INTERNATIONAL SERIES OF MONOGRAPHS ON

PURE AND APPLIED BIOLOGY

MODERN TRENDS IN PHYSIOLOGICAL SCIENCES

UNSATURATED FATTY ACIDS IN ATHEROSCLEROSIS

by

J. ENSELME

PROFESSEUR À LA FACULTÉ MIXTE DE MÉDECINE

ET DE PHARMACIE DE LYON

FRANCE

Translated by

R. CRAWFORD

PERGAMON PRESS

OXFORD · LONDON · NEW YORK · PARIS

1962

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London, W.1

PERGAMON PRESS INC.
122 East 55th Street, New York 22, N.Y.
1404 New York Avenue N.W., Washington 5 D.C.

PERGAMON PRESS S.A.R.L.
*24 Rue des Écoles, Paris V^e^*

PERGAMON PRESS G.m.b.H.
Kaiserstrasse 75, Frankfurt am Main

Library of Congress Card Number 61-18238

Set in Imprint 11 on 12 pt. and printed in Great Britain
J. W. ARROWSMITH LTD., BRISTOL 3

Preface

MANY observations and the generally consistent results of experiments suggest that certain fatty acids whose molecules contain several ethylenic bonds check the development of induced atherosclerosis in animals and have a favourable effect on the spontaneous atherosclerosis of man. As a result of their action hypercholesterolaemia is reduced, the fatty deposits in the vessels become both less extensive and less numerous and the histological lesions become less conspicuous.

The results can be summarized in the following way.

A saturated fatty acid produces atherosclerosis.

A mono-ethylenic fatty acid is without action, favourable or unfavourable.

A poly-ethylenic fatty acid checks the formation of atherosclerotic lesions and reduces cholesterolaemia and lipidaemia.

Consequently, there would not appear to be any legitimate grounds for hesitation when it is a question of advising patients with obvious vascular involvement to substitute poly-ethylenic fats for those generally used in their diet.

If, however, the improvements indicated are regarded as certain, it must be asked whether it would not be better to modify the diet of normal man from infancy, particularly in countries which have high mortalities from vascular disease. To accept such a conclusion would, however, be to upset the agricultural economy of certain regions, possibly even of some nations, to restrict the rearing of cattle and the use of butter, eggs and milk, to replace the prairies by areas for the cultivation of oil-yielding plants and to force the agricultural population to make an enormous effort of adaptation.

If, however, it is true that such a measure can reduce the ravages of atherosclerosis, which kills a considerable number of individuals at the age of greatest efficiency, then governments must bring about such a transformation. They have no right to hesitate and their role will be merely to minimize the economic consequences, which in many cases will be serious.

The aim of this book is to make a careful assessment of the results obtained so far and to determine how close we are to a definite solution. It is obvious that, in approaching such a problem, we must be conscious of the responsibilities which it involves. We shall examine the problem from two aspects, the medical, assessing the value of improvements obtained in patients, and the biochemical, affording a critical evaluation of responses in animal experiments.

The fatty acids are not the only unsaturated molecules which are active in atherosclerosis. Hormones and vitamins must also be included in this list. A study of these will suggest certain comparisons which we believe to be of interest.

In the first part of this work we shall describe the clinical and experimental facts without discussing the mechanism. In the second part, on the other hand, we shall try to explain the results obtained and, if possible, correlate them with definite molecular modifications. If we do not reach any definite conclusions, this evaluation will at least establish the points which still remain obscure and will afford us indications of the still unexplored regions to which our research should be directed.

CONTENTS

PART I

Facts relating to natural, atherosclerotic chemical elements

CHAPTER 1

Atherosclerogenic Food Substances

VARIOUS food substances have been found to give rise to atherosclerosis in man and animals. These are considered below, according to type, in relation to their dietary effect.

MILK AND MILK DERIVATIVES

The chemical similarity of these substances, due to their common origin, gives them the same effect on atherosclerosis, but an effect which varies in intensity.

