XCVIII. THE EFFECT OF THE ADMINISTRATION OF SQUALENE AND OTHER HYDROCARBONS ON CHOLESTEROL METABOLISM IN THE RAT

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IN a previous paper [Channon, 1926] it was reported that the inclusion of the hydrocarbon squalene in the diet of rats caused the amount of cholesterol in the livers to be approximately doubled. Two suggestions were made to account for this increase; either that the presence of the hydrocarbon in the liver from which it could be isolated caused a compensatory increase in the amount of liver sterol for reasons of physico-chemical nature, or alternatively that squalene was acting as a precursor of cholesterol. This latter possibility was mentioned at the same time by Heilbron, Kamm & Owens [1926] as a result of work concerned with the chemical structure of squalene. The more recently acquired knowledge of the structure both of squalene and cholesterol has led to further theoretical consideration of the problem of the conversion of the former into the latter. Thus Vanghelovici [1934] suggested that it was possible for cholesterol to be derived from carbohydrate through the intermediate stages of saturated and unsaturated fatty acids and terpenoid hydrocarbons, while Robinson [1934] pointed out that squalene contains the skeleton of cholesterol intact.

The rapidly increasing knowledge of the physiological importance of the large variety of compounds closely related to the sterols and polyene hydrocarbons led us to reinvestigate the question of the possible conversion of squalene into cholesterol. The method adopted has been to feed groups of rats on diets containing squalene and other compounds for varying periods and to study the sterol contents of the livers, carcasses and faeces with a view to relating any total sterol increase with the amount of squalene absorbed.

EXPERIMENTAL

A constant basal diet consisting of casein 20, wholemeal flour 50, beef fat 19, cod liver oil 1, marmite 5 and salt mixture 5 parts was used in all the experiments. Various supplements were incorporated in the diets after being stirred into the molten fat and the animals were allowed food *ad lib*. At the end of each experiment the animals were killed by guillotine in order to obtain the livers as equally free from blood as possible, and the gastro-intestinal tracts were removed.

The faeces were collected daily, stored under alcohol and ultimately extracted exhaustively with alcohol followed by ether. The material obtained by evaporation of the combined extracts to dryness *in vacuo* was saponified with *N* sodium ethoxide. The unsaponifiable fraction was then prepared in the usual way and its i.v. and sterol content determined by the methods of Rosenmund & Kuhnhehn [1923] and Windaus [1910] respectively.

The amount of each supplement ingested was determined by weighing the food remaining uneaten after the provision of a known weight at the beginning of each day. The excretion was estimated by a number of methods.

(a) In experiments in which different hydrocarbons were used, the difference in weights between the non-sterol fraction of the unsaponifiable matter of the faeces of the experimental and control groups was taken as the amount of the hydrocarbon excreted.

(b) The figure so obtained was confirmed by calculation of the amount of hydrocarbon excreted from the relationship

$$\text{wt. of hydrocarbon excreted} = \frac{\text{wt. of exp. non-sterol fraction } (I_e - I_c)}{I_s - I_c},$$

where I_e , I_c and I_s are the i.v. of the non-sterol fractions of the experimental and control groups and of the supplement respectively.

(c) In the case of groups which received cholesterol as a supplement, the amount of food sterol excreted was calculated as the difference between the weights of sterol in the faeces of the experimental and those of the control groups as determined by digitonin precipitation.

The method of preparation of the unsaponifiable fractions of the livers and carcasses varied in the different experiments. In Exps. 1 and 3 the livers were digested in 5% aqueous NaOH. The crude extract obtained by ethereal extraction was resaponified with *N* sodium ethoxide and the unsaponifiable fraction analysed by the usual methods. In the remaining experiments the livers were extracted by the alcohol-ether method, the extracts so obtained being saponified under strictly standard conditions as regards strength of ethoxide and time of heating, in order to minimize any possible effect of these factors on the amount of the sterol fractions.

