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Biosynthesis of Squalene and Cholesterol in the Fish

I. *In Vitro* Studies on Acetate Incorporation

Mitsu KAYAMA, S. Zafar W. RIZVI and Suezo ASAKAWA

Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

It has well been recognized that squalene is an important intermediate in the biosynthesis of cholesterol[1]. The intact rats[2,3], liver slices[4-6] and cell-free preparations [7-10] were capable of synthesizing radioactive cholesterol from ^{14}C-labelled acetate. BUCHER and MCGARRAHAN[9] carried out the experiment on the biosynthesis of cholesterol from acetate-1-^{14}C using rat liver homogenate, and reported the need of coenzymes and oxygen supply. The cofactor requirements were also demonstrated by POPJÁK *et al.*[10], while carrying out the synthesis of squalene and cholesterol from DL-3-hydroxy-3-methyl-(2-^{14}C)-pentano-5-lactone.

It must be noticed that, although the key intermediate squalene was first found in some shark liver oils[11], most of the experiments were performed on the liver of land animals, such as rats and mice. Hereupon, in the present study the authors used the liver or hepatopancreas of fish, such as rainbow trout and carp, to determine the cholesterogenesis of aquatic animals, and to understand the interesting phenomenon of deep sea shark liver oils, in which extraordinary high amounts of squalene are present while maintaining only slightly elevated cholesterol levels[12]. The liver homogenate supernatant of mice was also used as that of a typical land animal to compare with fish concerning the incorporation of acetate into the lipids. The coenzymes as well as molecular oxygen requirements were examined under aerobic conditions.

MATERIALS AND METHODS

The following abbreviations will be used in addition to those noted in the text: adenosine triphosphate (ATP); nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP) and its reduced form (NADPH).

Chemicals. The coenzymes (ATP, NAD, NADP, NADPH) of the Sigma Chemical Co., sodium acetate-1-^{14}C of the Daiichi Pure Chemicals Co., and silica gel G of the E. Merck AG. were purchased. Squalene was isolated from shark liver oil with a silicic acid (Mallinckrodt, 100 mesh) column after saponification, as described previously[12,13]. Lanosterol and cholesterol were obtained from the Katayama Chemical Co.

Animals. The one year old colored carp and rainbow trout, which had hatched out last April to May at the Umeda's carp breeding pond and last January

at the Kumano freshwater hatchery of Hiroshima University, originally derived from the stock fish and weighing approximately 100 g to 200 g, were sacrificed by cutting the caudal part. Besides the fish, NC strain mice were killed for source of the livers.

Preparation of homogenates. The hepatopancreases or livers taken out by the disembowelment from the anus of fish were weighed and rinsed *in situ* for a few seconds with ice-cold solution containing 0.044 *M* potassium phosphate buffer, pH 7.4, 0.028 *M* nicotinamide, 0.007 *M* magnesium chloride, and 0.126 *M* sucrose, and then homogenized for 30 seconds in a very loose-fitting chilled glass homogenizer containing 2.5 volumes of the same medium. By low speed centrifugation of 350 to 500 *g* for 5 minutes, the unbroken hepatic cells and tissue debris were eliminated. The resulted pink colored supernatant was used in all the incubation studies.

Incubation. In all the instances the incubation flask contained 2 m*l* of homogenate supernatant plus 20 μc of acetate-1-^{14}C with different combinations of cofactors dissolved in 20 μl buffer solution each as specified in the tables. The flasks were incubated aerobically under oxygen and anaerobically under nitrogen streams for different time intervals at 30°C in a Taiyo Monoshin shaker, Taiyo Bussan Co., Tokyo.

Extraction of lipids. Soon after the incubation, the enzymic reactions were terminated and the lipids were extracted with the addition of chloroform - methanol (2 m*l*, 1:1, v/v) into the flasks. The contents were stirred with a glass rod for about 1 minute, followed by the addition of another 2 m*l* portion of chloroform and 2 m*l* of distilled water, and shaken well. Then the whole content was transferred to the centrifuge tube and centrifuged for 3 minutes to separate hypophasic chloroform layer. The extract was then evaporated to dryness to obtain the lipids.

