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Sulphur Metabolism. VI.—The Effect of Various Levels of Fats and Proteins in the Ration on the Toxicity of Elementary Sulphur.

By J. H. KELLERMANN, Section of Biochemistry, Onderstepoort.

LEWIS AND LEWIS (1927) have found that the incorporation of one per cent. elementary sulphur in the milk powder-starch diet of Sherman and Merrill (1925) and in the low protein diet of Osborne and Mendel was definitely toxic. With a few exceptions, the livers of the rats which died showed a marked peripheral zonal necrosis. However, these authors found that the rats on the Osborne-Mendel diet were more resistant to the toxic effects of sulphur than were those on the Sherman-Merrill ration. The former diet is richer in fats and proteins than the latter and the authors expressed the opinion that the lower toxicity of sulphur in the Osborne-Mendel diet might be due to its higher fat and protein contents. Their suggestion is that the fat probably forms a coating around the sulphur particles which either prevented them from coming into intimately close contact with the intestinal mucosa or the bacterial action on them is thereby reduced. Furthermore, the higher protein level might lead to a better state of nutrition and hence a greater resistance to the toxic action. This supposition, as far as the writer is aware, has never been put to the test, and it was the object of the following experiments to determine its validity.

EXPERIMENTAL.

Young rats of the Wistar strain were used in these experiments. They were equally divided amongst the various groups with respect to litter, sex and weight. Each animal was kept in a separate cage on a raised screen bottom. In addition to the distilled water, to which the animals always had free access, each rat was fed daily its particular ration *ad libitum* with recorded food consumption for the experimental period. Owing to the slightly acid nature of the yeast extract on starch, to be described later, the diets rapidly developed a rancid odour. In order, therefore, to prevent rancidity in these diets, the yeast extract was withheld from the bulk of the diet and intimately mixed with each day's supply as practised by Gallup and Reder (1935). The composition of the rations is given in Table I.

The basal diets contained all the dietary essentials known. The vitamin B complex was given in the form of an extract prepared from brewers' yeast according to the method of Itter, Orent and

TABLE I.

Composition of Rations in Percentage by Weight.

	Low Protein.	High Protein.	Low Fat.		High Fat.	
"Vitaminized starch"	10	10	10	10	10	10
Dextrinized starch	44·7	15	49	43·6	31·4	26
Casein	10	40	20	20	20	20
Sucrose	12	12	12	12	12	12
Salt 40	4·5	4·5	4·5	4·5	4·5	4·5
Agar	2·5	2·5	2·5	2·5	2·5	2·5
Cod-liver oil	1	1	2	2	2	2
Elementary sulphur	3	3	—	3	—	3
Lard	—	—	—	2·4	17·6	20
Butter fat	12	12	—	—	—	—
l-cystine	0·3	—	—	—	—	—

McCollum (1935). This extract was evaporated on dextrinized starch subsequently designated "vitaminized starch". One gram of this starch was equivalent to 0·5 g. of yeast. The composition of Salt 40 was similar to that of Steenbock and Nelson (1923) as modified by Keenan and others (1933). However, in order to obtain a sulphur-free salt mixture the magnesium and manganese sulphates were replaced by equivalent amounts of their carbonate forms and the $CuSO_4 \cdot 5H_2O$ by cupric acetate. In addition to lard the effects of butterfat, beef dripping (M.P. 43-49° C.) and olive oil on the toxicity of elementary sulphur were studied. The rations containing these fats were identical in every respect to the lard ration except that the latter was replaced by an equivalent amount of these various fats. Furthermore, all the high fat diets with and without the addition of elementary sulphur were made equicaloric by replacement of some of the starch by isodynamic quantities of fat.* Similarly, the low fat diets, with and without added sulphur, were made isocaloric and the same applied for the low and high protein rations. After the rats had been on experiment for eight weeks they were killed by a blow on the head and various organs immediately removed and preserved in a formalin solution for histopathological examination.

