EVALUATION OF NICOTINIC ACID AS AN HYPOCHOLESTEREMIC AND ANTI-ATHEROGENIC SUBSTANCE *

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Despite the gaps existing in our contemporary understanding of the origin, the function, the mode of regulation and even the eventual fate of plasma cholesterol, various drugs have been introduced and applied to lower this particular lipid in the blood stream of man. It is perhaps suggestive of the clinical eagerness to accomplish this lowering that the precise *modus operandi* by which any of these substances effect their reduction of cholesterol in blood still remains to be determined.

One of these substances is nicotinic acid. Its administration and subsequent cholesterol-lowering effect in clinical subjects was first reported by Altschul, Hoffer and Stephen (1), thereafter to be confirmed by Parsons and co-workers (2), and by Miller, Hamilton and Goldsmith (3). Administration of this substance to rats (4) and rabbits (5) also has been followed by a reduction in serum cholesterol. Thus there appears to be little doubt that the oral administration of nicotinic acid is capable of reducing the serum cholesterol of both the normo- and the hypercholesteremic subject and laboratory animal when no dietary control is exercised.

Interested in the possible mechanism of this action, we performed various studies upon both the rat and the rabbit. The results, as we shall indicate below, suggest that the chief efficacy of the drug in regard to the cholesterol dynamics of the rat and rabbit appears to reside in its anoretic properties.

METHODS

A. Effect of nicotinic acid upon intestinal absorption of cholesterol and lipid by the rat. Ten rats (Long Evans strain) were maintained for 12 days on regular Purina Lab Chow® to which nicotinic acid (1 per cent) had been added. At the end of this period, each rat was given 150 mg. of cholesterol in 3 ml. of olive oil, anesthetized, and the intestinal lymph duct was cannulated (6). Lymph was collected for 24 hours and then analyzed for total cholesterol (7) and total lipid (8). Ten control rats given only regular Purina Lab Chow® were subjected to the same procedures. Plasma samples obtained on the first and twelfth day were analyzed for total cholesterol (9).

B. Effect of nicotinic acid on biliary excretion of cholesterol and cholate in the rat. Ten rats fed regular Purina Lab Chow® containing nicotinic acid (1 per cent) for 14 days were subjected to cannulation of their bile duct and the 24 hour volume of bile was analyzed for total cholesterol (10). An analogous bile collection was done upon 10 control animals given Purina Lab Chow® alone.

For the determination of biliary excretion of bile cholate, five rats were fed Purina Lab Chow® containing nicotinic acid (0.5 per cent) and in addition given 50 mg. of nicotinic acid by stomach tube daily for three days. The bile duct was then cannulated and the 24 hour bile was collected and analyzed for cholate (11). Control bile collections were done with four animals fed only Purina Lab Chow®.

C. Effect of nicotinic acid upon plasma cholesterol of rats fed a high fat-cholesterol diet. Four groups of 10 rats each were studied. The first group of rats (controls) was allowed to ingest *ad libitum* a high fat-cholesterol diet[1] which was slightly modified from that described by Hartroft (12). The second group of rats was allowed to ingest *ad libitum* the same diet containing in addition nicotinic acid (1 per cent). The third and fourth groups, however, were fed differently because the earlier experiment had indicated to us that rats fed a diet containing nicotinic acid rarely ingest more than 5 to 10 Gm. of food per rat per day. To be certain, therefore, that the control rats were not ingesting more food than the rats given nicotinic acid, both groups were allowed to eat only 5 to 6 Gm. of food per rat per day. The food, however, ingested by the fourth group contained in addition nicotinic acid (1 per cent). It was observed that the daily ration invariably was consumed completely by both groups.

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[1] Butter, 40 per cent; casein, 20 per cent; cholesterol, 5 per cent; cellulose, 4 per cent; salt mixture, 4 per cent; vitamin mixture, 2 per cent; choline chloride, 1 per cent; thiouracil, 0.2 per cent; cholic acid, 0.3 per cent; sucrose: sufficient to make 100 per cent.

TABLE I

Effect of nicotinic acid on cholesterol and total lipid absorption

No. of rats	Average wt.		Plasma cholesterol		Lymph				
	Beg.	12 Days	Beg.	12 Days	Vol.	Total cholesterol		Total lipid	
	Gm.		*mg./100 ml.*			*mg./100 ml.*	*mg./24 hrs.*	*mg./100 ml.*	*mg./24 hrs.*
A. Rats given nicotinic acid									
10	293	290	52	39	44.0	59	24.5	3,522	1,444
S.E. of mean			±3.2	±1.9	±5.1	±5.2	±2.1	±103	±99
B. Control rats									
10	293	318	53	49	33.0	75	25.1	4,084	1,367
S.E. of mean			±2.54	±2.76	±1.7	±3.3	±1.9	±110	±84

The feeding program was continued for four weeks with weighings and plasma cholesterol determinations obtained weekly.

