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Sulphur Metabolism. VIII.—The Effect of Incomplete Rations on the Toxicity of Elementary Sulphur.

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In a previous investigation the author (1938, 1) found that, although the incorporation of elementary sulphur in a stock ration slightly reduced food consumption and growth, it had no effect on food utilization. Furthermore, the feeding of sulphur had no appreciable influence on the cell structure of internal organs such as the liver and kidneys. This was true no matter whether the rations differed widely in their acid- and base potentialities (Kellermann, 1938, 3) and in their fat and protein contents (Kellermann, 1938, 2) and the findings do not support, therefore, the observations of Lewis and Lewis (1927) who found a greater resistance in rats to sulphur poisoning when fed the Osborne-Mendel low cystine diet as compared with animals fed the diet of Sherman and Merrill (1925) in which the concentrations of fats and proteins were lower than in the Osborne-Mendel diet. These rations are not only deficient in cystine, but also low in proteins. The latter value in the Osborne-Mendel ration is about 7·8 per cent. whereas that in the Sherman-Merrill diet only approximately 4·7 per cent. However, in addition to their low cystine and protein values these diets are also low in certain other constituents. For instance, the Osborne-Mendel diet is low in the vitamin B-complex whereas the Sherman-Merrill diet is low in mineral salts (except NaCl) and the vitamin B. However, these authors found that the addition of amounts of cystine adequate to produce good growth did not alter the toxicity of the sulphur. Nevertheless, even with the addition of cystine to the basal diets, the growth obtained was unsatisfactory and it is probable that the high toxicity of elementary sulphur, when incorporated in these diets, was due mainly to the lack of enough growth-promoting substances and not to that of any particular sulphur detoxifying agent. The experiments to be recorded in this paper were therefore carried out with the object of putting this supposition to the test.

Experimental.

Young white rats were used as experimental animals and they were kept under similar conditions as previously described (Kellermann, 1938, 2). The animals were weighed once weekly. They were given an excess of food and the daily consumption per rat recorded The composition of the rations is given in Table I.

Table I.

Percentage of Rations in Percentage by Weight.

	Normal diet.		Diet low in vitamins A and D.	Diet low in vitamin B–complex.	Diet low on vitamins.		Diet low in most minerals.		Diet low in proteins.		Diet low in proteins and low in most minerals.		Diet low in proteins, low on most minerals and low in vitamins.	
Designation of diet	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.	N.
"Vitaminized" starch	10	10	10	—	—	—	10	10	10	10	10	10	—	—
Dextrinized starch	39·4	34	34	44	49·4	44	45	39·6	53·5	48·1	59·1	53·7	69·1	63·7
Casein	20	20	20	20	20	20	20	20	5·6	5·6	5·6	5·6	5·6	5·6
Sucrose	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Salt 40	4·5	4·5	4·5	4·5	4·5	4·5	1·0	1	4·5	4·5	1	1	1	1
Lard	9·6	12	14	12	11·6	14	7·1	9·5	9·6	12	7·1	9·5	9·1	11·5
Agar	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Cod-liver oil	2	2	—	2	—	—	2	2	2	2	2	2	—	—
$NaCl$	—	—	—	—	—	—	·4	·4	—	—	·4	·4	·4	·4
l-cystine	—	—	—	—	—	—	—	—	·3	·3	·3	·3	·3	·3
Sulphur	—	3	3	3	—	3	—	3	—	3	—	3	—	3

All fourteen rations contained isodynamic quantities of energy* and the vitaminized starch " and salt 40 were prepared as described previously (Kellermann, 1938, 2). It was deemed advisable to make the rations low but not necessarily deficient in the various essential constituents because, although 3 per cent. of elementary sulphur had little effect on rats receiving normal diets, Lewis and Lewis obtained very detrimental results with rats on an inadequate diet (Sherman-Merrill) containing only 1 per cent. of flowers of sulphur. After the rats had been on the various diets for six weeks, they were killed by a blow on the head and certain internal organs immediately removed and preserved in a formalin solution for histopathological examination.