The results obtained on the low and high protein rations are presented in Tables II, III, and IV. Table II gives the original results, Table III the means and Table IV the analysis of variance. From Table IV it is clear that the growth-promoting value of the low protein ration was significantly greater than that of the high protein one; the probability P being ·01. This observation substantiates, therefore, the results of Osborne, Mendel and Ferry (1919), Mitchell (1923-24), St. John and others (1934), Chick and co-workers (1935) and Basu, Nath and Mukherpee (1937). However, the growth-promoting values for males and females were not significantly different and there was no interaction of sex with the protein content of the ration.

* The energy values of the diets were calculated on the basis of 4·0 Cal. per gram for the carbohydrates and casein, and of 9·0 Cal. per gram for the various fats and oils.

TABLE II.

The growth-promoting values of sulphur supplemented rations low and high in protein content as determined by the method of Osborne, Mendel and Ferry (1919).

PERIOD: 8 WEEKS.

Ration.	Sex.	Total Food consumed.	Protein consumed. P.	Weight increase. W.	Ratio W/P (or G–P value).
		g.	g.	g.	
Low Protein (8·5 per cent.)..	Male	363	30·85	69	2·24
	Male	265	22·52	27	1·20
	Male	396	33·66	76	2·26
	Female	371	31·54	72	2·28
	Female	322	27·37	41	1·50
	Female	292	24·82	34	1·37
High Protein (34 per cent.)....	Male	316	107·4	64	0·06
	Male	417	141·8	106	7·75
	Male	298	101·4	59	0·58
	Female	321	109·1	66	0·60
	Female	302	102·7	45	0·44
	Female	283	96·23	40	0·41

TABLE III.

Mean Growth-promoting Values.

	Males.	Females.	Total.
Low Protein	1·9	1·72	1·81
High Protein	0·64	0·48	0·56
TOTAL	1·27	1·1	1·19

TABLE IV.

Analysis of Variance.

	D.F.	S.S.	M.S.	S.D.	$\log_e$ S.D.	Z.
Low protein *vs*, high protein	1	4·6501	4·6501	2·156	0·7682	1·6895
Males *vs.* females	1	0·0884	0·0884	—	—	—
Interaction	1	0·0004	0·0004	—	—	—
Error	8	1·2578	0·1582	0·398	−0·9213	—
TOTAL	11	5·9967				

From Fisher's (1932) Table of "z":

	at $p = \cdot05$	at $p = \cdot01$
For $n_1 = 1$, $n_2 = 8$	$z = 0·8355$	1·2106

Hence this value of z is significant at $P = \cdot01$.

The results obtained on the low and high fat rations are given in Tables V and VI.

TABLE V.

Effect on Efficiency Quotient of adding 3 per cent. Elementary Sulphur to Synthetic Rations of Various Fat and Protein Content. Period 8 Weeks.

Ration.	Supplemented with.	Number of Rats.	Sex.	Mean Gain.	Mean total food.	Mean efficiency quotient.
Low fat	—	3	♂	143·5	670·5	3·2
Low fat	—	3	♀	98	673·5	5·41
Low fat	Sulphur	3	♂	102	554	4·65
Low fat	Sulphur	3	♀	46·8	383·6	6·29
High fat (lard)	—	6	♂	107·7	402·6	2·88
High fat (lard)	—	6	♀	85·5	395·7	3·88
High fat (lard)	Sulphur	3	♂	66	355	4·4
High fat (lard)	Sulphur	3	♀	55·7	306·3	5·46
High fat (butter)	Sulphur	3	♂	82	347	3·45
High fat (butter)	Sulphur	3	♀	66	324·3	4·62
High fat (dripping)	Sulphur	3	♂	83	352·3	3·63
High fat (dripping)	Sulphur	3	♀	64·7	331·2	4·91
High fat (olive oil)	Sulphur	3	♂	72·3	312·7	3·87
High fat (olive oil)	Sulphur	3	♀	59·3	313·5	5·25
All males	—	24	—	93·8	427·7	3·62
All females	—	24	—	68	389·7	4·96
All low fat diets	—	12	♂ & ♀	97·6	570·4	4·89
All high fat diets	—	36	♂ & ♀	74·2	344·1	4·10
Low fat diets	— Sulphur	6	♂ & ♀	120·7	672	4·30
Low fat diets	+ Sulphur	6	♂ & ♀	74·4	468·8	5·47
High fat diets	— Sulphur	12	♂ & ♀	96·6	399·1	3·38
High fat diets	+ Sulphur	24	♂ & ♀	68·6	330·3	4·45