In Exps. 1, 2 and 3, the "fat" of the carcasses was obtained by ethereal extraction of an aqueous alkaline (5% NaOH) digest, and the unsaponifiable fraction prepared as usual. In later experiments, the minced carcasses were extracted with alcohol followed by ether and the extract so obtained was saponified under the same standard conditions as already described for the liver extracts.

In order to present the extensive data as concisely as possible, all the figures recorded for the weights of sterol in the livers and carcasses, the amounts of the supplements ingested and excreted in the faeces daily, together with the faecal sterol excretion per day, have been calculated on the basis of the 100 g. rat. This method makes the assumption that the amount of unsaponifiable fraction excreted daily on a constant diet is proportional to the body weight. Study of all the results suggests that this assumption is probably justified. On the other hand this method of calculation will give for the supplement intakes figures which will not be strictly comparable, since the food intake of larger animals will not be strictly proportionally greater than that of smaller ones. This factor, however, appears to play no part in the interpretation of the results. The amount of sterol in the liver is thus calculated as the number of mg. per 100 g. of fresh liver multiplied by the average percentage which the liver constituted of the final body weight. The daily faecal excretion is the total sterol excretion in each experiment, divided by the number of 100 g. rat days (the 100 g. rat day is the mean body weight multiplied by the number of days of the experiment and divided by 100). The tables also record the average final body weights of the animals, the number of animals, the period of experiment and the liver as a percentage of the body weight. The recorded absorption of each supplement is

the difference between the weights of the substance ingested and excreted. In order to eliminate the factor of individual variation, the pooled tissues of groups of usually 10 animals were used in each experiment.

Exp. 1. The first experiment was carried out in order to obtain a sterol balance sheet after squalene feeding over varying periods of time. Since all subsequent experiments were carried out over 21 days, the result for that period only will be recorded.

The diet of the animals which received squalene contained 1 % of the hydrocarbon for the first 12 days and 0.5 % for the subsequent 9 days. The results are presented in Table I.

Table I. *Sterol balance in rats fed with squalene*

All figures are calculated on the basis of the 100 g. rat.

Period of feeding days	Group	No. of animals	Average final body wt. g.	Liver % of final body wt.	Sterol			Squalene	
					Carcass mg.	Liver mg.	Faeces mg./day	Absorption mg./day	Excretion mg./day
21	Control	5	184	4.15	167.4	11.05	4.84	—	—
	Squalene	5	193	4.98	153.5	26.34	8.66	28.2	38.8

Table I shows that the liver cholesterol has been increased by squalene feeding by 138 %, a figure which may be compared with increases previously obtained for the same period (102.5 % [Channon, 1926] and 66.2 % obtained by Devine [1934] in unpublished results from this laboratory). Further, the amount of sterol excreted by the control animals, 4.84 mg. per rat per day, has been increased by some 80 % as a result of squalene feeding. Consideration of the carcass figures shows that such difference as appears between the control and experimental groups is too small to be of significance.

Disregarding then the change in carcass sterol, squalene administration has caused an increase of 15.3 mg. in liver sterol and an increased excretion of faecal sterol of 3.82 mg. per day, i.e. 80.2 mg. over 21 days. During this period the average animal absorbed 28.2 mg. squalene per day. Thus an increased production of 95.5 mg. sterol has resulted from the absorption of 592 mg. squalene and squalene has thus apparently given rise to about one-sixth of its weight of sterol. It could be reasonably argued that if squalene were converted into sterol in molecular proportion, the sterol so produced would in part be broken down or excreted, for Page & Menschick [1932] have shown the ability of the animal to catabolize cholesterol. If an amount beyond this degradative capacity were produced, it might be stored in the liver, giving effectively the same result as cholesterol feeding, which many workers have shown to cause excess cholesterol to appear in the liver, with the production of the "cholesterol" fatty liver.

Exp. 2. In the second experiment therefore one group of animals served as a control, a second group received squalene, while two further groups received different percentages of cholesterol in the diet. The object was to attempt to equate the rise in liver sterol caused by squalene feeding with that caused by a known daily intake of cholesterol. While the squalene used had been carefully prepared by distillation from the unsaponifiable fraction of *Scymnorhinus lichia*, followed by chromatographic purification with alumina, the further precaution was yet taken of exhaustively extracting with hot methyl alcohol to remove possible traces of cholesterol.

