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Sulphur Metabolism. VII.—The Effect of the Acid-Base Balance of the Ration on the Toxicity of Elementary Sulphur.

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ELEMENTARY sulphur, when absorbed by the animal organism, is eventually excreted in the urine primarily, if not entirely, as sulphates. [Denis and Reed (1927), and Kellermann (1935)]: Evidently then, through the oxidation of sulphur, a large amount of acid is formed in the system. For instance, results (unpublished) obtained at this Institute show that 50 per cent., and even more, of elementary sulphur, when incorporated in the rations at a three per cent. level, can be absorbed. On this basis the sulphur in every 100 g. of food can give rise to about 936 c.c. of 0·1 N. acid which, together with the acid- and base-forming elements in the rest of the food will naturally determine the ultimate acid-base balance of the diet.

From the results of various investigators [Goss and Schmidt (1929), Mitchell and Miller (1931), and Lamb and Evvard (1932] it appears that the growth and reproduction of rats and pigs are not materially affected by diets with strongly acid-forming potentialities. Furthermore, Steenbock, Nelson and Hart (1914) observed that calves can tolerate large amounts of acid (220-500 c.c. N.HCl) without any ill effects. Similar results were obtained with dairy cows.

On the other hand, Addis, Mackay and Mackay (1926-27) found that by adding 2 grams of $CaCl_2$ to every 98 grams of control diet "the acid diet rats at first grew almost but not quite as quickly as the controls but from about the 120th day of age they ceased to gain and thereafter tended slowly to lose in weight. The food graph reveals the paradoxical circumstance that not only is this not to be explained by the insufficient food supply but that on the contrary the inhibition of growth and failure of maintenance occurs in spite of a food intake which considerably exceeds that of the controls." Similarly, Comes (1934), (1936) observed that the administration of not only acid but also alkaline diets caused a retardation in the growth of rats. Takahashi (1936) found that the feeding of sulphur to rats, pigeons, rabbits, guinea-pigs and frogs, for 2 to 3 months, produced severe acidosis and typical calcium metastases.

If, then, a diet is already potentially acid, which is likely and no doubt often the case in the nutrition of pigs, dogs and human beings, the incorporation of elementary sulphur may so enhance the potential acidity of the ration that it may prove detrimental to the growth and well-being of the individual. Therefore, in order to test this assumption experiments were conducted in which the toxicity of elementary sulphur in relation to the potential acidity and alkalinity of the ration was studied.

EXPERIMENTAL.

Young white rats were used in these experiments. The experimental procedure was the same as previously described (Kellermann, 1938, 2) with the only difference that the food was given in a moist instead of a dry form. In some groups, as shown in Table III, the food was moistened with fresh orange juice and in others with distilled water. Orange juice was selected on account of its alkalinizing properties as shown by Saywell and Lane (1933), Schuck (1934) and Clouse (1935). Furthermore, it was considered of interest to ascertain whether plant juices, with orange juice as representative, possess any specific properties in modifying the effects of sulphur as judged by the growth and other performances of the animals. In each case 2 c.c. of fluid was added to every gram of food and mixed thoroughly. Enough orange juice to last for a few days was squeezed out, strained and kept in a refrigerator. The dry matter of each batch so prepared was determined immediately and a record kept of the interval each batch was fed. The total amount of orange juice (dry) consumed added to that of the corresponding ration eaten constituted the total food intake for the experimental period.

Inasmuch as the results in the literature are not incontrovertible with respect to the effects of acid- and base-forming diets on growth and structure, it was necessary to feed such diets with and without the addition of sulphur in order to be able to distinguish between the effects of sulphur and those of the basal diets as such. The composition of the basal rations is given in Table I.

TABLE I.

Composition of Rations in Percentage by Weight.

	Acid-forming Diet.		Base-forming Diet.	
"Vitaminized starch"	10	10	10	10
Dextrinized starch	39·5	34·1	33·2	27·8
Casein	20	20	20	20
Sucrose	12	12	12	12
Butter fat	12	14·4	14·8	17·2
Agar	2	2	2	2
Cod-liver oil	1	1	1	1
Elementary sulphur	—	3	—	3
Acid salts	3·5	3·5	—	—
Basic salts	—	—	5	5
Sodium bicarbonate	—	—	2	2

The "vitaminized starch" was prepared as described previously (Kellermann, 1938, 2) and the composition of the acid and basic salt mixtures is given in Table II. The acid and base equivalents of the basal acid- and base-forming diets were 316·6 c.c. N/10 acid and 235·5 c.c. N/10 alkali, respectively, as determined by the method of Davidson and LeClerc (1935).

