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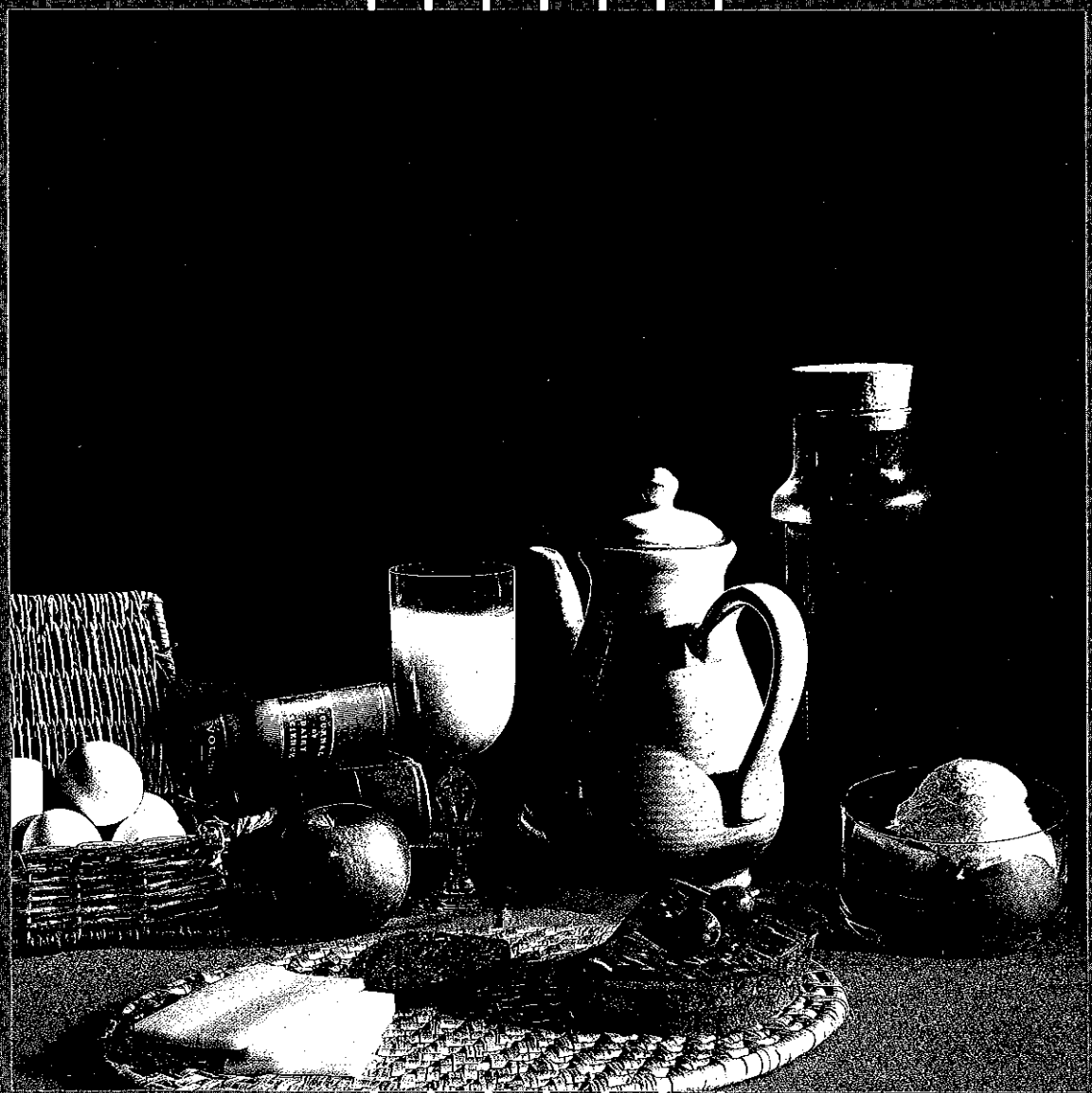

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Dietary Fatty Acids and Blood Lipids

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In the last thirty years there has been growing concern that the levels and types of fat in the diet may be related to the chronic degenerative diseases so prevalent in our affluent society, such as atherosclerosis, heart disease, cancer, diabetes, and arthritis. In the last two decades in particular, Australians have been exhorted to reduce the amount of fat in their diet, to reduce their intake of saturated fat and increase their intake of polyunsaturated fat, and to reduce their intake of cholesterol.

These recommendations date from the classic observations of Keys et al. (1957) and Ahrens et al. (1957) that serum lipid levels in man could be influenced by dietary fats, and the formulation of the 'lipid hypothesis' that excess dietary fats lead to raised serum lipid levels which instigate atherosclerosis. By adopting these recommendations, consumers have been led to believe that their serum lipid levels (especially serum cholesterol) would be reduced, and that consequently their risk of atherosclerosis and heart disease would also be reduced. Reduced-fat foods and polyunsaturated foods have thus become preferable substitutes for traditional foods, for example table margarines in place of butter, and skim milk in place of full-cream milk.

Over some three decades there has been an enormous amount of research predicated on the lipid hypothesis, but there appear to be many contradictory findings, as indicated in the review by Stehbens (1989). Certainly, within the last decade, the simplistic proposed relationships between dietary fatty acids and heart disease have been shown to be very much more complex. Taking the example of the effects of dietary fats on serum lipids, we have the following developments:

(a) Whereas total serum cholesterol was measured in the past, it is now possible to measure total serum cholesterol, high density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triacylglycerols (TAG) and other blood lipids. Current medical opinion is that total cholesterol and LDL levels should be low, but that HDL levels should be high, because this last form of cholesterol is involved in transporting cholesterol out of the arterial system.

(b) Not all saturated fatty acids have similar effects on serum lipids (see later).

(c) Monounsaturated fatty acids may be as effective as polyunsaturated fatty acids in lowering serum lipids (see later).

(d) Polyunsaturated fatty acids should be considered as two types, omega-6 or omega-3 (defined later). Polyunsaturated fatty acids of both types are potential precursors of powerful physiological regulating substances such as prostaglandins, thromboxanes, and leukotrienes, but those derived from omega-6 fatty acids

may be inhibited by those derived from omega-3 fatty acids and vice versa. Clearly a dietary balance between omega-3 and omega-6 polyunsaturated fatty acids has to be established.

A very brief outline of these recent developments is given below.

What Is Dietary Fat?

Scientists use the term 'lipid' to describe what the layman knows as a 'fat' or an 'oil'. This term covers a wide range of chemical compounds which are generally sparingly soluble in water, but soluble in solvents such as ether or chloroform.

The amounts and types of dietary fat consumed by individuals vary enormously, but a rough guide to an average daily per capita intake is that provided by Gurr (1988) for the United Kingdom. Triacylglycerols (TAG) account for about 90% of fat intake (about 90 g/d), with phospholipids (4-8 g/d), glycolipids (1 g/d) and cholesterol (0.5-1 g/d) making up the balance. Since TAG and phospholipids are largely composed of fatty acids (about 95% and about 70% respectively) it becomes clear that the bulk of our fat intake is fatty acids, and that it is the amounts and types of dietary fatty acids which are relevant to considerations of health and nutrition.

What Are Fatty Acids?

To the organic chemist fatty acids are long-chain monocarboxylic acids. Saturated fatty acids comprise a methyl group (CH_3-) at one end, a chain of methylene units (-CH_2- groups), and a carboxyl group (-COOH) at the other end (Table 1). They usually contain an even number of carbon atoms, and the term 'fatty acid' is applied to those containing six or more carbon atoms, and which are insoluble in water. Dietary fatty acids usually contain between 6 and 22 carbon atoms. For a layman the nomenclature of fatty acids is quite confusing. The organic chemist uses terms that indicate the structure of a particular fatty acid precisely, e.g. *n*-hexadecanoic acid indicates a straight-chain('*n*'-), sixteen-carbon ('hexadecan-'), carboxylic ('-oic') acid. However there co-exist a series of trivial names which are more commonly used, for example palmitic acid (*n*-hexadecanoic acid, above) or stearic acid (*n*-octadecanoic acid).

Because the chemical nomenclature is cumbersome, a shorthand notation has been developed which indicates immediately the number of carbon atoms, followed by the number of double bonds (see later), of the fatty acid in question.

Thus 16:0 (sixteen carbons, no double bonds) refers to *n*-hexadecanoic acid or palmitic acid. 16:0 and 18:0 are the most abundant SATURATED fatty acids in our diet, the term 'saturated' indicating the absence of 'unsaturated' double bonds in these compounds.

