cate that SKF 525-A has properties which differ markedly from those previously reported concerning the inhibition of microsomal detoxifying enzymes.

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Effect of Nicotinic Acid and Related Compounds on Incorporation of Mevalonic Acid into Cholesterol. (26565)

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The hypocholesterolemic effect of nicotinic acid administered orally to humans was first reported by Altschul *et al.*(1). Their results were confirmed by others(2,3). Nicotinic acid was later shown to lower serum cholesterol levels in cholesterol-fed rabbits(4), and a similar effect was observed in dogs(5). The mechanism by which nicotinic acid exerts the hypocholesterolemic effect has not been elucidated. Merrill(6) and later Hardy *et al.*(7) reported an increased incorporation of acetate-1-C^{14} into liver sterols in the presence of nicotinic acid, both *in vitro* and *in vivo*. However, Perry(8) found a decreased incorporation of acetate-2-C^{14} into cholesterol in rat liver slices incubated with nicotinic acid. Kritchevsky *et al.*(11) showed that nicotinic acid-treated rat liver mitochondrial preparations oxidize cholesterol to a greater extent than do similar preparations from control rats.

In the present experiments the effect of nicotinic acid and related compounds on incorporation of mevalonic acid-2-C^{14} (MVA-2-C^{14}) into non-saponifiable material (NSF, largely sterols) by rat liver homogenates was investigated. The afore-mentioned authors (6,7,8) had employed rat liver slices, while in the present studies rat liver homogenates were used. To permit comparison of our data with those of the other authors(6,7,8), the influence of nicotinic acid on conversion of acetate-1-C^{14} to sterols was tested in our system.

Methods and materials. The experimental procedure used to measure incorporation of MVA-2-C^{14} into sterols has been reported (9). Rat liver homogenates were prepared as described previously(9). Female and male rats weighing 200-360 g were employed. MVA-2-C^{14} (New England Nuclear Corp.) was isolated from the dibenzylethylenediamine salt (DBED); 0.5 mg MVA (0.05 μc) was added per flask. Where acetate was used, each flask contained 0.8 mg sodium acetate (1 μc). Nicotinic acid (General Biochemical Co.), isonicotinic acid, isonicotinic acid hydrazide, kynurenic acid, trigonelline, 6-OH nicotinic acid, and pyridine-3-sulfonic acid (the latter 6 compounds were obtained from Nutritional Biochemical Co.) were tested at levels of 1 mg/ml to 5 mg/ml. The excess acidity of these compounds was neutralized with KOH. Each flask contained 5 ml of rat liver homogenate, 1 mg ATP, 10 mg DPN, and 162 mg sodium succinate. Total volume per flask was 10 ml. Ribonuclease (RNAse), Worthington, 5 or 10 mg per flask and $CaCl_2 \cdot 2H_2O$ (7.5 mg per flask), where indicated, were used as "negative controls". Both of these materials have been shown previously to inhibit conversion of MVA to sterols(12,13). Homogenization and additions of ATP, DPN, and RNAse were at 4°C. The flasks were prepared by adding the homoge-

TABLE I. Effect of Various Compounds on Biosynthesis of NSF from MVA.

Exp.	Supplement	Amount, mg	NSF, cpm
1	None	—	4,370
	Nicotinic acid	1	4,259
	Idem	2	4,763
	"	5	4,557
	"	10	3,886
	"	20	3,914
	"	50	3,596
	Isonicotinic acid hydrazide	1	4,323
	Idem	2	4,326
	"	5	4,388
	"	10	5,533
	"	20	5,133
	"	50	6,109
	Pyridine-3-sulfonic acid	1	4,394
	Idem	10	4,233
	"	50	3,288
	Calcium chloride	7.5	3
2*	None	—	6,450
	Nicotinic acid	2	6,344
	Idem	10	6,290
	"	50	6,102
	Isonicotinic acid	1	6,284
	Idem	2	6,265
	"	5	6,347
	"	10	6,223
	"	20	6,179
	"	50	5,633
	Trigonelline	1	6,125
	"	10	6,321
	"	50	6,823
	6-OH Nicotinic acid	1	6,189
	Idem	10	6,177
	"	50	6,352
	Calcium chloride	7.5	0
3	None	—	4,394
	Nicotinic acid	2	4,115
	Idem	10	3,844
	"	50	2,973
	Kynurenic acid	2	4,572
	Idem	10	4,377
	"	50	2,519
	RNAse	5	181
	"	10	137

