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From the Department of Dairy Science, University of Alberta, Edmonton, Alberta (Canada)

The Free and Ester Cholesterol Content of Milk and Dairy Products

By J. M. DE MAN

With 2 figures and 1 table

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Cholesterol may be present in foodstuffs in the free alcohol form or esterified with fatty acids. Although it has been known that at least a good part of the total cholesterol in milkfat is present in the free form (1), there has been uncertainty as to whether any ester cholesterol is present in milk. NATAF et al (2) determined the free and total cholesterol contents of summer and winter milks of Holstein, Jersey and Guernsey cows and concluded that there was no reason to believe that any ester cholesterol was present in milk. NIEMAN & GROOT (3) concluded from their study of the free and ester cholesterol content of butter that there is esterified cholesterol present in butter. They reported that in one milk sample 9% of the total cholesterol present was in the ester form. It was also found that in some cases a larger proportion of the total cholesterol was present in the ester form in butter than in milk and it was suggested that esterification of cholesterol might take place during the manufacture of butter by an enzymic mechanism. The matter was further complicated by a report on the free and ester sterol content of foodstuffs by KRITCHEVSKY and TEPPER (4). They found no ester cholesterol in milk but large proportions of ester cholesterol in various dairy products, the ester content reported was as follows: thin cream 45%, thick cream 65%, butter 54%, American cheese 11%, blue cheese 10%, cream cheese 17% and Swiss cheese 0%. No explanation for these anomalous results was suggested and it is hard to understand how the simple technique of centrifugation could increase the ester cholesterol content to about half of the total value.

The present study was undertaken to establish whether any part of the cholesterol in milk is present in the ester form and whether any change in the free to ester cholesterol ratio occurs in the manufacture of dairy products.

Experimental

The dairy products tested were obtained from commercial sources except for the butterfat, which was prepared by melting of butter, followed by repeated washing with water until the fat became clear and drying under vacuum (5). The fat content of the products was determined by the Mojonnier method and the fat residue from these determinations was used for the free and total cholesterol determinations. For the colorimetric determination of cholesterol tomatine was used as precipitating agent as described by

KABARA et al. (6). The absorbance of the tomatinide solution was measured with a Bausch & Lomb Spectronic 20 instrument at a wavelength of 625 mμ. It was found that the readings should be made 30 minutes after preparation of the solution, as the colour is stable at this point.

Free cholesterol was determined in 200–250 mg of milkfat made to 10 ml in a volumetric flask with acetone-alcohol-ether 4-4-1. For the determination of total cholesterol 2 g of milkfat were saponified and the unsaponifiable fraction obtained by the A. O. A. C. method. The unsaponifiable fraction was made up to 100 ml in a volumetric flask with acetone-alcohol-ether 4-4-1 and a 10 tr. ml aliquot of this solution used for the preparation of the tomatinide. The standard curve obtained with pure cholesterol is given in fig. 1.

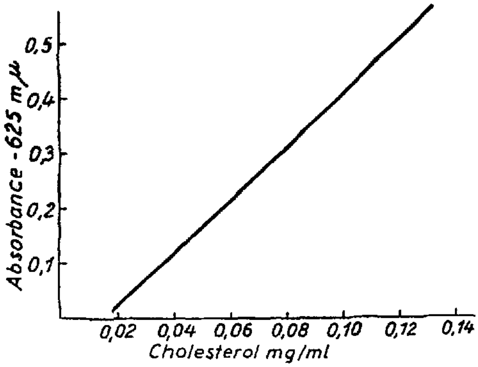

Fig. 1. Standard curve for the relation between cholesterol concentration and absorbance at 625 mμ

Thin layer chromatography was done using Silica gel G according to Stahl and the plates were developed in a mixture of petroleum ether, ethyl ether, acetic acid, 90/10/1, v/v. The plates were activated for 1 hour at 105 °C, the developing time was 40 minutes. The spots were made visible with a spray reagent consisting of a solution of 10% phosphomolybdic acid in methanol.

Table 1. Free and total cholesterol content of milk and dairy products

Product	Fat (%)	Free cholesterol (mg/100 g wet weight)	Total cholesterol (mg/100 g wet weight)
Milk	3.6	12.9	13.0
Milk	3.4	12.1	12.0
Milk	3.4	11.7	11.9
Milk	3.6	12.7	12.6
Milk	3.8	12.6	12.8
Skimmilk	1.2	6.1	6.1
Cream, thin	12.3	35.2	36.9
Cheddar cheese	32.7	103.0	108.0
Cheddar cheese	34.0	97.0	105.0
Cottage cheese	4.6	18.2	18.8
Butter	80.4	185.0	197.0
Butter	80.6	204.0	212.0
Butter	80.8	206.0	214.0
Butterfat	> 99.5	245.0	263.0
Butterfat	> 99.5	232.0	260.0
Butterfat	> 99.5	241.0	268.0

Results and Discussion

Free and total cholesterol contents of milk, Cheddar cheese, cottage cheese, cream and butterfat are listed in table 1. The figures for milk, skimmilk, cream and cottage cheese indicate that the total cholesterol content is slightly higher than the free cholesterol content but it is difficult to establish definitely the presence of ester cholesterol from these figures. However, the results obtained with Cheddar cheese, butter and butterfat give a definite indication of the presence of about 5–10% of the total cholesterol in the ester form.

