Plasma and Tissue Cholesterol and Lipid Levels in Rabbits on L-Histidine-Supplemented Diets[1] (34446)

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A marked hyperlipemia associated with dietary administration of histidine has been described in the Rhesus monkey (*Macaca mulatta*) (1, 2). Histidine was the only amino acid of nine studied which induced this hyperlipemia. The "creamy" appearance of the serum was due to the presence of triglyceride-laden chylomicrons. α-Lipoproteins were decreased, giving an elevated β/α lipoprotein ratio. These studies suggested that the hyperlipemia was probably derived from ingested rather than catabolic lipid. This report describes experiments designed to test these findings in the rabbit, which has been widely used in lipid studies.

Methods. Twelve male, 4-week-old, New Zealand rabbits were equally divided into three groups and placed on ground commercial rabbit chow[2], chow plus 5% excess L-histidine–HCl_3, and chow plus 8% excess L-histidine–HCl. Blood was periodically drawn by ear vein puncture, nonfasting or after a 4-hr fast. Plasma and tissue lipids were analyzed after chloroform–methanol extraction (3). Total cholesterol was determined (4) and phospholipids (25×phosphorus) were estimated by phosphorus analysis (5). Galactolipids in brain were determined by a modification of the procedure of Hess and Lewin (6). Histidine was determined enzymatically (7).

Results and Discussion. The growth curves are shown in Fig. 1. Of the original 12 rabbits at the start of the experiment, 8 survived. There was an initial growth depression in all groups. After acclimatization to the diets, the experimental groups still did not grow as well as the controls. A spurt in growth was noted when the 8% L-histidine supplemented group was removed from the diet and placed on chow diet.

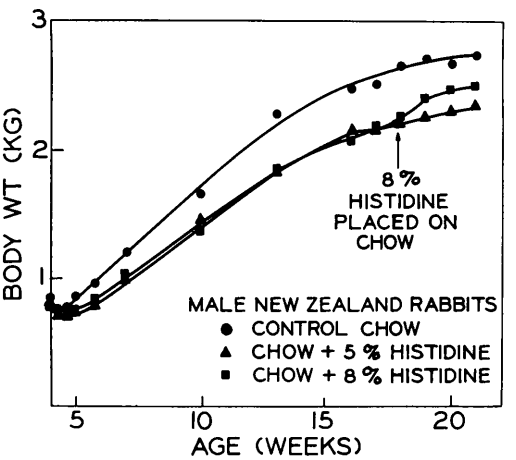

FIG. 1. Body growth curves of rabbits supplemented with excess L-histidine.

Plasma analyses are shown in Table I. Fasting histidine levels were near normal. Nonfasting levels, usually drawn in the early afternoon, in the 5% supplemented group varied from 20–50 mg/100 ml at the end of the experiment. In the monkey experiment (1) histidine serum levels of 20–60 mg/100 ml were observed 4 hr after feeding. Hematocrit values at the end of the experiment suggested the experimental rabbits were slightly anemic although the differences were not consistently significant. A recovery in red cell volume to normal values was noted in the 8% supplemented group after transfer to chow

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[2] Wayne rabbit ration, Allied Mills, Inc., Chicago, Illinois.

[3] Ajinomoto Co., Inc., Tokyo, Japan.

TABLE I. Rabbit Plasma Histidine, Phospholipid, Cholesterol, and Hematocrit Values while on Excess Dietary Histidine.

Age (days)	Diet	(mg/100 ml) Histidine (pooled)	Phospholipid	Cholesterol	Red cells (%)
56[a]	Chow (3)[b]	—	121 ± 32[c]	80.4 ± 3.3	—
	5%[d] (3)	—	198 ± 53 ns[e]	120 ± 8[f]] [f]	—
	8% (2)	—	—	184 (1 rabbit)]	—
77[a]	Chow	3.6	184 ± 14	65.7 ± 7.1	—
	5%	3.7	195 ± 30 ns	113 ± 30 ns] [f]	—
	8%	3.3	234 ± 3	115 ± 4]	—
105[a]	Chow	4.2	164 ± 31	57.3 ± 4.7	—
	5%	6.6	195 ± 27 ns	90.7 ± 21.1[f]] [f]	—
	8%	3.8	218 ± 29	100 ± 18]	—
125	Chow	3.0	—	48.9 ± 15.9	45 ± 8
	5%	19.2	—	71.5 ± 23.9 ns] ns	39 ± 2 ns
	8%	22.7	—	58.4 ± 13.2]	41 ± 3
125[a]	Chow	—	—	49.5 ± 5.2	—
	5%	—	—	68.5 ± 27.7 ns] ns	—
	8%	—	—	76.7 ± 7.6]	—
126	Chow	—	—	41.3 ± 4.1	40 ± 3
	5%	—	—	—	—
	8%	—	—	81.0 ± 15.6	34 ± 2
128	Chow	5.0	—	46.9 ± 3.0	42 ± 4
	5%	53.0	—	68.3 ± 26.6 ns	34 ± 3[f]
	8%	4.9	—	81.0 ± 11.4	37 ± 3
132	Chow	4.7	—	41.6 ± 8.6	47 ± 4
	5%	32.5	—	63.9 ± 36.7 ns	44 ± 5 ns
	8%	4.7	—	74.4 ± 20.2	46 ± 2
138	Chow	3.2	—	44.9 ± 1.2	46 ± 3
	5%	43.7	—	60.0 ± 28.3 ns	41 ± 6 ns
	8%	2.7	—	57.2 ± 11.9	48 ± 1
147	Chow	—	—	47.3 ± 5.9	46 ± 2
	5%	—	—	51.4 ± 17.5 ns	40 ± 2[f]
	8%	—	—	59.9 ± 6.1	54 ± 1