From the growth and food consumption records given in Table II it is evident that the incorporation of sulphur in rations, no matter whether they were physiologically complete or not, reduced food consumption and subsequent growth. In the case of the complete ration the addition of sulphur was of little effect inasmuch as the sulphur fed rats consumed on an average only 0·5 g. less food per day and gained 6 g. less over a period of six weeks with hardly any difference in the percentage gains. On a diet low in vitamins the animals still gained 13·3 per cent. but when sulphur was added to the same diet they lost 21·9 per cent. of their original weight. Similarly, when only the vitamin B-complex was low, the addition of sulphur resulted in a loss of 24·6 per cent. but, on the other hand, still gained 37·9 per cent. when the diet was lacking in vitamins A and D. This may be expected in view of the fact that the B-vitamins are water soluble, consequently their deposits in the body are sooner depleted, and their absence, therefore, sooner made to bear on the organism (Osborne and Mendel, 1919), than those of the fat soluble vitamins (Robertson, 1916). Likewise, on the average the rats gained on all the remaining incomplete rations but, with the exception of the low mineral diet, lost considerably when sulphur was added. The low mineral diet contained 1·98 per cent of ash (ashed at 450-500° C.), and it seems as if this concentration of ash was sufficient to support a fair amount of growth even though the diet contained 3 per cent. of elementary sulphur. However, when the difference in body gain of the animals on the control and sulphur low mineral rations is compared with the difference in gain and loss of the rats on the corresponding low protein and low vitamin rations, it is seen that the addition of sulphur to the low mineral diet resulted in a difference in gains of 66·7 per cent. whereas in the case of the low protein and low vitamin diets the differences were 67·5 and 35·2 per cent. respectively. This shows that not only proteins and vitamins, but also the essential minerals play an important rôle in counteracting the deleterious effects of elementary sulphur on growth. A multiplication of the defects, by limiting the proteins, vitamins and minerals, in one and the same diet, did not alter materially the final position in so far as change in weight was concerned.

In order to see whether the reduction in growth was due solely to a restriction of food consumption or whether it was accompanied

* The energy values of the diets were calculated as described previously (Kellermann, 1938, 2).

TABLE II.

Summary of Growth and Food Consumption of Rats on various incomplete Rations with and without the addition of 3 per cent. Elementary Sulphur.

Ration.	Supplemented with.	Number of animals.	Initial weight. g.	Gain + or loss − g.	Percentage gain + or loss −	Total food. g.	Food per rat per day. g.
Normal	—	6*	63	+ 77	+ 122·2	300	7·1
Normal	Sulphur	6	57	+ 71	+ 124·5	277	6·6
Low in vitamins A and D	Sulphur	6	66	+ 25	+ 37·9	220	5·2
Low in vitamin B-complex	Sulphur	6	65	− 16	− 24·6	137	3·3
Low in vitamins	—	6	60	+ 8	+ 13·3	177	4·2
Low in vitamins	Sulphur	6	73	− 16	− 21·9	155	3·7
Low in most minerals	—	6	68	+ 63	+ 92·6	333	7·9
Low in most minerals	Sulphur	6	77	+ 20	+ 25·9	191	4·5
Low in proteins	—	6	76	+ 35	+ 46·0	292	6·9
Low in proteins	Sulphur	6	79	− 17	− 21·5	176	4·2
Low in proteins and low in most minerals	—	6	77	+ 25	+ 32·5	284	6·8
Low in proteins and low in most minerals	Sulphur	6	75	− 25	− 33·3	161	3·8
Low in proteins, low in most minerals and low in vitamins	—	6	74	+ 7	+ 9·4	239	5·7
Low in proteins, low in most minerals and low in vitamins	Sulphur	6	73	− 24	− 32·9	151	3·6

* From the time that an animal dropped out of a group as the result of death (see graphs, Fig. I) the results represent the average of the remaining animals.

by a breakdown of tissues due to sulphur toxicosis, the average body weights and food consumption per 100 g. body weight were plotted against the days of the experiment. The results are depicted graphically in Fig. 1.

