

Lack of nicotinic acid effect on cholesterol metabolism of the rat*

CHARLES H. DUNCAN and MAURICE M. BEST

Department of Medicine, University of Louisville School of Medicine, Louisville 2, Kentucky

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SUMMARY

The effect of the addition of 1 per cent nicotinic acid to the diet of the rat on serum, liver, and carcass cholesterol, and on the incorporation of acetate-1-C¹⁴ into cholesterol by the intact animal were studied. The effect of nicotinic acid on absorption of cholesterol-4-C¹⁴ and on the elimination of labeled cholesterol from serum and liver were also studied. No significant effect of nicotinic acid, fed for either 8 or 42 days, on the incorporation of acetate-1-C¹⁴ into serum and liver cholesterol was observed. When weight gain of the control animals was limited to that of the nicotinic-acid-fed rats by pair-feeding, nicotinic acid had no significant effect on serum or liver cholesterol concentrations, nor on total carcass cholesterol. Absorption of cholesterol-4-C¹⁴ and the disappearance of labeled cholesterol from serum and liver were not influenced by nicotinic acid administration by stomach tube.

The addition of nicotinic acid to the diet for 8 days has been reported by Merrill to result in an increased incorporation of acetate-1-C¹⁴ into liver cholesterol by the rat (1). In a preliminary report we were unable to confirm this finding, the addition of 1 per cent nicotinic acid to the diet for 8 days having no effect on the incorporation of acetate into serum and liver cholesterol by the intact rat. After 42 days the mean incorporation of acetate into cholesterol was less in the nicotinic-acid-fed animals than in the controls, although the difference was not statistically significant (2).

In view of the widespread interest in nicotinic acid as a hypocholesterolemic agent in man (3, 4, 5), its effect on cholesterol metabolism of the rat has been further studied. Data have been obtained regarding its effect on the absorption and elimination of cholesterol as well as on the incorporation of acetate-1-C¹⁴ into cholesterol by the intact animal.

METHODS

Male albino rats (Holtzman) were employed in all studies, being housed in mesh-bottomed cages and kept

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in an air-conditioned animal room. Tap water and a balanced laboratory mash,¹ to which cottonseed oil, 5 ml. per 100 g., was added, were offered *ad libitum* except as noted below. When nicotinic acid was fed, it was incorporated into the diet in the amount of 1 per cent by weight.

The animals were killed under amobarbital sodium anesthesia, blood obtained by cardiotomy, and the livers removed, blotted, and weighed. Calculations were based on an assumed plasma volume of 3.42 per cent of body weight.

Serum cholesterol was determined by the method of Abell *et al.* (6). A 2-to-3 g. segment of liver was digested by refluxing for 2 hours in 10 per cent potassium hydroxide in 70 per cent ethanol. After neutralization with acetic acid and dilution to volume, an aliquot of the liver digest was taken for determination of total cholesterol by the method of Sperry and Webb (7).

Serum cholesterol was prepared for liquid scintillation counting of C¹⁴ as follows: Two ml. of serum was mixed with 8 ml. 95 per cent ethanol containing 2 per cent (w/v) potassium hydroxide and incubated at 37°C for 55 minutes. After cooling to room temperature, 5 ml. of distilled water was added and the mixture extracted four times with 5 ml. portions of

¹ Rat-Mouse Ration, Harlan Small Animal Industries, Cumberland, Ind.

TABLE 1. INCORPORATION OF SODIUM ACETATE-1-C¹⁴ INTO SERUM AND LIVER CHOLESTEROL*

Diet	Experimental Period	Number of Rats	Initial Weight	Weight Gain	Serum Cholesterol	Liver Cholesterol	Incorporation of C ¹⁴ into Cholesterol
	<i>no. of days</i>		<i>g.</i>	<i>g.</i>	<i>mg./100 ml.</i>	<i>mg./100 g.</i>	<i>percentage</i>
Control		8	288	35 ± 9	68 ± 7	251 ± 21	.151 ± .036
Nicotinic acid	8	8	288	24 ± 10 †	65 ± 6	251 ± 12	.163 ± .073
Control		4	269	137 ± 14	68 ± 3	256 ± 5	.174 ± .025
Nicotinic acid	42	4	266	92 ± 26 †	60 ± 4 †	282 ± 24	.123 ± .038
Control		7	361	32 ± 13	67 ± 10	272 ± 42	.227 ± .026
Nicotinic acid	42 ‡	7	361	32 ± 11	65 ± 9	295 ± 55	.219 ± .018

* Values given are the mean and standard deviation, estimated using (n-1).

