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THE EFFECTS OF SQUALENE ON THE INCORPORATION OF ACETATE INTO PLASMA CHOLESTEROL IN MAN¹

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Squalene is an unsaturated C30 H50 hydrocarbon found in the liver of the basking shark, and in human sebum and skin. Its relationship to cholesterol metabolism was first noted by Channon (1), who, in 1926, observed an increase in the cholesterol content of the livers of rats maintained on a squalene diet. Simultaneously, Heilbron, Kamm, and Owens (2), while studying the structure of this material, postulated that squalene might be an intermediate in the biosynthesis of cholesterol. More recently, with the aid of radioisotopes, Srere (3) demonstrated a significant decrease in the conversion of C¹⁴-acetate

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to cholesterol in liver slices prepared from rats fed 1 per cent squalene. Langdon and Bloch (4, 5) confirmed and expanded these studies and concluded that since labeled squalene is rapidly and efficiently converted to cholesterol, suppression of acetate incorporation into this steroid is the result of the preferential utilization of squalene as a cholesterol precursor.

The present investigation was undertaken to elucidate the role of squalene in the synthesis of plasma cholesterol from acetate in man.

MATERIALS AND METHODS

This study was performed on eight subjects with limited life expectancy. All were considered to be in a good nutritional state and on the basis of clinical and laboratory criteria were free from apparent metabolic disturbances. Pertinent data on these patients are listed in Table I.

Two hundred microcuries of sodium acetate-1-C¹⁴ with a specific activity of 1.0 mc per mM were given orally in the postabsorptive state to all subjects.⁴ Patient

⁴ This was authorized by the Isotopes Division, U. S. Atomic Energy Commission.

	Patient	Patient Age S		Weight kilograms	Diagnosis	Total cholesterol mg. %		Free cholesterol <i>mg</i> . %	
A	F. J. A. M. B. F.	56 45 54	M M M	65.5 74.6 56	Carcinoma of lung Carcinoma of lung Carcinoma of lung	200 230 173		55 67 45	
						Before Squa	After llene	Before Squ	After alene
В	{H. F. {J. M. J. R.*	35 62 47	F M M	60 58 70	Carcinoma of breast Carcinoma of tonsil Carcinoma of lung	253 178 135	248 180 174	70 49 44	76 47 50
с	J. R.† N. D.	47 25	M M	70 55.5	Carcinoma of lung Functioning carcinoma of adrenal—removed	164 176	179 184	51 50	58 49
	J. G.	63	М	62.7	Carcinoma of colon	152	164	42	46

TABLE I Clinical and laboratory data

* Control study—Group B. † Repeat study—Group C.

J.R. was used as his own control and received a second dose of sodium acetate-1-C¹⁴ eight weeks after the initial dose. Since approximately 56 per cent of C¹⁴ given as acetate is eliminated as expired C¹⁴O₂ within the first 24 hours (6), it was felt that the radiation resulting

from the retained isotope was far below the accepted radiation tolerance level of 0.3 rep per week.

The subjects were divided into three groups:

Group A (Pts. F. J., A. M., B. F.) received 200 microcuries of sodium acetate-1-C⁴⁴.

	F . J.			А. М.			B. F.	
Time	Free	Ester	Time	Free	Ester	Time	Free	Ester
				Group A				
Hours			Hours			Hours		
1	509	26	1	—	82	1	851	119
2	576	93	2	1,105	75	2	903	218
8	420	197	8		269	8		420
24	384	249	24	672	278	24	773	513
40	348	280	40	605	377	40	498	550
48	337	295	48	511	400	48	402	430
34	290	301	54	400	442	54	437	420
Days			Days			Days		
3	258	290	3	472	467	3	379	384
5	218	259	_	_	—	5	337	380
7	176	208	7	300	329			
9	154	180	. 9	211	275			
13	125	150	13	179	199			
17	112	141	17	150	109			
22	104	125	22	141	139			
29	109	119	29		149			
				Group B				
	H. F.			J. M.			J. R.	
Hours			Hours			Hours		
1	1,121	52	1	511	39	1	882	93
2	1,354	83	2	724	99	2	1,012	88
8	701	207	8	402	135	8	033	280
24 19	121	300 430	44	312	233	2 4 48	430	332
54		430	5 4	238	260	48 54	343	374
Days			Days			Days		
3	456	374	3	220	239	3	358	327
4	342	370	4	206	206	4	270	301
5	259	332	5	170	210	5	249	260
7	228	305	7	135	167	7	197	` 234
9	238	270	9	134	151	9	166	239
11	202	238	11	110	142	11	156	173
14	181	215	14	110	121	14	151	161
17	176	155	17	85 80	109	17	141 140	154
	150	152			30		140	140
				Group C				
	J. R.	<u> </u>	····	N. D.			J. G.	
Hours 1	204	36	Hours 1	125	119	Hours	00	4.2
2	324	30 26	1	133	110	1	88 120	13
8	320 244	20	2	145	304	2	03	26
24	171	88	24	104	65	24	73	33
Davs			Dave			Davs		
2	145	104	2	125	78	2.5%	92	96
3	120	107	3	134	87	3	90	90 94
4	115	119	, 4	139		4	90 90	<u> </u>
7	97	111	7	69	112	7	84	91
10	75	84						

 TABLE II

 Specific activity* of plasma cholesterol

* Specific activity = disintegrations per mg. plasma cholesterol.