TABLE 1
Some straight-chain saturated fatty acids, $C_nH_{2n+1}COOH$

Shorthand Desig,	Recc'd name	Alternative name	Systematic name	Formula	Occurence
6:0	Hexanoic			$CH_3(CH_2)_4COOH$	Butter, palm oil, coconut oil
8:0	Octanoic			$CH_3(CH_2)_6COOH$	Butter, palm oil, coconut oil
10:0	Decanoic			$CH_3(CH_2)_8COOH$	Butter, whale oil, coconut oil
12:0	Lauric		Dodecanoic	$CH_3(CH_2)_{10}COOH$	Coconut oil
14:0	Myristic		Tetradecanoic	$CH_3(CH_2)_{12}COOH$	Widespread
16:0	Palmitic		Hexadecanoic	$CH_3(CH_2)_{14}COOH$	Widespread
18:0	Stearic		Octadecanoic	$CH_3(CH_2)_{16}COOH$	Widespread
20:0	Eicosanoic	Arachidic		$CH_3(CH_2)_{18}COOH$	Seed oils, fish oils

TABLE 2
Some straight-chain monounsaturated fatty acids, $C_nH_{2n-1}COOH$

Short-hand Desig,	Recc'd name	Alternative name	Systematic name	Formula	Occurrence
14:1	*cis*-9-Tetradecenoic	Myristoleic		$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$	Widespread
16:1	*cis*-9-Hexadecenoic	Palmitoleic		$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	Widespread
18:1	Oleic		*cis*-9-Octadecenoic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Widespread
18:1	Elaidic		*trans*-9-Octadecenoic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Ruminants
18:1	*cis*-11-Octadecenoic	Vaccenic		$CH_3(CH_2)_5CH=CH(CH_2)_9COOH$	Widespread
20:1	*cis*-9-Eicosenoic	Gadoleic		$CH_3(CH_2)_9CH=CH(CH_2)_7COOH$	Fish oils
22:1	*cis*-11-Docosenoic	Cetoleic		$CH_3(CH_2)_9CH=CH(CH_2)_9COOH$	Fish oils
22:1	Erucic		*cis*-13-Docosenoic	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	Rapeseed oil

Fatty acids containing one double bond are called MONOUNSATURATED fatty acids, a typical example being oleic acid, 18:1 (eighteen carbon atoms, one double bond) (Table 2). Because the double bond may be present between any pair of methylene units in the carbon chain (positional isomerism), and because the double bond may be of the *cis*- or *trans*- configuration (geometrical isomerism), an exact description of oleic acid would be *cis*-9-octadecenoic acid; the double bond is *cis*-, located between carbon atoms 9 and 10 (numbered from the carboxyl end of the chain), 'octadec-' signifying eighteen carbon atoms, '-en-' signifying a double bond, '-oic acid' indicating a carboxylic acid. Often this information is added to the shorthand notation, thus oleic acid is *cis*-9-18:1. Oleic acid is by far the most common monounsaturated fatty acid in our diet, with palmitoleic acid (16:1, *cis*-9-hexadecenoic acid) the next most common. Most dietary monounsaturated fatty acids have the *cis*- type double bond, but *trans*- fatty acids occur in foodstuffs derived from ruminants, such as beef, veal, lamb, milk, butter, cheese and other dairy products, as a result of partial hydrogenation within the rumen of the fatty acids of grasses, etc., consumed by the ruminant animal. Ruminant-derived foodstuffs also contain minor quantities of branched-chain saturated fatty acids, and saturated fatty acids containing an odd number of carbon atoms, as a result of microbial fermentation in the rumen. Other major sources of dietary *trans*- monounsaturated fatty acids are margarines and other fats containing hydrogenated vegetable or marine oils.

POLYUNSATURATED fatty acids (often abbreviated to PUFA) is the term applied to fatty acids containing two or more double bonds (Table 3). With these the nomenclature in use becomes even more complex. The basic shorthand notation 18:2 is quite inadequate to describe linoleic acid, the most common PUFA. As with monounsaturated fatty acids, the positions and configurations of each double bond must be specified, and so linoleic acid becomes *cis*-9, *cis*-12-octadecadienoic acid. The most common forms of 18:3 are γ-linolenic acid (all-*cis*-6, 9, 12-octadecatrienoic acid) and α-linolenic acid (all-*cis*-9, 12, 15-octadecatrienoic acid). In the above names the double bonds are still numbered in relation to the carboxyl group. Sometimes a capital delta (Δ) is used ahead of the numerals to emphasise that numbering relates to the carboxyl group.

Problems with unwieldy correct names for PUFA have been overcome by modifying the shorthand notation to take into consideration three observations. The first is that most naturally occurring PUFA have a methylene-interrupted sequence of double bonds, that is to say that each pair of double-bonded carbons is separated from the next pair by a single methylene group, thus: $-CH=CH-CH_2-CH=CH-CH_2-$. Secondly, most natural PUFA contain only *cis*- double bonds. Thirdly, the natural PUFA in foods mostly fall into

TABLE 3

Some straight-chain polyunsaturated fatty acids, $C_nH_{2n-x}COOH$

Short-hand Desig,	Recc'd name	Alternative name	Systematic name	Formula	Occurence
18:2	Linoleic		*cis*-9, *cis*-12-Octadecadienoic	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7$-COOH	Widespread
18:3	α-Linolenic		*cis*-9, *cis*-12,*cis*-15-Octadecatrienoic	$CH_3CH_2CH=CHCH_2CH=CHCH_2$-$CH=CH(CH_2)_7COOH$	Widespread
18:3	*cis*-6, *cis*-9, *cis*-12-Octa-decatrienoic	γ-Linolenic		$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2$-$CH=CH(CH_2)_4COOH$	Evening primrose oil
20:3	*cis*-8, *cis*-11, *cis*-14-Eico-satrienoic	Dihomo-γ-linolenic		$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2$-$CH=CH(CH_2)_6COOH$	Animals
20:4	Arachidonic		*cis*-5, *cis*-8, *cis*-11, cis-14-Eicosatetraenoic	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2$-$CH=CHCH_2CH=CH(CH_2)_3COOH$	Widespread in animals

20:5, 22:5, and 22:6 acids occur widely in fish oils

two series depending on whether they are derived from linoleic acid or from α-linolenic acid (Figure 1). If, instead of numbering the double bond positions in relation to the carboxyl group, as in the conventional organic chemical nomenclature, we were to reverse the procedure and number from the last or omega carbon, i.e. the methyl group, it becomes apparent that linoleic acid *and all its derivatives* have the double bond nearest the methyl group at the omega-6 (ω6) position. In contrast, α-linolenic acid and all of its derivatives have this double bond at the omega-3 (ω3) position. In a more recent variant of this the terms (n-6) and (n-3) have been used in place of ω6 and ω3 respectively. These types of notation are used together with the shorthand; thus 18:3ω3 or 18:3n-3 indicates α-linolenic acid, numbered 9,12,15- in the classical (Δ) way (see earlier), or 3, 6, 9 from the methyl end (ω notation).

What are Essential Fatty Acids?

Humans and other mammals obtain their fatty acid requirements not only from the diet but also from biosynthesis within the body. The body can make all the fatty acids it requires except for two, namely linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3). In the strictest sense of the word 'essential', these are the only two essential fatty acids (EFA) since they can only be obtained from the diet, but the term 'essential' is also loosely applied to those fatty acids which the body can make from these two EFA (Figure 1). Linoleic and α-linolenic acids must be obtained directly from plant foods, or indirectly by consuming foods (meat, fish, etc.) from animals whose food chain includes plant foods. Figure 1 shows that, in

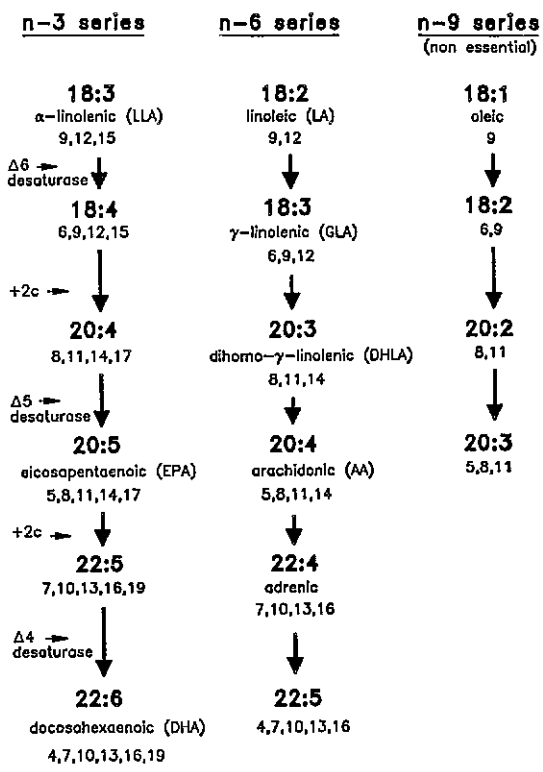

Fig. 1 The pathways for the desaturation and elongation of long-chain unsaturated fatty acids

building up to higher fatty acids, the (n-6) configuration of linoleic acid remains intact. Likewise the (n-3) configuration of α-linolenic acid is present in all its higher derivatives. In cases of EFA-deficiency, the body strives to make higher fatty acids from oleic acid by the (n-9) pathway, but the end-product, 20:3n-9, Figure 1, has no EFA-activity. Of the products from linoleic acid, 20:3n-6 and 20:4n-6 are important precursors of prostaglandins, etc., whereas of the products from α-linolenic acid, 20:5n-3 is also a prostaglandin precursor. The 20-carbon derivatives of these fatty acids (prostaglandins, thromboxanes, and leukotrienes) are often called 'eicosanoids' after eicosanoic acid (20:0).

