Dietary Interrelationships of Ethionine and Methionine in the Weanling Rat

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Excessive dietary concentrations of methionine are toxic to growth in the rat and produce haemosiderosis, particularly in the spleen (Earle, Small & Victor, 1942; Kade & Sheperd, 1948; Brown & Allison, 1948; Van Pilsum & Berg, 1950; Stekol & Szaran, 1962). Cohen, Choitz & Berg (1958) suggested that the inhibition of growth is primarily an effect of the homocysteine moiety of the methionine molecule and that the deposition of haemosiderin is associated with the methyl group. Therefore an agent which interfered with the catabolism of methionine might reduce both the retardation of growth and the deposition of haemosiderin in animals given an excess of methionine.

Evidence is available that ethionine fed at a dietary concentration of 0.5% will decrease transmethylation from methionine to choline by as much as 20% (Simmonds, Keller, Chandler & Du Vigneaud, 1950). Ethionine sulphur may be available to the rat for cysteine synthesis, implying that a de-ethylation occurs *in vivo*, and that the ethyl groups are available for the synthesis of ethyl analogues that enter into 'transmethylation' reactions (Stekol & Weiss, 1950). Ethionine has been reported to be 'activated' to *S*-adenosylethionine by the same fraction of rat liver as is methionine (Glenn, 1961). Mody, Perry & Stekol (1961) noted the incorporation of both ^{14}C and ^3H from the ethyl group of ethionine into liver DNA and RNA, choline, creatine and proteins.

Since physiological concentrations of methionine alleviate or prevent the toxic effects of ethionine (Dyer, 1938; Stekol & Weiss, 1949; Simpson, Farber & Tarver, 1950; Rice, Boulanger, Plummer & Annai, 1953; Farber, Simpson & Tarver, 1950; Jensen, Chaikoff & Tarver, 1951; Stein, Kent, Barka & Popper, 1960), it was decided to determine whether or not ethionine could interfere with methionine metabolism to an extent sufficient to prevent the depression of growth or the deposition of haemosiderin produced by high dietary concentrations of methionine. It was also decided to determine the effects that non-physiological concentrations of methionine might have on the toxicity resulting from ethionine.

EXPERIMENTAL

The basal diet contained 12% of casein and was similar to that employed by Cohen & Berg (1956) except for the substitution of Alphacel (Nutritional Biochemical Corp.) for Cellu flour and the omission of the supplement of 0.6% of methionine from the basal diet.

In all experiments young male albino rats were employed. Two strains of animals, 'local' and 'Holtzman', were used. The starting weights of the animals varied from 40 to 65 g. All animals were individually housed and unlimited food and water were supplied. Weight change and food consumption were measured every 4 days. Differences in weight gained between various experimental groups have been analysed statistically. Groups are stated to differ only when *P* is less than 0.01.

At the end of the 28-day experimental period, the animals were killed and dissected. The blood was collected and the liver, spleen and kidneys were weighed. Histological sections of the spleens were stained for haemosiderin by Turnbull's Blue method (Mallory, 1957).

RESULTS

The growth response of animals on various combinations of methionine and ethionine are shown in Table 1. Animals which received the diet containing 0.6% of methionine grew somewhat more rapidly than those which received no methionine supplement (Table 1, groups 1*a* and 1*b*, *P* = 0.025). Higher dietary concentrations of methionine (1.8 and 3.6%) depressed growth under the experimental conditions described (*P* = 0.0025 in all comparisons against the diet supplemented with 0.6% of methionine). These findings are in complete harmony with those reported by other investigators (Earle *et al.* 1942; Kade & Sheperd, 1948; Brown & Allison, 1948; Van Pilsum & Berg, 1950).

The addition of ethionine to the basal diet in concentrations as small as 0.02% was sufficient to depress the rate of growth (groups 1*a* and 3*a*, *P* = 0.0025). Higher concentrations of ethionine were even more detrimental. Ethionine, when given at a dietary concentration of 0.01%, appeared to augment the growth of all 'local' animals that were receiving methionine supplements. However, when 'Holtzman' rats were employed and the size

of the experimental group was increased, these suggested differences disappeared. Half of the animals given a supplement of 0.64 % of ethionine died before the end of the experiment.

The protective effect of methionine against ethionine poisoning with respect to growth is apparent from an examination of Table 1. Supplementation of the basal diet with 0.6 % of methionine made it difficult to detect the presence of ethionine until the concentration of the latter reached 0.16 %. Even with 0.64 % of ethionine, moderate growth was obtained and none of the experimental animals died.

In animals whose diets were supplemented with 1.8 % of methionine, ethionine either did not affect the growth appreciably or produced further retardation (Table 1, rows *c* and *d*). Ethionine did not appear to protect the animals against methionine poisoning at any of the dietary concentrations employed. Thus the protective effect of methionine against ethionine poisoning does not have its

counterpart in the protection by ethionine from methionine poisoning.