Group B (Pts. H. F., J. M., J. R.) were fed 25 grams of squalene per day in divided doses for five days beginning 72 hours after the administration of 200 microcuries of $1-C^{14}$ acetate.

Group C (Pts. J. R., N. D., J. G.) received 25 grams of squalene per day in divided doses for five days. On the morning of the fourth day of squalene feeding, 200 microcuries of sodium acetate- $1-C^{14}$ were given as described.

All subjects were maintained on normal dietary intakes during the course of the investigation.

Serial blood samples were taken in heparinized syringes at measured intervals beginning one hour after the administration of the acetate. In some cases the sampling continued for as long as 29 days. The specific activity of the plasma C¹⁴ cholesterol fractions was determined according to the method of Rosenfeld, Hellman, Considine, and Gallagher (7). Radioactive assay was done in a windowless flow proportional counter. All counts were corrected for self absorption and by the use of absolute standards, converted to disintegrations per minute per mgm. cholesterol. The standard error in the net sample measurement was no greater than 5 per cent. This corresponded to a 3.4 per cent probable error. Blood for measurements of quantitative plasma cholesterol fractions was obtained at the beginning of each study and analyzed by the method of Man and Peters (8). In the two squalene fed groups (B and C) the cholesterol content of the plasma was determined at the beginning and end of the feeding period.

The squalene administered in this study was redistilled 'natural' squalene ⁵ emulsified with an equal volume of acacia and flavored with spearmint. It was estimated that the quantities fed per day comprised approximately 4 per cent of the daily dietary solids. There were no ill effects following the ingestion of this substance.

RESULTS

The serial specific activities of the free and esterified plasma C^{14} cholesterol of the control subjects (Group A) are given in Table IIA. The variations in activity of a typical patient (A. M.) followed for twenty-nine days are depicted as a

⁵ Squalene supplied by Dr. Stanley Ames of Distillation Products Industries, Rochester, N. Y.



FIG. 1. SEMILOGARITHMIC PLOT OF THE INCORPORATION OF CARBON 14 INTO PLASMA FREE AND ESTERIFIED CHOLESTEROL FOLLOWING THE ADMINISTRATION OF 200 MICROCURIES OF ACE-TATE-1-C¹⁴ TO SUBJECT A. M. OF GROUP A

	Patient	% in free cholesterol*	% in ester chol estero l†	% in total cholesterol	
A	{F. J. 8. F. A. M.	0.212 0.227 0.565	0.0905 0.1600 0.0992	0.3025 0.3870 0.6640	Groups A and B Bange: 0 3025 to 0 6930
B	{H. F. {J. M J. R.	0.603 0.209 0.322	0.0931 0.0755 0.0575	0.6930 0.2845 0.3775	Average $\%$ in total circulating plasma cholesterol = 0.4514
с	{J. R. {N. D. J. G.	0.1198 0.0503 0.0342	0.0234 0.0298 0.0036	0.1428 0.0798 0.0376	Group C Range: 0.0376 to 0.1428 Average % in total circulating plasma cholesterol = 0.0867

TABLE III Acetate utilization in the biosynthesis of plasma cholesterol

* Per cent incorporation of radioactivity into total circulating free cholesterol at two hours.

† Per cent incorporation of radioactivity into total circulating ester cholesterol at two hours.

semilogarithmic plot in Figure 1. These curves closely resemble those described by Hellman, Rosenfeld, and Gallagher (9) using similar techniques. In this series, the peak activity of the plasma free C¹⁴ cholesterol invariably occurred in the two-hour sample. The peak activity for the ester C¹⁴ cholesterol fraction was usually reached between two and two and one-half days. At this point, the specific activity curve of the ester cholesterol intersected that of the free cholesterol and then diminished at a comparable rate of decay.

In the Group B subjects who received squalene three days after the administration of $1-C^{14}$ acetate, the curves of the serial specific activities of the free and esterified plasma cholesterol (Table IIB) were essentially identical with those of the control group. It should be noted that squalene was not introduced until maximum labeling of both cholesterol fractions had already occurred and at a point where the activities of these steroids were decaying in an exponential fashion.

The most striking results were noted in the subjects who had ingested squalene prior to and after the administration of radioactive acetate (Table IIC). In comparison with Groups A and B, there was an average five-fold decrease in the per cent of acetate-1-C¹⁴ entering the total circulating plasma as labeled cholesterol at two hours (Table III). This reduction in incorporation varied from two to twenty-three-fold.

