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Quality & quantity of dietary protein & acute endosulphan metabolic toxicity in rat liver microsomes

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The effect of feeding rice diets with and without lysine and threonine supplementation and endosulphan administration was studied on rat liver microsomal lipids, and two mixed function oxidases (*viz.*, aniline hydroxylase and aminopyrine N-demethylase). These groups were compared with rats fed 20 per cent casein diet and given endosul phan. Endosulphan administration increased liver microsomal protein in animals fed *ad libitum*. Activities of both enzymes were more in rats fed *ad libitum*, as compared to pair-fed rats. Endosulphan administration increased the activity of aniline hydroxylase more in the deficient group, as compared to the supplemented group. The activity of aminopyrine N-demethylase was increased significantly in the supplemented and deficient groups following endosulphan administration. Total phospholipids, phosphatidylcholine and phosphatidylethanolamine contents were higher in pair-fed rats as compared to rats fed *ad libitum*. The cholesterol content in the supplemented and casein groups of *ad libitum* fed rats were nearly comparable. However, in the endosulphan treated deficient group cholesterol level was significantly higher than the untreated deficient group.

Quality and quantity of dietary protein have been shown to affect the activities of hepatic microsomal drug metabolizing enzymes leading to a modified toxicity of certain drugs[1-3]. Boyd *et al*[4] reported that the toxicity of endosulphan was inversely related to the dietary protein contents. Endosulphan toxicity stimulated the nervous system leading to irritant gastroenteritis and congestion of brain and liver[4]. Dorough *et al*[5] observed that the administration of endosulpan to rats did not induce liver oxidases. Agarwal *et al*[6] however reported increased activities of aniline hydroxylase, aminopyrine N-demethylase and tyrosine amino-transferase in rats given endosulphan. The important role of membrane phospholipids, especially phosphatidylcholine, in the microsomal drug metabolizing system has been demonstrated[7]. Feeding of inadequate dietary proteins to experimental animals has been shown[3] to alter hepatic phospholipids.

Rice is a cereal used commonly in many countries as a major staple food. The most practical way of improving the nutri-

tive value of rice diet is by supplementation with food containing nutritionally well balanced proteins[8]. Protein efficiency ratio of poor rice diet can be increased by supplementation of lysine or threonine or both[9,10]. Rats fed basal rice diet alone or with lysine supplemented grew poorly and were anaemic and hypoproteinemic in contrast to rats receiving combined lysine-threonine supplemented diets[11]. In view of the possible relationship between the activities of drug metabolizing enzymes and the requirement of membrane phospholipids for their maximal activity, we studied the effect of administration of endosulphan—a chloroinsecticide of cyclodiene group, on the activities of liver microsomal aniline hydroxylase, aminopyrine N-demethylase and phospholipids of rats fed rice diets with and without lysine, threonine supplementation.

Material & Methods

Male-Wistar rats (80-100 g), divided into three groups of 12 rats each were housed in individual cages. To one group of rats rice diet without supplementation of lysine and threonine (D-diet) was fed for seven days. The second group of rats was fed rice diet supplemented with lysine and threonine (S-diet) for the same period. The third group of rats was fed 20 per cent casein diet (C-diet) *ad libitum* for seven days. After feeding the diets for five days, each group was subdivided into groups of six rats each. To one subgroup endosulphan (5 mg/kg body weight) was given orally in groundnut oil for the last two days. The control group was given only groundnut oil for the same period. In another experiment supplemented and casein diets were pair fed against deficient group (*ad libitum*). Each dietary group had 8 rats. Endosulphan treatment was the same as described before. The diets were prepared as described by Viviani *et al*[12] and their formulation is shown in Table I.