Table II shows that the absorption of no more than 9.25 mg. of squalene per day has caused an increase of 5.95 mg. sterol in the liver, a 55 % increase on the

Table II. *Sterol balance in rats fed with squalene or cholesterol (21 days)*

All figures are calculated on the basis of the 100 g. rat

Group	No. of animals	Average final body wt. g.	Liver % of final body wt.	Sterol			Supplement	
				Carcass mg.	Liver mg.	Faeces mg./day	Absorption mg./day	Excretion mg./day
Control	10	151	3.99	178.9	11.09	5.1	—	—
0.25% squalene	10	164	4.47	199.3	17.04	5.0	9.25	1.65
0.05% cholesterol	10	159	4.55	199.6	14.49	5.6	2.82	0.71
0.2% cholesterol	10	164	4.53	190.6	19.42	6.9	9.46	2.13

control figure. The increases in liver sterol caused by the daily absorption of 2.82 and 9.46 mg. of cholesterol are 3.40 and 7.33 mg. respectively. This increase in liver sterol caused by 9.25 mg. of squalene thus lies between that caused by 2.82 and 9.46 mg. of cholesterol and approximates more closely to that caused by the latter amount. This implies a conversion of squalene into cholesterol in something approaching molar proportions. Two further points need mention. Firstly, the absorption of squalene in this experiment has caused no increase in the amount of the faecal sterol excretion, a result which is in contrast to the finding in Exp. 1. This point will be elucidated later, but meanwhile it is to be emphasized that the daily squalene excretion in Exp. 1 was 38.77 mg., 24 times greater than that in the present experiment. Secondly, it appears that in both the groups receiving cholesterol, some of the administered cholesterol was unabsorbed.

Investigation was made of the nature of the cholesterol increase in the liver by analysis of the total ethereal extract. The amounts of free cholesterol in the control, squalene and two cholesterol groups were 9.3, 9.2, 9.7 and 10.9 mg. per 100 g. rat respectively, while the amounts of cholesteryl ester were 1.8, 7.9, 4.9, 8.2 mg. These findings show firstly that the increase in liver sterol caused by squalene is confined to the ester fraction, and secondly the close resemblance of the figures obtained by squalene administration to those resulting from the absorption of 9.46 mg. cholesterol. The changes in the amount of carcass sterol are regarded as insignificant for reasons mentioned later.

At this stage therefore the evidence seemed to be increasingly great that squalene was converted into cholesterol in the tissues of the rat and the next experiment was designed to equate more accurately the increase in liver sterol caused by varying amounts of squalene with that resulting from different amounts of cholesterol. The results did not, however, provide the answer sought and accordingly a further method of approach was investigated.

Exps. 3 and 4. If squalene were converted into cholesterol by a process of direct cyclization, hydrogenation of the squalene would prevent the conversion, unless the rat possessed the ability to desaturate the hydrogenated terpene. Accordingly Exps. 3 and 4 were carried out in order to compare the increases in sterol caused by the administration of similar amounts of squalene and hydro-squalene. The degree of hydrogenation adopted was decided from consideration of the known influences of mol. wt. and i.v. on the degree of absorption of hydrocarbons generally. It appeared unlikely that fully hydrogenated squalene, squalane, would be absorbed at all and therefore squalene was hydrogenated to the point where the product had i.v. approximately 100; such material would be a mixture of partially hydrogenated squalenes and no unhydrogenated squalene would be present [Heilbron, Hilditch & Kamm, 1926].