Fatty acids in the diet

Depending on the types of fat in the diet, the amounts and types of fatty acids in the diet will vary enormously. The fatty acid composition of adipose tissue, however, has often been used as an index of long-term dietary consumption of fatty acids, particularly of linoleic acid (Field et al., 1985). In 1981 Thomas et al. compared the percentage fatty acid composition of the average UK dietary fat with the average fatty acid composition of adipose tissue from 95 human subjects and showed that there was a remarkable similarity between the two (Table 4), allowing for the conversion of 16:0 to 16:1 and of 18:0 to 18:1 within the body. When positional and geometrical isomers, branched-chain and odd-carbon-number fatty acids are considered as well as the common fatty acids, it becomes apparent that dietary fat and body fat may contain over 100 different fatty acids, though most of these would only be present in minute amounts. Six fatty acids, however, account for more than 90% of the fatty acids in the diet and in human adipose tissue, namely 14:0, 16:0, 18:0, 16:1 and 18:1 (the last two including small amounts of isomers) and 18:2 (Table 5). The data in Table 5 show a close similarity in the fatty acid composition of adipose tissue of UK subjects and Australian subjects in the period 1975 to 1980, and hence by inference (Thomas et al (1981), see Table 4) in the average fatty acid composition of the UK and Australian diets at that time. The increasing use of polyunsaturated fat in Australia is indicated by a rise in the level of 18:2 in adipose tissue fatty acids in subjects studied in 1984-1988 (Table 5).

Saturated fatty acids & blood lipids

Dietary saturated fats are major sources of energy, and the simplistic notion that all saturated fats are undesirable

TABLE 4

Comparison of percentage fatty acid composition of the UK diet with that of the adipose tissue in 95 subjects

Fatty acid	Dietary Fat		Adipose tissue fat	
14:0	5.9		3.8	
16:0	25.3	29.2	22.3	29.3
16:1	3.9		7.0	
18:0	12.3	48.3	5.1	50.4
18:1	36.0		45.3	
18:2	8.6		8.3	
18:3	1.0		0.7	
Trans isomers	5.4		5.2	
PUFA	10.6		9.9	

(after Thomas et al. 1981)

elements of the diet is clearly erroneous. Firstly, the metabolism of ingested medium-chain saturated fatty acids (6:0 to 12:0) differs from that of long-chain ones (14:0 and beyond). After ingestion and hydrolysis from parent TAG, the former are transported as free acids directly to the liver via the portal system and there they are oxidised. The saturated (and unsaturated) fatty acids from 14:0 and beyond are re-esterified and the newly-formed TAG transported as chylomicrons in the blood stream. Clearly the ability of the medium-chain saturated fatty acids to affect blood lipid levels will differ from that of long-chain saturated fatty acids as a consequence of these differences in absorption.

The second aspect of dietary saturated fatty acid intake which is often ignored is that humans and other animals synthesize considerable quantities of saturated fatty acids, especially 16:0 and 18:0, from two-carbon or three-carbon units within the body.

This is analogous to the dietary cholesterol story, in which the ability of the body to synthesize its own cholesterol is often overlooked. As Stehbens (1989) notes, it is incongruous that exogenous animal fat (equated with saturated fat) should be regarded as noxious while the body synthesizes its own saturated fat.

More recently Bonanome and Grundy (1988) showed that dietary 18:0 had little effect on serum cholesterol levels, possibly because it was rapidly desaturated to 18:1. Over 20 years earlier Keys et al. (1965) and Hegsted et al. (1965) had similarly reported that 18:0 and 18:1 were 'neutral' in their effect on serum cholesterol levels.

TABLE 5

Major fatty acids of human adipose tissue (percent of total fatty acids)

Country	UK[1]	Australia[2]	Australia[3]	Australia[4]	Australia[5]
Period (estimated)	1977-1980	1975-1976	1975-1978	1984-1985	1987-1988
Number of subjects	95	58	421	200	266
Sex of subjects	All male	18 male	241 male	92 male	110 male
Fatty acid/percent					
14:0	3.8	3.5	3.6	2.5	3.4
16:0	22.3	21.4	22.9	23.4	23.0
16:1	7.0	9.6	7.4	6.7	5.1
18:0	5.1	4.2	5.3	5.6	5.8
18:1	45.3	48.6	48.6	47.9	45.6
18:2	8.3	9.4	8.1	11.2	12.2
Total	91.8	96.7	95.9	97.3	95.1

[1] Thomas et al. 1981
[2] Goldrick et al. 1976
[3] Fogerty et al. 1987 (raw data)
[4] Mackie et al. 1987
[5] Roberts et al. 1989

Kinsella (1988) has suggested that, since much of the data regarding the hyperlipidemic effects of saturated fatty acids are derived from studies using solely one type of fat, usually at 40% of dietary energy requirements, the results are of questionable significance when applied to humans who consume a heterogeneous mixture of fatty acids. In the light of current knowledge, the recommendation that the diet should include a moderate fat intake (30% of dietary energy), with the fatty acids evenly balanced between saturated, monounsaturated, and polyunsaturated would seem to be sound advice.

Monounsaturated fatty acids & blood lipids

Much of the research relating to the lipid hypothesis has concentrated on the effects of polyunsaturated and saturated fatty acids on serum total cholesterol levels:- PUFA were considered to lower cholesterol levels, saturated fatty acids to raise them. In consequence the ratio P/S became a convenient way to describe the proportions of PUFA (P) and saturates (S) in edible oils, foods and so on.

Monounsaturated fatty acids were considered 'neutral', neither raising or lowering

serum cholesterol levels, although early work of Keys et al. (1965) and Hegsted et al. (1965) had shown that substitution of oleic acid for saturated fatty acids resulted in a lowering of serum cholesterol levels.

Since 1985, interest in the monounsaturated fatty acids, especially oleic acid, has again surfaced (Mattson and Grundy, 1985; Grundy, 1989). Grundy and his colleagues claim that dietary oleic acid lowers total serum cholesterol as much as linoleic acid, without causing the reduction in HDL levels sometimes observed with linoleic acid. Naturally these claims have been enthusiastically taken up by producers of high-oleic oils (such as olive oil, or high-oleic varieties of safflower oil or sunflower oil) in order to market their products. One of the problems however in comparing different edible oils is that their cholesterol-lowering effects may be obscured by minor components, e.g. phytosterols in polyunsaturated oils, or squalene in olive oil, which may also affect serum cholesterol levels. Moreover the study of Mattson and Grundy (1985) was based on twenty subjects, eight of whom had high blood TAG levels, using liquid formula diets. Further studies are currently being undertaken by other research groups to establish whether the findings of Mattson and Grundy (1985) are applicable in larger populations on more realistic diets. Support for their findings has recently been provided by a study by Mensink and Katan (1989). Until further scientific evidence is available it would seem that the main dietary value of oleic acid is as a replacement for saturated fatty acids in the diet, thus ameliorating whatever hyperlipidaemic effects the latter may have.

Essential fatty acids & blood lipids

When discussing dietary polyunsaturated fatty acids and their effects on serum lipids, researchers are usually referring to the essential fatty acids derived from linoleic and α-linolenic acids. During the first twenty of the thirty or so years in which the lipid hypothesis has existed most studies have focussed almost exclusively on linoleic acid and the vegetable oils rich in this fatty acid. During that time there was not much interest in other (n-6)PUFA, nor in (n-3)PUFA. In the last ten years, however, there has been an enormous surge of interest in these other PUFA, as a result of the realisation that the eicosanoids which regulate so many of our physiological responses are derived from the 20-carbon (n-6) or (n-3)PUFA. Since the fatty acids and eicosanoids of the (n-6) pathway are completely separate from those of the (n-3) pathway, i.e. no interconversion between pathways, and since eicosanoids from the (n-6) pathway may compete with, or even inhibit those derived from the (n-3) pathway, it has been inferred that changing the dietary intake or balance of (n-6)PUFA and (n-3)PUFA can affect human health.

Within the last ten years, therefore, research has extended into several new areas, for example into the use of fish or fish oil preparations as sources of pre-formed (n-3) fatty acids such as 20:5n-3 or 22:6n-3 (for review, see Kinsella [1986], or Harris [1989]). The consumption of (n-3) fatty acids results in a lowering of serum triacylglycerol levels as well as serum cholesterol, in contrast to 18:2(n-6) which only lowers serum cholesterol.

Research is being undertaken to determine whether there are undesirable effects of over-consumption of fish oils (Ackman, 1988), but there is little doubt that occasional inclusion of fish in a varied diet is likely to be beneficial.

There has been increasing interest in the metabolism and effects of dietary α-linolenic acid (18:3n-3), the precursor of the (n-3) essential fatty acids, and in the optimum balance between dietary intakes of linoleic and α-linolenic acids. It is generally felt that 1 to 2% of caloric intake as linoleic acid, and 0.5% as α-linolenic acid, is sufficient to provide the daily requirement of these essential fatty acids; in both cases the average daily consumption is usually well in excess of requirements (Kinsella, 1986), and essential fatty acid deficiency in humans is extremely rare.