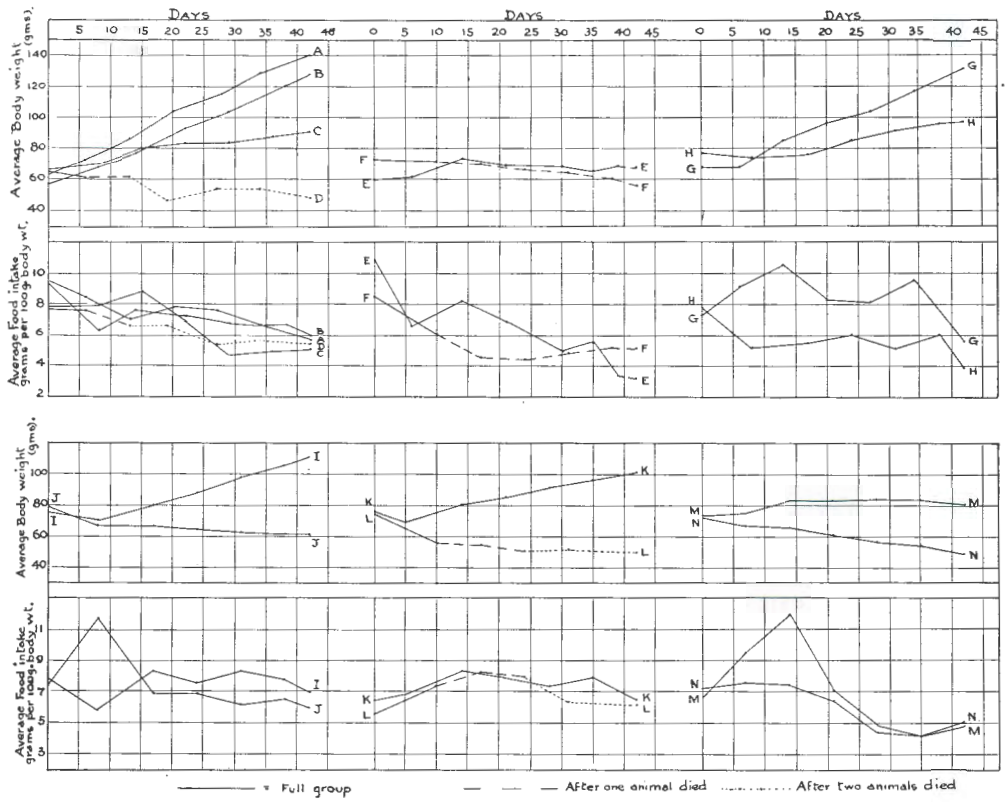

Fig I

Average body weight and food consumption of rats on a normal and various incomplete rations with, and without, the addition of 3% elementary sulphur.

The graph shows that the addition of elementary sulphur to the various diets invariably reduced the growth rate. In most of the incomplete rations the incorporation of sulphur actually resulted in a loss of weight. From the graphs giving the food consumption per 100 g. body weight it is evideut that, with the exception of the low mineral diet plus sulphur (graph H), the inhibition of growth was not due to a lowered food intake per unit of body weight. As might be expected there was a gradual drop in the food intake per 100 g. body weight as the animals grew older [Macallum (1919), and Levine and Smith (1927)]. The deleterious effects of sulphur on growth should then mainly be ascribed to the reduced food intake per rat per day as shown in the last columns of Table II. This observation substantiates the results of Franke (1934) and Franke and Potter (1934) who showed that the poor growth of animals on a toxic diet was mainly due to a restriction of food intake.