Table III. *Sterol balance in rats fed with squalene or hydrosqualene (Exps. 3 and 4, 21 days)*

Group	No. of animals	Average final body wt. g.	Liver % of final body wt.	Sterol			Supplement	
				Carcass mg.	Liver mg.	Faeces mg./day	Absorption mg./day	Excretion mg./day
Control	9	271	3.82	158.6	13.3	5.1	—	—
0.5% squalene	10	263	3.81	141.3	15.2	6.4	11.3	8.45
0.5% hydro-squalene	9	255	3.58	154.7	12.1	6.6	3.2	19.3
Control	10	226	3.39	—	9.46	5.3	—	—
1% squalene	10	224	3.48	—	13.08	5.9	17.5	28.7
1% hydrosqualene	10	230	3.38	—	9.73	5.9	1.4	46.1

The findings recorded in Table III show that the apparent daily absorption of hydrosqualene, 3.2 mg., is much less than that of squalene itself, 11.3 mg. This finding, so far from nullifying the effect of the experiment, has thrown considerable light on the problem. The absorption of 11.3 mg. of squalene has increased the liver sterol to 15.2 mg., whereas the absorption of 3.2 mg. of hydrosqualene has if anything caused a decrease. In contrast to the different influences of the two compounds on the amount of liver sterol, their effects on the amount of faecal sterol have been identical, for the control excretion, 5.1 mg., has been increased to 6.4 mg. by squalene and 6.6 mg. by hydrosqualene. Because the hydrosqualene liver figure, 12.1 mg. was below that of the control, 13.3 mg., by an amount which appeared possibly outside experimental error, this experiment was repeated in Exp. 4. Its results confirm those of Exp. 3 in showing that whereas both hydrocarbons cause similar increases in faecal sterol excretion, squalene alone causes the liver sterol to increase.

Exps. 3 and 4 therefore suggest that the increase in liver sterol caused by squalene administration is to be dissociated from the rise in faecal sterol, for hydrosqualene is unabsorbed and yet causes the same increase in faecal sterol as does squalene itself, without causing any increase in liver sterol. This naturally suggested that the presence of unabsorbed hydrocarbons in the intestine was the cause of the increased faecal excretion, and that their effect was independent of their chemical nature.

Exp. 5. This experiment was carried out therefore to confirm this possibility by the use of a chemically dissimilar hydrocarbon, *n*-hexadecane, which was prepared from cetyl alcohol. At the same time opportunity was taken to investigate the possibility that some contaminant present in the squalene was the effective agent causing the increase in liver sterol, rather than the squalene itself. For this experiment, 6 groups of 10 animals each received the following diets: (a) control diet; (b) to (f) received supplements of 1% of the following hydrocarbons: (b) "pure" squalene; (c) insoluble fraction obtained after exhaustive extraction of "pure" squalene with methyl alcohol; (d) squalene fraction soluble in methyl alcohol; (e) hexadecane; (f) hydrosqualene (i.v. 100). In this experiment the carcasses, livers and faeces were extracted by the alcohol-ether method and the unsaponifiable fraction prepared under standard conditions, refluxing with *N*/2 sodium ethoxide for 1½ hr. The results are recorded in Table IV.

Table IV shows that the administration of each of the three squalene preparations has caused a significant increase in the liver sterol. While the effects of the different preparations on the liver sterol show some variation, the

Table IV. *Sterol balance in rats fed with various squalene preparations, hydrosqualene or hexadecane (Exp. 5, 21 days)*

Group	No. of animals	Average final body wt. g.	Liver % of final body wt.	Sterol			Supplement	
				Carcass mg.	Liver mg.	Faeces mg./day	Absorption mg./day	Excretion mg./day
Control	9	232	3.40	186.5	9.53	5.4	—	—
1% puresqualene	10	218	3.39	186.1	13.93	6.4	17.0	27.8
1% MeOH-insoluble	10	225	3.72	179.8	13.27	7.0	18.4	28.0
1% MeOH-soluble	10	219	3.57	184.9	14.98	6.6	22.0	24.5
1% hexadecane	10	204	3.75	193.4	10.21	6.9	40.2	10.1
1% hydrosqualene	5	222	3.38	175.1	9.29	6.7	3.9	43.8

variations, the maximum of which is 1.71 mg., are small. These results suggest therefore that the squalene effect on the liver is not due to a contaminant. In contrast, neither hexadecane nor hydrosqualene has caused an increase in liver sterol. These results thus confirm the previous findings that, whereas squalene causes an increase in liver sterol, hydrosqualene fails to do so, and they show that hexadecane behaves like hydrosqualene in this respect. The faecal sterol figures also confirm the previous observations for squalene and hydrosqualene and they show in addition that hexadecane also causes an increased faecal excretion. This latter result is confirmed by previous work [Mahdi & Channon, 1933], although in the absence of knowledge at that time as to the constancy of faecal excretion, the increase was not discussed. Table IV also shows that none of the substances administered has significantly affected the amount of carcass sterol.

