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Alimentary production of gallstones in hamsters

28. Influence of isomerized squalene on gallstone production

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With 1 table

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In our study (1) it was shown that dietary squalene counteracts formation of cholesterol containing gallstones in young hamsters. Addition of 1 % squalene to the gallstone producing diet used in that study resulted in complete or almost complete protection against formation of such gallstones in hamsters of both sexes.

Inhibition of biosynthesis of cholesterol in the liver, an effect which has been proved for rats by *Langdon* and *Bloch* (2), was suggested as the most likely explanation of the protective effect of squalene against production of cholesterol containing gallstones in young hamsters.

Alternatively, it might be thought that the effect of squalene on gallstone-production was due to a general non-specific effect of substances containing many double bonds, since also the ethyl esters of the fatty acids from a fish oil, menhaden oil, exhibited a similar effect on gallstones production, although the arrangement of the double bonds in fish oil fatty acids is different from the arrangement of the double bonds in squalene.

According to *Langdon* and *Bloch* (2), an isomerized form of squalene in which the arrangement of the double bonds has been changed from all-trans in the natural form to a more or less randomized arrangement is no longer able to interfere with the biosynthesis of cholesterol in the liver, but the question whether the isomerized Squalene can protect against formation of gallstones has hitherto not been examined.

In the present study, we have therefore subjected natural squalene to the isomerization procedure described by *Langdon* and *Bloch* (3) and compared the influence of the isomerized and the natural squalene on gallstone production in young hamsters.

The squalene used in the present study was Sigma, grade 1, of the same degree of purity as that used in our previous study (1).

Several portions, each of 30–60 g of squalene, totalling 256 g, were dissolved in diethyl ether. Dry HCl gas was bubbled through the solutions under reflux for about two hours. The HCl gas was produced by dropwise addition of conc. sulfuric acid to solid ammonium chloride, and dried by bubbling through two wash bottles with conc. sulfuric acid. The reaction mixture was left standing at 0° C over night, whereafter the crystallized squalene hexahydrochloride was separated from the solution by filtration,

washed with cold dry diethyl ether, evaporated to dryness and refluxed with pyridine for 16 hours under a stream of nitrogen. The resulting hydrocarbon was isolated by washing of the reaction mixture with water and extraction with diethyl ether. The ether was removed *in vacuo*, first by a water jet pump, thereafter by an oil pump. Yield 93 g.

Both the unchanged and the isomerized squalene were tested by thin layer chromatography as described in our paper (1). Both migrated with the same Rf value of 0.86 during 45 minutes, and were followed by traces of impurities.

Further, both products, in petroleum ether solution, were analyzed by gas liquid chromatography in a Beckman GC 4 apparatus, with a column of diethyleneglycol-succinate phase coated on acid washed Chromosorb W (15 % by weight, 80–100 mesh), temperature 175° C. The natural squalene showed an asymmetric top, retention time 40.9 cm. The isomerized squalene showed 6 tops with retention times 27.7 cm, 30.3 cm, 34.0 cm, 37.7 cm, 41.8 cm, and 44.1 cm, or in per cent: 6.0, 12.6, 22.2, 23.6, 22.0 and 13.6.

The iodine values determined as in our paper (1) were: for the unchanged squalene 385; for the isomerized squalene 384.

The test for ability to prevent production of cholesterol containing gallstones was carried out as described in our paper (1), using hamsters of the origin and preexperimental feeding described therein. The basal diet was the glucose diet containing 7 % palmkernel oil and 0.05 % of the non-absorbable antioxidant Ionox 330. Three groups of animals, each comprising 10 males and 10 females, were used. One group of animals received the basal diet without addition, a second group received the diet with addition of 1 % of natural squalene, and a third group received the diet with addition of 1 % of "isomerized squalene". The animals were 38 to 48 days old at start of the experimental feeding which lasted 42 to 44 days.

Determinations of cholesterol in serum and liver were carried out as described previously (1).