The histological examination of the livers showed that, on the whole, the degenerative changes varied not only in degree and distribution in the various groups, with and without the administration of sulphur, but also in animals fed the same diet. These changes consisted of cloudy swellings and vacuolation in groups of hepatic cells situated at the periphery of some lobules. Furthermore, in a number of cells, the protoplasm had a tendency to disintegrate and to droplet (hyalin) formation. In addition to the above changes observed in the livers of rats that were killed at the conclusion of the experiment, the livers of animals that died showed haemorrhagic necrotic areas with slight or more advanced interstitial hepatitis.

Discussion.

Whether the hepatic necrosis was the ultimate cause of death is difficult to say. However, inasmuch as no bacteria were seen in these lesions together with the fact that all the animals that succumbed, received elementary sulphur in their food, seem to show that the animals died from sulphur toxicosis. These results therefore substantiate the observation of Lewis and Lewis (1927) that the ingestion of appreciable amounts of elementary sulphur by rats can exert toxic and even fatal effects on the animals. However, this statement should be accepted with some reservation because, in the writer's experience, elementary sulphur will have definitely toxic effects only under certain conditions. One of these conditions is that the basal ration should be deficient in one or more respects so as to retard or stop growth. As already pointed out in Table II, one of the main effects of sulphur was to reduce food consumption and the subsequent gain in weight. As a matter of fact in some cases the addition of sulphur resulted in a loss of 30 per cent. or more of the animals' initial weight, and it would seem, therefore, that under the experimental conditions, the greater the inhibition of growth the more susceptible the animals become to sulphur toxicosis.

If this viewpoint is correct it will help to explain why the animals of Lewis and Lewis were more resistant to the toxic effects of sulphur on the Osborne-Mendel diet than on the Sherman-Merrill (1925) one in view of the fact that the former diet is the better balanced of the two being richer in proteins, minerals and vitamins A and D. However, Lewis and Lewis suggested that the high fat content of the Osborne and Mendel diet might be partly responsible for the lessened toxicity as "the fat might form a coating around the particles of sulphur and thus make more difficult the intimate contact of the sulphur with the intestinal mucosa or the action of bacteria." This explanation seems to be untenable as the writer did not find (unpublished data) any difference in the absorption of elementary sulphur on diets low and high in fat content. Furthermore, young rats fed these respective diets, containing 3 per cent. of elementary sulphur, over a period of eight weeks, did not reveal any difference in their general well-being (Kellermann, 1938, 2).

Another factor would seem to be the method of sulphur administration. Swart (1936) expressed the opinion that the feeding of sulphur mixed with the feeds might be less toxic than when it is dosed as he fed his animals 9·5 g. of flowers of sulphur per sheep

per day over a period of 10 months without any ill-effects, whereas Steyn (1931) observed that the dosing of an appreciably smaller amount of flowers of sulphur, namely 45 g. per sheep per week, over a period of thirty-five days caused symptoms of poisoning and high mortality. Therefore, it would be of interest to investigate this problem further, especially with the view of ascertaining whether rabbits and guinea-pigs are actually as susceptible to sulphur poisoning as found by Lawson, Redfield and Boyd (1934) whose animals all died within twenty-four hours after receiving doses of from 0·5 to 2·0 g. of either colloidal or flowers of sulphur per Kg. of body weight into the stomach through a small stomach tube.

Summary.

1. Experiments are described in which were studied the effects of incomplete rations on the toxicity of elementary sulphur.

2. The incorporation of sulphur in the diet exerted toxic effects only when the basal ration itself could not support normal growth as the result of one or more deficiencies. The chief effect of sulphur under such conditions was to reduce food consumption per animal but not per unit body weight.

3. It would seem that vitamins, proteins and minerals were all equally important in counteracting the deleterious effects of the sulphur.

4. Although the rats lost considerably in weight when sulphur was added to the defective diets, the livers of the animals that were killed at the conclusion of the experiment did not show any constant pathological change whereas the same organs of those animals that died during the experiment exhibited haemorrhagic necrotic areas with slight or more advanced interstitial hepatitis.

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