Animals whose diets were supplemented with a concentration of ethionine permitting virtually no growth grew moderately well in the presence of methionine supplements which themselves produced retardation of growth (Table 1, groups 8*a* and 8*c*).

The degree of haemosiderosis that developed in animals given various diets is presented in Table 2. In addition to the marked inhibition of growth 1.8 % of methionine also induced the deposition of relatively large amounts of haemosiderin in the spleen. The haemosiderosis was neither increased nor decreased in such animals in the presence of even large dietary concentrations (0.64 %) of ethionine.

In general, the efficiency with which the animals utilized a given diet was proportional to the rate of growth on that diet. In all cases the 'Holtzman' animals exhibited a higher efficiency of food utilization than did the corresponding 'local' animals.

Table 1. *Growth response of rats receiving various dietary concentrations of methionine and ethionine, singly and in combination*

'Holtzman' rats were animals obtained directly from the Holtzman Rat Co. 'Local' rats were animals obtained from a colony maintained at the University of Texas Medical Branch, Galveston, Texas. The values given (with numbers of animals in each experimental group in parentheses) represent the average percentage change in weight in 28 days.

Group	Methionine added (%)	Ethionine added (%)							
		0.0	0.01	0.02	0.04	0.08	0.16	0.32	0.64
		Group							
		1	2	3	4	5	6	7	8
'Holtzman' rats									
(a)	0.0	297 (9)	262 (6)	246 (6)	273 (6)	225 (6)	178 (6)	135 (5)	-13 (6)
(b)	0.6	387 (11)	401 (8)	—	—	—	286 (6)	229 (4)	194 (6)
(c)	1.8	231 (12)	216 (8)	—	—	—	154 (6)	147 (6)	97 (6)
(d)	3.6	39 (7)	32 (8)	—	—	—	—	—	—
'Local' rats									
(e)	0.6	204 (16)	294 (4)	180 (4)	185 (4)	206 (3)	180 (8)	140 (4)	—
(f)	1.8	124 (14)	130 (3)	110 (3)	98 (3)	94 (2)	99 (7)	96 (4)	—
(g)	3.6	17 (4)	30 (4)	—	—	—	—	—	—

Table 2. *Deposition of haemosiderin in rats receiving various dietary concentrations of methionine and ethionine, singly and in combination*

'Holtzman' and 'local' rats were as described in Table 1. The values given represent the severity of haemosiderin deposition, which is indicated as: 0, negative; 1+, slight; 2+, moderate; 3+, severe.

Methionine added (%)	Ethionine added (%)							
	0.00	0.01	0.02	0.04	0.08	0.16	0.32	0.64
'Holtzman' rats								
0	0	0	0	0	0	0	0	0
0.6	0	—	—	—	—	0	0	0
1.8	3+	—	—	—	—	3+	3+	3+
'Local' rats								
0.6	0	0	0	0	0	0	0	—
1.8	2+—3+	3+	1+—2+	2+—3+	2+—3+	2+—3+	2+—3+	—
3.6	2+	3+	—	—	—	—	—	—

Animals given the diet containing 0.64 % of ethionine did not grow, i.e. they exhibited an efficiency of zero. Peak efficiency was achieved in those animals whose diet was supplemented with 0.6 % of methionine. The presence of traces of ethionine was not detectable under these circumstances. Regardless of the diet given, there were no significant deviations from the normal in the ratios of organ weight to total body weight.

Determination of the blood concentrations of prothrombin and of accelerator globulin revealed a decrease of the former by as much as one-half in the presence of higher dietary concentrations (0.16 % or more) of ethionine. Methionine, at a concentration of 0.6 %, completely alleviated this toxic manifestation of ethionine. However, a higher concentration of methionine (1.8 %) was almost without effect.

DISCUSSION

The effect of supplementary methionine on the growth of rats given a 12 % casein diet varied, depending on the concentration given, from beneficial to harmful. These findings are in harmony with those of Kade & Sheperd (1948), Van Pilsum & Berg (1950), Cohen *et al.* (1958) and Hardin & Hove (1951). Stekol & Szaran (1962) have reported that the depression of growth and the pathological organ changes effected by high concentrations of methionine are reversed with time even in the continuing presence of an excess of methionine.

The reversal of ethionine toxicity by methionine, originally observed by Dyer (1938) and subsequently reported by others (Stekol & Weiss, 1949; Simpson *et al.* 1950), was easily demonstrated. The protective relationship of methionine has been extended to include a dietary concentration of methionine that is itself markedly inhibitory to growth. At this toxic concentration (1.8 %), methionine permitted nominal growth on a diet containing 0.64 % of ethionine, which without added methionine caused animals to lose weight rapidly and die.