As indicated earlier, there has been an enormous amount of scientific literature published on the subject of dietary fats and health.

This article has given only a bare outline of fatty acid chemistry and the effects of fatty acids on blood lipids, but the effects of dietary fatty acids are currently being studied in relation to a wide variety of diseases, such as cancer, heart

disease, atherosclerosis, arthritis, and so on, and each of these areas has resulted in a great deal of scientific publication.

The reader is referred to the many, much more detailed, reviews which have appeared periodically in the literature, which cover most of the aspects of dietary fatty acids so briefly alluded to above.

These include those by Kinsella (1986, 1988), Beare-Rogers (1988), Gurr (1988), Sanders (1988), Nelson and Ackman (1988), Simopoulos (1989), Harris (1989), and Stehbens (1989).

That so many reviews should have appeared in the 1988-1989 period is a good indication of the current world-wide research interest in dietary fatty acids.

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Changing Perceptions of Foodborne Botulism

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Recent research on the characteristics of *Clostridium botulinum* and the epidemiology of botulism has challenged some well-established assumptions about botulism and the problems that it presents for the food industry. Botulism has traditionally been perceived as a problem for food processors, associated principally with improperly canned or preserved low-acid foods (pH >4.6). However foods subjected to temperature abuse in food service operations have now been implicated as the cause of several large outbreaks of botulism, and *C. botulinum* has been shown to grow at pH levels below 4.6. The belief that foodborne botulism is always a result of ingestion of preformed toxin has been challenged. There is evidence that botulism may also result from colonisation of the adult intestinal tract by *C. botulinum*. The production of botulinum toxin may not be restricted to the organisms usually included within the species *C. botulinum*. Other species of *Clostridium* appear to have acquired the ability to produce botulinum toxin. The implications of these findings are discussed in relation to trends in food processing and packaging.

Introduction

Food-borne botulism is a severe and often fatal type of food poisoning which has traditionally been regarded as an intoxication. The causative organism, *Clostridium botulinum*, grows and produces toxin in a food which is subsequently ingested. The toxin blocks the release of acetylcholine at neuromuscular junctions, thereby effecting a flaccid paralysis. If untreated, the muscles controlling breathing are no longer stimulated and death occurs from suffocation. There are seven types of *C. botulinum*, classified A-G according to the serological specificity of the type of toxin they produce. Types A, B and E commonly cause illness in man and type F has been implicated occasionally. Many reviews have described the disease, the organism and its toxins (see Hauschild 1989; Hazzard and Murrell 1989).

Soil is the natural reservoir for *C. botulinum*. Most surveys for *C. botulinum* have been carried out in the northern hemisphere, where it is found frequently in soils from the USA, USSR, Europe and Japan. There is comparatively little information about *C. botulinum* in the southern hemisphere. Types A, B, C and D have been found in Australian soils and may therefore find their way onto raw agricultural products.

Traditionally, foods known to be potential sources of *C. botulinum* have been treated to destroy the spores of the organism (e.g. the 'Botulinum Cook' for canned foods), or conditions that prevent the outgrowth of spores have been created within the

Table 1
Examples of the Incidence of Foodborne Botulism

Country	Period	Outbreaks		Cases	
		Total	Per year	Total	Per year
Poland	1979-83	-	-	2390	478
China	1958-83	986	38	4377	168
USSR	1958-64	95	14	328	47
France	1978-84	115	16	217	31
USA	1971-85	210	14	485	32
Japan	1951-84	96	3	478	14
Britain	1949-84	3	0.1	11	0.3
Australia	1942-84	5[1]	0.1	53	1.3
New Zealand	1940-82	0			

[1] Four associated with vegetables, 1 with canned tuna

Data from Hauschild (1989).

food (Hauschild 1989). Methods for the control of *C. botulinum* in processed foods are well established, and commercially processed foods have a good safety record (Anon 1984; Tompkin 1980; Hauschild 1989). Botulism has been a problem principally with home-processed foods in the USA and improperly cured meat products in Europe. Fermented fish products in Japan and fermented marine products from arctic regions of Alaska and Canada are also often associated with botulism. Worldwide, large numbers of outbreaks occur, although the incidence varies considerably in different regions (Table 1; Hauschild 1989). Outbreaks have not been common in Australia.

This review examines some changes in our perceptions of food-borne botulism that have resulted from research over the past 10-15 years. Conventional assumptions about the range of clostridia that are capable of producing botulinum toxins, the mechanisms by which botulism occurs, and the environmental conditions that permit the growth of *C. botulinum* are being questioned. With the advent of new processing methods and novel food preparations, concern is being expressed about the risk of botulism from foods not previously thought to be potential vehicles. Increasing numbers of outbreaks are originating in food service establishments.

Botulinum toxins and taxonomy

The inclusion of Gram positive, anaerobic, spore-forming rods within the species *C. botulinum* is based solely on an organism's ability to produce botulinum toxin. This practice has been questioned frequently and appears to have little justification, other than convenience. From DNA binding experiments Lee and

Table 2

Sub-grouping Within the Species *C.botulinum*

Type	Proteolytic (meat)	Saccharolytic	Nucleic Acid Studies	Somatic Antigens[1]	Suggested Grouping
A	+			x	I
B	+		all	x	I
F	+		closely	x	I
C.sporogenes	+	-	related	x	I
Putrefactive anaerobes	+	-		x	I
B	-	+		x	II
E	-[2]	+	all	x	II
F	-	+	closely	x	II
Atoxic variants	-	+	related		II
C	+/-	-			III
D	+/-	-			III
G	+	-	homology		IV
Non-toxic *?C.subterminale*					IV

[1] x indicates cross-reaction between members of same group, there is no cross-reaction between groups.

[2] Proteolytic mutants of *C. botulinum* type E have been reported (Nakane and Iida 1977).

Riemann (1970) concluded that the species *C. botulinum* could be divided into three groups:

I Proteolytic strains, including *C. sporogenes*

II *C. botulinum* types C and D strains

III Non-proteolytic strains.

Type G had not been isolated at that time. DNA homology between the proteolytic *C. botulinum* and *C. sporogenes* confirmed that toxic and nontoxic strains were closely related (Joyce *et al.* 1972).

At present, the division of *C. botulinum* strains into four groups or species according to their proteolytic characteristics appears a logical proposal (Table 2). The physiological characteristics of *C. botulinum* strains vary according to the same phenotypic groups (Table 3, see Hazzard and Murrell 1989; Hauschild 1989; Lynt *et al.* 1982).

The production of botulinum toxin might not always be a stable characteristic of clostridia or a suitable basis for the definition of a species. There is evidence that the ability to produce botulinum toxin, at least in some types, may be carried by bacteriophages or plasmids. It also appears that other species of clostridia, which are quite different from the classical *C. botulinum* strains described above, may acquire the ability to produce the toxin. These findings could have important implications for the food industry.

During the 1970s toxigenicity of *C. botulinum* types C and D and *C. novyi* was shown to be due to the presence of specific bacteriophages (Eklund and Poysky 1981). *C. botulinum* type C cured of its bacteriophage could be converted to *C. novyi* or to *C. botulinum* type C or D by growth in the presence of the appropriate phage. The possibility that types C and D *C. botulinum* may have arisen from a common non-toxic strain which became either a type C or type D toxin producer according to the type of phage that infected it was also suggested.

The role of plasmids has been investigated to a limited extent. Plasmids have been found in many strains of *C. botulinum* and closely related non-toxic species. Four proteolytic type F strains carried a single plasmid of the same size and four of six non-proteolytic type F strains carried a single plasmid which was of a similar size to but different from the plasmids of the proteolytic strains. However other groups of toxic *C. botulinum* were not always found to carry plasmids (Strom *et al.* 1984; Weickert *et al.* 1986).

Table 3

Growth Requirements and Characteristics of *C.botulinum* Types A, B, E and F

	Group	
	I (proteolytic)	II (non-proteolytic)
Toxin types	A,B,F	B,E,F
Inhibitory pH (see text)	4.6	5.0
Minimum a_w[1]	0.94	0.97
Inhibitory NaCl concentration	10%	5%
Minimum temperature	10°C	3.3°C
Maximum temperature	45-50°C	40-45°C
Spore heat resistance	High[2]	Low[3]

[1] Varies with the controlling solute. Data are for NaCl.
[2] $D_{100°C}$ about 25 min
[3] $D_{100°C}$ <0.1 min

Recent studies have indicated that toxin production by *C. botulinum* type G is plasmid mediated (Eklund *et al.* 1988).

In 1985 a case of infant botulism attributed to type F toxin was described, yet the causative organism was identified by its biochemical characteristics as *C. barati.* This organism was quite different from the proteolytic types A and B normally associated with infant botulism, and it produced botulinal toxin which was neutralised by type F antibodies (Hall *et al.* 1985). In 1986 two separate incidents of infant botulism, both attributed to type E toxin, occurred in Italy. The causative organisms were identified by biochemical tests as *C. butyricum,* yet they produced type E botulinum toxin (Aureli *et al.* 1986; McCroskey *et al.* 1986). The type E toxin produced by these strains was compared with that produced by typical strains of *C. botulinum* type E and found to be very closely related but not identical. It was suggested that the *C. butyricum* strains had acquired the toxin gene of *C. botulinum* type E (Gimenez and Sugiyama 1988).