* No preincubation — substrate and compound tested were both added before incubation. Preincubation in all other experiments.

nate and above indicated constituents. They were aerated with O_2, stoppered tightly, and incubated at 37°C in a shaker bath (50 oscillations per min). Preincubation was for 30 min, after which the substrate was added and the aeration repeated. The flasks were then reincubated for 3 hours. When no preincubation was employed the flasks were incubated for 3½ hours. The reaction was stopped by addition of 10 ml of alcoholic KOH, and the mixtures saponified on a steam bath overnight. The NSF was extracted, and counted by means of a Packard Tricarb-Scintillation Counter as reported previously (10). The data are recorded as counts per min per flask.

Results. Nicotinic acid, isonicotinic acid, isonicotinic acid hydrazide, pyridine-3-sulfonic acid, trigonelline, and 6-OH nicotinic acid each had no significant effect on incorporation of MVA to NSF (Table I). At the highest level tested, 50 mg/flask in one experiment, a small decrease (about 25%) in incorporation of MVA was observed with nicotinic acid and pyridine-3-sulfonic acids. This effect is not considered significant since the decrease occurred only at this high level; moreover, in another experiment the same level of nicotinic acid had no effect (Table I).

Isonicotinic acid hydrazide seemed to cause a slight increase in MVA conversion to NSF at the higher levels (Table I). Kynurenic acid had no effect, except at the highest level, 50 mg/per flask in which case there was a decrease (Table I). RNAse and $CaCl_2$ were each tested in representative experiments and inhibition of MVA incorporation into NSF occurred with each compound (Table I). The data in Table II represent the averaged results of experiments with acetate-1-C^{14}. Considerable variation occurred in the observed values, hence the data were averaged. A 34% average decrease in acetate incorporation was observed at the 10 mg level of nicotinic acid. This finding is in agreement with that of Perry (8) who obtained approximately 25% reduction with the same level of nicotinic acid.

Discussion. Since it had been reported

TABLE II. Effect of Nicotinic Acid on Biosynthesis of NSF from Acetate-1-C^{14}.

Supplement	Amount, mg	NSF, cpm	Decrease in biosynthesis, %
Nicotinic acid	0	1032	0
Idem	1	1104	0
"	10	684	34
"	20	464	55
"	50	351	66

that nicotinic acid influences incorporation of acetate in sterols, it seemed desirable to determine whether or not nicotinic acid has any effect on conversion of MVA to NSF. The data indicate that nicotinic acid does not influence formation of sterols from MVA. Several nicotinic acid-related compounds likewise have little or no influence on MVA conversion to NSF. The previous experimenters had employed rat liver slices to investigate acetate incorporation into sterols. In the present experiments rat liver homogenates were used. Therefore the effect of nicotinic acid on acetate-1-C^{14} conversion to NSF was reinvestigated with homogenates. Considerable variation in ability of control homogenates to convert acetate to NSF was observed, in contrast to their ability to convert MVA to NSF, and consequently the data (Table II) were averaged. The resulting data indicated a decrease in amount of acetate converted to NSF as the concentration of nicotinic acid increased. These findings are in agreement with those of Perry who found a decrease in conversion of acetate (approx. 25%) to cholesterol using 1 mg per ml of nicotinic acid. Hardy *et al.* have reported an increase in acetate incorporation into sterols using 0.05 M nicotinic acid (6.15 mg/ml) but a decrease at 0.10 to 0.25 M (about 12 to 30 mg/ml) nicotinic acid. The present data indicate that the locus of the nicotinic acid effect on cholesterol metabolism, at least in homogenates, is before MVA or at some other position which indirectly influences cholesterol metabolism. The findings with acetate suggest that the effect takes place somewhere between acetate and MVA, since nicotinic acid inhibited acetate incorporation into NSF but does not inhibit MVA incorporation into NSF.

Summary. Nicotinic acid and related compounds (0.1 to 5 mg per ml) have no significant effect on conversion of mevalonic acid to non-saponifiable material by rat liver homogenates. The incorporation of acetate into non-saponifiable material (largely cholesterol) decreases with increasing amounts of nicotinic acid. At 1 mg per ml of nicotinic acid a 34% average decrease in biosynthesis was observed. The results suggest that the locus of the nicotinic acid effect may be somewhere between acetate and mevalonate.

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