I. *Cow's Milk*

Cow's milk is, in effect, what concerns us. It has the following average composition per hundred millilitres:

Proteins 3·5; glucides, etc. 4·2; salts 0·70; water 88 and—what is the important point—lipids 3·60. A person who drinks 3 l. of milk daily, which is quite a large quantity, thus absorbs about 100 g lipids; as these lipids are, in fact, those found in butter, we can, therefore, reckon that the subject absorbs about 30–40 g of saturated fatty acids. Preformed cholesterol is minimal and of little account.

It must also be noted that milk contains 3·18 per cent casein among its proteins. The subject who absorbs 3 l. of milk thus has an absorption of 96 g casein. We shall see that casein, even when freed from all milk fats, is atherosclerogenic [12, 37] and increases blood cholesterol [1, 35, 40].

The saturated fatty acid content, and possibly the considerable quantity of casein in milk, are probably responsible for many very important phenomena.

Various authors [49, 50] have observed that, among subjects with gastric ulcer submitted to various dietetic regimes, clinical accidents (infarct) were statistically much more frequent among those patients who had been on exclusively milk diets. In 1960 Briggs *et al.* [10] examined necropsy reports and showed that myocardial infarct was much more frequent (more than twice as frequent) in subjects who had been on milk diets than in ulcer subjects treated differently or in subjects free from ulcer. There was no significant difference between the last two types. This enquiry covered fifteen hospitals in the United States and Great Britain.

II. *Butter*

Butter is the food substance which has been most fully examined.

A. *Butter is rich in saturated fatty acids, a considerable fraction of these being short-chain acids.* Its iodine index is about 40.

Its (percentage) composition is the following:

Proteins 0·8; salts 0·07; water 6–20; but lipids are about 83 per cent and cholesterol 0·3 per cent.

The quantity of preformed cholesterol introduced by a diet rich in butter is still, therefore, minimal. The quantity of glycerides is considerable. Phosphatides represent only 0·03–0·1 per cent and are not of any importance.

The fatty acids of butter are distributed in the following manner:

(1) saturated fatty acids with short chains (from C_4 to C_{10})	10 per cent
(2) myristic acid	10 per cent
(3) palmitic acid	20–30 per cent
(4) stearic acid	10 per cent
This gives a total of 50–60 per cent saturated fatty acids	
(5) oleic acid	30–40 per cent

In addition, there are very small quantities of acids with one unsaturated bond (C_{10}, C_{12}, C_{14}, C_{16}, C_{20}, C_{22}), with two unsaturated bonds (C_{18}, C_{20}), with three (C_{18}, C_{20}, C_{22}) and with four and five unsaturated bonds (C_{20}, C_{22}). There are also some fatty acids with odd numbers of carbon atoms. The points of essential importance are:

The large quantity of saturated fatty acids.

The presence among them of an appreciable quantity of short-chain fatty acids.

These two features are not balanced by the presence of poly-ethylenic acids in sufficient quantity. Oleic acid, which is neutral in relation to atherosclerosis, is the only acid present in considerable quantity.

B. *Butter increases blood cholesterol in animals and man.*

(1) Butter increased blood cholesterol in fowls [36].

(2) In 1960 Wigand [52] gave 13 rabbits a semi-synthetic diet containing no cholesterol but 25 per cent of casein and 8 per cent of butter. The butter had the following composition: saturated fatty acids 58·6 per cent; acids with one unsaturated bond 38 per cent; with several unsaturated bonds 3·4 per cent; cholesterol 0·27 per cent. The diet was maintained for eighteen weeks. The results are shown in the following table.

TABLE I

SERUM CONTENTS (mg/100 ml)

	Free cholesterol	Bound cholesterol	Total cholesterol	Triglycerides
Before	11·2	17·6	28·8	148·6
After	93·9	249·5	343·4	130·3
Difference	82·8	231	314·6	−18·3
p	<0·001	<0·001	<0·001	>0·05

	Phospholipids	Total lipids	Total cholesterol/ phospholipids
Before	60·3	249·7	0·49
After	180·2	823·5	1·94
Difference	119·9	573·9	1·45
p	<0·001	<0·001	<0·001

Blood cholesterol and total lipids were thus considerably increased. Triglycerides were unchanged. Phospholipids were increased but to a much less extent than cholesterol. Esterized cholesterol was increased most.

