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By

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In relation to the essentiality of methionine in supporting the growth of animals it seems to be a problem to ascertain whether S-alkyl derivatives other than methyl could serve in this capacity. On the ethyl derivative, ethionine, in 1938 Dyer (1) indicated its toxity to the rat. By feeding experiments using S^{35} ethionine Stekol and Weiss (2) (3) suggested that ethionine sulfur was available to the rat for cystein synthesis, implying deethylation of ethionine in vivo, and that the ethyl group of ethionine was avaliable to the rat for the synthesis of ethyl analouges of substances which were known to participate in the so-called transmethylation reactions. Moreover, they showed that triethyl choline inhibited the growth of rats. Simpson (4) demonstrated that ethionine inhibited the incorporation of methionine into the proteins of intact rats. Farber and Popper (5), Oda and Naito (6) reported pancreatic damage due to ethionine. There are a few studies on the biological effect of methionine homologues other than ethionine. Oda and Naito observed pancreatic damage due to propionine and isoamilonine which was milder than that due to ethionine. In an attempt to obtain additional information concerning the relation between biological effects of methionine homologues and their alkyl carbon numbers, a study was undertaken, in which ethionine, propoionine and buthionine were prepared and administered to rats.

Experimental

Synthesis of ethionine, propionine and buthionine ..... These S containing amino acids were synthesized from ethyl sodium phtalimido malonate and β-chloro-ethyl alkyl sulfide in a manner analogous to that employed in the preparation of methionine (7). The preparations were recrystallized from 50 per cent alcohol and their melting points are shown in table 1.

Feeding experiment .....

To study the biological effects of ethionine, propionine and buthionine, the growth method and the repletion method were used.

Table 1. Melting Points of synthesized methionine homologues

Substance	Formula	mP (by authors)	mP (in literatures)
DL-Ethionine	$C_2H_5 \cdot S(CH_2)_2 \cdot CH \cdot COOH$ NH_2	273°–274°C(d)	272°C–274°C (1) 257°C–260°C (3)
DL-Propionine	$C_3H_7 \cdot S(CH_2)_2 \cdot CH \cdot COCH$ NH_2	252°C(d)	249°C (9) 250°C–252°C (8)
DL-Buthionine	$C_4H_9S(CH_2)_2 \cdot CH \cdot COOH$ NH_2	253°–254°O(d)	254°C (9) 244°C–246°C (8)

a) Growth method : Grouping of rats and the composition of the basal diet are shown in table 2. Table 3 shows the daily average gain and food consumption.

Table 2. Grouping of animals

Group	No. of male rats	Diet
Control	3	*Basal (20% casein)
Methionine	5	Basal+0.23% Methionine
Ethionine	5	Basal+0.25% Ethionine
Propionine	5	Basal+0.27% Propionine
Buthionine	5	Basal+0.29% Buthionine

*Each kilo gram of basal diet contained casein 200 gm., sucrose 150 gm., starch 460 gm., salts 40 gm., lard 150 gm., thiamine 1.25 mg., riboflavin 2.5 mg., pyridoxine 1 mg., and calcium pantothenate 10 mg. Amount of supplements is molecular equivalent each other.

In the ethionine group three of the five animals died within four to nine days after the experiments began and the survivals showed a marked loss of appetite and very slight gain. This is in accord with the result of Stekol and Weiss (10) who observed slow growth in rats fed 25 per cent casein diet supplemented with 0.275 per cent ethionine. In propionine or buthionine group the animals showed normal appetite and such toxity as shown in the ethionine group was not observed in our experimental condition, but it was demonstrated that their growth were inhibited appreciably when compared with the control or methionine group.

b) Repletion method : Recently the so-called rat repletion method has been employed by some investigators (11) to evaluate the nutritive value of protein or related substances. In our experiment, the rats weighing 170 to 220 gm were fed non-protein diet for 11 days and when the animals lost an average of 20 to 25% of body weight, they were fed protein diet supplemented with methionine and its homologues and subsequent recovery of body weight