Saponification. A few mg of the lipids were dissolved in 1m*l* of 5% alcoholic potassium hydroxide under nitrogen stream, and saponified over night at room temperature. The hydrolysate was diluted with 1m*l* of distilled water and the nonsaponifiable materials were extracted three times by the successive addition of 1.5 m*l* of petroleum ether. The combined petroleum ether fraction was washed with 3×1.5 m*l* of distilled water, dehydrated with anhydrous magnesium sulphate, and the solvent was removed under nitrogen stream. From the remaining hydrolysate the mixed fatty acids were extracted after acidifying the solution.

Counting of radioactivity. The radioactivities were measured at the Faculty of Agriculture, Tohoku University by a liquid scintillation spectrometer with an efficiency of approximately 90% each time for ^{14}C. Aliquots of samples were dried in the low-potassium vials and dissolved in 15 m*l* of toluene containing 45mg of 2, 5-diphenyloxazole (PPO) and 4.5 mg of 1, 4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). All the vials and scintillators were obtained from Packard Instrument Co.

Thin-layer chromatography and radiochromatography. The glass plates of 5×20 cm were coated with a layer of silica gel G about 250 μ in thickness, dried at 120°C for 30 minutes, and washed with chloroform - methanol (2:1). The total lipids or nonsaponifiable materials were diluted to around 5% in chloroform, and

were spotted on the plates in a 1-cm-wide line at 2.5 cm from the glass bottom edge. The spotted plates were developed with petroleum ether - diethyl ether - acetic acid (87.5:12.5:1, v/v/v). After drying, the plates were scanned on an Aloka thin-layer radiochromatogram scanner (Nippon Musen, Model JTC-201). The scanning was carried out at the speed of 12.5 mm/min with the range of 300-10 K cpm scale and 10 seconds of time constant. The flow rate of Q-gas was about 150 bubbles per minutes. The plates were then sprayed with 50% H_2SO_4 solution and charred. The detected spots coincided with the peaks obtained from scanning.

RESULTS

Three sets of experiments were performed on the homogenate supernatant of carp hepatopancreas. The requirement of cofactors was observed for the synthesis of both squalene and sterols from acetate-1-^{14}C.

Table 1. Incorporation of acetate-1-^{14}C into lipids, squalene and sterols by homogenate preparation of carp hepatopancreas.

Addition	Condition	Total activity incorporated into lipids (Percentage incorporation)		Radioactivity (Percentage activity in lipids) present in			
				Squalene		Sterols	
		$\times 10^3$dpm	%	$\times 10^3$dpm	%	$\times 10^3$dpm	%
ATP+NAD+NADP	Aerobic	1,767	(4.0)	37	(2.1)	103	(5.8)
ATP+NAD	Aerobic	5,256	(11.8)	185	(3.5)	108	(2.1)
ATP+NAD+NADP	Anaerobic	2,686	(6.1)	142	(5.3)	66	(2.5)
ATP+NAD	Anaerobic	1,920	(4.3)	361	(18.8)	158	(8.2)
None, boiled*	Aerobic	10	(0.02)	—		—	
None, boiled*	Anaerobic	12	(0.03)	—		—	

Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14} C, 2 m*l* of homogenate supernatant (34.4 mg of protein/m*l*) of carp hepatopancreas, and the following coenzymes as indicated: ATP, 2.5 μmoles; NAD, 0,5 μmole; and NADP, 0.5 μmole. The total volume in each flask was 2.1 m*l*, and the reaction mixture was incubated at 30°C for 4 hr under oxygen or nitrogen streams.
* These flasks were kept in boiling water for 1 minute. Almost negligible activity was observed.

The data listed in the table show that the sterol synthesis is rather high when the cofactors ATP, NAD and NADP are added under aerobic condition. However, if NADP is not added in the incubation the percentage incorporation into sterols decreases, although there is an increase in squalene synthesis. This explains that NADP favours the conversion of squalene to cholesterol. Under anaerobic condition the synthesis of squalene was expected to be high[9], and it is found that squalene synthesized under anaerobic condition and in the absence of NADP was maximum i.e. the highest count of 361×10^3 dpm. Considering from the conversion ratio (cholesterol activity to squalene activity), it is apparent that the conversion from squalene to cholesterol proceeds rapidly under aerobic condition in comparison with anaerobic. This shows that molecular oxygen is required for the cyclization of

squalene to sterols[10, 14, 15] though the squalene formation can be carried out under anaerobic condition.