In subject J. R. who served as his own control (Figure 2) a two and one-half-fold decrease was seen at two hours. At the intersects, when the activity of the free cholesterol begins to decay at a rate comparable to that of the ester, similar degrees of depression prevailed.

Patients in Group C, particularly N. D. and J. G., exhibited atypical specific activity curves. This probably reflects irregularities in squalene absorption from the gastrointestinal tract with subsequent alterations in size of the squalene pool.

In four of the six patients who received squalene, small rises in the total plasma cholesterol were seen (Table I). Except for J. R., these results are of doubtful significance. However, these findings are in the same direction as those of Srere (3) who observed elevations of total cholesterol in the plasma of squalene fed dogs.

DISCUSSION

Acetate molecules apparently provide the major source of carbon for the synthesis of cholesterol in man and animal; however the mechanism by which 2 C fragments condense to form cholesterol precursors of high molecular weights has only recently begun to be understood. Although Zabin and Bloch (10, 11) showed that carbon atoms derived from isovalerate and butyrate were efficiently incorporated into the sterol nucleus, these compounds are not true intermediates since the conversion probably took place through the formation of "active acetate." The isoprenoid triterpene squalene has been proposed as an intermediate of cholesterol (1-3). Langdon and Bloch (4, 5) isolated radioactive squalene from the tissues of squalene fed rats who received C14 labeled acetate. When this tagged substance, which had all the biochemical characteristics of natural squalene, was fed to rats, it was found to be the most potent cholesterol precursor known to date.

If the absorption data obtained from these ex-

periments are applied to our studies (see Appendix), it can be roughly estimated that about 20 per cent of the liver cholesterol formed during the feeding period was derived from squalene. Since squalene has not as yet been isolated from the liver of humans, if it exists as an obligatory intermediate, it must occur in very small quantities and turn over with great rapidity. Thus in the subjects who were prefed squalene (Group C) the two-to twenty-three-fold depression in incorporation of acetate to cholesterol may be attributed to a dual mechanism. Exogenous squalene probably dilutes the "higher intermediate" pool between acetate and cholesterol thus displacing acetate as a source of cholesterol and permitting it to be rerouted and utilized along other metabolic path-



FIG. 2. SEMILOGARITHMIC PLOT OF THE INCORPORATION OF CARBON 14 INTO PLASMA FREE AND ESTERIFIED CHOLESTEROL OF PATIENT J. R. FOLLOWING THE ADMINISTRATION OF 200 MICROCURIES OF ACETATE-1-C⁴⁴ Showing the Effect of Prefeeding with Squalene

ways. Moreover the plasma cholesterol rose slightly in four of the six subjects receiving squalene. Since the liver is the major source of plasma cholesterol (14) it seems reasonable to assume that similar changes occurred in this organ. Indeed, both Channon and Srere (1, 3) observed considerable increases in the total hepatic cholesterol content of squalene fed rats. Squalene, by causing a "piling up" of hepatic cholesterol, may therefore evoke a homeostatic reduction in cholesterol synthesis from all sources (15).

The lack of appreciable changes in the specific activities of carbon ¹⁴ cholesterol in the patients who comprised Group B can be readily explained. Since these subjects received their squalene at a time when all of the available radioactive acetate had already been assimilated into the cholesterol molecule, no depression in peak activities could occur. Moreover, the increases in cholesterol caused by five days of squalene feeding at the 4 per cent dose level were insufficient to produce a detectable "dilution effect." The segment of the curve that is decaying exponentially (about the 4th to the 15th day) thus remains unaltered.

SUMMARY

The daily administration of 4 per cent squalene for five days to three subjects who received sodium acetate- $1-C^{14}$ in the middle of the feeding period resulted in a two- to twenty-three-fold depression in the synthesis of C^{14} plasma cholesterol from this 2 carbon fragment.

Suppression of acetate incorporation by squalene feeding is probably the result of dilution of the higher intermediate pool between acetate and cholesterol.

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APPENDIX

An approximation of the quantity of liver cholesterol derived from squalene in man

50 per cent or 12.5 gm.
8 per cent or 1.0 gm.
ave. 4.04 gm.
83.8 per cent or 3.38 gm.
0.84 gm.

In comparison to dogs, if it is assumed that the delay in appearance of the peak specific activity in the plasma free cholesterol in man is also reflected in the hepatic fraction, then the half life of the free cholesterol in the liver of man is approximately twice that found in dogs (13) or about 14 hours.

Thus, the turnover time $\overline{T} = t_{1/2}^{1/2} \times k$

$$\overline{T} = 14$$
 hrs. $\times 1.44 = 20$ hours

then per cent turnover per hour $= 1/\overline{T}$ or 5 per cent; therefore the absolute turnover expressed as mg. of free cholesterol of the liver = 5 per cent of 3.38 grams or 169 mg. per hr. or 4.06 grams per day.

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