TABLE II.

*Composition of Acid and Basic Salt Mixtures.**

	Acid Salt Mixture.	Basic Salt Mixture.
$CaCO_3$	—	1·627
Na_3PO_4	—	2·781
$CaHPO_4.2H_2O$	2·8	—
NaCl	0·3	0·3
KCl	0·2	0·2
$MgCl_2.6H_2O$	0·16	—
Iron citrate	0·08	0·08
$MgCO_3$	—	0·0662
$MnCl_2$	0·0058	0·0058
KI	0·0023	0·0023
$ZnCl_2$	0·0007	0·0007
$CuCl_2.2H_2O$	0·0005	0·0005
TOTALS	3·5493	5·0635

* Roughly 3·5 g. of the acid salt mixture is equivalent to 5·0 g. of the basic mixture in so far as the metals, except sodium, are concerned.

TABLE III.

Comparison of Efficiency Quotients of Rats fed Acid- and Base-Forming Diets with and without the addition of 3 per cent. Elementary Sulphur and Orange Juice. Period, 8 Weeks.

Ration.	Supplemented with.		Number of Rats.	Sex.	Mean Gain.	Mean total Food.	Mean Efficiency Quotient.
					g.	g.	
Acid	—	—	3	♂	89·3	464·3	4·78
Acid	—	—	3	♀	61·3	401·3	7·26
Acid	—	Orange juice	3	♂	97·3	453·0	4·31
Acid	—	Orange juice	3	♀	72·3	447·0	6·39
Acid	Sulphur	—	3	♂	79·7	392·3	4·85
Acid	Sulphur	—	3	♀	45·7	324·3	9·14
Acid	Sulphur	Orange juice	3	♂	82·7	412·7	4·90
Acid	Sulphur	Orange juice	3	♀	64·3	432·9	7·33
Alkaline	—	—	3	♂	118·3	494·3	3·35
Alkaline	—	—	3	♀	68·7	416·0	6·06
Alkaline	—	Orange juice	3	♂	129·3	557·0	3·43
Alkaline	—	Orange juice	3	♀	80·3	446·8	5·57
Alkaline	Sulphur	—	3	♂	91·3	427·0	4·26
Alkaline	Sulphur	—	3	♀	60·7	383·2	6·63
Alkaline	Sulphur	Orange juice	3	♂	119·3	540·0	3·69
Alkaline	Sulphur	Orange juice	3	♀	70·7	421·5	6·26
All acid diets			24	♂ & ♀	74·1	416·0	6·12
All alkaline diets			24	♂ & ♀	88·6	460·7	4·91
All sulphur diets			24	♂ & ♀	76·8	416·7	5·88
All no-sulphur diets			24	♂ & ♀	89·6	459·9	5·15
All no-orange juice diets			24	♂ & ♀	76·9	412·8	5·79
All orange juice diets			24	♂ & ♀	89·5	463·9	5·24
All females			24	♀	65·5	409·1	6·83
All males			24	♂	100·9	467·6	4·20

All four diets were made equicaloric by replacement of some of the starch by isodynamic quantities of fat.* After the rats had been on experiment for 8 weeks they were killed by a blow on the head and various organs immediately removed and preserved in a formalin solution.

TABLE IV.

Analysis of Variance of Efficiency Quotients.