D. The effect of nicotinic acid upon plasma cholesterol and aortic atherosclerosis of rabbits fed a high fat-cholesterol diet. Twenty male rabbits, California Standard strain (approximately 10 weeks old), were given Purina Rabbit Chow® enriched with cottonseed oil (2 per cent) and cholesterol (1 per cent). Ten of these rabbits also received nicotinic acid (0.5 per cent) in their diet. Each series of animals was pair-fed as described previously (13) to ensure equality of ingestion. This program of feeding was continued for three months with weighings and plasma cholesterol determinations obtained monthly. At the end of three months, all animals were sacrificed. The aorta was assessed grossly for degree of atherosclerosis (13) and then an 8 cm. segment of aorta immediately distal to the attachment of the semilunar valves was analyzed for its cholesterol content (13).

RESULTS

A. Effect of nicotinic acid upon intestinal absorption of cholesterol and lipid of the rat

Rats given nicotinic acid in their regular chow for 12 days exhibited a moderate decline in their average serum cholesterol as compared to that of the controls (see Table I). Such rats, however, also failed to gain weight, an observation which led to the later dietary studies described below.

Regardless of the decline in serum cholesterol observed, the rats maintained on the nicotinic acid containing diet were found (see Table I) to absorb exogenously derived cholesterol and total lipid as well as the control animals.

B. Effect of nicotinic acid upon biliary excretion of cholesterol and cholate in the rat

Here again, the rats given the nicotinic acid containing diet for 14 days exhibited a slightly lower serum cholesterol and a lesser gain in weight than the controls (see Table II). However, the daily biliary excretion of cholesterol was almost identical in the experimental and control series.

Similarly, the biliary excretion of cholate in the rats given nicotinic acid for 72 hours prior to cannulation of the bile duct was approximately the same as that observed in the controls (see Table III). It is of course possible that the changes effected in cholate metabolism and excretion by the method of collection itself might have been of an order to obscure the possibly slight changes pro-

TABLE II

Effect of nicotinic acid on bile cholesterol

No. of rats	Average wt.		Plasma cholesterol		Bile		
	Beg.	14 Days	Beg.	14 Days	Volume	Cholesterol	
	Gm.		*mg./100 ml.*		*ml./24 hrs.*	*mg./100 ml.*	*mg./24 hrs.*
A. Rats given nicotinic acid							
10	295	310	56	47	19.5	15.7	3.0
S.E. of mean			±2.2	±3.5	±1.1	±1.6	±0.37
B. Control rats							
10	298	330	49	55	16.6	19.5	3.1
S.E. of mean			±3.0	±2.9	±1.7	±1.3	±0.21

TABLE III

Effect of nicotinic acid on bile cholate

No. of rats	Average weight	Bile		
		Vol.	Cholate	
	Gm.	*ml.*	*mg./100 ml.*	*mg./24 hrs.*
A. Rats on nicotinic acid				
5	290	18.4	212	39.0
S.E. of mean				(±1.3)
B. Control rats				
5	288	17.5	241	42.1
S.E. of mean				(±3.2)

duced by nicotinic acid administration. We believe, however, that this is most unlikely in view of the rather profound changes observed in cholate excretion in rats fed excess unsaturated oils (14) when studied by the same technique.

C. Effect of nicotinic acid upon plasma cholesterol of rats fed a high fat-cholesterol diet

Rather surprising results were observed in the feeding studies. Rats ingesting, *ad libitum,* the high lipid diet containing nicotinic acid exhibited throughout the period of feeding (see Table IV) a significantly reduced plasma cholesterol as compared with that observed in the control rats eating the same diet without nicotinic acid. However, these experimental rats were observed not only to eat less than the control rats but also to lose weight more sharply, and to fail to regain their original weight during the four weeks.

On the other hand, the series of rats that were pair-fed, and thus ingesting a limited quantity of this same diet plus nicotinic acid equal to that ingested by a control series of the same diet without nicotinic acid, after the first week exhibited (see Table IV) approximately the same weight and cholesterol changes as observed in the controls. Even the difference observed the first week is of doubtful statistical significance. Therefore, when intake of food was equalized, the addition of nicotinic acid did not exhibit a chronic hypocholesteremic effect.