The production of botulinal toxin by clostridia that do not have the physiological characteristics traditionally associated with *C. botulinum* is of great potential significance to the food industry. The growth of these strains may not be prevented by the pH levels and other factors normally used to control *C. botulinum.*

Minimum pH for growth of *C. botulinum*

It has long been considered that spores of *C. botulinum* cannot germinate and grow at pH

Table 4

Reported Minimum pH Levels Permitting Growth of *C.botulinum*

Type	pH	Acidulant	Medium	Other bacteria present	Other significant factors
A & B[1]	4.18	HCl	15% pork	*B.subtilis*	Localised high pH ?
A & B[2]	4.2	HCl	5.5% soy protein	Bacilli	Low Eh Acid type
A[3]	4.11	HCl	3-5% soy	-	Titratable acidity Controlled low Eh
A[4]	3.73	HCl	3% beef	-	Controlled low Eh Titratable acidity Acid & protein type

[1] Tanaka (1982)
[2] Smelt *et al.* (1982)
[3] Young-Perkins and Merson (1987)
[4] Wong *et al.* (1988)

levels below 4.6 (Townsend *et al.* 1954; Ito and Chen 1978) and that figure has become enshrined in food processing regulations throughout the world. Canned foods with pH levels <4.6 are not required to be heat processed to kill *C. botulinum* spores; a lesser treatment, sufficient to destroy only vegetative bacteria, is used.

In 1978, Ito and Chen reviewed the effect of pH level on outgrowth of *C. botulinum.* They stated that generally a pH of 4.6 inhibits outgrowth of spores, although many factors such as inoculum size, number of strains or replicates studied, medium and growth conditions, affect this minimum level. Under sub-optimal conditions the minimum pH level is higher for some foods; inhibitory factors act in combination to control growth of *C. botulinum* (Gibson *et al.* 1987; Hauschild 1989; Roberts and Gibson 1986). Early incidents in which *C. botulinum* apparently grew in foods with pH levels below 4.6 were explained by localised areas of raised pH, created by mould growth in products such as tomato juice (Huhtanen *et al.* 1976; Odlaug and Pflug 1979). Experiments in several laboratories have now shown that *C. botulinum is* able to germinate and produce toxin at pH levels below 4.6 under certain circumstances (Table 4).

Several factors are important determinants of the ability of *C. botulinum* to grow at low pH. A low redox potential (Eh) in the growth medium is one such factor (Wong *et al.* 1988). In early studies with mixtures of *C. botulinum* and other spore-formers, such as *B. subtilis* and *B. licheniformis,* these other organisms were believed to play an important role in reducing the Eh to a level at which *C. botulinum* could grow (Raatjes and Smelt 1979). Other studies have used controlled, low Eh media in combination with low pH.

The presence of high concentrations of protein in the growth media has been an important feature of these studies, but the role of the protein is not clear. The proteins have included soy, beef, and pork. Smelt *et al.* (1982) postulated that soy proteins provide essential nutrients and reducing conditions that permit growth at low pH. They did not obtain reproducible results, since the batches of soy protein tested were not equally capable of supporting growth at low pH. The theory that germination occurs in localised areas of higher pH, e.g. in high concentrations of precipitated protein (Tanaka 1982), was not supported by studies in which toxin was formed in acidified media that were agitated to disrupt clumps and maintain soy protein in suspension (Young-Perkins and Merson 1987).

The type of acidulant used in low pH media is important. In one study hydrochloric and citric acids were less inhibitory than lactic or acetic acids (Smelt *et al.* 1982). Under different conditions citric acid was less inhibitory than hydrochloric acid (Wong *et al.* 1988). It has been suggested that titratable acidity is more important than pH in defining endpoints (Young-Perkins and Merson 1987; Wong *et al.* 1988).

Many food processors have not considered growth of *C. botulinum* at pH levels below 4.6 to be a problem, because most studies have indicated that growth occurs at low pH only in the presence of high concentrations of proteins. Traditional acid foods such as canned fruit products do not contain high concentrations of protein. However some new products, such as pasta sauces containing meat, which rely on low pH combined with a mild heat treatment for stability, may pose potential botulinal hazards.

Spoilage of canned, brined mung bean sprouts acidified with citric acid has been attributed to acid-tolerant clostridia including *C. barati, C. butyricum* and *C. perfringens.* These organisms can cause spoilage at pH levels as low as 3.7 for *C. barati* and *C. perfringens* and pH 4.0 for *C. butyricum* (de Jong 1989). Growth of such organisms in acid products is of concern, since they could reduce the Eh sufficiently to allow growth of *C. botulinum.* The possibility that strains of *C. barati* and *C. butyricum* might produce botulinum toxin has already been discussed.

Toxico-infection

Toxico-infection with *C. botulinum* differs from classical foodborne botulism in that the causative toxin is produced during an infection of the intestinal tract by *C. botulinum.* The mere presence of the organism in a food, without an opportunity for growth, can lead to botulism.

The first evidence for toxico-infection came when Minervin (1967) described

cases in the USSR. Patients suffering from botulism recovered, were released from hospital, and then became ill again with severe symptoms of botulism. At that time the idea of toxico-infection in humans was not well accepted. It was not until the infant botulism syndrome was recognised in 1976 (reviewed by Sugiyama 1982; Hazzard and Murrell 1989) that the occurrence of toxico-infections was accepted. Even then the syndrome was thought to occur only in infants of six months or less.

In cases of infant botulism the source of *C. botulinum* is for the most part unknown. Honey has been implicated as a source, but less than one third of all patients worldwide were fed honey. The organism has also been isolated from the domestic environment (household dust, water, etc) where its presence may be connected with incidents of infant botulism (Murrell and Stewart 1983). Generally, *C. botulinum* types A and B are responsible, although types C, F and G have also been implicated (Anon 1986).

Experiments with rats and mice have shown that once the normal intestinal flora has developed, young animals are no longer susceptible to *C. botulinum*. Germ-free adult mice and rats are susceptible to infection by 10 spores of *C. botulinum*, but they become resistant to infection after they develop a 'normal gut flora', indicating that infant botulism results from opportunistic growth of *C. botulinum* in a gut lacking certain bacteria (Sugiyama 1981).

The possibility exists that botulism may occur in adults as a result of intestinal colonisation with *C. botulinum* following ingestion of spores at a time when the gut flora is altered. Four cases of toxico-infection of adults, supported by laboratory evidence, were identified retrospectively from USA records dating back to 1966 (McCroskey and Hatheway 1988). In each case preformed toxin was not detected in any foods, although in two cases the organism was detected.

The first incident involved an elderly couple. Botulism was not considered early in their illness and the woman died before botulism was diagnosed. Toxin was not found in any foods in the house but *C. botulinum* spores were detected in home-made blackberry jam. Botulinum toxin was found in the man's serum 30 days after onset of symptoms and the organism was isolated from faeces on day 22 and 32.

The second case was a 33-year-old woman who had undergone intestinal surgery for obesity three years before the illness. Toxin was detected in serum and *C. botulinum* was isolated from faeces on the second day of the illness. She died later from respiratory complications. There was no evidence of wound botulism, neither *C. botulinum* nor its toxin was detected in foods tested from her home, and there was no food history consistent with food-borne botulism.

In the third incident, a family outbreak of food-borne botulism occurred, with the father not affected initially. Serum from the asymptomatic father was negative for botulinum toxin 19 days after the outbreak, but type B toxin was detected in an enrichment culture of his faeces, although *C. botulinum* was not isolated. On the 44th day after the outbreak the father developed botulism. Type B toxin was detected in his serum and *C. botulinum* was found in his faeces on day 47. Botulism was attributed to a gradual build-up of toxigenic organisms in his intestine.

The patient in the fourth incident, who had undergone intestinal surgery five weeks earlier, suffered from botulism and finally died from complications 240 days after onset of the illness. Type A toxin was detected in the serum and the patient was given antitoxin on day 15. The toxin was detected in stools up to day 20 but not afterwards. *C. botulinum* type A cells were detected in stools intermittently for 119 days. The persistence of the organism indicated colonisation of the gut. Prior surgery had probably provided the conditions necessary for establishment of the organism (McCroskey and Hatheway 1988).

Production of toxin *in vivo* was also shown in a 27 year old adult who suffered two episodes of botulinum toxaemia, with recovery in between. Both episodes were associated with severe illness requiring hospitalisation. Toxin and *C. botulinum* were found in faecal specimens during a three-month stay in hospital. Between episodes neither toxin nor C. *botulinum* could be demonstrated in clinical specimens (Sonnabend *et al.* 1987).

Evidence for toxico-infection has also been

presented in a variety of animals, including birds such as chickens and turkeys (Smart and Roberts 1977; Smart *et al.* 1983) and horses (Swerczek 1980; MacKay and Berkhoff 1982). Growth of *C. botulinum* in chicken caeca has been demonstrated (Miyazaki and Sakaguchi 1978).