(3) Bragdon *et al.* [9] showed that butter, added to the regular diet, increased blood cholesterol in pigs.

(4) Two series of experiments concern monkeys. In 1956 Galapan *et al.* [21] studied the changes in blood cholesterol produced by large quantities of butter (56 per cent of the lipids in the diet) administered over a period of two months. Initially 0·14 per cent, the blood cholesterol reached 0·24 per cent at the end of the experiment. There was no increase from sesame oil, used as a control.

In 1959 Emerson *et al.* [19] studied the effect of a butter-rich diet in monkeys which also received 50 mg pyridoxine daily. The butter still increased the blood cholesterol.

(5) The results were similar in the case of man. In 1956 Beveridge *et al.* [8] administered quite large quantities of butter to 48 normal subjects over a period of eight days. Blood cholesterol rose. The "most volatile" fractions of butter, which were isolated by a process which is not described by the authors, were the most active. These were undoubtedly the short-chain acids.

In 1957 Keys *et al.* [33] administered 100 g butter daily (40 per cent of the total lipids in the diet) to 26 normal subjects. Their blood cholesterols were increased and were above those of controls given vegetable oils.

In 1957 Horlick and Graig [28] observed a reduction in the blood

cholesterol of subjects deprived of butter, whether replaced by vegetable oils or not.

In 1957 Armstrong *et al.* [5] demonstrated on 122 subjects that butter produced a moderate, yet statistically significant, increase of blood cholesterol.

In 1958 Schettler *et al.* [45] showed that all lipids in the blood were increased in students who had consumed large quantities of butter, and that only the phosphatides were unchanged.

In 1959 Hammerl *et al.* [23] arrived at a similar conclusion in respect of total lipids in subjects who had consumed fairly large quantities. Various oils tested gave lower values.

In 1960 Hashim *et al.* [24] gave their subjects first butter and then synthetic saturated triglycerides. The latter, which are more saturated than butter, lowered the blood cholesterol. They concluded that butter had a specific action, increasing the blood cholesterol.

C. *Butter produces the lesions of atherosclerosis in the pig and rabbit*, quite apart from any direct introduction of cholesterol.

(1) In 1959 Rowsell *et al.* [44] studied 12 pigs given a diet rich in butter and observed some development of atherosclerosis, although the blood cholesterol was only slightly increased.

(2) Post-mortem examination in the experiment of Wigand *et al.* [52], referred to earlier, revealed single or numerous plaques (type + and + +, but no type + + +) in 12 of the 13 rabbits. There were no lipid deposits in the control rabbits (type 0).

In practice it will be remembered that, in the case of man, butter rations of 100 g daily will raise the blood cholesterol rapidly but that a similar effect can undoubtedly be produced by smaller doses if repeated daily over a number of years. Furthermore, in the animal butter itself can produce a type of atherosclerosis.

III. *Cream*

Cream has a fat content of from 30–50 per cent which is approximately half that of butter. Cholesterol is only 0·15 per cent. There have been no special investigations on cream but, from the practical standpoint, it probably behaves in the same way as butter, as cooks often use a weight of cream which is twice that of butter for preparations equivalent to those obtained with butter.

IV. *Cheeses*

Cheeses are rich in lipids, 30–35 per cent and in casein, about 25–30 per cent. The lipids are those of butter.

They have not been studied as their importance in human nutrition is minimal. A reasonable portion of cheese, 50 g, is only the equivalent of 15 g of butter.

Eggs, Normal or Modified

Eggs have been extensively used for fifty years to induce atherosclerosis in rabbits. Their atherosclerogenic role would appear to be beyond doubt. In order that they may be used without danger, attempts had to be made to give fowls a diet rich in ethylenic fatty acids. We shall now describe these investigations.

I. *Normal Hens' Eggs*

Their composition affords part of the explanation of their activity.