† Values that differ significantly from the Control (p < .05).

‡ Paired weight gain.

TABLE 2. ABSORPTION OF CHOLESTEROL-4-C¹⁴*

Gavage Solution	Number of Rats	Weight	Serum Cholesterol	Liver Cholesterol	Administered C ¹⁴ in Serum and Liver Cholesterol
		<i>g.</i>	<i>mg./100 ml.</i>	<i>mg./100 g.</i>	<i>percentage</i>
Control	6	321	55 ± 6	198 ± 23	4.83 ± 1.28
Nicotinic acid	6	316	54 ± 5	220 ± 39	5.77 ± 1.19

*Values given are the mean and standard deviation, estimated using (n-1).

petroleum ether. The combined petroleum ether extracts were evaporated to dryness under nitrogen. The residue was redissolved in 1:1 acetone-ethanol, acidified with acetic acid, filtered, digitonin added, an estimated 50 per cent excess being employed, and the tube allowed to stand overnight at room temperature. After washing with 1:2 acetone ether and then ether, the cholesterol digitonide was dissolved in 10 ml. of methanol, 10 ml. of toluene added, and the digitonide decomposed by evaporation to dryness with heat and a stream of nitrogen. The residue was then redissolved in 14 ml. of toluene, two 1.5 ml. aliquots taken for duplicate determination of cholesterol, and 9 ml. added to the 20 ml. counting vial containing 30 mg. of the scintillator, 2,5-diphenyloxazole, in 1 ml. of toluene.

An aliquot of the neutralized liver digest, representing approximately 1 g. of liver, was extracted with petroleum ether, and the cholesterol prepared for counting in the same manner as described for serum

cholesterol. In the Packard Tri-Carb liquid scintillation counter the background was approximately 8 cpm. and the counting efficiency, determined with a C¹⁴-benzoic acid standard, 44 per cent.

Incorporation of Sodium Acetate-1-C¹⁴ into Serum and Liver Cholesterol. Control and 1 per cent nicotinic acid diets were fed *ad libitum* to groups of rats for 8 and 42 days. Two hours before sacrifice each rat received an intraperitoneal injection of carboxyl labeled sodium acetate, 2 μ c. per 100 g. body weight. Labeled acetate with a specific activity of 25 μ c. per mmole was used, each animal receiving 25 to 30 mg. of sodium acetate in aqueous solution.

The 42-day feeding of nicotinic acid was also carried out, using seven pairs of rats housed in individual cages. The rats given nicotinic acid were fed *ad libitum* and weighed every other day; the control rats were given just sufficient food to maintain the same weight gain as the paired experimental animals. In addition

TABLE 3. DISAPPEARANCE OF CHOLESTEROL-C¹⁴ FROM SERUM AND LIVER *

Group	Number of Rats	Initial Weight	Serum Cholesterol	Liver Cholesterol	Administered C ¹⁴ in Serum and Liver Cholesterol
		<i>g.</i>	<i>mg./100 ml.</i>	<i>mg./100 g.</i>	<i>percentage</i>
2 hours after mevalonic acid administration	4	369	53 ± 5	224 ± 13	5.14 ± 1.19
6 days after mevalonic acid administration: control gavage†	4	359	64 ± 7	218 ± 12	.679 ± .066
6 days after mevalonic acid administration: nicotinic acid gavage†	4	348	65 ± 6	228 ± 11	.684 ± .227

* Values given are the mean and standard deviation, estimated using (n-1).

† Third through sixth day.

to the other observations, the oxygen consumption of each of these rats was determined in a closed-circuit respirometer, and at sacrifice the thyroid gland was weighed (8).