Table 2. Incorporation of acetate-1-^{14}C into lipids, squalene and sterols by homogenate preparation of carp hepatopancreas with different incubation time.

Incubation time	Total activity incorporated into lipids (Percentage incorporation)		Radioactivity (Percentage activity in lipids) present in			
			Squalene		Sterols	
hr	$\times 10^3$ dpm	%	$\times 10^3$ dpm	%	$\times 10^3$ dpm	%
2	1,638	(3.7)	8	(0.5)	202	(12.3)
4	2,614	(5.9)	26	(1.0)	376	(14.4)
8	1,896	(4.3)	32	(1.7)	235	(12.4)
16	1,967	(4.4)	26	(1.3)	218	(11.1)

Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14}C, 2 m*l* of homogenate supernatant (34.4 mg of protein/m*l*) of carp hepatopancreas, and the following coenzymes: ATP, 2.5 μ moles; NAD, 0.5 μmole; and NADP, 0.5 μmole. The total volume in each flask was 2.1 m*l*, and the reaction mixture was incubated at 30°C for the indicated hours under aerobic condition.

Next experiment was carried out to find the effect of incubation time as shown in Table 2. It is noteworthy that 4 hours incubation shows the maximum activity of lipids and also of sterols. The percentage incorporation of sterols slightly decreased as the incubation period increased, while the correspondent squalene synthesis showed a little increase with the incubation time. This may be explained on the basis that the synthesis of squalene could be continued because of the formation of acetate pool due to the oxidation of the fatty acids which were majorly synthesized. It can be thus considered from the above data that the cholesterol synthesis proceeds rather fast under aerobic conditions even in the fish.

In the following experiment, the effects of NADP and its reduced form NADPH, which is supposed to be one of the most important coenzymes for cholesterogenesis, were compared. The incubation was carried out under aerobic condition for 8 hours. The results are listed in Table 3.

The addition of the reduced form favours the cyclization of squalene to cholesterol. As to the lipids the separation between squalene and sterol esters was not so good enough to be measured respectively, however, in the unsaponifiable matter the relation of squalene and sterols could be indicated for reason of the complete separation between them. The percentage activity 12.8% of squalene in the NADP flask was reduced to 8.1% in the NADPH flask, and oppositely 40.3% of sterols was increased to 47.0%.

Another set of experiments was performed using the rainbow trout livers. The homogenate supernatants were incubated for different time intervals with acetate-1-^{14}C and coenzymes under aerobic and anaerobic conditions as shown in Table 4.

It will be of interest to note that under aerobic conditions the synthesis of sterols is in direct proportion to the duration of incubation. The percentage activity of sterols

Table 3. Distribution of percentage activity in various lipid classes synthesized from acetate-1-^{14}C by homogenate preparation of carp hepatopancreas with different combination of NADP and NADPH.

Addition	Total activity incorporated into lipids (Percentage incorporation)		Lipids, %						Nonsaponifiable*, %			
			Phos.	Part. glyc.	St.	F.A.	Trig.	Sq. + St. est.	Polar lipids	St.	F.A.	Sq.
	$\times 10^3$ dpm	%										
NADP	1,228	(2.8)	27.6	6.1	1.6	25.7	31.4	7.7	21.1	40.3	25.8	12.8
NADPH	1,122	(2.5)	35.4	7.9	1.0	33.7	20.0	2.0	33.2	47.0	11.7	8.1

Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14}C, 2 m*l* of homogenate supernatant (34.4 mg of protein/m*l*) of carp hepatopancreas, and the following coenzymes: ATP, 2.5 μ moles; NAD, 0.5 μmole ; and NADP, 0.5 μmole or NADPH, 0.5 μmole as indicated. The total volume in each flask was 2.1m*l*, and the reaction mixture was incubated at 30°C for 8 hr under aerobic condition. Abbreviations: Phos., phospholipids; Part. glyc., partial glycerides; St., sterols; F.A., fatty acids; Trig., triglycerides ; Sq., squalene; St. est., sterol esters.