A. *Composition of eggs.* Different analyses gave results which differ considerably, the eggs used being of widely varying origin.

(1) According to Terroine and Belin [48], the general composition of an egg is as follows:

White 56·7 per cent; yolk 31·3 per cent and shell 11·4 per cent. As the weight of an egg is only very slightly above 50 g, it can be assumed that the yolk weighs about 17 g.

(2) According to Terroine and Belin [48], the fatty acids are, for 100 g of yolk, 28·4 per cent of the fresh weight; an egg yolk contains, therefore, about 4·8 g of fatty acids.

(3) The distribution of the fatty acids (percentages of total fatty acids) is presented in Table II.

Table II

	Cruickshank [17]	James [32]
Saturated fatty acids	31·4%	43·12%
Unsaturated fatty acids	68·6%	56·88%
One egg therefore contains:		
Saturated fatty acids	1·5 g	2·07 g
Unsaturated fatty acids	3·28 g	2·73 g

According to James [32], the saturated fatty acids are distributed as shown in Table III.

Table III

Acid	Percentage
Branched fatty acids	7·45
Myristic	0·33
n-Hexadecanoic	26·7
Stearic	8·64
Total	43·12

The unsaturated fatty acids are distributed as shown in Table IV (percentage of total fatty acids):

TABLE IV

Acid	Cruickshank [17] (percentage)	James [32] (percentage)
Palmitoleic		3·08
Oleic	46·7	46·9
Linoleic	19	
Linolenic	2·9	5·65
Arachidonic		1·28

Confining ourselves to the more recent analysis, we have the following values for an egg yolk (Table V).

TABLE V

Acid	Weight (g)	Acid	Weight (g)
Branched fatty acids	0·357	Palmitoleic	0·148
Myristic	0·016	Oleic	2·25
Hexadecanoic	1·28	Linoleic Linolenic	0·27
Stearic	0·41	Arachidonic	0·06
Total saturated acids	2·063	Total unsaturated acids	2·73

An analysis by Fisher and Leveille [20] gave the results listed in Table VI.

TABLE VI

Acid		for one egg (g)	
Linoleic	8·9		0·43
Linolenic	3·8		0·18
Total	12·7	Total	0·61

(4) The distribution between the constituent lipids was determined by Reiser [43] in 1951 (Table VII).

TABLE VII

Acids	Saturated	Oleic	Ethylenic				
			Di-	Tri-	Tetra-	Penta-	Hexa-
Glycerides	31	53	14·4	0·18	1·43	2·61	1·12
Phosphatides			20·1		9·1	9·2	3·79

The poly-ethylenic fatty acids would thus appear to be present in larger quantities in the phosphatides.

(5) Egg lipoproteins. In 1956 Schmidt *et al.* (36) centrifuged egg yolk at 20,000 *g*.

There were two phases (see Table VIII).

TABLE VIII

Phase	Proteins (percentage of total)	Phospholipids (percentage of total)
Supernatant, clear yellow liquid	60	90
Granular	40	10

McIndoe [34] isolated from the upper phase a lipoprotein which represented almost the entire yolk lipids. A very closely related lipoprotein is present in the serum of hens during the egg-laying period.

It yields two components on ultracentrifugation. It may possibly stand in some relationship to the spontaneous atherosclerosis which is frequent in the hen. It may also constitute an atherosclerogenic element in the subject who consumes eggs.

(6) The sterols of egg have been fairly well studied. A typical egg yolk contains 0·25–0·30 g sterol [14].

These sterols are: cholesterol, Δ^7 cholesterol (1 per cent of the total sterol, 3 mg for one egg yolk) [38, 39], a sterol with two conjugated bonds [54], $\Delta^{5,24}$ cholestadienol and its isomers [47].

B. *Experimental work with eggs* showed that atherosclerosis was produced by regular use of increased quantities of cholesterol.

Chalatow [11] fed rabbits with enormous quantities of egg yolk. He found lipid deposits in the intima.

In 1913 Wesselkin [51] used a mixture of egg yolk and milk (one egg yolk daily for six months). He found crystalline lipid deposits in the intima.