Results and discussion

The results are shown in tables 1 and 1A.

As expected, none of the animals in the group receiving the diet with 1 % squalene had cholesterol containing gallstones whereas in the group receiving the unsupplemented diet 70 percent of the males and 80 percent of the females had such gallstones.

In the group receiving the diet with 1 % of "isomerized squalene", 10 percent of the males and 60 percent of the females had cholesterol containing gallstones. Thus, the diet with "isomerized squalene" has behaved almost like the unsupplemented diet toward the females and almost like the diet with natural squalene toward the males. This result is most likely due to the presence of a small amount of unchanged squalene in the "isomerized squalene". From our study (1) it is known that under the experimental conditions used it is much easier to reduce formation of cholesterol containing gallstones in the males than in the females, wherefore a partial ability to prevent formation of cholesterol containing gallstones is likely to manifest itself primarily among the males.

Table 1. Exp. Series G 164. Summary of occurrence and non-occurrence of gallstones

Group no.	Diet characteristics	Number of animals in group	Animals having C gallstones only		Animals having C and A gallstones		Animals having A gallstones only		Animals having no gallstones	
			no.	%	no.	%	no.	%	no.	%
1432	7% palmkernel oil	10 m	7	70	0	0	0	0	3	30
	0.05% Ionox	10 f	7	70	1	10	0	0	2	20
1436	7% palmkernel oil	10 m	0	0	0	0	0	0	10	100
	0.05% Ionox 1% squalene	10 f	0	0	0	0	2	20	8	80
1437	7% palmkernel oil	10 m	1	10	0	0	0	0	9	90
	0.05% Ionox 1% "isomerized squalene"	10 f	6	60	0	0	1	10	3	30

Age of the animals at start of the experiment 38–48 days. Duration of the feeding period 42–44 days.
Abbreviations: C-gallstones = Cholesterol gallstones,
A-gallstones = Amorphous gallstones

Serum cholesterol was somewhat increased in the male – but not in female hamsters – receiving natural squalene and "isomerized squalene" compared to the serum cholesterol of the hamsters of the same sex in the group receiving the unsupplemented diet.

Total cholesterol in the liver (g/hg) and percentage of esterified cholesterol in total cholesterol were somewhat increased in the males receiving the diet with natural squalene but not in the females in the same group and not in males and females receiving the diet with "isomerized squalene".

Summary

Squalene was treated by addition of dry HCl and subsequent removal of HCl from the addition product. The resulting "isomerized squalene" was compared with natural squalene with respect to the ability to prevent formation of cholesterol containing gallstones in young hamsters reared on a gallstone producing diet. At the 1% dietary level, natural squalene afforded complete protection against formation of cholesterol containing gallstones whereas "isomerized squalene" had almost no protective effect in the female hamsters but largely prevented formation of cholesterol containing gallstones in the male hamsters. Since under the experimental conditions used, protection against cholesterol containing gallstones is much easier to obtain in the male than in the female hamsters, the result may be due to the presence of a small amount of unchanged squalene in the "isomerized squalene".

Zusammenfassung

Squalen wurde durch Anlagerung von trockenem HCl und darauffolgende Abspaltung von HCl behandelt. Das dadurch erhaltene „isomerisierte Squalen"

Table 1A. Exp. Series G 164. Mean values of body weight, liver weight and cholesterol in liver and serum with st. d.

