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Research Article

THE EFFECTS OF SQUALENE ON THE INCORPORATION OF ACETATE INTO PLASMA CHOLESTEROL IN MAN [1]

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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 C^{14}-acetate 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-C^{14} with a specific activity of 1.0 mc per mM were given orally in the postabsorptive state to all subjects.[4] Patient

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[2] This article represents work done in part as a James Hudson Brown Memorial Junior Fellow, in fulfillment of the thesis requirement for the degree of Doctor of Medicine at the Yale University School of Medicine.

[3] U. S. Public Health Service Postdoctorate Research Fellow, National Heart Institute, 1952–1954. At present, Postdoctorate Research Fellow of the National Foundation for Infantile Paralysis.

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

TABLE I

Clinical and laboratory data

	Patient	Age	Sex	Weight *kilograms*	Diagnosis	Total cholesterol *mg. %*		Free cholesterol *mg. %*	
A	F. J.	56	M	65.5	Carcinoma of lung	200		55	
	A. M.	45	M	74.6	Carcinoma of lung	230		67	
	B. F.	54	M	56	Carcinoma of lung	173		45	
						Before Squalene	After Squalene	Before Squalene	After Squalene
B	H. F.	35	F	60	Carcinoma of breast	253	248	70	76
	J. M.	62	M	58	Carcinoma of tonsil	178	180	49	47
	J. R.*	47	M	70	Carcinoma of lung	135	174	44	50
C	J. R.†	47	M	70	Carcinoma of lung	164	179	51	58
	N. D.	25	M	55.5	Functioning carcinoma of adrenal—removed 1 month prior to study	176	184	50	49
	J. G.	63	M	62.7	Carcinoma of colon	152	164	42	46

* 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-C^{14} eight weeks after the initial dose. Since approximately 56 per cent of C^{14} given as acetate is eliminated as expired $C^{14}O_2$ 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-C^{14}.

TABLE II

Specific activity of plasma cholesterol*

F. J.			A. M.			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	406	48	462	436
54	290	301	54	480	442	54	457	426
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	169			
22	104	125	22	141	159			
29	109	119	29	130	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	—	207	8	402	135	8	633	286
24	721	368	24	312	235	24	436	332
48	477	430	48	222	276	48	363	327
54	—	430	54	238	260	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	109	17	141	154
22	150	152	22	80	90	22	140	148
Group C								
J. R.			N. D.			J. G.		
Hours			*Hours*			*Hours*		
1	324	36	1	135	118	1	88	13
2	326	26	2	176	42	2	129	5
8	244	6	8	145	304	8	93	26
24	171	88	24	104	65	24	73	33
Days			*Days*			*Days*		
2	145	104	2	125	78	2	92	96
3	120	107	3	134	87	3	90	94
4	115	119	4	139	91	4	90	99
7	97	111	7	69	112	7	84	91
10	75	84						

* 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 C^{14} 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.

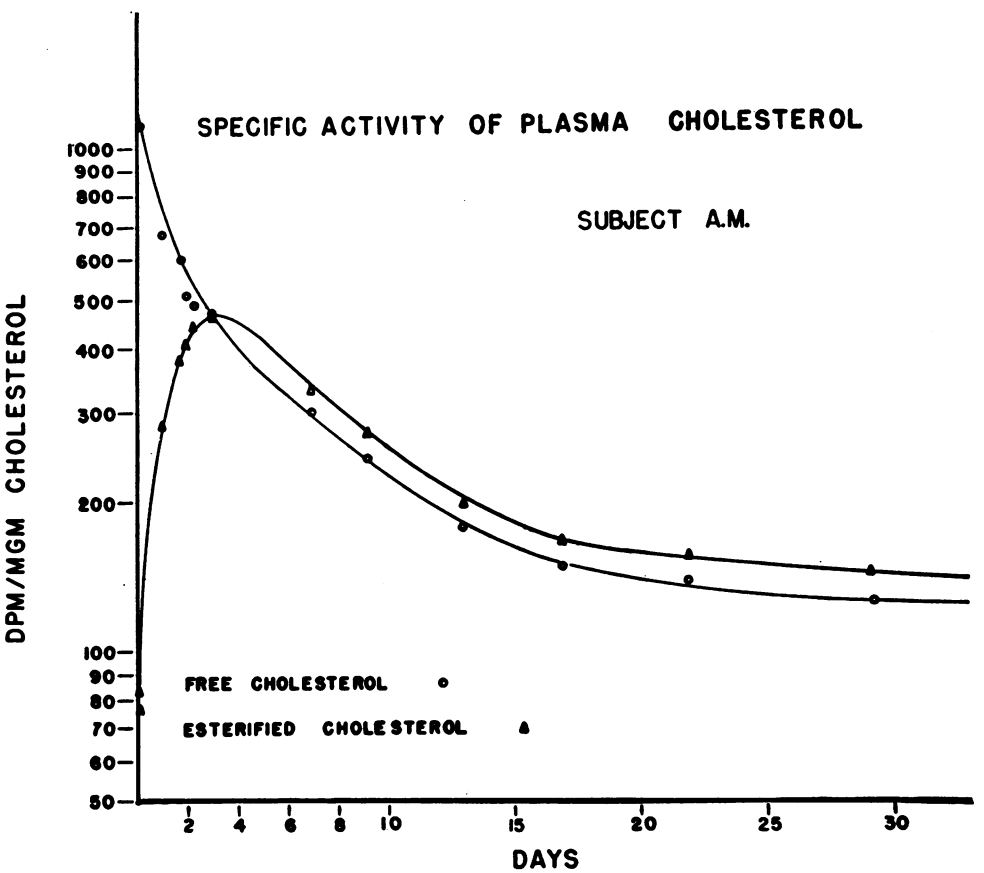

FIG. 1. SEMILOGARITHMIC PLOT OF THE INCORPORATION OF CARBON 14 INTO PLASMA FREE AND ESTERIFIED CHOLESTEROL FOLLOWING THE ADMINISTRATION OF 200 MICROCURIES OF ACETATE-1-C^{14} TO SUBJECT A. M. OF GROUP A

