Brewer's yeast	2	5
Yellow maize meal	—	68
Linseed oil meal	—	6
Milk powder (Nestle's, S. Africa)	—	4
Lucerne meal	—	3
Butter fat	—	5
Beef liver (dried at 70° C.)	—	2
Bone ash	—	1
Calcium carbonate	—	0·5
Sodium chloride	—	0·5

* The $MgSO_4 \cdot 7H_2O$ in Steenbock and Nelson Salts 40 (1923) was replaced by the same amount of magnesium carbonate.

TABLE II.

Summary of Growth and Food Consumption.

Rat. No.	Sex.	Diet.	Initial Weight.	Final Weight.	Gain or Loss.	Total Food.	Gain per 100 gm. Food.
			Gm.	Gm.	Gm.	Gm.	Gm.
11	Female	Basal	43	56	+13	—	—
12	,,	,,	52	84	+32	—	—
13	,,	,,	45	72	+72	—	—
14	,,	,,	50	69	+19	—	—
15	,,	,,	53	73	+20	—	—
		MEAN	48·6	70·8	+22·2	262	8·5
16	Male	Basal + 0·8 per cent. S.	50	47	− 3	—	—
17	,,	—	44	54	+10	—	—
18	,,	—	41	43	+ 2	—	—
19	,,	—	46	57	+11	—	—
20	,,	—	36	57	+21	—	—
		MEAN	43·4	51·6	+ 8·2	226	3·6
21	Female	Basal + 0·8 per cent. S. plus orange juice *ad lib.*	48	78	+30	—	—
22	,,	—	40	54	+14	—	—
23	,,	—	40	35	− 5	—	—
24	,,	—	47	54	+ 7	—	—
25	,,	—	44	44	± 0	—	—
		MEAN	43·8	53	+ 9·2	222*	4·1

* The food value of orange juice was not taken into consideration.

In view of the fact that the groups were too small and, because the food consumption of each animal was not measured separately, no statistical analysis can be made of the results. However, the results seem to show that the incorporation of 0·8 per cent. of sulphur in the basal ration retarded the rate of growth, and that the administration of orange juice had, under the experimental conditions, no beneficial effect on the rate of growth of the second sulphur group. Over a period of 60 days the average gain in weight, the total food consumed and the gain per 100 gm. food were 22·2, 8·2 and 9·2; 262, 226 and 222, and 8·5, 3·6 and 4·1 gm. for the basal, sulphur and sulphur plus orange juice groups respectively.

In a second experiment the effect of different concentrations of flowers of sulphur was studied on the growth of young rats fed a basal ration, which in the light of present knowledge, is complete in all respects for this species of animal. The diet was a slight modification of the stock ration (Kellermann, 1934) used in this laboratory. Its composition is given in Table I. The sulphur rations had the same composition as the basal except that sulphur was used in the place of like amounts of yellow maize meal in the diets. The paired-feeding method of Mitchell and Beadles (1930) was employed, and all pair-mates were matched for litter and sex. There were six pairs to each group. The initial and final weights of the rats were the average of weighings taken on three consecutive days. During the rest of the experimental period they were weighed once a week. Fresh distilled water was given in addition to the ration. The animals were fed according to the method of Mitchell (1933). The results are given in Table III.

It will be seen that on the whole the control rats gained highly significantly more in weight than those receiving the sulphur supplement. The number of weeks, however, during which the control rats gained more in weight than their mates is reasonably near 50 per cent. and therefore, indicates no such difference as reflected by the gains in weight for the entire period. Furthermore, the various treatments show no significant difference amongst themselves.

It is also of interest to note that on the whole the rats in this experiment, including those that received rations containing added sulphur, made as good gains as those obtained by Greenman and Duhring (1923) for the albino rat under normal conditions. As a matter of fact even the 11 male and 13 female rats fed the basal ration in which was incorporated elementary sulphur in such high concentrations as 2·0, 2·5, 3·0 and 3·5 per cent. reached at approximately 82 days of age average weights of 206·5 gm. and 148·2 gm., respectively, which were 60·8 gm. and 11·2 gm. above the respective normal weights given by Greenman and Duhring.

Discussion.

Lewis and Lewis (1927) advanced the theory that the lessened toxicity of sulphur on the Osborne-Mendel diet as compared with the Sherman-Merrill ration might be explained by its higher fat and protein contents. However, it should be pointed out that, as a

TABLE III.

Growth Records of Rats on Basal Ration with and without Different Percentages of Elementary Sulphur.

(All periods, 8 weeks, and all weights in grams).