Table 3. Result of growth experiment

Group	Daily gain*	Daily food eaten
Control	3.8±0.26 gm.	17.8gm.
Methionine	4.2±0.26	14.0
Ethionine	0.8±0.02	7.5
Propionine	3.0±0.19	13.4
Buthionine	2.7±0.24	13.6

*Mean ± standard error of the mean experimental period was 18 days

was compared. The compositions of non-protein and protein diet were as follows :

Non-protein diet : sucrose 83, agar 1, lard 5, sesame oil 5, salts 4, $CaHPO_4 2H_2O$ 1, vitamin mixture 1. The latter consisted of pure vitamins mixed with sucrose to supply the following levels per 100 gms of diet : thiamine and pyridoxine, 0.6 mg each ; riboflavin 1.2 mg ; niacin 3.7 mg ; calcium pantothenate 5 mg ; and choline chloride 100 mg.

Protein diet : non-protein diet 94 and casein 6

Table 4. Result of repletion method

Group	Supplements added to basal diet*	Gain for 10 days♯	Daily food eaten
Control		13.5±1.5 gm	14.2 gm
Methionine	0.25% Methionine	24.7±1.7	14.3
Ethionine	0.27% Ethionine	−11.3±0 7	8.3
Propionine	0.30% Propionine	11.3±0.9	14.4
Buthionine	0.32% Buthionine	9.3±1.7	13.1

*6% casein diet as shown in text

♯ Mean±standard error of the mean

Amount of supplements is molecular equivalent each other.

Table 4 shows the gain of each group for 10 days and the daily average food comsumption. As shown with the minus figure, the animals of the ethionine group lost appetite and continued to lose body weight in the repletion period. It is clear that the gains of propionine and buthionine groups are smaller than in the methionine group. The differences between the control and propionine or buthionine groups are not significant in this experiment. Figs. 1 and 2 illustrate the results by growth and repletion methods, respectively.

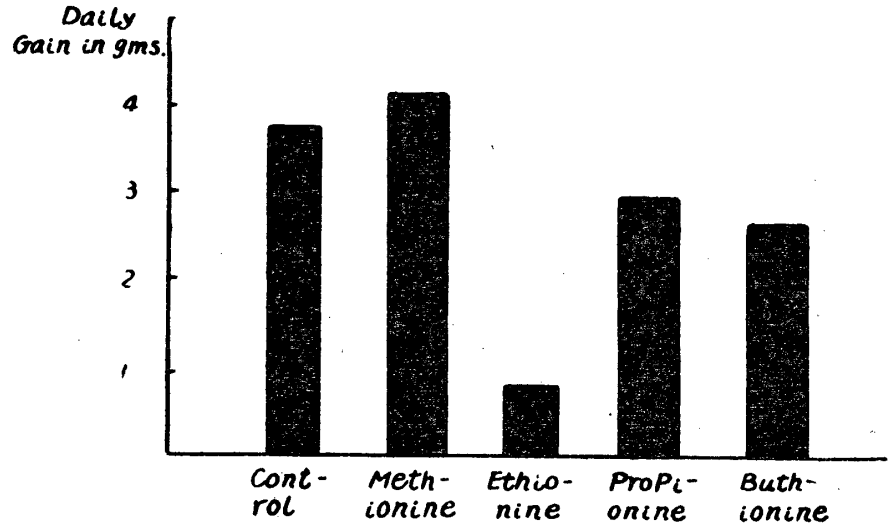

Fig. 1. Influence of methionine homologues upon growth of rats fed 20 percent casein diet.