Cholesterol Absorption. After fasting overnight, each of 12 rats was fed by gavage with 1 ml. of cottonseed oil in which was dissolved 10 mg. of cholesterol containing 0.8 μ c. of cholesterol-4-C¹⁴. At the same time six of the animals also received by gavage a solution of sodium nicotinate equivalent to 65 mg. of nicotinic acid. The remaining six were given an equal volume of water by gavage. The basic laboratory diet and water were offered *ad libitum* until the animals were killed 24 hours later, and cholesterol-C¹⁴ content of serum and liver determined.

Cholesterol Excretion. The cholesterol of 12 rats was labeled by the intraperitoneal injection of mevalonic acid-2-C¹⁴, 0.1 μ c. per 100 g. body weight. Two hours later four rats were killed and the incorporation of C¹⁴ into serum and liver cholesterol determined. The remaining animals were divided into two groups, each receiving the basic diet. From the third through the sixth day the rats in one group were given sodium nicotinate equivalent to 25 mg. nicotinic acid per 100 g. body weight twice daily by gavage; the remaining animals received a like volume of water by gavage. The animals were then killed and the C¹⁴ remaining in serum and liver cholesterol determined.

Total Rat Cholesterol. Two groups of four rats were maintained for 42 days, one on 1 per cent nicotinic acid diet *ad libitum*, and the other on the basic diet, weight gain of paired animals being controlled as previously described. Serum and liver cholesterol were determined in the usual manner. In addition, the resid-

ual carcass, including the gastrointestinal tract, from which the contents had been removed by irrigation, was digested by refluxing overnight in alcoholic potassium hydroxide, and total carcass cholesterol determined by the same method used for liver cholesterol.

RESULTS

Incorporation of Sodium Acetate-1-C¹⁴ into Serum and Liver Cholesterol. The addition of 1 per cent nicotinic acid to the *ad libitum* diet resulted in a significant inhibition of weight gain, apparent in both the 8- and the 42-day experiments (Table 1). A modest but statistically significant reduction of serum cholesterol was noted in the nicotinic-acid-fed animals receiving the *ad libitum* diets for 42 days. The most pronounced difference between these animals and their control group is in the partition of cholesterol between serum and liver, the ratio of liver cholesterol (mg. per 100 g.) to serum cholesterol (mg. per 100 ml.) being $3.79 \pm .21$ in the control group and $4.72 \pm .13$ in the nicotinic-acid-fed group ($p < .01$).

In the final experiment, in which the weight gain of the control group was restricted to that of the nicotinic-acid-fed group, no significant effect of nicotinic acid on serum or liver cholesterol was observed. Nor was any significant effect of nicotinic acid on oxygen consumption observed, the mean oxygen consumption of the seven control animals being 27.4 ± 4.0 ml. per 10 minutes per 100 g. to the two-thirds power, and that of the nicotinic-acid-fed group, 28.5 ± 2.9 . Thyroid weights were also essentially identical, $4.8 \pm .6$ mg. per 100 g. body weight in the control group and $4.7 \pm .7$ in the nicotinic-acid-fed group.

TABLE 4. TOTAL CHOLESTEROL CONTENT OF SERUM, LIVER, AND RESIDUAL CARCASS.
42-DAY EXPERIMENTAL PERIOD—PAIRED WEIGHT GAIN *

Diet	Number of Rats	Initial Weight	Weight Gain	Total Cholesterol Content			
				Serum	Liver	Carcass	Total
		<i>g.</i>	<i>g.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Control	4	281	103 ± 9	8.7 ± 1.3	23.5 ± 2.6	501 ± 56	533.2 ± 57.9
Nicotinic acid	4	284	103 ± 2	9.0 ± 1.7	25.4 ± 2.1	508 ± 37	542.4 ± 40.2

* Values given are the mean and standard deviation, estimated using (n-1).

In none of the experiments did nicotinic acid significantly influence the incorporation of sodium acetate-1-C¹⁴ into serum and liver cholesterol.