Percentages of Elementary Sulphur incorporated.	Data.	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
		Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.
0·1	Initial weight	54	52	54	55	60	62	61	61	65	64	62	61
	Final weight	166	162	153	153	158	158	149	161	292	283	235	223
	Total gain	112	110	99	98	98	96	88	100	227	219	173	162
	Total food	559	558	522	520·5	522	522	513	513	816	816	668	668
	Comparison of weekly gains	4	4	3	5	5	3	3	5	5	3	4	4
0·25	Initial weight	55	56	59	59	73	69	80	79	65	68	57	57
	Final weight	162	167	268	282	287	279	298	303	173	172	167	163
	Total gain	107	111	209	223	214	210	218	224	108	104	110	106
	Total food	534	533·5	757	757	769	768·5	795	795	570	570	540	537·5
	Comparison of weekly gains	4	4	3	5	5	3	3·5	4·5	4·5	3·5	5	3
0·5	Initial weight	61	62	61	61	74	76	71	71	74	77	75	77
	Final weight	169	171	252	245	265	261	287	278	243	251	218	221
	Total gain	108	109	191	184	191	185	216	207	169	174	143	144
	Total food	539	539	673	673	708	708	768	768	698	696	654	654
	Comparison of weekly gains	6	2	5·5	2·5	5·5	2·5	5	3	3	5	4·5	3·5
0·75	Initial weight	76	75	62	63	88	90	66	65	58	58	60	61
	Final weight	240	250	143	140	285	288	160	154	171	170	164	167
	Total gain	164	175	81	77	197	198	94	89	113	112	104	106
	Total food	726	726	483	483	775	775	550	550	596	596	562	562
	Comparison of weekly gains	2	6	4	4	4	4	5	3	4	4	3	5
1·0	Initial weight	53	54	66	69	80	78	73	73	68	70	53	56
	Final weight	150	148	147	158	246	242	240	227	248	233	247	234
	Total gain	97	94	81	89	166	164	167	154	180	163	194	178
	Total food	518	518	529	529	685	685	651	651	742	742	669	669
	Comparison of weekly gains	4	4	2	6	3	5	5	3	7	1	5	3
1·25	Initial weight	56	57	67	69	66	65	50	50	47	51	63	66
	Final weight	159	153	179	195	153	150	145	136	132	130	228	228
	Total gain	103	96	112	126	87	85	95	86	85	79	165	162
	Total food	523	521	593	593	492	492	229	230	468·5	469	657	657
	Comparison of weekly gains	4	4	3	5	4·5	3·5	5	3	5	3	4	4

TABLE III—(*continued*).

Percentages of Elementary Sulphur incorporated.	Data.	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
		Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.	Control.	Sulphur.
1·5	Initial weight	61	61	50	53	49	51	40	40	52	55	54	54
	Final weight	162	154	210	195	151	157	142	136	152	166	228	225
	Total gain	101	93	160	142	102	106	102	96	100	111	174	171
	Total food	530	530	586	586	516·5	517	481	481	537	537	638	637·5
	Comparison of weekly gains	4·5	3·5	6	2	3·5	4·5	4·5	3·5	3	5	5	3
2·0	Initial weight	56	57	62	63	59	59	48	49	73	72	48	50
	Final weight	164	166	271	252	147	152	128	131	222	216	219	203
	Total gain	108	109	209	189	88	93	80	82	149	144	171	153
	Total food	607	607	750	750	483	483	468	471	675	675	594	594
	Comparison of weekly gains	3·5	4·5	6·0	2·0	3·5	4·5	5·5	2·5	4·5	3·5	5·5	2·5
2·5	Initial weight	62	62	64	62	65	65	61	60	80	80	69	70
	Final weight	207	192	183	184	145	131	151	139	234	215	148	139
	Total gain	145	130	119	122	80	66	90	79	154	135	79	69
	Total food	563	563	564	564	462	462	522	523	635	635	481	481
	Comparison of weekly gains	6·0	2·0	4·0	4·0	6·0	2·0	6·0	2·0	5·5	2·5	5·5	2·5
3·0	Initial weight	69	68	66	67	58	58	60	61	72	73	63	63
	Final weight	150	147	158	151	179	163	160	145	258	246	155	156
	Total gain	81	79	92	84	121	105	100	84	186	173	92	93
	Total food	537	537	553	553	593	593	506	506	762	762	540	540
	Comparison of weekly gains	4·0	4·0	4·5	3·5	6·0	2·0	6·0	2·0	5·5	2·5	4·0	4·0
3·5	Initial weight	78	79	72	69	71	70	79	79	78	78	61	62
	Final weight	141	152	185	172	159	158	245	224	240	221	134	144
	Total gain	63	73	113	103	88	88	166	145	162	143	73	82
	Total food	453	453	501	501	522	522	688	688	655	655	480	482
	Comparison of weekly gains	3·0	5·0	4·0	4·0	5·0	3·0	6·5	1·5	4·5	3·5	2·0	6·0

whole, the Osborne-Mendel diet is better balanced than the Sherman-Merrill one. Not only are its protein and salts about twice as high as those of the latter diet but it is also rich in both vitamins A and D. The Osborne-Mendel diet, on the other hand, is decidedly lower in the vitamin B complex than the Sherman-Merrill diet, consequently, in this particular case vitamin B can be ruled out as an agent in the detoxification of sulphur. Furthermore, it is very improbable that the fat in the Osborne-Mendel diet played any appreciable rôle in the detoxification of the sulphur in view of the fact that it could not be concluded from previous results (Kellermann, 1935) that lard had an inhibitory effect on the absorption of sulphur. If, as suggested by Lewis and Lewis, the fat formed a coating around the particles of sulphur and thus made more difficult the intimate contact of the sulphur with the intestinal mucosa or the action of bacteria, one would have expected a lower concentration of hydrogen sulphide in the digestive tract and consequently a lower absorption of sulphur.