[a] Blood drawn after 4-hr fast, the remaining bloods were drawn nonfasting.
[b] No. of rabbits per group.
[c] Mean ± 1 SD for chow and 5% group; mean ± range for 8% group.
[d] Percentage excess dietary histidine.
[e] ns = not significantly different from chow group ($p > 05$).
[f] Significantly different from chow group ($p < 05$).

diet. Plasma total phospholipid levels, although slightly elevated, did not show any significant variation from control in contrast to the 2-fold increase obtained in serum from monkeys on L-histidine diets. Cholesterol levels, however, were significantly elevated 50% or more above control values initially. The magnitude and statistical significance of this elevation decreased toward the end of the experiment. This may be related to diminished intake of histidine or adaptation to the diets. At 125 days of age the magnitude of elevated levels was similar fasting or nonfasting although significance was not achieved in either case. Serum cholesterol was elevated 2–3-fold in the monkeys. At no

TABLE II. Liver and Brain Lipid Composition after Excess Dietary Histidine.

		Histidine (%)	
	Control	5	8[a]
Body wt (g)	2728 ± 62[b](3)[c]	2353 ± 294 (3)	2480 ± 186 (2)
Liver wt (g)	80 ± 9	69 ± 3	82 ± 1
Liver components (mg/g)			
Total lipid	39.3 ± 3.6	40.9 ± 2.8 ns[d]	36.0 ± 1.5
Phospholipid	31.1 ± 4.5	30.9 ± 2.6 ns	25.7 ± 4.3
Cholesterol	2.73± 0.58	2.62± 0.32 ns	2.42± 0.10
Cerebrum components (mg/g)			
Nonlipid residue	99.9 ± 5.4	101.9 ± 8.5 ns	90.0 ± 4.1
Total lipid	86.6 ± 6.6	86.2 ± 2.6 ns	88.8 ± 0.5
Phospholipid	47.4 ± 3.4	45.0 ± 1.9 ns	47.5 ± 2.0
Cholesterol	19.3 ± 1.2	19.0 ± 0.9 ns	19.6 ± 0.6
Galactolipid	16.8 ± 1.2	16.7 ± 1.8 ns	15.7 ± 0.5
Cerebellum components (mg/g)			
Nonlipid residue	97.4 ± 4.2	107.6 ± 1.8[e]	101.4 ± 0.3
Total lipid	90.4 ± 2.8	90.7 ± 3.8 ns	88.5 ± 2.7
Phospholipid	49.4 ± 0.7	46.0 ± 2.3 ns	47.3 ± 0.5
Cholesterol	18.8 ± 0.4	16.9 ± 1.3 ns	17.2 ± 0.2

[a] 8% histidine group analyzed 22 days after starting control chow.
[b] Mean ± 1 SD for control and 5% histidine groups; mean ± range for 8% histidine group.
[c] No. of rabbits per group.
[d] ns = not significantly different from chow group ($p>05$).
[e] Significantly different from chow group ($p<05$).

time did the rabbits have "creamy" plasma suggesting no marked effect on triglyceride levels such as the 2–8-fold increases seen in monkeys coincident with "creamy" serums. The rabbits on 8% L-histidine had cholesterol values within 25% of control 3 weeks after being returned to chow diet. These data plus experiments with rats[4] suggest several days are required for recovery to normal cholesterol levels after removal from histidine supplementation. The persistent elevated cholesterol levels when histidine levels are normal after fasting or after removal of the supplemented diet suggest an indirect causal relationship of endogenous origin.

Lipid analyses of brain and liver at the termination of the experiment are shown in Table II. The values obtained in the experimental groups do not suggest a significant variation from control.

The data indicate that excessive administration of L-histidine to the rabbit at levels which just start to reduce growth do not result in the profound hyperlipemia found in monkeys fed L-histidine. However, significant elevation of plasma cholesterol levels occurred, the effect being localized to the plasma for no changes in liver or brain cholesterol were noted. The fat content of the monkey diet was 3.5% while the rabbit chow had a 2% fat content. A higher dietary fat content may be necessary to induce more significant effects in the rabbit.

The other lipid components were also near normal in plasma as well as brain and liver. The lack of any marked effect on brain galactolipid and cholesterol concentrations indicate that the excesses of histidine produced in the rabbit at the ages studied in these experiments do not markedly influence or retard myelin deposition in the areas analyzed.

The mechanism of these histidine effects are not known but since the effects are limited to blood, it suggests that the mechanisms governing blood lipid and lipoprotein levels are altered. Further studies should aid the

[4] R. L. Geison, unpublished data.

understanding of these mechanisms of control in this animal.

Summary. Feeding 5 and 8% excess L-histidine to rabbits caused significantly elevated plasma cholesterol levels. Phospholipid levels were slightly but not significantly elevated. The 5% histidine-supplemented diet produced plasma levels of 20–50 mg/100 ml of histidine. After 17 weeks on diet, no effects on brain cerebrum and cerebellum or liver cholesterol concentrations were found. Brain and liver total lipid and phospholipid and cerebrum galactolipid concentrations were also normal. The effect of excess dietary histidine on lipid components is apparently localized in the circulatory system. The effects noted in rabbits are much less than those previously observed in monkeys.

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