New preservation processes

The ways in which we preserve food are changing. There is more emphasis on convenience, elimination of preservatives and use of minimal heat processes. These changes must be fully evaluated in terms of botulinal hazards otherwise disaster lies ahead. A recent outbreak of 36 cases (including 11 deaths) in Japan illustrated the risk of botulism when a food processor enhances rather than excludes the growth of *C. botulinum* by applying the wrong technology. A local product made from lotus roots was fried, then vacuum packed and sold unrefrigerated. In some areas of Japan the product was eaten without further cooking. *C. botulinum* was detected in 13 of 42 packs of the product, and type A toxin was detected in 11. In other areas of Japan this product is made in the home and eaten immediately after cooking (Otofuji et *al.* 1987).

Modified atmosphere packaging is a preservation process which is being applied in many new ways and which is cause for some concern. Even if a modified atmosphere does not directly affect growth of *C. botulinum,* changes in the microbial flora may result in less competition from other organisms. The extended shelf life may also provide additional time for growth of *C. botulinum* to take place. For example, if fish are stored in a modified atmosphere, it is possible that growth and toxin production can occur before the fish is spoiled sufficiently to cause consumer rejection, as occurs in air.

Trials with various types of fish which were inoculated with *C. botulinum,* packed in modified atmospheres, and incubated at 8, 12 and 26°C have illustrated the potential hazards (Post et *al.* 1985). Toxin was produced in cod and whiting before or simultaneously with sensory rejection, irrespective of storage temperature. Flounder was rejected before toxin was detected when stored at 8 or 12°C but at 26°C flounder was not rejected until after toxin production occurred (Table 5).

Lindroth and Genigeorgis (1986) investigated the probability of outgrowth and toxin production of non-proteolytic *C. botulinum* types B, E and F in fish stored in

Table 5

Storage of Cod Fillets in Different Atmospheres at 8°C

	Days to	
	Spoilage	Toxin production
Air	6	>10
Vacuum	16	20
N_2	17	17
100% CO_2	23	19
65% CO_2	16	9

Data from Post *et al.*, 1985.

modified atmospheres. The probability of toxin production was affected significantly by storage temperature but not by atmosphere alone, although the probability of toxin production was significantly affected by an interaction between atmosphere and temperature. They concluded that naturally occurring levels of *C. botulinum* would not initiate toxigenesis in red snapper stored at 4°C for up to 21 days, but at an abuse temperature of 8°C one spore could initiate growth and toxigenesis, occasionally before spoilage made the fish unacceptable.

There is concern that a variety of other packaged, non-sterile foods might be able to cause botulism. Cooked vacuum-packed potatoes, a convenience product with a suggested shelf-life of 6 weeks, were inoculated with about 10^6 spores of *C. botulinum* (types A, B and C) prior to sealing, evacuating and cooking. Type E spores were inoculated after heating. Cooking of potatoes in the bag did not destroy inoculated spores, which were subsequently able to grow and produce toxin at 10, 15 and 20° C. Toxin was produced before the product spoiled (Notermans *et al.* 1981). Inoculated pack studies with a double pasteurisation process showed a high proportion of spores remained viable in vacuum packed potatoes after the process. The spores were able to grow and produce toxin within 5-9 days at 25°C (Lund *et al.* 1988).

A study of potential hazards associated with packaging of mushrooms showed that mushrooms overwrapped with PVC utilised O_2 inside the packs faster than it diffused through the film. Spores of *C. botulinum* type A or B inoculated into the packaged mushrooms grew at 20°C and produced toxin before the mushrooms appeared inedible (Sugiyama and Yang 1975). Mushrooms are often contaminated with *C. botulinum* spores, e.g., about 10-100 spores/100g were present in each of 12 retail samples of fresh mushrooms (Hauschild *et al.* 1975). In a separate study, botulinum toxin was not detected in 1078 samples of fresh PVC-wrapped mushrooms, although spores inoculated into packaged mushrooms grew and produced toxin in 14 of 250 samples (Kautter *et al.* 1978).

The growth of *C. botulinum* in vacuum-sealed sandwiches packed in nitrogen has been investigated. The sandwich fillings had a pH of 5.9-6.6 and the product was known to be subjected to temperature abuse on occasions. Sandwiches inoculated with type E spores remained organoleptically acceptable but were toxic after 30 days at 12°C. Adequate refrigeration prevented the growth of types A and B. Hamburgers inoculated with types A and B became toxic on day 4 when stored at room temperature, although they were still visually acceptable (Kautter *et al.* 1981).

The absence of spores of *C. botulinum* from raw foods cannot be guaranteed. The food industry must evaluate new products and processes carefully to take full account of the potential botulinal hazards. Prepared chilled foods, particularly those in which mixed food types are packaged together, foods packaged in modified atmospheres, and other semipreserved products may cause botulism if subjected to temperature abuse. The form in which some of these products are packaged may lead some consumers to believe that the products are shelf-stable or can tolerate temperature abuse.

Botulism outbreaks associated with food service operations

Data on botulism outbreaks from the USA are summarised in Table 6. There were 175 outbreaks of foodborne botulism during the years 1957-1975 and information is available on 125 of those. Of these, 88% were related to home canned/processed foods, particularly vegetables, and 12% to commercially processed foods. The commercially processed foods were predominantly canned foods, although commercially packaged fish was implicated in several outbreaks, potato salad in one outbreak, and one case was associated with frozen pot pies which were mishandled in the home (MacDonald *et al.* 1986). There was only one small restaurant-associated outbreak during that period (Ryan and Cherrington 1971).

More recent data suggest that this pattern may be changing. From 1976 to 1984, 124 outbreaks involving 308 persons were identified. Vegetables (usually home canned) were implicated as

Table 6

Sources of Food-borne Botulism in the USA

Source	1957-1975[1] outbreaks		1976-1984[2] % of total	
	Number	% of total	Outbreaks	Cases
Commercially processed foods	15	12	3	2
Home processed foods	110	88	90[3]	
Restaurant associated	1	0.8	4	42

[1] Information was available for 125 outbreaks
[2] Information was available for 124 outbreaks
[3] Data for 1971-1985
Data from MacDonald *et al.* (1986), Hauschild (1989).

vehicles in 70% of outbreaks and meat/fish in the remaining 30%. These data show that *C. botulinum* toxins in restaurant foods can cause substantial public health problems. Although only 4% of these outbreaks were associated with food service operations, they accounted for 42% of cases. Four of these five outbreaks were large. The fifth involved only one person and was attributed to a left-over baked potato eaten in a restaurant (MacDonald *et al.* 1986). The restaurant-associated outbreaks have shown that foods not previously considered as potential vehicles for botulism can cause outbreaks if mishandled in ways that allow *C. botulinum* to grow and produce toxin. Restaurant-associated botulism can occur in the same way as some more common but less severe forms of food-poisoning - i.e. *C. botulinum* survives cooking and grows in food during temperature abuse.

In 1978, seven cases of type A botulism were caused by potato salad eaten at a restaurant. At this restaurant, unwashed whole potatoes were baked in aluminium foil for serving with dinners. Leftover potatoes were stored at room temperature and used in the next batch of potato salad, which was normally prepared every 5-7 days. The implicated potato salad was discarded before it could be tested for toxin, and toxin was not detected in baked potatoes from the restaurant. However, raw potatoes were found to carry *C. botulinum* spores. Spores of the outbreak strain inoculated into raw potatoes survived baking and were able to germinate and produce toxin within the cooked potato (Seals *et al.* 1981). Contaminated potato salad was also the vehicle for an outbreak of type A botulism in Colorado in 1969 (Ryan and Cherrington 1971) and potato salad was one of three foods implicated in an outbreak involving 34 persons in New Mexico in 1978 (Seals *et al.* 1981).

Sauteed onions, made from fresh raw onions and

served with a cheeseburger were implicated as the vehicle of transmission for type A botulism in 1983. Twenty eight people were affected over a three-day period. Investigations showed that raw onions had been sliced and cooked with spices, then covered with molten margarine and left warm on top of a grill for use throughout the day. The onions were not reheated before serving. The implicated batches of cooked onions were not available for testing, but 5 of 75 raw onions from the restaurant contained *C. botulinum*. The pH of the mixture of spiced onions and margarine kept on the grill was 5.6 and the temperature 41°C - ideal for the growth of *C. botulinum* (MacDonald *et al.* 1985; Solomon and Kautter 1986).

The second-largest outbreak in North America occurred in 1985. Thirty seven cases were reported among persons who ate at a restaurant in Vancouver BC. The victims included three from the USA and one from the Netherlands. Commercially-bottled chopped garlic in soybean oil served on sandwiches was implicated (Solomon and Kautter 1988). A notable feature of this outbreak was the slow development and progression of symptoms. Cases were widely dispersed and the early manifestations were not typical of botulism, therefore initial diagnosis was not always accurate. It is possible that even more cases with atypical symptoms went undiagnosed (St. Louis *et al.* 1988; Blatherwick *et al.* 1986). It was more than two months after the beginning of the outbreak and several days after the last patient had eaten at the restaurant that botulism was first diagnosed.

