Effect of Dietary Monosodium Glutamate and Cholesterol on Growth and Lipid Metabolism in the Rat[1] (37301)

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A hypocholesterolemic effect has been reported when glutamic acid provided the sole source of nonessential nitrogen in chemically defined diets fed to gerbils and chicks (1) and to human volunteers (2, 3). A diet of similar composition when fed to adult male rats, however, was found to be hypercholesterolemic (1). The addition of glutamic acid to an ordinary mixed diet was without effect on serum cholesterol concentrations of human subjects (4).

The purpose of the study reported here is to evaluate further the effects of glutamic acid, in the form of monosodium glutamate (MSG), on growth, organ weights, and lipid metabolism of the male weanling rat fed a semisynthetic diet with or without the addition of cholesterol.

Materials and Methods. Forty male weanling rats of the Carworth strain were randomly divided into 4 groups of 10 rats each and housed individually in stainless steel cages.

During the 28-day experiment each group was allowed *ad libitum* access to one of the isonitrogenous diets shown in Table I. All of the dietary nitrogen (N) and glutamic acid in diets A and B was derived from casein whereas 20% of the N and 65% of the glutamic acid in diets C and D were derived from MSG.

At the end of the 4-wk feeding period all rats were fasted overnight, then allowed access to 10 g of diet for 1 hr before ip injection of 5 μCi of Na acetate-2-^{14}C (S.A. 59.2 mCi/mM)/100 g body weight. Ninety minutes post-injection each rat was killed using a decapitator[3] and a blood sample was obtained. The liver, kidney, and spleen were removed from each animal, weighed and frozen for further analysis.

TABLE I. Composition of Diets.

	A	B	C	D
	(Percentage)			
Sucrose	60.8	59.8	57.7	56.7
Casein	25.0	25.0	20.0	20.0
Monosodium glutamate	—	—	8.1	8.1
Soybean oil	8.0	8.0	8.0	8.0
Salt mixture U.S.P. XIV	4.0	4.0	4.0	4.0
Vitamin mixture[a]	2.2	2.2	2.2	2.2
Cholesterol U.S.P.	—	1.0	—	1.0
Total	100.0	100.0	100.0	100.0
Nitrogen	3.3	3.3	3.3	3.3
Glutamic acid	4.8	4.8	10.9	10.9

[a] Vitamin Diet Fortification Mixture, Nutritional Biochemicals, Cleveland, Ohio 44128.

Serum concentrations of cholesterol and total protein were determined by automated methods (5). Separation of serum protein fractions was done on cellulose acetate[4]. After saponification and pentane extraction of total nonsaponifiable (TNS) lipid, hepatic cholesterol was determined by the same automated procedure used for determination of serum cholesterol (5). An aliquant of the pentane extract was evaporated to dryness in a liquid scintillation counting vial, redissolved in 15 ml of a solution consisting

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[3] Model RM130 Decapitator, Harvard Apparatus Company, Inc., Millis, Massachusetts 02054.

[4] Microzone Electrophoresis Cell, Model R-101, Beckman Instruments Co., Fullerton, California 92634.

TABLE II. Effect of Dietary Nitrogen Source and Cholesterol on Body and Organ Weights.

Parameter	Treatment effects: — MSG + (A&B)	(C&D)	— Cholesterol + (A&C)	(B&D)	Standard error of the mean
Body wt (g)					
Final	203[a]	183	196	191	3.7
Initial	49	50	49	50	1.3
Gain	154[a]	133	147	141	3.1
Organs (% of body wt)					
Liver	4.28	4.17	4.08	4.37[a]	0.27
Kidney	0.90	0.95[a]	0.94	0.91	0.02
Spleen	0.35[a]	0.29	0.33	0.31	0.02

[a] $p < 0.01$.

of 4 g 2,5-diphenyloxazole (PPO) and 300 mg 1,4-bis-2-(5-phenyloxazole)-benzene (POPOP) in 1 liter toluene and counted in a liquid scintillation counter[5] to determine the amount of Na-acetate-2-^{14}C incorporated into hepatic TNS lipid.

Samples equivalent in weight to 20% of each liver within each treatment group were pooled and prepared for thin-layer chromatography (TLC) by the method of Folch *et al.* (6). Approximately 100 mg of the lipid extracted from each pooled liver sample were applied to precoated TLC plates[6] ($\sim$ 20 mg/plate) and developed as described by French and Andersen (7). Each plate was developed to 19.5 cm using a two-phase solvent system: hexane–benzene (85:15) followed by hexane–diethyl ether–acetic acid (69:29:2). The lipid bands, made visible by brief exposure to iodine vapors, were scraped into flasks and eluted alternately with diethyl ether and a 4:1 mixture of chloroform–methanol. Elution of the phospholipid fraction was accomplished by two extractions with a 2:1 mixture of chloroform–methanol alternated with two extractions with a 5:3:2 mixture of methanol–chloroform–water. The eluted bands from each lipid class were evaporated to dryness in liquid scintillation counting vials and redissolved in the fluor and toluene scintillation solvent system previously described for counting ^{14}C activity in hepatic TNS lipid. The data were subjected to analysis of variance applicable to a 2 $\times$ 2 factorial design as described by Snedecor (8).