		D.F.	S.S.	M.S.	S.D.	Log_e S.D.	Z.
	Acid or Alkaline..........	1	—‡	17·6540	4·202	1·435	1·282††
	Sulphur *vs.* no Sulphur.....	1	—	6·5940	2·559	0·940	0·787†
	Orange juice *vs.* no orange juice	1	—	3·7130	1·927	0·656	0·503
	Male *vs.* female...........	1	—	83·2924	9·126	2·212	2·059††
	First Order Interactions.....	—	—	—	—	—	—
	Acidity *vs.* sulphur.........	1	—	0·2067	—	—	—
	Acidity *vs.* orange juice.....	1	—	0·5830	—	—	—
	Acidity *vs.* sex............	1	—	0·4200	—	—	—
Treats 15 D.F.	Sulphur *vs.* orange juice.....	1	—	0·1622	—	—	—
	Sulphur *vs.* sex............	1	—	0·9605	—	—	—
	Orange juice *vs.* sex........	1	—	1·3101	—	—	—
	Second Order Interactions—						
	Acid *vs.* Sulphur *vs.* orange juice	1	Up to this point the S.S. total = 115. Since all Treats S.S. = 117·3, only 2·3 is left for the remaining 5 D.F. Even if all this is allotted to a particular D.F. it would be insignificant. The remaining S.S. need therefore not to be calculated and apportioned.				
	Acid *vs.* Sulphur *vs.* sex.....	1					
	Acid *vs.* orange juice *vs.* sex..	1					
	Sulphur *vs.* orange juice *vs.* sex	1					
	Third Order Interactions—						
	Acid *vs.* Sulphur *vs.* orange juice *vs.* sex	1					
	Error	32	42·1010	1·3581	1·165	0·153	
	Total	47	159·4498				

From Fisher's (1932) Table of Z :—

	At P = ·05	AT P = ·01.
For $n_1 = 1$, $n_2 = 30$.	Z = 0·7141	1·0116

And hence the significance of the Z's is as above.

† Denotes significance at P = ·05. †† Denotes significance at P = ·01.

‡ These S.S. values are the same as the M.S. values in the next column since D.F. in each is unity. They have only been omitted to avoid duplication.

* The energy values of the diets were calculated as described in the previous article.

The results obtained on the acid and alkaline rations are given in Tables III and IV. Table III gives the mean values and Table IV the analysis of variance of the efficiency quotients. The data in the last table show that the efficiency quotient, calculated according to the method of Palmer and Kennedy (1929),* of the males was significantly superior to that of the females; the probability P being ·01. Similarly, the efficiency index on the base-forming rations was significantly better than that on the acid-forming ones at probability P = ·01. Consequently this observation substantiates the results of various authors already quoted in the introduction. Furthermore, the efficiency quotient on the basal rations was significantly better than that on the diets supplemented with elementary sulphur; the probability P being ·05. 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.

Likewise, even greater effects on the efficiency index were produced by sulphur when added to synthetic rations of various fat content (Kellermann, 1938, 2). However, the addition of sulphur to a stock ration had no such influence on food utilization no matter whether equalised food consumption (Kellermann, 1936) or *ad libitum*, recorded food consumption (Kellermann, 1938, 1) was practised and it seems, therefore, that the stock ration was more effective in counteracting the detrimental effects of sulphur on food utilization and subsequent growth than the synthetic diets used under the experimental conditions.

There was no significant difference between the diets with and without orange juice. The same held for all interactions and by stating, for example, that there was no significant interaction of the acid-base balance with sulphur, is meant that the difference between acid- and base-forming rations is relatively the same in the presence or absence of sulphur.

Histopathological examination of the livers showed no unusual changes no matter whether the acid- and base-forming diets were supplemented with elementary sulphur and orange juice or not. The fatty changes noted varied somewhat in degree and distribution not only in the various diet groups, but also in individual rats kept on the same diet. However, these changes did not differ much from those encountered in the livers of rats fed a normal ration, and it can, therefore, be concluded that, under the experimental conditions, the feeding of acid- and base-forming diets, with and without the addition of elementary sulphur, had no ill-effects on the cell structure of the liver.

Summary.

1. Data are presented on the relation of the acid-base balance of the ration to the effects of elementary sulphur on food utilization and the well-being of rats.

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

2. The efficiency quotient on the base-forming rations was significantly better than on the acid-forming ones, and the addition of elementary sulphur to these rations significantly lowered the efficiency index on both base- and acid-forming diets.

3. The efficiency quotient of the males was superior to that of the females.

4. No interaction between diets or between sex and diets appeared to be significant.

5. No pathological changes could be found in the livers of rats fed the various acid- and base-forming rations no matter whether they were supplemented with elementary sulphur or not.

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