Apart from the possible loss of life, the costs of large outbreaks of botulism caused by food service operations are enormous. Total expenses for one restaurant-associated outbreak, involving 34 victims, where a commercially-produced three-bean salad was implicated, were estimated at US$5.8 million in 1978. This included the cost of control, medical care, and legal settlements. The median period for hospitalisation was 89.5 days for severe cases and 7.5 days for moderate cases (Mann *et al.* 1983).

Conclusion

The food industry is adopting a variety of new techniques for processing, packaging, preserving, and distributing foods. These advances offer substantial benefits for both industry and consumers. However they may also introduce new microbiological hazards, which must be evaluated and understood. History has shown clearly that the microbiological safety of new food products and processes must be validated adequately if public health problems are to be avoided. In addition, our understanding of the ways in which *C. botulinum* can create hazards is changing, and it is possible that the organisms capable of causing botulism are changing. The industry should maintain an awareness of these developments and their implications for industry practices.

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Rice Bran

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Rice bran is being promoted as 'The Whole Body Bran'. Like oat bran, it is claimed, rice bran is potent in reducing the risk of coronary heart disease. Like wheat bran, it is a good laxative. How good is the scientific basis of these claims? The short answer, I believe, is that these claims are premature. In particular, the scientific evidence that rice bran can reduce the risk of heart disease is too flimsy to justify the claims that have been made (*e.g. The Sun-Herald,* Sydney, October 29, 1989; *The Age,* Melbourne, November 21, 1989).

Bran

Bran is the outer layer, but not the husk, of cereal grains. All brans are more or less concentrated sources of dietary fibre and the potential health benefits from fibre are now well known. Fibre has beneficial effects on bowel action and may have a protective effect against heart disease and colon cancer (Royal College of Physicians 1980). There is no doubt whatsoever that fibre is effective in easing laxation. Wheat bran is particularly effective, provided that it is not too finely milled (Oakenfull and Topping 1987). In contrast, though, wheat bran appears to be totally ineffective in reducing the risk of heart disease. In controlled feeding trials with humans, most studies have shown no change in low density lipoprotein cholesterol in response to wheat bran, sometimes even a small increase (Truswell and Kay 1976).

With oat bran, the picture is quite different. Numerous feeding trials, with both animal and human subjects, have, with one notable exception (Swain *et al.* 1990), demonstrated a cholesterol-lowering effect - provided the daily intake is high enough (Oakenfull 1988).

The cholesterol-lowering action of oat bran appears to be related to its high level of soluble fibre. It is important to remember that dietary fibre is a complex and variable mixture of plant materials with different functions at different stages in its passage through the gut. Fibres from different sources are different mixes. It is hardly surprising that we should experience different bodily responses from consuming them. We should probably think of fibre in much the same way as vitamins - all types are necessary, but have different roles in the prevention of disease (Oakenfull and Topping 1987).

Rice Bran

Approximately 4.5 million tonnes of rice bran are produced in the world per year. Much of this is used as stock feed - it is rich in protein, lipids, vitamins and trace minerals (Saunders 1985). The chemical composition of rice bran depends both on the rice variety

and the milling procedure, so it varies widely (Saunders 1985). Much of the world's rice crop is still milled in a one-stage process which removes a mixture of hull, bran and germ as a single by-product in the production of white rice (Grist 1965). However, the use of two-stage or multistage mills in which hulls and bran are removed and recovered separately is increasing (Saunders 1985). It is this bran which is a potentially valuable product for human consumption.

The approximate composition of rice bran in comparison with oat bran and wheat bran, is shown in Table 1. there are two points to be emphasised from these data. Firstly, rice bran and oat bran both have a lower fibre content than wheat bran, but rice bran more resembles wheat bran in composition, having only a small proportion of soluble fibre. Secondly, rice bran has a much higher lipid content than the other brans.

This 'rice bran oil' creates technological problems because it rapidly turns rancid. Rice bran contains highly active lipases which convert neutral lipids into free fatty acids causing off flavours to develop within a few days. For this reason, rice bran has in the past been used exclusively for stock feed (Barber and de

Table 1.

Comparison of the major components (g/100g) of rice bran, oat bran and wheat bran (Saunders 1985,86; Oakenfull 1988).

	Rice Bran	Oat Bran	Wheat Bran
Protein	14.1	15.3	14.1
Lipid	20.4	6.0	5.5
Starch	24.0	38.4	23.0
Dietary fibre -			
soluble	2.5	10.5	2.8
insoluble	23.0	11.7	39-9
total	25.5	22.2	42.7

Table 2.

Phytosterol content of vegetable oils (Gordon, 1986).

Oil	Total phytosterols (mg/100g oil)
Corn	1452
Olive	300
Palm	157
Rapeseed	520
Soya bean	352
Rice bran	3645

Barber 1980) and the oil extracted from it has been suitable only for lubricating machinery. Recently, though, an economically viable process has been developed which uses extrusion cooking to deactivate the enzymes, producing a stable rice bran product acceptable for human consumption (Randall et al. 1985). The oil in rice bran may also have important nutritional consequences because of its unusual composition. It contains a much higher proportion of phytosterols than other vegetable oils (Table 2) and this component of the oil appears to have cholesterol-lowering activity in rats (Sharma and Rukmini 1986; 1987).

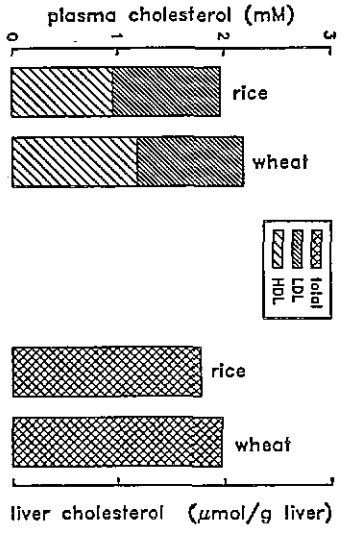

Fig. 1 Plasma and liver cholesterol concentrations in rats fed rice bran or wheat bran (Topping *et al., 1990).*

Effect of rice bran on plasma lipids & laxation

Compared with wheat bran and oat bran, very few studies have so far been carried out with rice bran.

(1) Animal Feeding Studies:
(a) Whole Rice Bran: Rice bran has been compared with wheat bran in an experiment with rats designed primarily to study the interactive effect of the bran with fish oil (Topping et al. 1990). The animals were fed enough of either of the two brans to provide dietary fibre at a level of 70 g/kg of diet. Other dietary components were appropriately adjusted. In the absence of fish oil, the total plasma cholesterol after 10 days of the diet was 10% lower in the animals fed rice bran than in those fed wheat bran; liver cholesterol was similarly lower in the rice bran group (Fig. 1). Rice bran also influenced the distribution of cholesterol between its carrier molecules in the blood plasma. There are two main types of carrier - high density lipoprotein (HDL) and low density lipoprotein (LDL). It is only the LDL-cholesterol which is associated with increased risk of heart disease and with rice bran most of the reduction in plasma cholesterol was in the LDL fraction.

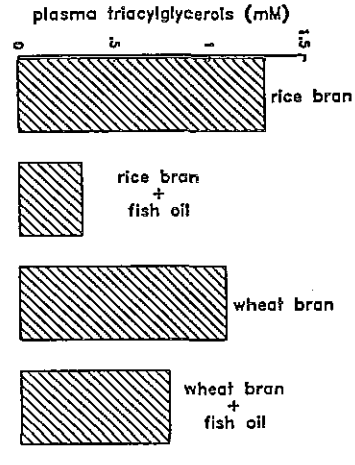

Fig. 2. Plasma triacylglycerols in rats fed rice bran or wheat bran, with or without fish oil (Topping *et al., 1990).*

Even more interestingly, rice bran dramatically enhanced the effect of fish oil on the triacylglycerols (Fig. 2). The triacylglycerols are the major component of the body fat. Plasma and liver concentrations of triacylglycerols were substantially lower in the animals fed fish oil with rice bran than in those fed fish oil with wheat bran (Roach et *al.* 1987).

Thus rice bran appears to have a modest cholesterol-lowering activity and, perhaps more importantly, a very definite effect on fat metabolism in the liver. Topping et *al.* (1990) consider it most likely that the soluble fibre fraction of the rice bran is responsible for these effects. It is possible, though, that the unusual composition of the rice bran oil may also be a significant factor.

(b) Rice Bran Oil: Sharma and Rukmini (1986), again using rats, compared rice bran oil with groundnut oil. The oil was fed at a level of 100 g/kg of diet and the diets were in other respects equivalent. In animals fed a cholesterol-free diet, similar to that used in the experiment reported by Topping *et al.* (1990), there was again a significant (14%) reduction in total plasma cholesterol in the rice bran oil group, most of this reduction being in the LDL-cholesterol (Fig. 3). (A bigger reduction in plasma cholesterol was seen when the diet included cholesterol.)