Group no.	Diet characteristics	No. of animals in group	Body weight at start	Body weight after 6 weeks	Weight gain after 6 weeks	Weight of liver	Cholesterol in liver total	Cholesterol in liver ester	Cholesterol in liver free	Cholesterol in liver per cent ester in total	Serum cholesterol
			g	g	g	g	mg	g/hg	g/hg	g/hg	mg/dl
1432	7% palmkernel oil 0.05% Ionox	10 m	60.3 ±3.0	89.4 ±2.8	29.1 ±4.1	4.55 ±0.22	11.1 ±0.5	244.7 ±6.1	39.4 ±1.3	205.3 ±5.9	16.1 ±0.4	199.8 ±6.5
		10 f	58.0 ±2.2	88.8 ±4.1	30.8 ±4.6	4.63 ±0.29	13.2 ±0.9	286.6 ±10.1	47.9 ±2.2	238.7 ±9.8	16.8 ±0.6	261.1 ±7.9
1436	7% palmkernel oil 0.05% Ionox 1% squalene	10 m	60.1 ±2.3	93.4 ±2.3	33.3 ±3.1	4.88[9] ±0.14	15.7[9] ±2.0	321.1[9] ±36.8	113.8[9] ±36.5	207.4[9] ±5.6	30.4[9] ±5.6	230.5 ±5.6
		10 f	57.9 ±3.3	92.6 ±4.3	34.7 ±5.4	4.69[9] ±0.36	12.7[9] ±0.8	273.7[9] ±9.9	49.3[9] ±4.6	224.4[9] ±8.8	17.9[9] ±1.1	280.1 ±8.1
1437	7% palmkernel oil 0.05% Ionox	10 m	60.1 ±2.5	88.6 ±4.6	28.5 ±5.0	4.34 ±0.24	10.4 ±0.6	240.6 ±6.8	36.4 ±1.4	204.2 ±6.8	15.2 ±0.6	219.4 ±4.8
	1% isomerized squalene	10 f	58.1 ±3.0	83.9 ±2.1	25.8 ±3.6	4.29 ±0.19	12.5 ±0.6	292.6 ±11.2	60.2 ±3.5	232.4 ±10.6	20.6 ±0.9	261.6 ±12.2

In cases where weight of liver and cholesterol in liver were not determined in all the animals in a group, the number of animals on which the mean values of these data are based is indicated by superscripts.

wurde in bezug auf die Fähigkeit, die Bildung von cholesterinhaltigen Gallensteinen zu verhindern, mit natürlichem Squalen verglichen, indem man beide Substanzen als Zulage in der Höhe von 1 Prozent zu einer Gallensteine hervorrufenden künstlichen Nahrung für junge Hamster prüfte.

Unter diesen Umständen verhinderte das natürliche Squalen völlig das Auftreten von cholesterinhaltigen Gallensteinen in Hamstern beider Geschlechter. Das „isomerisierte Squalen" war fast ohne Schutzwirkung gegen das Auftreten solcher Gallensteine in den weiblichen Hamstern, vermochte aber weitgehend die männlichen Hamster gegen Bildung von cholesterinhaltigen Gallensteinen zu schützen. Unter den benutzten Versuchsbedingungen ist es besonders leicht, die männlichen Hamster gegen Bildung von cholesterinhaltigen Gallensteinen zu schützen. Das Ergebnis ist deswegen wahrscheinlich durch die Anwesenheit einer geringen Menge unveränderten Squalens in dem „isomerisierten Squalen" bedingt.

References

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MITTEILUNGEN

Unter der Schirmherrschaft der Arbeitsgruppe Lebensmittel der Europäischen Föderation für Chemie-Ingenieurwesen und der Internationalen Vereinigung für Lebensmittel-Wissenschaft und -Technologie (IUFoST) veranstalten die französischen Vereinigungen STELA (Comité Français de Science, Technologie et Economie des Industries Alimentaires) und Société de Chimie Industrielle am 13. und 14. März 1975 in Paris ein **internationales Symposium über Trennprozesse durch Membranen, Ionenaustausch und Gefrierkonzentrieren in der Lebensmittelindustrie.**

Insgesamt sind rund 15 Vorträge auf dem Gebiet moderner Trennverfahren vorgesehen.

Das Programm sowie die Anmeldeformulare zu diesem Symposium können beim Sekretariat APRIA *M. Bragadir,* 35 rue du Général Foy, F-75008 Paris, Frankreich, von Interessenten angefordert werden.

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