Results and Discussion. As shown in Table II, rats fed the 25% casein diet gained significantly more weight than did those fed the 20% casein diet supplemented with nonessential N in the form of MSG. Since all diets were isonitrogenous the depressed weight gain observed in the MSG-fed rats may have been the result of a deficiency of methionine, the first limiting amino acid in casein. The significantly greater kidney weight in the rats fed MSG is thought to be due to a comparatively greater renal solute load (urea and sodium) resulting from ingestion of these diets. Since MSG is 13.6% Na by weight, the diets containing MSG provided approximately 10 times the amount of Na (1230 mg) per 100 g diet as did the 25% casein diets (128 mg). Daily Na intake of rats fed the MSG-containing diets was approximately 130 mg greater than that of rats fed the 25% casein diets.

The reason for the significant treatment effect on spleen weight is not known but could be related to an undetermined difference in hematologic status between the MSG- and

[5] Model LS-100, Beckman Instruments Co., Fullerton, California 92634.

[6] Eastman Chromatogram Sheet, silica gel absorbent without fluorescent indicator, Eastman Kodak Company, Rochester, New York 14650.

TABLE III. Effect of Dietary Nitrogen Source on Serum Protein and Serum and Hepatic Cholesterol.

Parameter	Treatment effects: — MSG + (A&B)	(C&D)	— Cholesterol + (A&C)	(B&D)	Standard error of the mean
Serum (100 ml)					
Protein (g)					
Albumin	3.02	3.26[a]	3.09	3.20[b]	0.02
Globulin	2.50[a]	2.39	2.34	2.54[b]	0.03
Total	5.54	5.65	5.44	5.74[b]	0.02
Cholesterol (mg)	80	68	76	72	6.2
Liver (100 g)					
Cholesterol (mg)	452	556	271	737[a]	41.8
Lipid (g)	4.48	4.45	3.85	5.08	NA[c]

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] Not applicable, pooled samples.

casein-fed groups. Livers of rats fed cholesterol were significantly heavier than those of animals not receiving a dietary source of cholesterol.

Concentration of albumin was significantly greater and that of globulin significantly less in the serum of MSG-fed rats than in serum of rats fed 25% casein diets (Table III). In rats fed cholesterol-containing diets, serum concentrations of albumin, globulin and total protein were significantly greater than those of rats fed the cholesterol-free diets.

The previously described hypercholesterolemic effect of feeding MSG to rats (1) was not observed under the conditions of this study. Feeding of the cholesterol-containing diets did not produce significant alterations in serum cholesterol; however, hepatic cholesterol concentrations of the rats fed cholesterol-containing diets were significantly elevated and analysis of the pooled liver samples indicated that the greater liver weights of the cholesterol-fed rats (Table II) were due in part to an increased lipid content. Concentrations of cholesterol were approximately 20% greater in livers of rats fed MSG than those of rats fed the 25% casein diets.

The treatment effects on the incorporation of Na-2-^{14}C-acetate into TNS lipid are shown in Table IV. The feedback mechanism described by Gould (9) was clearly demonstrated by the greater incorporation of Na-

TABLE IV. Effect of Nitrogen Source and Cholesterol on the Incorporation of Na Acetate-2-^{14}C into Hepatic Total Nonsaponifiable Lipid.

Parameter	Treatment effects: — MSG + (A&B)	(C&D)	— Cholesterol + (A&C)	(B&D)	Standard error of the mean
CPM/g liver	1307	1642	1668	1280	144
CPM/mg hepatic cholesterol	361	438	616[a]	183	48
% Dose $\times 10^{-3}$/g liver	57.2	80.6[b]	76.3	61.5	6.5

[a] $p < 0.01$.
[b] $p < 0.05$.

2-^{14}C-acetate into TNS lipid by rats fed the cholesterol-free diets. When expressed in terms of cpm/mg of hepatic cholesterol, incorporation of ^{14}C acetate into TNS lipid was only 30% as great by rats receiving cholesterol-containing diets as by those fed cholesterol-free diets. Rats fed MSG incorporated significantly more of the dose of ^{14}C acetate into TNS lipid than did those fed the 25% casein diets. Although mean body weight of the rats fed MSG was 10% less than that of rats fed the 25% casein diets, ^{14}C activity in TNS lipid (cpm/g liver) was approximately 25% greater in the MSG-fed groups.