The rice bran oil and groundnut oil were very similar in composition except for the

substantial non-saponifiable fraction (4.1% compared with 0.3% in groundnut oil) which consists mainly of the phytosterols mentioned earlier. Sharma and Rukmini (1987) have identified this non-saponifiable fraction as having the cholesterol-lowering activity. Phytosterols have been identified as having cholesterol-lowering activity in humans (Lees *et al.* 1977), probably by inhibiting absorption of cholesterol from the gut. But the level known to be effective is greater than that present in the diets used by Sharma and Rukmini (1986) and they suggest that an as yet unidentified component of the non-saponifiable fraction may also be active.

The level of rice bran oil in the diets used by Topping et *al.* (1990) would have been about 60 g/kg of diet - i.e. approaching the 100 g/kg of diet used by Sharma and Rukmini (1986). Thus an unidentified component of the oil may be at least partly responsible for the lipid lowering activity of rice bran.

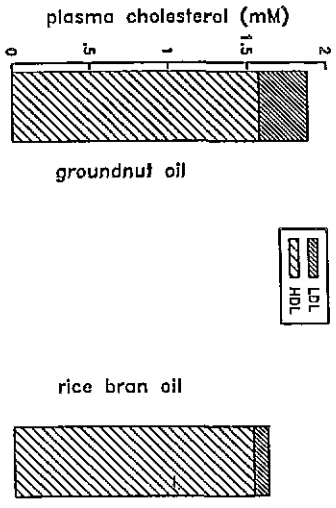

Fig. 3 Plasma cholesterol concentrations in rats fed cholesterol-free diets containing either groundnut oil or rice bran oil (Sharma and Rukmini, 1986).

(2) Human Feeding Study

Only a single clinical trial has so far been reported (Inge 1989). This study compared the effects of rice bran, wheat bran and oat bran on laxation and plasma lipids in a group of 24 men with mildly elevated plasma cholesterol levels. The experimental diets provided 22-23g dietary fibre daily, of which 12 g was supplied by the respective bran. The diets were otherwise matched for dietary fibre and also matched for fatty acid content and fatty acid profile. The experiment was conducted in three four-week periods in which the three test brans were allocated to each subject in a predetermined random order. Looking first at plasma lipids, the results are summarised in Table 3. Rice bran had no significant effect on the *total* plasma cholesterol whereas oat bran gave a modest 5% reduction.

The claim that rice bran reduces the risk of heart disease is based on the effect of the bran on the ratio of HDL-

Table 3.

Effects of wheat bran, rice bran and oat bran on plasma lipids, lipoprotein lipids and apoproteins in human volunteers. Means and standard deviations are given; in each row, values with different superscripts differ significantly.

	Baseline	Wheat Bran	Rice Bran	Oat Bran
Total cholesterol (mM)	6.34[a] ±0.84	6.39[a] ±1.07	6.27[a] ±1.01	6.03[b] ±0.87
Ratio of HDL to total cholesterol	0.166[a] ±0.046	0.171[a] ±0.048	0.175[b] ±0.051	0 179[b] ±0.049
Ratio of apoprotein A1 to apoprotein B	1.29[a] ±0.29	1.28[a] ±0.28	1.33[b] ±0.28	1.34[b] ±0.27

cholesterol to total cholesterol and on the ratio of apoprotein A1 to apoprotein B. Both these quantities were significantly greater when the diet included rice or oat bran and there are indications from epidemiological studies, in particular the Framingham study, that these ratios are more powerful predictors of heart disease risk than the total plasma cholesterol (Naito 1987).

The claim that rice bran has a beneficial effect on laxation is more strongly based, but not from this particular study. The subjects were only asked to keep a log book of their daily number of stool motions. Counting numbers of bowel movements certainly provides some indication of laxation but it is more usual to weigh the faecal material produced *(e.g.* Stephen and Cummings 1979). The results of these counts are shown in Table 4. The number of motions per day was significantly increased by both rice bran and wheat bran. Although this particular study was not rigorous, it is indicative, and there is additional and independent evidence of the effectiveness of rice bran in easing laxation (Tomlin and Read 1988).

'One swallow does not make a Summer' - Aristotle

As Sir Karl Popper has observed (1959), 'We do not take even our own observations quite seriously, or accept them as scientific observations, until we have repeated and tested them.' In nutrition, individual observations are even less repeatable than in the physical sciences. We are dealing with whole organisms whose behaviour is complex and still poorly understood. It is important to keep this fact in mind before making definite claims or specific dietary recommendations.

The claim that rice bran 'is potent in reducing the risk of coronary heart disease' is based on a single observation that has not yet been repeated. In contrast, the claim that oat bran can lower plasma cholesterol is based on repeated observations in a number of different laboratories (Oakenfull 1988). The claim for rice bran may ultimately be substantiated - particularly as the phytosterols present in the oil suggest a plausible explanation for any cholesterol-lowering activity. But a single observation does not provide scientific grounds for recommending rice bran as a dietary means of reducing the risk of heart disease. More research is needed.

We are still a long way from seeing enough evidence to justify the claim that rice bran is 'The Whole Body Bran'.

Table 4.

Effects of wheat bran, rice bran and oat bran on laxation. (Mean number of stools passed daily; values with different superscripts differ significantly.)

Baseline	Wheat Bran	Rice Bran	Oat Bran
1.23^{a}	1.42^{b}	1.46^{b}	1.31^{a}

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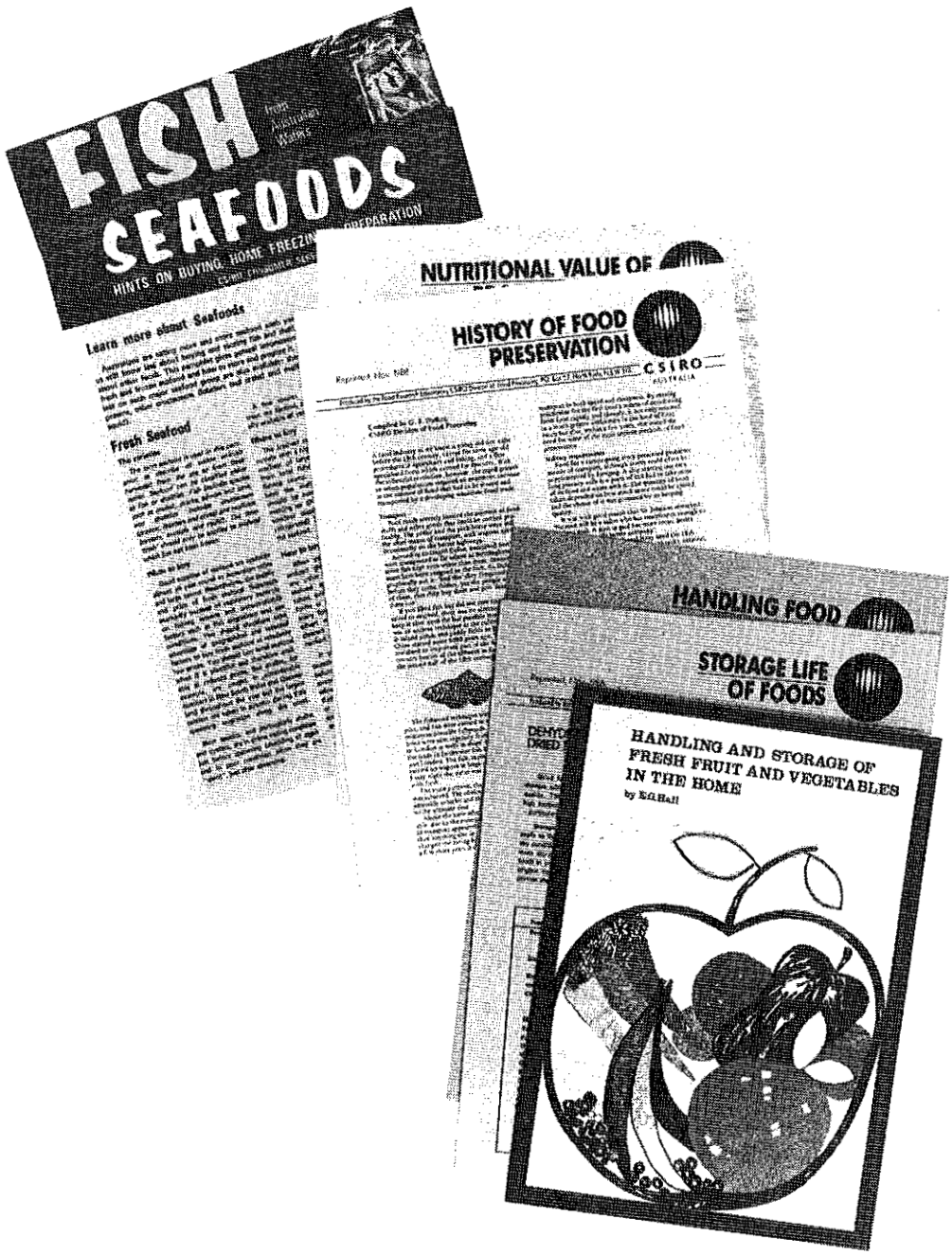

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