* Approximately 15% radioactivity of the lipids were found in the nonsaponifiable fraction, in which still some activities due to free fatty acids and polar lipids were present.

Table 4. Incorporation of acetate-1-^{14}C into lipids and nonsaponifiable materials by homogenate preparation of rainbow trout liver, and activity distribution of squalene and sterols in nonsaponifiable fraction.

Incubation time	Condition	Total activity incorporated into lipids (Percentage incorporation)	Total activity of fatty acids (Percent of lipids)	Nonsaponifiable materials*		
				Total activity (Percent of lipids)	Squalene (Percent in nonsap. mat.)	Sterols (Percent in nonsap. mat.)
hr		$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm
4	Aerobic	20,875 (47.0%)	17,654 (84.6%)	3,221 (15.4%)	1,891 (58.7%)	544 (16.9%)
4	Anaerobic	4,234 (9.5%)	4,067 (96.1%)	167 (3.9%)	87 (52.1%)	29 (17.1%)
8	Aerobic	16,449 (37.1%)	9,763 (59.4%)	6,686 (40.6%)	3,664 (54.8%)	1,518 (22.7%)
8	Anaerobic	4,900 (11.0%)	4,111 (83.9%)	789 (16.1%)	337 (42.7%)	115 (14.6%)
16	Aerobic	18,254 (41.1%)	12,787 (70.1%)	5,468 (29.9%)	2,493 (45.6%)	1,487 (27.2%)
16	Anaerobic	5,027 (11.3%)	3,342 (66.5%)	1,686 (33.5%)	234 (13.9%)	319 (18.9%)

Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14}C, 2 m*l* of homogenate supernatant (61.3 mg of protein/m*l*) of rainbow trout liver, and the following coenzymes: ATP, 2.5 μ moles; NAD, 0.5 μmole; and NADP, 0.5 μmole. The total volume in each flask was 2.1 m*l*, and the reaction mixture was incubated at 30°C for the indicated hours under oxygen or nitrogen streams.

* Still some activities due to free fatty acids and polar lipids were present in this fraction.

rose from 16.9% in 4 hours to 22.7% in 8 hours and to 27.2% in 16 hours, while there was a corrspondent fall in the percentage activity of squalene from 58.7% to 54.8% and to 45.6%. It may be of further interest to note that 8 hours incubation under the flow of oxygen (aerobic condition) provides the best condition for the

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	$\times 10^3$ dpm	%										
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				Total activity (Percent of lipids)	Squalene (Percent in nonsap. mat.)	Sterols (Percent in nonsap. mat.)
hr		$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm
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Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14}C, 2 m*l* of homogenate supernatant (61.3 mg of protein/m*l*) of rainbow trout liver, and the following coenzymes: ATP, 2.5 μ moles; NAD, 0.5 μmole; and NADP, 0.5 μmole. The total volume in each flask was 2.1 m*l*, and the reaction mixture was incubated at 30°C for the indicated hours under oxygen or nitrogen streams.

* Still some activities due to free fatty acids and polar lipids were present in this fraction.

rose from 16.9% in 4 hours to 22.7% in 8 hours and to 27.2% in 16 hours, while there was a corrspondent fall in the percentage activity of squalene from 58.7% to 54.8% and to 45.6%. It may be of further interest to note that 8 hours incubation under the flow of oxygen (aerobic condition) provides the best condition for the

incorporation of acetate into the unsaponifiable materials in this incubation system. The total activity of unsaponifiable fraction, in which there remained some activities ($100-54.8+22.7=22.5\%$) due to the fatty acid residues and polar lipids, was 40.6% (31.5% on the base of the fatty acids and polar lipids free, the highest in the fish experiment, and the same was true with the acetate incorporation into squalene and