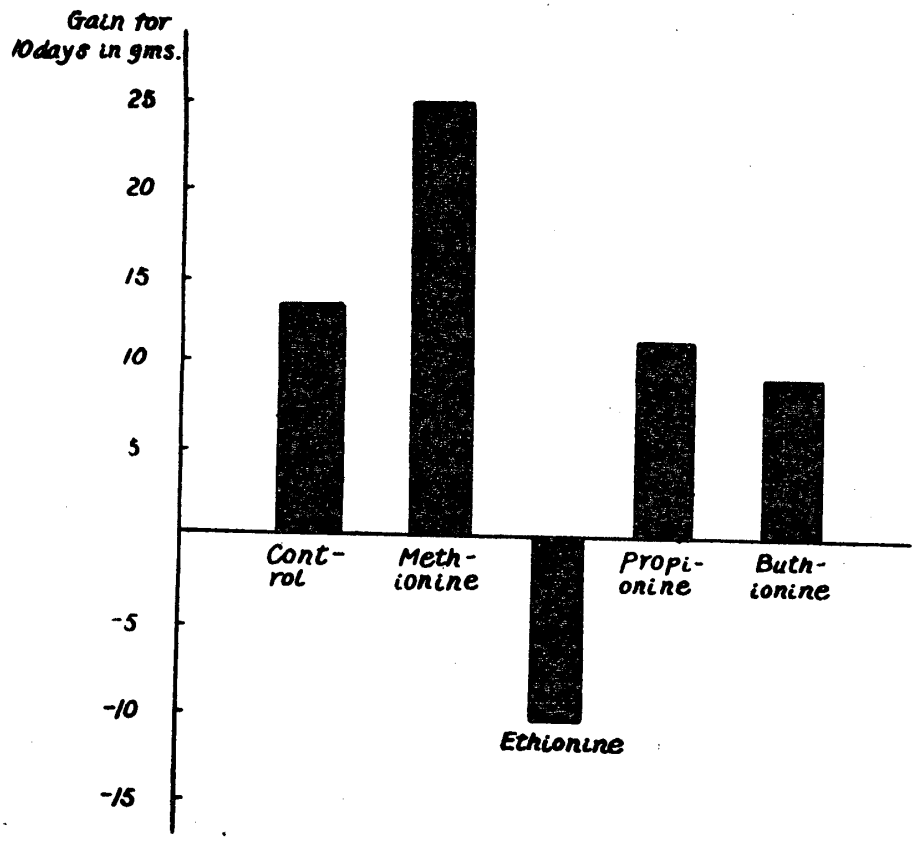

Fig. 2. Influence of methionine homologues upon weight recovery of protein depleted adult rats fed 6 percent casein diet.

c) Determination of methionine and cystine in liver protein of the rats receiving methionine homologues in the diets: Levine and Fopeano (12) postulated that ethionine in the diet resulted in the formation of abnormal protein which accumulate in the liver. Levine and Tarver (13) showed an incorporation of the ethionine itself into the proteins. If the amount of such abnormal products is large, it is expected that the amino acid composition of the liver protein will differ from that of the normal constant. We determined chemically the methionine (14) and cystine (15) content of the liver protein of the rats in the repletion experiment. As table 5 shows, no significant difference was found between each groups. This suggests that the amount of the so-called abnormal protein produced in the repletion period may be insignificant.

Table 5. Methionine and cystine content of liver protein of the experimental animals

Group	Protein*	Methionine #	Cystine #
Control	0.99±0.15%	2.83±0.02%	1.14±0.04%
Methionine	0.81±0.01	2.42±0.04	0.95±0.02
Ethionlne	0.55±0.01	2.32±0.16	1.05±0.07
Propionine	0.88±0.04	2.42±0.18	1.01±0.01
Buthionine	0.92±0.05	2.23±0.10	0.05±0.01

*% in body weight
#% in liver protein

Summary

Ethionine, propoionine and buthionine were synthesized and tested as to their biological effect using both the growth and repletion methods. The results indicated that propionine and buthionine had also inhibitory action for growth or repletion, but it was far milder than the toxity of ethionine in our experimental condition.

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