DISCUSSION

The results of Exps. 3, 4 and 5 appear to show clearly that the increased faecal sterol excretion occurs irrespective of the chemical nature of the hydrocarbon administered, for squalene alone causes an increase in liver sterol, and possible causes of these faecal increases will now be discussed. The simplest explanation of this finding would be that the hydrocarbons exercised a solvent action on cholesterol in their passage through the intestinal tract, and that the increased sterol appearing in the faeces is sterol which would normally have been absorbed. It was of interest therefore to attempt to determine the solubility of cholesterol in the hydrocarbons used. The low solubilities of cholesterol in squalene, hydrogenated squalene and hexadecane, as well as the relative insolubility of these hydrocarbons in the 95% alcohol used for the digitonin precipitation, render figures obtained by the digitonin method somewhat uncertain. As a check on these, determinations of the i.v. of solutions of cholesterol in hexadecane and hydrogenated squalene were carried out. This method will be accurate for the former hydrocarbon, of some value with the latter, but is inapplicable to squalene itself (i.v. 371). Excess of finely powdered cholesterol was therefore shaken with each hydrocarbon for 16 hr. at 37°. The solubilities determined on the filtrates are recorded in Table V.

Table V. *Solubility of cholesterol in squalene, hydrosqualene and hexadecane at 37°*

Hydrocarbon	(a) i.v. method %	(b) Digitonin method %
Squalene	—	2.57
Hydrosqualene	2.16	1.37
Hexadecane	1.96	1.92

While the figures for the first two hydrocarbons are not accurate because of the difficulties mentioned, they will serve for the purpose of the present discussion. These solubilities could account for a few of the results, but they will not explain the majority of them, as will be seen by inspection of Table VI,

Table VI. *Faecal sterol excretion (mg./100 g. rat/day)*

Exp.	Control	Experi- mental	Increase <i>I</i>	Increase %	Supplement excretion <i>S</i>	$\frac{I}{S} \times 100$
Squalene						
1	4.8	8.7	3.9	78.9	38.8	9.9
2	5.1	5.0	-0.1	—	1.7	—
3	5.1	6.4	1.3	25.5	8.5	15.4
4	5.3	5.9	0.6	11.3	28.7	2.1
5 (b)	5.4	6.4	1.0	18.5	27.8	3.6
(c)	5.4	7.0	1.6	29.6	28.0	5.7
(d)	5.4	6.6	1.2	22.2	24.5	4.9
Hydrosqualene						
3	5.1	6.6	1.5	29.4	19.3	7.8
4	5.3	5.9	0.6	11.3	46.1	1.5
5	5.4	6.7	1.3	24.1	43.8	3.0
Hexadecane						
5	5.4	6.9	1.5	27.8	10.1	14.8

where are recorded the increased sterol excretions, together with the corresponding hydrocarbon excretion. In the last column of the table are recorded also the solubilities which would be required to explain entirely the increased faecal excretion.

These figures show that while a solvent effect might explain the excretion in Exp. 4 of the squalene series, it will not suffice for the other findings with this hydrocarbon, for solubilities as high as 15.4 % would be necessary. For hydrosqualene, solvent action will again explain only one result (Exp. 4), while it will not explain the finding with hexadecane. Nevertheless such an action appears likely to be responsible in part and might be expected in minor degree perhaps from the studies of Rowntree [1930] and Jackson [1931] on the effect of hydrocarbon administration on vitamin A absorption in the intestine, and it is possible that the effect of the hydrocarbon is much greater *in vivo* than in simple solution *in vitro*.