Cholesterol Absorption. Twenty-four hours after the administration of cholesterol-4-C¹⁴ approximately 5 per cent of that administered was present in serum and liver (Table 2). There was no significant difference between the control and nicotinic acid groups.

Cholesterol Excretion. The incorporation of mevalonic acid-2-C¹⁴ into cholesterol was much more efficient than that of acetate, approximately 5 per cent of the administered C¹⁴ being present in serum and liver cholesterol 2 hours after its injection (Table 3). Six days later essentially identical amounts of labeled cholesterol remained in serum and liver of the control and nicotinic-acid-treated rats.

Total Rat Cholesterol. As compared to their weight gain paired controls, the four rats fed 1 per cent nicotinic acid for 42 days displayed no significant difference in total cholesterol content or in its distribution between serum, liver, and residual carcass (Table 4).

DISCUSSION

The addition of 1 per cent nicotinic acid to a balanced laboratory mash consistently exerted an inhibitory effect on weight gain. Although only an approximate measure of daily food intake was made, it was apparent that the nicotinic-acid-fed animals consumed less than the controls, and it is assumed that the decreased rate of growth was a consequence of decreased food intake. That it was not due to hypermetabolism is suggested by the results of the oxygen consumption measurements, the mean oxygen consumption of the nicotinic-acid-fed rats not differing significantly from that of the controls. This finding in the rat is in disagreement with the reported increase in basal metabolic rate of the human produced by nicotinic acid (5, 9).

As previously reported in a larger series (2), the mean serum cholesterol of rats fed nicotinic acid for 42 days was slightly but significantly lower than that of controls maintained on *ad libitum* diet. However, when the food intake of the controls was restricted so that their rate of weight gain was the same as that of the nicotinic-acid-fed animals, there was no significant difference in serum cholesterol levels. It would thus appear that in the rat the hypocholesterolemic effect of nicotinic acid is due at least in large part to its anorectic effect. That the value which best differentiates the nicotinic-acid-fed animals from their controls is the ratio of liver to serum cholesterol also suggests that the reduced serum cholesterol is the result of a shift of the plasma-liver pool of cholesterol from plasma to liver, rather than any net change in cholesterol balance.

No consistent effect of nicotinic acid on the incorporation of acetate-1-C¹⁴ into cholesterol was observed. The reason for the failure to observe the increased incorporation in the nicotinic-acid-fed groups reported by Merrill is not apparent (1). Since he reported only relative values for incorporation, exact comparison of the data is not possible.

The greater incorporation of acetate into cholesterol in both control and nicotinic-acid-fed animals in the experiment with paired weight gain may be the result of either their greater age and weight or of a difference in the experimental design. In the groups allowed *ad libitum* diet, no period of fasting was imposed, and the acetate was injected between 11:00 A.M. and noon. Because the control rats on restricted food intake consumed all their food by early evening, while the nicotinic-acid-fed rats continued to eat during the night, it was necessary to amend this procedure to avoid differences in acetate incorporation into cholesterol due to differences in immediate nutritional state of the animals. Both groups were fasted overnight prior to acetate injection. At 8:00 A.M. each animal

was given 5 g. of food, all of which was consumed by both control and nicotinic-acid-fed rats within 1 hour. Acetate was then injected 2 to 3 hours later.

With regard to the absorption of cholesterol, no acute effect of nicotinic acid was observed. Nor did the administration of nicotinic acid for 4 days have any effect on the disappearance of cholesterol-C¹⁴ from serum and liver. Assuming that the disappearance of tagged cholesterol from serum and liver is a valid reflection of disappearance of cholesterol from all the other body pools, this indicates a lack of nicotinic acid effect on cholesterol excretion.

That the more prolonged ingestion of nicotinic acid (42 days) has no measurable net effect on cholesterol balance in the rat, providing weight gain of experimental and control animals is matched, is indicated by the final experiment.

The hypocholesterolemic effect of nicotinic acid in man cannot be due solely to any effect it may have on quantity or type of food selected, since Goldsmith *et al.* have observed it to effect a lowering of serum cholesterol in patients maintained on constant diets

(10). It thus seems that the rat differs from man in its lack of response to nicotinic acid, provided diet is controlled.

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