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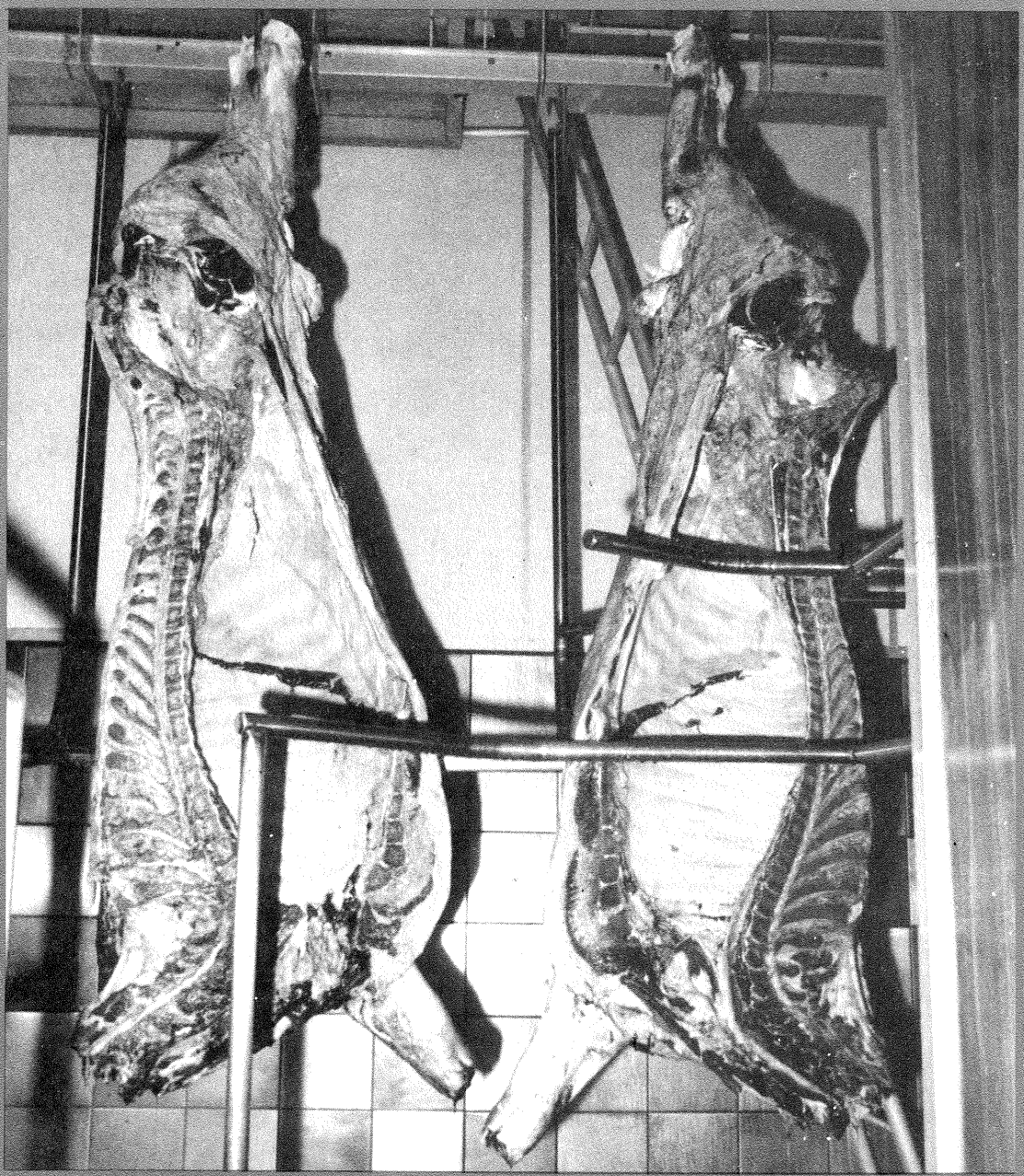

Beef tenderness: the influence of animal age and postmortem treatment

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Introduction

Tenderness is a very important attribute of meat and much is now known about it.

Scientists in the early part of this century were convinced that tenderness was associated with marbling, which they knew to be governed by pre-slaughter nutrition. The thinking of that time can be summed up with a quote from Bull (1916): "The main object in fattening is to improve the flavour, tenderness, and quality of lean meat by deposition of fat between the muscle fibres."

The early beliefs were based solely on subjective observations and in the absence of anything more convincing, were accepted by scientists and laymen alike. It is no wonder, therefore, that the perceived importance of marbling has become so widespread. Doubts seem to have occupied some minds, because in 1956 Cover *et al.*, in discussing the marbling/tenderness relationship, observed "It is disconcerting that something which has appeared so obvious to so many for so long should be so extraordinarily difficult to prove in the laboratory." Subsequently, research in the 1970s demonstrated only a weak relationship. This work has been reviewed recently by Tatum (1981).