TABLE III

Acetate utilization in the biosynthesis of plasma cholesterol

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

* 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 C^{14} cholesterol invariably occurred in the two-hour sample. The peak activity for the ester C^{14} 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-C^{14} 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 C^{14} 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-

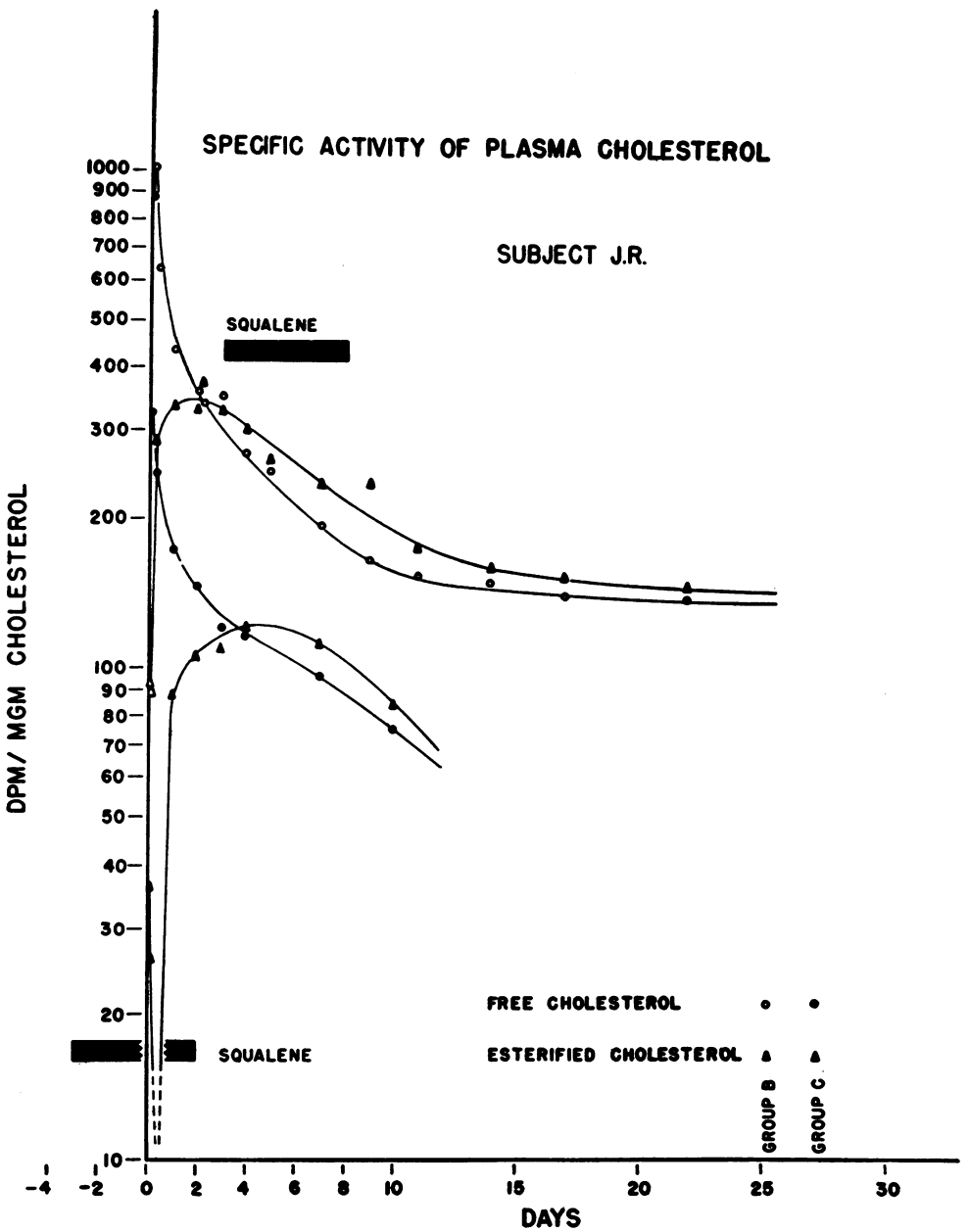

FIG. 2. SEMILOGARITHMIC PLOT OF THE INCORPORATION OF CARBON 14 INTO PLASMA FREE AND ESTERIFIED CHOLESTEROL OF PATIENT J. R. FOLLOWING THE ADMINISTRATION OF 200 MICROCURIES OF ACETATE-1-C^{14} SHOWING THE EFFECT OF PREFEEDING WITH SQUALENE

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

Absorption of squalene from GI tract (5)	50 per cent or 12.5 gm.
Per cent of absorbed squalene going to liver cholesterol (5)	8 per cent or 1.0 gm.
Total liver cholesterol in man—two cases at necropsy (12)	ave. 4.04 gm.
Per cent of total liver cholesterol as the free fraction (12)	83.8 per cent or 3.38 gm.
Quantity of free cholesterol derived from squalene	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 $\overline{T} = t_{1/2} \times k$

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

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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