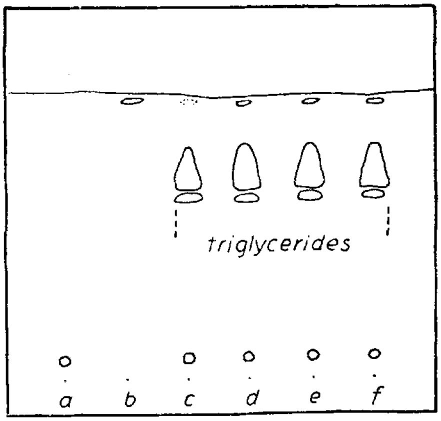

Fig. 2. Thin layer chromatography of cholesterol and cholesterol esters in milkfat. Solvent: petroleum ether-ethyl ether-acetic acid, 90/10/1, v/v. Activation 1 hour at 105 °C, developing time 40 min. Spot detection reagent: 10% phosphomolybdic acid in methanol. Amounts of 5 λ of 1% solution. a) cholesterol, b) cholesterol palmitate, or stearate, or oleate, c) milkfat, d) milkfat with 1/4% cholesterol palmitate added, e) milkfat with 1/4% cholesterol stearate added, f) milkfat with 1/4 % cholesterol oleate added

There is no indication that this ratio is different from one product to another as was found by Kritchevsky & Tepper (1961). These results can be further substantiated by thin layer chromatography, as indicated in fig. 2; butterfat obtained from milk, cheese or butter gave completely identical chromatograms indicating the presence of small amounts of ester cholesterol. The presence of small proportions of cholesterol in the ester form has been taken for granted by dairy research workers (7) and the present work does not lend support to the idea that changes in the ratio of free to ester cholesterol might occur during manufacturing of dairy products.

Summary

Free and total cholesterol contents of milk and a number of dairy products were determined. 5–10% of the total cholesterol was found present in the ester form in Cheddar cheese, butter and butterfat, and there is no evidence that this ratio was different from one dairy product to another. The results of these analyses were confirmed by thin layer chromatography.

References

1. Jack, E. L. & L. M. Smith, J. Dairy Sci. **39**, 1 (1956). — 2. Nataf, B., O. Michelsen, A. Keys & W. E. Petersen, J. Nutrition **36**, 495 (1948). — 3. Nieman, C. & E. H.

GROOT, Acta Physiol. Pharmacol. Neerl. **1**, 488 (1950). — 4. KRITCHEVSKY, D. & S. A. TEPPER, J. Nutrition **74**, 441 (1961). — 5. DEMAN, J. M., J. Dairy Research **28**, 81 (1961). — 6. KABARA, J. J., J. T. MCLAUGHLIN & C. A. RIEGEL, Anal. Chem. **33**, 305 (1961). — 7. PATTON, S. & R. D. MCCARTHY, J. Dairy Sci. **46**, 396 (1963).

Author's adress:
J. M. DEMAN. Ph. D., Associate Professor of Dairy & Food Chemistry, University of Alberta, Edmonton, Canada

From the Department of Hygiene, University of Iceland, Reykjavik (Iceland)

Adeqate Allowance for Vitamin C*)

By JULIUS SIGURJONSSON

With 1 figure

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It appears to be generally accepted that less than 10 mg. per day of ascorbic acid – perhaps not more than about 5 mg. – is needed to prevent manifest scorbut in the adult. This is some times referred to as the minimum requirement.

It is also generally assumed that an amount of ascorbic acid which is just about enough to prevent the occurrence of clinical deficiency symptoms may not be sufficient to ensure full physical fitness. An adequate allowance, therefore, should provide for such extra amount as needed for full benefit and for a reasonable safety margin on top of that.

The question then is, how much would be needed to fulfill these requirements. It is here that opinions diverge widely, and thus "recommended allowances" range from 20–30 mg. on the lower side to 75 mg. and upwards.

The highest levels of intake recommended appear to be based on the assumption that a state of saturation or near saturation is required for full benefit to be attained from vitamin C. This has, however, not been shown conclusively to be the case, nor is it possible to point out any particular level of ascorbic acid in blood as the lower limit of normality.

A priori it would seem unlikely that an increase to more than tenfold the minimum requirement dose is needed for optimum results. Anyhow, this would put vitamin C in a rather unique position among the known nutritional essentials. But more significant is that experience does not seem to substantiate the highest claims in so much as one can point out population groups which, for all evidence, have thrived well at vitamin C intake levels of not more than $^1/_3$ to $^1/_4$ of that required for saturation.

As an example we may take the population of Iceland. In bygone ages, and before the introduction of potatoes, milk was the all-important source of vitamin C and the intake level has probably not been much above 10 mg. Scurvy occurred at times but apparently only when milk was not available or where it was scarce as in the sea coast villages.

*) Read at the 6th International Congress on Nutrition, Edinburgh, August 9–15, 1963.