Influence of Dietary Cod-liver Oil and Some Fractions of Cod-liver Oil on Serum Cholesterol-level of Rats

FROM numerous experiments with humans and experimental animals it is known that the cholesterol content of the blood is greatly influenced by the amount and kind of dietary fat. In general, the relatively unsaturated vegetable fats decrease the serum cholesterol-level, while an increasing influence is observed with the relatively saturated animal fats. The highly unsaturated oils from marine animals, however, exert a potent cholesterol-lowering activity. This effect was observed in humans following the consumption of seal oil[1], sardine oil[2], whale oil[3] and pilchard oil[1,4] and also in rats fed sardine oil[5].

So far it is not yet decided what compounds of the fats are responsible for the action on cholesterol. The polyenoic acids, linoleic acid and arachidonic acid, applied in pure form, were found to be very active in lowering serum cholesterol-levels[6], whereas hydrogenation of highly unsaturated fats was accompanied by a decrease in activity[3,7]. These findings suggest that the fatty acid fraction is responsible for the effect observed. However, other experiments suggest that the unsaponifiable fraction of vegetable oils possesses cholesterol-lowering properties[8,9].

We have studied the cholesterolemic properties of cod-liver oil and some fractions thereof by feeding them to groups of 10–12 newly weaned rats in a hypercholesterolemic basal diet of the following percentage composition: casein 15, potato starch 10, wheat starch 57·5, minerals 4, vitamin mixture 2·1, choline chloride 0·2, hydrogenated coconut oil 10, cholesterol 1·0 and cholic acid 0·2. Different fats or fractions of cod-liver oil were added at the expense of equal amounts of wheat starch. Fractions of cod-liver oil were prepared after saponification with potassium hydroxide in the presence of ethanol. The soap was split by the addition of hydrochloric acid and the resulting fatty acids washed and dried in a centrifugal separator. The fatty acids were then allowed to crystallize for 24 hr. at 15° C. and filtered through a bag filter at the same temperature. The fatty acid fractions were esterified with ethyl alcohol in the presence of sulphuric acid and the resulting esters refined, washed and dried under vacuum. The unsaponifiable matter was isolated by extracting a diluted solution of cod-liver oil soap with methylated ether. After three extractions, the solvent was removed from the washed extract by evaporation, the residue re-saponified and extracted with ether. The ethereal extract was then washed with dilute potassium hydroxide solution, followed by water washes until neutral, evaporated and dried under vacuum. The aqueous soap solution from which the unsaponified matter had been extracted was freed from dissolved ether, the soap split with acid and the fatty acids purified and esterified as above to give the fatty ester fraction free of unsaponifiable matter.

After feeding the experimental diets *ad lib.* over four weeks, the amount of total serum cholesterol of each rat was determined by the Liebermann–Burchard reaction.

The results presented (Table 1) show that the growth-rate was very poor, that is, only half that obtained on a stock ration. This may be ascribed to the sub-optimal protein content and the high fat content of the diet and to the presence of cholesterol and cholic acid. With corn oil in the diet, growth-rate was highest and somewhat higher than with cod-liver oil. The esterified fatty acid fractions caused a decrease in food intake which was paralleled by a slower growth-rate.

Table 1. MEAN GAIN IN WEIGHT, FOOD CONSUMPTION AND SERUM CHOLESTEROL

Addition to basal diet	Per cent	Daily weight increase (gm.)	Daily food consumption (gm.)	Total serum cholesterol (mgm./100 ml.)
Hydrogenated coconut oil	10	1·6	6·5	784
Corn oil	10	2·3	7·2	430
Cod-liver oil	10	2·1	7·0	196
Ethyl esters of fatty acids liquid at 2° C.; iodine value 189	10	1·6	5·9	241
Ethyl esters of fatty acids liquid at 15° C.; iodine value 161	10	1·7	6·3	240
Ethyl esters of fatty acids solid at 15° C.; iodine value 135	10	1·7	6·3	277
None		2·0	8·8	884
Cod-liver oil	10	1·7	6·6	205
Unsaponified matter	0·4	2·0	8·0	750
Ethyl esters of fatty acids	10	1·2	5·9	281
Unsaponified matter + ethyl esters of fatty acids	10	1·5	6·2	321

The serum cholesterol-level obtained with corn oil was much lower than with hydrogenated coconut oil. With cod-liver oil a significantly lower value was obtained than with corn oil ($P = 0{\cdot}003$ according to Wilcoxon's test). The ethyl esters of cod-liver oil fatty acids decreased the cholesterol content to nearly the same degree as did whole cod-liver oil. The mean serum cholesterol values obtained with the cod-liver oil fractions with different melting points were not significantly different. This may be the result of incomplete separation or of equal cholesterol-lowering properties of the various fatty acids present in the different fractions. The unsaponifiable fraction, in an amount equivalent to the level present in the whole cod-liver oil, exerted no distinct hypocholesterolemic action. The combination of unsaponified fraction with the esterified fatty acid fraction resulted in a mean value not significantly different from that obtained with whole cod-liver oil ($P > 0{\cdot}05$).

From these results it is concluded that cod-liver oil has a higher cholesterol-lowering activity than corn oil. The fatty acid fraction accounts for most if not all of the activity. The unsaponifiable fraction shows no distinct activity.

A. P. DE GROOT

Central Institute for Nutrition and Food Research, T.N.O., Utrecht.

S. A. REED

Marfleet Refining Co., Ltd., Hull.

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