The rats were sacrificed by decapitation, liver removed quickly, immersed in ice-cold saline, cleaned, wiped and weighed. Microsomes were prepared as described by Zanoni[13]. The aniline hydroxylase activity was assayed by the method of Imai *et al*[14] and aminopyrine N-demethylase

Table I. Composition of diets (g/100 g diet)

Ingredient	Deficient	Supplemented	Casein
Casein (fat free)	—	—	20·00
Rice flour	89·00	89·215	—
L-lysine HCl	—	0·425	—
DL-threonine	—	0·360	—
Ammonium citrate	1·00	—	—
Ground nut oil	4·50	4·50	4·50
Potato starch	—	—	70·00
Salt mixture*	4·00	4·00	4·00
Vitamin mixture**	1·50	1·50	1·50

*Salt mixture (g/kg salt mixture): K_2HPO_4, 322; $CaCO_3$,300; NaCl,167; $MgCl_2 7H_2O$,102; $CaHPO_4$ $2H_2O$, 75; $FeSO_4$, 27·5; $MnSO_4$ $4H_2O$, 5·1; KI, 0·8; $CuSO_4 5H_2O$, 0·3; $ZnCl_2$, 0·25; $CoCl_2$, $6H_2O$, 0·05

**Vitamin mixture (mg/kg diet): Thiamine HCl, 5; riboflavin, 5; niacinamide, 25; Ca pantothenate, 20; pyridoxine HCl, 5; folic acid, 0·5; menadione, 0·5; biotin 0·2; B_{12} (cyanocobalamine) 0·03; para-minobenzoic acid, 100; ascorbic acid (vitamin C), 50; vitamin A acetate, 5·00 (14500 IU); vitamin D, 5·00 (2000 IU); alpha-tocopherol, 10, (150 mg choline chloride was added to 231·23 mg vitamin mixture and made up to 1·5 g with dextrose and added at 1·5 per cent level to the diet)

activity was determined by the method of Kato and Gillette[15]. Protein was estimated by the method of Lowry *et al*[16].

The lipids from microsomal fractions were extracted by the method of Folch *et al*[17]. Thin layer chromatographic method was used to separate phospholipid contents of microsomal lipids unidimensionally[18]. Phospholipid phosphorus was estimated by the method of Bartlett[19]. Cholesterol was estimated by the method of Zlatkis *et al*[20].

The results were statistically analysed using Student's 't' test.

Results

The average daily food consumption in rats fed *ad libitum* was : Deficient group 8·0 g; supplemented 10·7 g; and casein group 11·2 g. The growth of rats was highest in rats fed casein diet and lowest in rats fed rice diet deficient in lysine and threonine. Administration of endosulphan (5 mg/kg body weight) caused a reduction in the body weights of rats of all dietary groups but the reduction was more in the deficient group (Table II). The growth of rats appeared to be influenced by the quality and quantity of dietary protein. Calorie intake of the experimental animals also appear to affect endosulphan toxicity in this parameter. The weight loss in pair fed rats was slightly more than *ad libitum* fed rats (Table II).

Liver weights of rats of the different dietary groups regardless of *ad libitum* or pair feeding, were not statistically significant. Endosulphan administration did not affect liver weights of rats fed different dietary proteins (Table II). Liver microsomal protein contents of rats pair-fed different dietary proteins were not statistically significant and endosulphan treatment did not show any significant effect on the contents of microsomal proteins (Table III). The microsomal protein contents of rats fed *ad libitum* rice diet deficient and supplemented with lysine and threonine were significantly lower than the rats fed casein diet. Administration of endosulphan significantly increased microsomal protein contents of the supplemented and the casein group as compared to their respective untreated controls (Table III). The results indicated that the calorie intake of animals appeared to influence the effects of endosulphan on liver microsomal protein.

In pair-fed rats, endosulphan did not show any discernible effect on microsomal protein, but in rats fed these diets *ad libitum*, endosulphan administration increased the microsomal protein contents in rats of S-group and C-group. The effect of feeding different dietary proteins either *ad libitum* or by pair-feeding mode, on the microsomal drug metabolizing enzymes (aniline hydroxylase and amino pyrine-N-demethylase) is shown in Table III. The activities of these two enzymes were lower in pair-fed rats as compared to those fed *ad libitum*, possibly due to the overall impaired protein metabolism in pair-fed rats. The order of activities of these two enzymes in rats fed different dietary protein *ad libitum* was C-group > S-group > D-group. Endosulphan administration increased the activities of these enzymes in rats fed *ad libitum* in all the dietary groups though this was statistically significant in only a few cases (Table III), but the increase was more pronounced in the D-group followed by S-group and C-group. In contrast to rats fed *ad libitum* endosulfan administration to pair-fed rats did not affect the