It will be observed from Table VI that while the increased sterol excretion is not strictly related to the amount of non-absorbed squalene, the figures suggest a parallel as good as can perhaps be expected, bearing in mind the experimental difficulties. They show that no extra excretion occurs when 1.65 mg. squalene only are unabsorbed, but that with 8 mg. the increase becomes apparent. Whether the hydrocarbons exercise their effect by causing an increased output of cholesterol into the intestine via the bile or otherwise, or whether they partially inhibit cholesterol absorption cannot be decided from the available data.

The next question for discussion is the increase in liver sterol caused by squalene administration, and the relevant figures are recorded in Table VII, together with the degree of absorption of the hydrocarbon. It will be seen that in all cases there have been increases in liver sterol ranging from 14.3 % in Exp. 3 to 138.3 % in Exp. 1. While the percentage increases in the liver sterol are in general related to the amount of hydrocarbon absorbed, Exp. 2 is a marked exception, for an absorption of 9.2 mg. only has caused a 53.7 % increase.

Table VII. *Liver sterol (mg./100 g. rat)*

Exp.	Control mg.	Squalene mg.	Increase mg.	Increase %	Supplement absorption mg.
1	11.05	26.34	15.29	138.3	28.2
2	11.09	17.04	5.95	53.7	9.25
3	13.3	15.20	1.90	14.29	11.3
4	9.46	13.08	3.62	38.26	17.5
5	9.53	13.97	4.40	46.20	17.0
		13.27	3.74	39.20	18.4
		14.98	5.45	57.20	22.0

There seems little doubt that the amount of sterol in the carcasses of animals receiving squalene does not undergo change, for while certain differences have appeared in some of the later experiments, they are too small and irregular to be of significance. No conclusions are to be drawn from the carcass sterol values of Exps. 1, 2 and 3, since subsequent work has emphasized the difficulty of complete extraction of "fat" by the method used.

Hence, if the correct interpretation has been given to the increases in faecal sterol, the increase in liver sterol, together with the results of Exp. 2 in which the increase in liver sterol caused by squalene was equated against that caused by cholesterol, are the only facts on which a judgment may be formed as to whether squalene is converted into cholesterol.

With regard to the first, it can be stated that in the present work, in spite of the use of a variety of substances other than those recorded here such as higher alcohols and cetyl iodide, squalene alone, apart from cholesterol itself, causes an increase in liver sterol. It has to be stated, however, that in a previous paper [Channon & Collinson, 1928] there were recorded certain increases in the percentage of cholesterol in the liver of animals which received either oleyl alcohol or phytol. In that work the absolute amount of cholesterol in the liver was not calculated and therefore the absolute increase cannot be stated. Apart from this, the influence on the amount of liver cholesterol of factors such as the dietary history and the necessity of using large groups of animals had not been appreciated at that time. For these reasons it would be unwise to place reliance on the increases recorded without more extensive investigation of such increases. In view of the capacity of tissues to degrade cholesterol, the fact that any increase occurs could be taken as strong evidence in favour of conversion. In this connexion, an interesting experiment was carried out by Dr J. Devine in this laboratory a few years ago. In view of the synthesis of squalene by condensation of two molecules of farnesyl bromide [Karrer & Helvenstein, 1931] farnesol was administered to rats. In spite of the fact that it was absorbed far better than squalene, it caused no increase in liver sterol. Hence, if the sterol increase in the liver is regarded as demonstrating the conversion of squalene into cholesterol, it implies a direct cyclization which in turn requires a number of further unusual reactions. From present knowledge, both of the latter phenomena seem improbable. Again, while the equating experiment with cholesterol (Exp. 2) seems strong evidence for conversion of squalene into cholesterol, it is possible that it also may be of less value than is at first sight apparent. It is common knowledge from work from various laboratories that, when cholesterol is administered in the diet, it appears in the tissues chiefly in the liver in the early stages and that its amount progressively increases. It might be anticipated therefore that if squalene were absorbed in sufficient amount to cause an increase in liver sterol after 3 weeks, its amount would increase progressively with

time. Such is not the case, for in experiments designed to investigate this point, no greater increase was observed after 20 weeks.