Stein *et al.* (1960) have reported that ethionine appears to behave as an antimetabolite of methionine and possesses little inherent toxicity. They observed that toxic manifestations of ethionine to hepatic cells and to ductular cells in the livers of experimental rats could be prevented completely by elevating the methionine content of the diet.

If the relationship existing between methionine and ethionine were purely one of a metabolite and its antimetabolite, one could reasonably expect ethionine to antagonize the toxic manifestations of methionine. It might be predicted that both the haemosiderosis (due to methyl groups) and the retardation of growth (due to homocysteine) seen in animals given diets with a high methionine content

(Cohen *et al.* 1958) could be alleviated by the addition to the diet of appropriate amounts of ethionine. Stekol & Weiss (1950) have indeed reported that ethionine interferes not only with the transmethylation from methionine but also with the conversion of methionine sulphur into cysteine sulphur.

We found no experimental conditions under which ethionine provided a statistically significant protection to animals whose growth was inhibited by excessive amounts of dietary methionine. Neither was there any prevention of methionine-induced haemosiderosis by ethionine. Our findings indicate that, in the presence of excessive amounts of dietary methionine, ethionine apparently does not interfere with the rate of formation of homocysteine and methyl groups to a degree sufficient to prevent either the inhibition of growth or the haemosiderosis. These findings may reflect a greater affinity of the enzyme systems involved for methionine than for ethionine. This suggestion is supported by the observation of Glenn (1961) that little or no activation of ethionine is accomplished by an enzyme system from rat liver capable of activating either methionine or ethionine if saturation concentrations of L-methionine are present.

Ethionine apparently provides no catabolic products which will produce haemosiderosis. In the absence of methionine, haemosiderin granules were not observed. Ethionine did not induce the deposition of a haemosiderin, even when given at the highly toxic dietary concentration of 0.64 %, nor did it prevent the development of haemosiderosis in the spleens of animals given an excess of dietary methionine.

The hepatotoxic effects of ethionine responsible for the depressed formation of prothrombin were readily prevented by 0.6 % of methionine. These observations with rats are in agreement with the findings of Rice *et al.* (1953) in guinea pigs. However, toxic dietary concentrations (1.8 %) of methionine did not prevent the decrease in the blood concentration of prothrombin that occurred in the presence of ethionine. DeGowin, Sheets, Hamilton & Carter (1955) reported that, in man, an excess of dietary methionine results in the depression of the blood concentrations of prothrombin and accelerator globulin to haemorrhagic levels. These observations with methionine and ethionine are probably manifestations of alterations in the overall economy of the liver rather than the result of any direct action of either of these amino acids on prothrombin synthesis.

SUMMARY

1. The observations of others with regard to (a) the improved growth response obtained by supplementation of a 12 % casein diet with 0.6 % of

methionine, (b) the toxicity of ethionine and the inhibition of growth by an excess of dietary methionine, and (c) the reversal of ethionine poisoning by methionine, are confirmed.

2. These findings have been extended to show that (a) even toxic concentrations of dietary methionine reverse ethionine toxicity, (b) ethionine is without effect on the depression of growth or on the deposition of haemosiderin induced by high dietary concentrations of methionine, and (c) prothrombin deficiency induced by dietary ethionine is readily reversed by optimum dietary concentrations of methionine but not by methionine given in excess.

3. It is concluded that the relationship between methionine and ethionine is not simply one of an essential metabolite to its antimetabolite.

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Carbohydases of the Rumen Ciliate *Epidinium ecaudatum* (Crawley)

2. α -GALACTOSIDASE AND ISOMALTASE*

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The presence in many pasture plants of α -linked galactose in galactosylgalactosyl glycerides (Weenink, 1961) and oligosaccharides such as raffinose suggest that the rumens of grazing sheep and cattle should be a good source of micro-organisms producing α -galactosidases. Because of the apparent rarity of bacterial α -galactosidases an investigation of the presence of these enzymes in rumen bacteria has been undertaken and the α -galactosidase produced by a rumen strain of *Streptococcus bovis* has

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already been described (Bailey, 1963). In studies on the carbohydase activity of members of the other class of rumen organisms, namely, the protozoa, α -galactosidase has been shown to be absent from the holotrichs (Howard, 1959) and virtually absent from the oligotrich *Entodinium caudatum* (Abou-Akkada & Howard, 1960). However, Bailey, Clarke & Wright (1962) reported that extracts of the rumen oligotrich *Epidinium ecaudatum* (Crawley) were able to hydrolyse melibiose in addition to other carbohydrates. An investigation of the α -galactosidase present in these extracts has, therefore, been undertaken in order to compare it with