Table II. Effect of endosulphan administration on growth and liver weight of rats fed different dietary proteins

(Data are mean±SE)

Dietary group	Liver weight (g)	Body weight (g)		
		Initial	Terminal	Gain in body weight
ad libitum :				
D	3·81±0·23	73·6±4·6	76·6±8·3	3·0
D-End	3·82±0·31	73·6±4·3	71·0±3·8	—2·6
S	4·47±0·69	73·6±6·7	97·0±3·0	23·4
S-End	4·34±0·21	73·6±5·9	92·3±6·3	18·7
C	4·64±0·49	73·3±3·2	104·3±6·0	31·0
C-End	4·47±0·19	74·3±3·4	99·3±5·10	25·0
pair fed :				
S	4·37±0·08	87·2±1·6	101·0±1·8	13·8
S-End	4·52±0·10	87·0±1·8	98·5±2·1	11·5
C	4·68±0·07	87·3±1·55	110·75±2·49	23·45
C-End	4·92±0·23	89·2±1·87	109·0±2·34	19·8

The *ad libitum* groups had 6 animals in each group and the pair fed groups had 4 in each.
D=rats fed deficient diet; D-End=D group given endosulphan; S=rats fed supplemented diet; S-End=S group given endosulphan; C=rats fed casein diet; C-End=C group given endosulphan.

activity of aniline hydroxylase in any group (Table III). However, the activity of aminopyrine N-demethylase was increased by endosulphan more in S-group than in C-group, though these were statistically not significant.

The contents of liver microsomal total phospholipids, *viz.*, phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) *i.e.*, μg phospholipid/mg protein) were higher in pair-fed rats as compared to those fed *ad libitum* (Table IV). However, cholesterol contents in the two groups were nearly comparable. Endosulphan treatment of the deficient group significantly increased liver microsomal total phospholipids, PC, PE and cholesterol as compared to the untreated deficient group. Microsomal total phospholipids of the deficient group were significantly lower than the casein group. PC and PE of supplemented groups given endosulphan were significantly increased as compared to their respective untreated controls. Endosulphan administration in rats fed casein diet did not affect microsomal cholesterol content as compared to the untreated casein group. The microsomal cholesterol content of supplemented group was significantly increased as compared to deficient group. In pair-fed rats, endosulphan treatment of casein group significantly increased microsomal PC and PE contents.

Table III. Effect of endosulphan treatment on the activities of liver microsomal aniline hydroxylase and aminopyrine N-demethylase of rats fed different dietary proteins
(Data are mean±SE)

Dietary group	Microsomal protein (mg/g liver)	Aniline hydroxylase (n moles of p-aminophenol formed/mg protein/h)	Aminopyrine N-demethylase (n moles of formaldehyde formed/mg protein/h)
ad libitum (*AL*) :			
D	10·60±1·27*	19·75±1·01	45·87±3·08*
D-End	11·09±1·66	25·17±1·02†	60·06±4·40†
S	12·46±0·59*	23·27±0·26†	59·71±3·46†
S-End	16·52±1·11**	26·54±2·18	77·52±5·21**
C	15·40±0·35	24·33±2·54	85·56±5·75
C-End	20·11±1·38*	25·12±1·58	90·60±4·33
pair fed (*PF*) :			
S	12·12±0·75	16·95±1·36	49·41±6·40
S-End	12·90±0·53	17·69±1·84	62·33±4·50
C	14·89±0·91	17·29±1·74	66·68±3·33
C-End	14·81±0·39	18·05±1·83	73·13±9·24

* $P<0·05$ as compared to C group (AL); ** $P<0·05$ as compared to S group (AL); † $P<0·05$ as compared to D group (AL). The *ad libitum* groups had 6 animals in each group and the pair fed groups had 4 in each.

Discussion

The results of our study showed that acute effects of endosulphan were profoundly affected both by quality and quantity of dietary proteins and the mode of ingestion. The effects were more pronounced in rats fed *ad libitum* than in pair fed rats. This is perhaps related to the availability of surplus dietary nutrients in rats fed *ad libitum*. The results also show that the adverse effects of endosulphan in parameters of growth and the activities of aniline hydroxylase and aminopyrine N-demethylase and microsomal phospholipids are inversely related to quality and quantity of dietary proteins.