Other possible causes for the sterol increase in the liver are, however, difficult to visualize. In earlier work [Mahdi & Channon, 1933; Channon & Devine, 1934] it was found that *n*-hexadecane which was fairly well absorbed could not be isolated from the livers of rats and did not cause a sterol increase. It could, however, be obtained from the unsaponifiable fraction of the carcasses. It is possible that squalene appearing in the liver itself for some reasons yet to be discovered, possibly along the lines of the preferential absorption of highly unsaturated acids which Sinclair [1929] has shown to occur, has an effect by virtue of its unsaturated nature on the permeability of the cells and that this in turn is compensated by an increase in the liver sterol content.

Another attempt was made to obtain further evidence on the problem of the conversion of squalene into cholesterol by administering squalene to cod. It will be remembered that Drummond *et al.* [1925] and Drummond & Baker [1929] showed that squalene occurred in traces in cod liver, and it seemed possible that further evidence might therefore be obtained by a study of this fish. Cod of approximately 2 kg. in weight were caught and transferred to the tanks of the Marine Biological Station, Port Erin. They refused to eat for about 3 weeks. After this, squalene was administered to them by insertion in capsules into mussels, which were readily consumed, and after the administration of an average amount of 8.5 g. of squalene in this way to each cod over a period of 21 days, they were killed and their livers analysed. The results are recorded in Table VIII, calculated on the basis of 1 kg. body weight.

Table VIII. *The unsaponifiable fractions of the liver of the cod, per kg. body weight*

Exp.	Fish no.	Wt. of liver g.	Unsap. matter mg.	Sterol mg.	Non-sterol mg.	I.v. of non-sterol calculated
Control	1	18.8	182.0	84.1	97.9	132.6
Control	2	27.3	199.5	88.6	110.9	148
Squalene	1	24.6	253.0	83.4	168.6	286.8
Squalene	2	46.4	286.9	94.1	192.8	301.3
Squalene	3	28.2	347.9	106.2	241.4	267.7

While the data in Table VIII are limited, they are sufficient to show that there has been little, if any, increase in the cholesterol content of the livers, although the i.v. clearly demonstrated that squalene itself had been deposited. The amount of squalene calculated from the i.v. represents, however, no more than 3.2% of the squalene administered.

SUMMARY

1. When squalene is administered to rats in amounts of the order of 1% of the diet for 21 days, there results an increase in liver sterol averaging about 50%, an increase in faecal sterol averaging 33%, with no change in the carcass sterol. The sum of the liver and faecal sterol increases is equivalent on the average to one-eighth of the squalene administered.

2. The increase in liver sterol was equated against that caused by cholesterol feeding and it was found that the amount of the food cholesterol necessary to cause the same liver cholesterol increase was roughly molecularly equivalent to the squalene. The liver sterol increase was confined to the cholesterol ester fraction.

3. When partly hydrogenated squalene, i.v. 100, is administered, little absorption occurs and there is no increase in liver sterol. The faecal increase is, however, again apparent, suggesting that the faecal excretion caused by squalene is to be dissociated from the liver increase and is due to the unabsorbed hydrocarbon passing through the intestine.

4. This suggestion received confirmation in that *n*-hexadecane had the same effects in increasing faecal excretion without increasing liver sterol.

5. The solubility of cholesterol in partly hydrogenated squalene and hexadecane is too low to account for the increased faecal excretion and it appears unlikely that the effects of the hydrocarbons are thus due entirely to solvent action. This suggests that the hydrocarbons cause increased production of cholesterol in the intestine or interfere with the absorption of cholesterol.

6. Squalene was administered to captive cod, but did not cause a sterol increase in the liver, although the hydrocarbon itself appeared in the liver.

7. While no definite conclusion is possible, the evidence on the whole suggests that squalene is not converted into cholesterol.

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