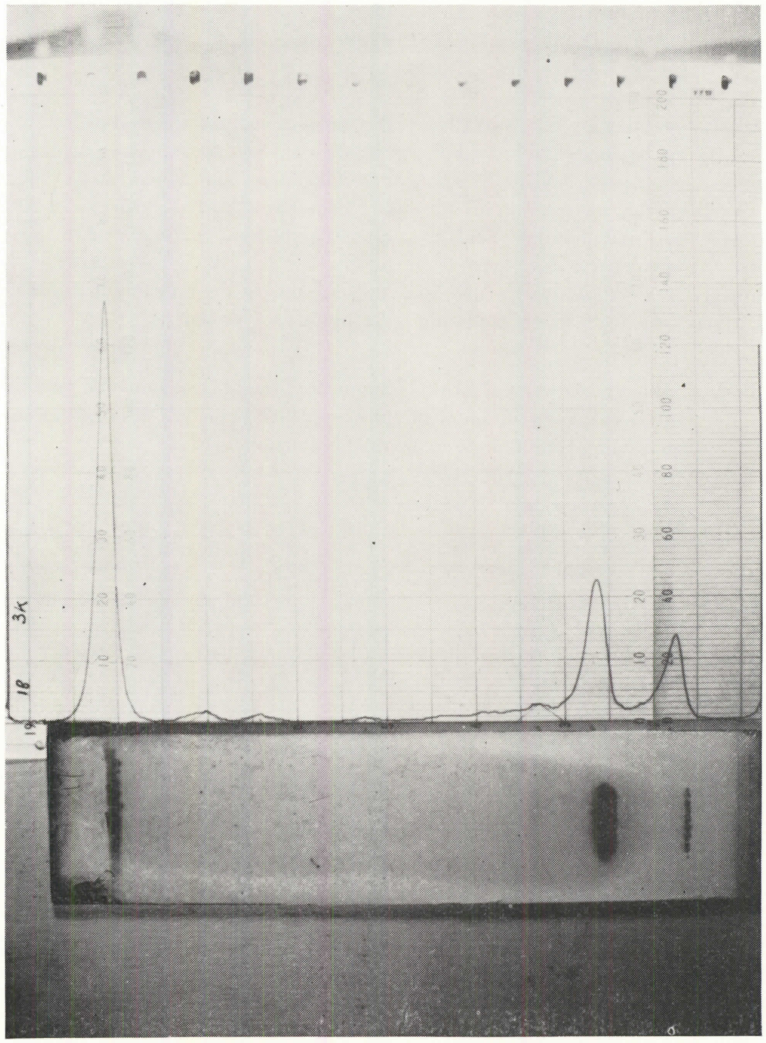

Fig. 1. Radiochromatogram and thin-layer chromatogram of the unsaponifiable fraction obtained from an incubation mixture of rainbow trout liver homogenate with sodium acetate-1-^{14}C and cofactors, as indicated in Table 4. The reaction mixture was incubated for 8 hours at 30°C under aerobic condition. The chromatogram was developed on silica gel G layers in a solvent system of petroleum ether-diethyl ether-acetic acid (87.5: 12.5: 1, V/V/V). Charring with sulphuric acid was carried out after scanning on the radiochromatogram scanner. Scanning condition for radiochromatogram: high voltage, 1080 V; full scale, 3000 cpm; time constant, 10 sec; scanning speed, 12.5 mm/min; chart speed, 12.5 mm/min; slit width, 1.5 mm.

sterols. It might be further mentioned from the table that anaerobic conditions did not favour the synthesis of both squalene and sterols, although squalene formation was relatively appreciable during 8 hours incubation.

A representative thin-layer chromatogram and its radiochromatogram of the unsaponifiable fraction obtained from the rainbow trout liver incubation is presented in Fig. 1.

Several peaks can be observed in the plate: a high squalene peak around the solvent front; a quite large peak located at sterols which consist mostly of cholesterol; between these two major peaks a few small peaks derived from possibly oxidosqualene, free fatty acids acids and lanosterol *etc.*; and a fairly large peak due to polar lipids near the original spotted line. It clearly appears from this figure that *de novo* synthesis of squalene and cholesterol does occur even in the liver homogenate of fish.