Bio-transformation of endosulphan in rat liver has been reported. It has been shown that biotransformation of xenobiotics is directly related to the quality and quantity of dietary protein[1-4]. The enhanced activities of aniline hydroxylase in the deficient group suggest the production of higher amounts of hydroxylated derivatives of endosulphan *e.g.*, diols. Most of the deleterious effects of xenobiotics are generally due to their metabolites. Observations similar to ours have been made by Boyd *et al*[4]. The present findings on the induction of the hepatic mixed function oxidase are similar to those reported by some authors[6] but different from those of others[5] who did not find any increase in liver oxidases.

Table IV. Effect of endosulphan administration on hepatic microsomal phospholipids and cholesterol contents of rats fed different dietary proteins

(Data, expressed as μg/mg microsomal protein, are mean±SE)

Dietary group	Total phospholipids	Phosphatidyl-choline (PC)	Phosphatidyl-ethanolamine (PE)	Cholesterol
ad libitum :				
D	287·98±18·10[a]	119·63±12·50	41·22±6·63	52·25±4·08
D-End	386·84±17·66[e]	161·69±7·03[c]	72·86±5·09[c]	80·21±7·73[c]
S	293·98±14·41	141·32±2·49	42·00±1·99	67·40±3·06[c]
S-End	342·57±35·42	149·67±7·22[b]	50·31±1·24[b]	76·92±5·89
C	357·96±13·72	144·79±12·63	45·93±1·23	61·98±4·47
C-End	370·76±12·43	189·52±6·48[a]	55·29±3·31[a]	59·58±3·26
pair fed :				
S	540·04±37·44	261·20±28·06	128·06±11·54	78·88±6·31
S-End	555·30±47·32	268·19±12·87	114·34±3·79	72·22±2·27
C	558·64±18·14	247·64±13·44[a]	70·46±9·47[a]	64·51±0·59
C-End	574·50±11·21	291·90±8·50	132·04±6·66	67·80±3·70

[a]significantly different at 5 per cent level as compared to C group; [b]significantly different at 5 per cent level as compared to S group; [c]significantly different at 5 per cent level as compared to D group; [a]significantly different at 5 per cent level as compared to C End group (PF). The *ad libitum* groups had 6 animals in each group and the pair fed groups had 4 in each.

Dorough *et al*[5] suggested that because of rapid conversion of endosulphan to polar products in the liver in contrast to apolar products which are characteristic of many cyclodiene insecticides, the induction of hepatic mixed function oxidases was not observed with endosulphan. However, Gupta and Gupta[21] reported that administration of endosulphan (5mg/kg body weight) for 7-15 days decreased pentobarbital induced sleeping time and the levels of pentobarbital in blood and brain. Agarwal *et al*[6] have reported that endosulphan increased the activities of aniline hydroxylase, aminopyrine-N-demethylase and tyrosine aminotransferase in rats. The finding that administration of endosulphan did not influence the liver weight of rats fed different dietary protein given either *ad libitum* or pair-fed supports the observations of Dorough *et al*[5]. The increase noted in microsomal proteins of the supplemented and the casein group rats fed *ad libitum*, suggests either weak induction of microsomal protein synthesis or reduced catabolism of microsomal protein by endosulphan. The effect of endosulphan in relation to quality and quantity of dietary protein and caloric in takeis different on the two liver mixed function oxidases *viz.*, aniline hydroxylase and aminopyrine N-demethylase studied. The

observed differential effect of endosulphan on the activities of the two membrane-bound enzymes may possibly be attributed to weak inductive capacity of endosulphan and the microenvironment of the membrane. Membrane phospholipids are absolutely essential for the function of membrane bound enzymes.

In the present study the increase in enzyme activities was followed by an increase in endoplasmic reticulum phospholipids and variations in the magnitude were influenced by the quality and quantity of dietary protein and the caloric intake. Thus our results suggest that factors which influence membrane micro-environment may also affect the activities of membrane bound enzymes. The increase in microsomal phospholipids of pair fed rats as compared to those fed *ad libitum* may be due to a reduced catabolism of microsomal phospholipids in pair fed rats.

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