D. Effect of nicotinic acid upon plasma cholesterol and aortic atherosclerosis of rabbits fed a high fat-cholesterol diet

Although it is not unusual for a third of a group of rabbits fed a high cholesterol-oil diet to succumb

TABLE IV

The effect of nicotinic acid on plasma cholesterol of rats given limited and unlimited intakes of high fat-cholesterol diet

Type of feeding	No. of rats	Av. daily food consumption throughout exp.	Onset		1 Week		2 Weeks		3 Weeks		4 Weeks	
			Wt.	Plasma cholest.	Wt.	Plasma cholest.	Wt.	Plasma cholest.	Wt.	Plasma cholest.	Wt.	Plasma cholest.
		Gm.	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*
1. Unlimited intake + 1% nicotinic acid	10	8.3	304	62	261	144	267	330	277	346	274	284
S.E. of mean				±4.1		±8.4		±10.4		±12.3		±24
2. Unlimited intake	10	12.8	295	63	280	190	302	515	304	559	302	480
S.E. of mean				±3.8		±12.2		±12.1		±14.2		±33
3. Limited intake + 1% nicotinic acid	10	5.8	287	56	246	110	218	194	240	242	238	358
S.E. of mean				±3.2		±8.6		±11.1		±8.2		±21
4. Limited intake	10	5.8	286	59	255	148	225	162	220	242	227	317
S.E. of mean				±4.2		±9.4		±8.3		±9.2		±40.0

TABLE V

The effect of nicotinic acid on plasma and aortic cholesterol of pair-fed rabbits given high fat-cholesterol

No. of rabbits		Weight and plasma cholesterol						Aorta	
		Onset		1 Month		2 Months		3 Months			
Beginning	End	Wt.	Plasma cholest.	Wt.	Plasma cholest.	Wt.	Plasma cholest.	Wt.	Plasma cholest.	Grade atherosel.	Cholesterol
		Gm.	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	*Gm.*	*mg./100 ml.*	(0–4)	*mg./100 Gm.*
A. Rabbits given 1% cholesterol—2% cottonseed oil + nicotinic acid (0.5%)											
10	5	1,712	71	2,089	387*	2,489	810*	2,510	749*	0.9	1,812*
S.E. of mean			±6.1		±39.6		±96		±51		±184
B. Rabbits given 1% cholesterol—2% cottonseed oil only											
10	7	1,668	76	2,106	429	2,244	1,035†	2,436	1,020†	0.8†	1,950†
S.E. of mean			±4.9		±69.1		±66		±157		±220

* Five surviving animals.
† Seven surviving animals.

to intercurrent infections during a period of three months, half of the rabbits fed the nicotinic enriched experimental diet died of intercurrent infections manifested chiefly by nasal discharge, middle ear disorder and weight loss. Only three of the 10 control rabbits similarly succumbed. Certainly it was our impression that the rabbits fed nicotinic acid, as a group, presented a far less healthy and vigorous appearance than the controls.

The series of rabbits ingesting the high lipid diet with nicotinic acid had a monthly average plasma cholesterol which was consistently lower than that of the control rabbits. However, this difference could not be adjudged significant when subjected to statistical analysis. Thus for the three successive months (see Table V) the standard error of the difference of means was only 0.52, 1.92 and 1.7 times the difference of means, respectively. Similarly, the gross degree of aortic atherosclerosis and the average cholesterol content of the aortic samples were approximately the same in both series.

DISCUSSION

The results of the above studies are remarkably similar to those we recently obtained (13) in our study of the mode of action and possible effectiveness of potassium iodide as a hypocholesteremic and anti-atherogenic agent. Both substances were found ineffective in influencing the intestinal absorption of either cholesterol or total lipid when both experimental and control animals were given *precisely* the same amount of cholesterol and olive oil by stomach tube. Apparently, then, the hypocholesteremic effect of nicotinic acid does not stem from any interference with the absorption of either cholesterol or total lipid.

Again, similar to potassium iodide, the administration of nicotinic acid to the rat was not observed to influence the hepatic rate of cholesterol turnover as determined by the biliary cholesterol assay method (15). We employed this technique, rather than any radioactive tracer method, because at the time this work was done such methods were still yielding equivocal results when used as an indicator of the rate of synthesis of cholesterol (16, 17). The conflicting findings of Duncan and Best (4), compared with those of Merrill (18) (both employed radioactive tracers), concerning the rate of hepatic synthesis of cholesterol after adminis-

tration of nicotinic acid, serve to stress this difficulty in employing tracers for this particular determination. Nicotinic acid also was not found to effect the biliary excretion of cholate in our rats.