TLC separation of hepatic lipids revealed that the greatest ^{14}C activity (% dose/mg hepatic lipid) in all treatment groups was found in the cholesterol fraction (Table V); however, this fraction appeared to be less affected by treatment than was the cholesterol ester fraction. The ^{14}C activity in hepatic lipid of rats fed MSG was greater in each of the 5 TLC lipid class fractions than in the corresponding fractions of rats fed the 25% casein diet. Compared with rats fed the 25% casein diets the ^{14}C activity in hepatic lipid of MSG-fed rats was highest in the cholesterol ester fraction, followed by phospholipid, free fatty acid, cholesterol and triglyceride. The ^{14}C activity in the cholesterol ester fraction was 85% less in livers of rats fed cholesterol-containing diets than in livers of those fed the cholesterol-free diets. Rats fed the cholesterol-containing diets had slightly greater ^{14}C activity in the phospholipid and triglyceride fractions and less activity in the cholesterol and free fatty acid fractions.

The mechanism by which large intakes of glutamate affect cholesterol metabolism is not clear. Bazzano *et al.* (10) theorize from results of their *in vitro* studies with acetate-1-^{14}C that gerbils fed amino acid formula diets containing glutamic acid as the sole source of nonessential N have a block in hepatic cholesterol synthesis between acetate and mevalonate. The hypocholesterolemic effect of glutamic acid in man appears to be associated with an increased half life of serum cholesterol, a decrease in total fecal sterols and a reduction in sterol biosynthesis (4).

Gerbils fed amino acid formula diets containing either glutamic acid or α-ketoglutarate lost from 15 to 22% of their body weight during a 1-wk feeding period (11). These results are difficult to interpret since it has been well established that weight loss, in itself, will lower serum concentrations of cholesterol (12, 13).

D'Elia *et al.* (14) fed 30% casein diets to adult male mongolian gerbils for 2 wk and observed an increase of 33 mg/100 ml in serum cholesterol. Since glutamic acid makes up about 23% of the protein in casein, the diet contained approximately 7% glutamate. When a 10% casein diet (~2% glutamate) was fed for 2 wk, weight loss occurred and a significant decrease of 91 mg/100 ml was seen in serum cholesterol. It would appear that loss of body weight in the gerbil also markedly affects the serum cholesterol level.

TABLE V. Effect of Nitrogen Source and Cholesterol upon Hepatic Lipid-^{14}C as Determined by Thin-Layer Chromatography.

	Treatment effects			
	— MSG +		— Cholesterol +	
TLC fraction	(A&B)	(C&D)	(A&C)	(B&D)
Phospholipid	3.0 (47)[a]	7.0 (49)	4.3 (53)	5.7 (43)
Free fatty acid	0.9 (20)	1.9 (19)	1.5 (18)	1.3 (21)
Cholesterol	5.7 (16)	9.5 (17)	8.7 (18)	6.5 (15)
Cholesterol ester	1.7 (10)	5.3 (10)	6.1 (6)	0.9 (15)
Triglyceride	4.4 (7)	5.5 (5)	4.6 (5)	5.4 (6)
Total lipid	2.8 (100)	6.0 (100)	4.7 (100)	4.1 (100)

[a] % Dose × 10^{-3}/mg lipid; figures in parentheses denote % of total lipid.

The increased incorporation of acetate-2-^{14}C into hepatic TNS lipid and into various hepatic lipid fractions by rats fed diets containing approximately 20% of the N as MSG might logically be explained as follows: significant amounts of the dietary glutamate would be metabolized to α-ketoglutarate, thence into citric acid cycle intermediates. Acetyl CoA, formed by action of ATP citrate lyase (E.C. 4.1.3.8) on citrate, would be available to enter rapidly into the various lipid biosynthetic pathways. The ip injection of acetate-2-^{14}C would be metabolized most readily via those enzymatic pathways stimulated by the presence in the diet of an appropriate substrate.

Summary. Male weanling rats were divided into 4 isonitrogenous dietary treatment groups, all of the dietary N and glutamic acid in groups A and B being derived from casein whereas 20% of the N and 65% of the glutamic acid in diets C and D were derived from MSG. Diets B and D contained 1% cholesterol. At the end of a 28-day feeding period, the rats receiving MSG (groups C and D) exhibited the following statistically significant differences from the rats receiving N only from casein (groups A and B): depressed weight gain, heavier kidneys, higher levels of serum albumin, lower levels of serum globulin, and a greater percent incorporation of an ip injection of ^{14}C acetate into hepatic TNS lipid. Rats fed the diets containing 1% cholesterol (groups B and D) exhibited the following statistically significant differences from those fed the cholesterol-free diets (groups A and C): heavier livers, higher levels of serum albumin, globulin and total protein, greater concentration of hepatic cholesterol, and lower percent incorporation of an ip injection of ^{14}C acetate into hepatic cholesterol.

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