Finally, the same type of experiments were carried out using the liver homogenate supernatant of mice to compare the incorporation of acetate into the lipids for aquatic and terrestrial animals. The results the authors obtained from the twice experiments were quite reproduciable, and were in due accordance with the results using rat liver homogenate of other studies[7-10] except for the poor incorporation owing to the lower incubation temperature of 30°C.

Table 5. Incorporation of acetate-1-^{14}C into lipids and nonsaponifiable materials by homogenate preparation of mouse liver, and activity distribution of squalene and sterols in nonsaponifiable fraction.

Addition	Condition	Total activity incorporated into lipids (Percentage incorporation)	Total activity of fatty acids (Percent of lipids)	Nonsaponifiable materials*		
				Total activity (Percent of lipids)	Squalene (Percent in nonsap. mat.)	Sterols (Percent in nonsap. mat.)
		$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm	$\times 10^3$ dpm
All cofactors	Aerobic	106 (0.2%)	29 (27.4%)	77 (72.6%)	2.7 (3.5%)	64.8 (84.2%)
No cofactors	Aerobic	43 (0.1%)	18 (41.8%)	25 (58.2%)	2.0 (8.0%)	15.3 (61.2%)
All cofactors	Anaerobic	33 (0.07%)	21 (63.6%)	12 (36.4%)	7.9 (65.8%)	2.1 (17.5%)
No cofactors	Anaerobic	17 (0.04%)	9 (52.9%)	8 (47.1%)	—	—

Each incubation mixture contained 20 μc (0.66 μmole) of sodium acetate-1-^{14}C, 2 m*l* of homogenate supernatant (76.9 mg of protein/m*l*) of mouse liver, with or without following cofactors: ATP, 2.5 μmoles; NAD, 0.5 μmole; and NADP, 0.5 μmole. The total volume in each flask was 2.1 m*l*, and the reaction mixture was incubated at 30°C for 4 hr under oxygen or nitrogen streams.

* Still some activities due to free fatty acids and polar lipids were present in this fraction.

The data listed in Table 5 show that under aerobic conditions with the addition of cofactors, ATP, NAD and NADP, the sterol synthesis was at a maximum of 84.2% (64.8×10^3 dpm) and the squalene at a minimum 3.5% (2.7×10^3 dpm) of the nonsaponifiable matter. Moreover, without the addition of cofactors, the acetate

incorporation lowers down, not only into the total lipids but also into the squalene or sterols, which proves the need of cofactors. In comparison with the aerobic conditions, under anaerobic conditions with the cofactors, the formation of squalene showed an increasing tendency in the activity percentage. Thus squalene percentage was 65.8% (7.9×10^3 dpm), while sterols was only 17.5% (2.1×10^3 dpm). Under the same conditions, without adding the cofactors, the total activity of lipids and thus of both fatty acids and unsaponifiable fractions decreased considerably. In this case almost no peaks were obtained during the scanning process, showing negligible activity in squalene and sterols.

DISCUSSION

For the three kinds of animals with different environmental temperature, all the incubations of homogenate supernatants were performed at the constant temperature. The incorporation of acetate-1-^{14}C into the total lipids was very active in the rainbow trout liver homogenate, mediocre in the carp, and poor in the mouse, at 30°C. It must be considered that there might be an optimum temperature in the incubation of homogenate of the respective animals, however, the incorporation percentage of acetate into the rainbow trout lipids was remarkable. In the mouse the comparatively lower temperature incubation resulted in rather poor incorporation. In any case, the data obtained from the present study as well as from the mevalonic-2-^{14}C acid of us[16] show that the homogenate supernatant not only of mouse liver but also of fish, such as carp hepatopancreas and rainbow trout liver, can synthesize sterols *via* squalene, although the cholesterol biosynthesis is heavily blocked between squalene and its cyclization to lanosterol.