Table V gives the mean values and Table VI the analysis of variance of the efficiency quotients. The data in the latter table show that the efficiency quotients, calculated according to the method of Palmer and Kennedy (1929)* of the males were significantly better than those of the females. The probability P was ·01 which substantiates the results of Palmer and Kennedy (1931). On the high fat rations efficiency quotients were obtained that were significantly superior (P = ·01) to those on the low fat rations. This result might be expected in view of the great difference in caloric value between the two types of diet. Within both low and high fat rations the addition of elementary sulphur significantly lowered the animals' efficiency to utilize their food; the probability P being ·01. The lower food consumption on the sulphur rations might have contributed to the inferior utilization of food inasmuch as Palmer and Kennedy (1931) found that a restriction of food intake lowered the index of food utilization. However, the efficiency quotients on the butterfat, lard, dripping and olive oil rations were not significantly different, and the same was true for the interaction of sex with the various fats.

* The dry matter only, not the digestible dry matter, was used in these calculations.

TABLE VI.

Analysis of Variance of Efficiency Quotients.

	D.F.	S.S.	M.S.	S.D.	$\log_e$ S.D.	Z.
Male *vs.* female.....	1	21·6008	21·6008	4·648	1·5364	2·0540
Low fat *vs.* high fat...	1	5·6486	5·6486	2·377	0·8658	1·3834
Within low fat : Sulphur *vs.* no S.	1	4·0950	4·0950	2·024	0·7051	1·227
Within high fat : S. *vs.* no S.	1	9·1022	9·1022	3·017	1·1041	1·627
Within S. butter *vs.* lard *vs.* dripping *vs.* olive oil	3	2·6819	0·8940	0·946	—·0555	0·4621
Between all fats.....	6	21·5277	3·5879	1·894	0·6387	1·1563
Interaction of sex with fats	6	1·8009	0·3002	0·548	—	—
Treatments.....	13	44·9294	3·4561	1·859	0·6201	1·1377
Error.....	34	12·0773	0·3552	0·596	—0·5176	—
TOTAL....	47	57·0067				

From Fisher's (1932) Table of z:

		at P = 0·5	at P = ·01
for n_1 = 1,	n_2 = 30,	z = 0·7141	1·0116
n_1 = 3,	n_2 = 30,	z = 0·5632	0·7531
n_1 = 6,	n_2 = 30,	z = 0·4420	0·6226
n_1 = 12,	n_2 = 30,	z = 0·3691	0·5224

A comparison of the z values from the above table, with these criterions indicate that all, except the " Butter *vs.* lard *vs.* dripping *vs.* olive oil " z, are significant at P = ·01.

On the whole, the histopathological changes noted in the livers of rats fed the various diets, with and without the addition of elementary sulphur, did not vary much from those seen in the livers of normal animals. The most common observations were fatty changes which varied from slight to fairly advanced and diffuse. The kidneys of the animals on the low and high protein rations were also examined. However, except for slight fatty changes, these organs did not show any constant pathological changes, and one is therefore led to the conclusion that elementary sulphur has no deleterious effects on the structure of livers and kidneys, which is most probably also true for all other organs of rats as long as the animals are fed a ration that contains all the essential constituents for growth.

SUMMARY.

1. The extent to which various levels of fats and proteins in rations could influence the effects of elementary sulphur on rats as judged from growth, food utilization and pathological lesions, was studied.

2. The growth-promoting value (gain per unit protein consumed) of the low protein ration was significantly greater than that of the high protein diet.

3. On the same ration the males utilized their food significantly better than the females.

4. Within both low and high fat rations the addition of elementary sulphur significantly lowered the animals' efficiency of food utilization.

5. Irrespective of the fat and protein contents of the diets the livers and kidneys of the sulphur fed rats, as compared with those of the control groups, did not exhibit any constant pathological changes.

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