There remains one more point of difference in the composition of the Osborne-Mendel and Sherman-Merrill diets that deserves to be pointed out, namely, their contents in easily available " food sulphur ". In view of the fact that the composition of the Osborne-Mendel salt mixture (1913) conforms closely to that of milk salts as such, it is evident that the sulphur content of the Osborne-Mendel basal diet must have been at least four times as high as that of the Sherman-Merrill one. Furthermore, it will be remembered that the inference was drawn in a previous publication (Kellermann, 1935) that the presence of easily available " food sulphur " in a ration might exert a depressing effect upon the toxicity of elementary sulphur, and it is possible that the greater content of " food sulphur " of the Osborne-Mendel diet accounted partly for the greater antitoxic effect of sulphur poisoning.

From a perusal of the results given in Tables II and III, it is evident that, on the basis of every 100 gm. of food consumed, the rats on the modified Osborne-Mendel diet gained over 50 per cent. more in weight than those on the same diet supplemented with 0·8 per cent. sulphur; whereas in no case were such great differences observed in the growth of pair-mates fed the modified stock ration with and without sulphur. As a matter of fact, in only a few instances did the control rats gain as much as 12 to 13 per cent. over their " sulphur " mates on the same amount of food, and in 28·8 per cent. of the total number of pairs, the sulphur-fed animals gained even more than their control mates. Furthermore, it should be borne in mind that the sulphur-containing rations had a lower energy value than the basal diet, and the rats in the sulphur groups were, therefore, put at a disadvantage to those in the basal groups in so far as growth was concerned.

In the 0·75 and 1·0 per cent. sulphur groups, in contrast to those that received 0·8 per cent. sulphur with the Osborne-Mendel diet, there was practically no difference in the average gains made by the control and " sulphur " groups. It is evident, therefore, that the stock ration has much more antitoxic effect to sulphur poisoning than the Osborne-Mendel diet. The latter diet, as modified in this experiment, meets, as far as is known, all the essential requirements

for growth except that it is low in protein and cystine contents. It would seem that its low cystine content could be ruled out as a cause of the high toxicity of sulphur, in view of the fact that Lewis and Lewis (1927) found that the toxicity of sulphur, when incorporated in the cystine deficient diet of Osborne and Mendel, was not affected by the addition of amounts of cystine adequate to produce good growth. However, this diet is also low in easily available " food sulphur " for the reason that the Osborne-Mendel salt mixture was replaced by the same amount of Steenbock and Nelson salts 40 (1923), modified slightly so as to contain no sulphur. Furthermore, it was found by the author (1935) that the absorption of sulphur in a ration containing 10 per cent. of casein and 2 per cent. of brewer's yeast, as the only sources of food sulphur, was rather low with a subsequently high absorption of elementary sulphur and it might be, therefore, that the high toxicity of sulphur when fed with the Osborne-Mendel diet as compared with its low deleterious effect in the stock ration, was partly due to the low protein and " food sulphur " contents of the former diet.

Summary.

1. Experiments are described in which were studied the effect of flowers of sulphur on the growth of young white rats maintained on two types of basal diets, one a modification of the low protein diet of Osborne and Mendel and the other a ration complete in all respects from a nutritional standpoint for this species of animal.

2. Sulphur, when added to the amount of 0·8 per cent. to the low protein diet which was also low in cystine and " food sulphur ", caused a marked retardation of the rate of growth but no deaths during an experimental period of 60 days.

3. The toxicity of sulphur when added to a well balanced ration in concentrations varying from 0·1 to 3·5 per cent. was, comparatively speaking, rather low. A comparison of the rate of growth of young rats by the paired-feeding method of Mitchell and Beadles showed that the number of weeks during which the control rats gained more in weight than their litter mates on the sulphur rations, is reasonable near 50 per cent., which indicates that the different treatments show no significant difference amongst themselves.

4. The solution of the problem as to why certain rations show greater antitoxic effect to sulphur poisoning than others should probably be looked for in the difference in their protein and " food sulphur " contents. The results to date seem to show that rations, complete in all respects for growth with optimum protein and easily available " food sulphur " contents, possess greater antitoxic effect to sulphur poisoning than rations that are low in these constituents.

5. Large amounts of flowers of sulphur can be given to animals (rats) without marked deleterious effects on their rate of growth when optimum amounts for growth of a well-balanced ration (stock) is given at the same time.

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