The pathways from acetyl CoA to polyketides and isoprenoids diverge at the early stage. The balance between these important biosynthetic pathways is at least partly controlled by two reactions: on the one hand, the carboxylation of acetyl CoA to malonyl CoA, and on the other, the reduction of hydroxymethyl-glutaryl CoA to mevalonic acid[17]. In the fish represented by carp and rainbow trout it is conceivable that *de novo* synthesis of the fatty acids exceeds the sterol formation. Therefore, though the high incorporation of acetate into the total lipids was measured, most of the activity was present in the saponifiable fraction, and comparatively little in the nonsaponifiable materials in which squalene and cholesterol are contained. In the mouse, on the contrary the cholesterogenesis surpasses the lipogenesis.

In general, the cofactor requirement for cholesterol synthesis in fish is not markedly different from those required by liver homogenate of land animals. Moreover, it is difficult to obtain clearcut evidence in a crude multi-enzyme system, such as the one used by us, as to the reactions in which the pyridine nucleotides participate. Nevertheless, the reduced form such as NADPH effects the cyclization of squalene to lanosterol under the existence of molecular oxygen. The introduction of oxygen at C_3 of squalene gives an intermediate 2,3-oxidosqualene which is capable of cyclization from squalene to lanosterol[14,15].

In our previous experiment[13] on the incorporation of acetate-1-^{14}C into lipid classes of liver oil by intact leopard shark, acetate actually incorporated into the various lipid classes. The metabolic relation between squalene and cholesterol would be justified also in leopard shark by considering the specific activities, high in the former and relatively low in the latter, measured in both fractions. However, the fact that some deep sea sharks contain large amounts of squalene and a little cholesterol in their liver oils must be explained by their environmental factors[12]. It is supposed from the present study that as one of the cofactors affecting on the cholesterogenesis the dissolved oxygen in deep sea is less than the surface layer, hence the cyclization of squalene to sterols must be heavily blocked.

SUMMARY

The biosynthesis of squalene and cholesterol from acetate-l-^{14}C has been studied in the homogenate supernatant of carp hepatopancreas, rainbow trout liver and mouse liver. The incubation was carried out with the addition of ATP, NAD, NADP and NADPH at 30°C for certain periods of time under aerobic and anaerobic conditions.

It was shown that the incorporation of acetate-1-^{14}C into the total lipids was very active in the preparation of rainbow trout liver, less active in carp hepatopancreas, and low in mouse liver. In the fish preparation represented by carp and rainbow trout, the *de novo* synthesis of the fatty acids exceeds of the sterols. On the contrary, the cholesterogenesis surpasses the lipogenesis in the mouse liver preparation.

In general, the coenzyme requirements for the cholesterol synthesis in the fish are not markedly different from those needed by land animals.

As one of the cofactors required for the cyclization of squalene to lanosterol, the molecular oxygen is very important as shown in the case of aerobic condition. Moreover, it was discussed why certain deep sea sharks contain extraordinarily high amounts of squalene and little cholesterol in their liver oils.

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魚類におけるスクワレンおよびコレステロールの生合成

I. アセテートのとり込みに関する *in vitro* の研究

鹿山　光・S. Z. W. リズビイ・浅川末三

アセテート -1-^{14}C からのスクワレンおよびコレステロールの生合成に関し，コイの肝膵臓，ニジマス肝臓およびマウス肝臓のホモジネート上澄液を用いて研究した．補酵素 ATP, NAD, NADP および NADPH を添加し，30°C で好気的・嫌気的条件下に培養を行なった．

アセテートの総脂質中へのとり込みは，ニジマス標品を用いた場合は非常に活潑で，コイ標品でもかなりな程度行なわれたが，マウス標品では著しく低い結果を得た．コイおよびニジマスで代表される魚類標品での脂肪酸合成はステロール合成を凌駕していたが，マウス標品では逆にコレステロール合成が優れていた．

一般的に魚類におけるコレステロール合成に関する補酵素要求性は陸上動物のそれとあまり相違してはいない．

スクワレンからコレステロールへの閉環に関し要求される補助因子の一つとして，好気的条件下の合成にみられたように，分子状酸素が重要に作用する．さらに，深海産のサメ肝油中にみられる異常な程の大量なスクワレンとそれ程でもないコレステロールの含量に関して考察を加えた．