Unlike the administration of potassium iodide, that of nicotinic acid was found to lower the plasma cholesterol slightly but significantly in normocholesteremic rats when ingesting ordinary laboratory rat chow *ad libitum*. Such rats, however, lost weight, a phenomenon we concluded to be due to their decreased intake of food. The importance of this latter phenomenon in effecting the comparative hypocholesteremia observed was made clear in our subsequent feeding experiments with high fat-high cholesterol diets. In these experiments, when the control rats were allowed to ingest no more food than the experimental rats, no sustained hypocholesteremic effect resulted from the administration of nicotinic acid. However, the comparative hypocholesteremic effect of nicotinic acid promptly reappeared in the series of experimental and control rats that were allowed to ingest *ad libitum* the same type of diet. But here again, the rats on the nicotinic acid regimen ate less and lost weight. The likely conclusion thus appeared to be that nicotinic acid was acting as a hypocholesteremic agent because of its anoretic properties.

Finally, the results of nicotinic acid administration in the rabbit appeared similar to those obtained after the administration of potassium iodide. When the intakes of both the experimental and control series were made the same, the hypocholesteremic effect of nicotinic acid did not appear to be statistically significant. Also, no prevention of atherosclerosis was observed in the experimental animals.

The preceding results are opposite to those found both by Altschul and associates (1) and Merrill and Lemley-Stone (5). Neither of these authors, however, pair-fed their animals, although Altschul administered equal quantities of cholesterol to both experimental and control animals. We believe that in their studies, the anoretic properties of nicotinic acid reduced the quantity of either cholesterol or fat taken in by their experimental animals, hence effected the plasma, hepatic and aortic cholesterol values observed. Certainly the comparatively small amount of excess cholesterol present in the liver of nicotinic-acid-fed-rabbits of Merrill and Lemley-Stone (5) suggests that these animals were absorbing less exogenous cholesterol because this organ, at least as observed in the rat (19, 20), serves as the initial site of deposition of intestinally absorbed excess cholesterol. It also should be stressed, as we earlier observed (13), that the weights of rabbits cannot be employed as an exact indicator of their food intake.

If, then, nicotinic acid appears to act as an anoretic agent in its relationship to hypocholesterolemia and subsequent prevention of atherosclerosis in the experimental animal, can it exert a more specific effect in human lipid metabolism? In this connection, although Altschul and co-workers (1) reported a fall in the serum cholesterol of man a few hours after administration of a single dose of nicotinic acid, this could not be confirmed by Parsons and associates (2). Moreover, the clinical studies to date of which we are aware (1–3, 21, 22) have not subjected the patient to a strictly controlled regimen in which the actual quantity of the food ingested before, during and after nicotinic acid administration has been accurately determined. In addition, the weight changes of these patients have not been published, although Galbraith, Perry and Beamish (22) state that, without giving any actual data, their series of subjects showed no weight changes. Finally, in view of the frequency with which nicotinic acid induces nausea and other gastrointestinal effects (2, 21), it is of paramount interest to determine any possible spontaneous *qualitative* changes in food intake induced by its administration. It is a well-known clinical truism that a potential nauseant operating possibly still at a subclinical level might induce a reversion to foods of a lighter, less lipid containing nature. If this possible factor were in action, then the moderate hypocholesteremia ensuing after the administration of nicotinic acid becomes readily understandable. Certainly the relatively tremendous quantity of nicotinic acid needed for effective lowering of plasma cholesterol militates against the view that it functions in its usually accepted vitamin function. Even more disturbing is the observation that its use appeared to be of doubtful or slight value in a severe hypercholesteremia stemming from an undoubted endogenous defect such as idiopathic familial hypercholesteremia with xanthoma tuberosum (2).

When substances which are potential, as well as actual, disturbants of the gastrointestinal sys-

tem are under investigation, scrutiny of the sequences of events occurring in this system is justified fully as much as more detailed investigations of intracellular mechanisms residing in the tissues of more distant organs.

SUMMARY

Ingestion of nicotinic acid was not found to alter the rate of intestinal absorption of cholesterol or total lipid in the rat. No change either was observed in the rate of hepatic synthesis of cholesterol (as measured by the biliary cholesterol assay method) or in the biliary secretion of bile acid in the rat administered nicotinic acid. Finally, when the intake of a high fat-cholesterol diet was controlled in both the treated and control animal, the ingestion of nicotinic acid did not hinder the expected onset of hypercholesteremia.

Rabbits pair-fed a high cholesterol-fat diet with and without nicotinic acid failed to exhibit a significant difference either in their average plasma cholesterol or in their degree of aortic atherosclerosis.

It is suggested that the previously observed hypocholesteremic and atherosclerosis preventing properties of nicotinic acid may be due to an anoretic effect. The possible influence of this substance both upon the quantity and the *quality* of food taken by human subjects eating under noncontrolled conditions is discussed.

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