Our present understanding of the factors that influence meat tenderness began to evolve in the early 1960s (Locker 1960) when closer observations were made of the behaviour of muscle as it goes into rigor. Marsh and Leet (1966) commented that, given the extensive series of biochemical changes taking place in the 12-24 hours after slaughter, it was surprising that few attempts had been made to relate meat tenderness to these conditions. Extensive research done since that time has led to a better understanding of the rigor processes and how they influence meat tenderness. This paper discusses post-mortem muscle metabolism, electrical stimulation, tenderstretch, ageing, animal age and the degree to which we now understand their influences on tenderness.

Causes of meat toughness

Meat toughness is attributable to three main factors. The first is loosely termed "age-induced toughening," although a more accurate description is connective tissue toughening. The connective tissue component of most interest to us is collagen because it makes a significant contribution to the toughness of meat (Marsh 1974). The crosslinks between the molecules which comprise collagen are responsible for the strength of connective tissue. In young animals the crosslinks are weak enough to be broken when muscle is heated. These crosslinks strengthen as the animal gets older, resulting in an increase in resistance to thermal breakage and therefore meat toughness. Increasing connective tissue toughness is probably not commercially significant until a beast is about four years old (i.e. full mouth or 8-tooth).

Whereas the age of the animal determines the strength of the connective tissue, the actual amount of connective tissue will also have an effect on toughness. Amount of connective tissue varies with the anatomical location of the muscle and is a reflection of the function of that muscle. Muscles that do a lot of work have more connective tissue than muscles that do not do a lot of work. Increasing toughness is therefore observed as we go from fillet to rump to chuck, irrespective of animal age.

The second, and probably the most important, cause of toughening is cold shortening. The cold shortening phenomenon affects the contractile structure of the muscle as distinct from the connective tissue. The contractile structure or the basic components of the muscle fibres, namely the actin and myosin filaments, move with respect to one another during normal contraction and relaxation of the muscle in the live animal.

Cold shortening was first described by Locker and Hagyard (1963) and it has been widely studied since (Marsh *et al.* 1968; Bendall 1971; Harris 1976). In the pre-rigor state the muscle fibres, including actin and myosin, are free to move. During rigor at room temperature (approximately 15 °C) the fibres contract by about 10% of their rest length. If the pre-rigor muscle is subjected to more rapid cooling, a much larger contraction is experienced. The

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more a muscle contracts the tougher the meat will be. A muscle shortened 40% of its length will be about four times as tough as the original (Marsh and Leet 1966).

The third, generally least, significant factor affecting tenderness is pH. Toughness increases as the ultimate pH (i.e. that value reached after post-mortem glycolysis has ceased) increases from 5.4 to about 6.0, then decreases with further increase in ultimate pH.

Cold shortening

Rigorous hygiene standards require fresh carcasses to be chilled as rapidly as possible. This is in conflict with the desire to preserve tenderness. If a dressed side of beef is rapidly chilled, many of the surface muscles will reach temperatures below 10°C within 10 to 12 hours after slaughter. Lean, light bodies are more susceptible to toughening than are fat light bodies and fat heavier bodies, because the latter will tend to cool more slowly. Bodies of similar weight and fat cover may have different rates of cooling due to different chilling conditions (e.g. position in the chiller). This, too, will lead to differences in tenderness. Recent work by the CSIRO (Powell and Walker 1984) in Victorian and Queensland abattoirs has demonstrated that even with relatively slow chilling of carcasses an unacceptable amount of cold shortening has resulted. The effect of post-mortem changes (including those influenced by chilling) was reviewed by Newbold and Harris (1972), and they concluded that processing methods should incorporate means of preventing cold shortening.

Cold shortening can be prevented by physical restraint of muscle as it goes into rigor. Shortening can also be prevented if rigor is complete before the muscle is subjected to rapid chilling. Muscles that are in rigor will not cold shorten, regardless of temperature. In fact, they will begin to tenderize due to "ageing" whilst held in a chilled state.

Physical restraint of muscles can be achieved by hanging a side in the "tenderstretch" (sacrosciatic ligament or aitch bone hung) position as it goes into rigor. Electrical stimulation can be used to accelerate the rigor process, thus removing the ability of a muscle to shorten under chiller conditions. Each method achieves the result via a different mechanism. However, the end result is the preservation of inherent tenderness.

Tenderness preservation

Influence of animal age

Provided a muscle is not toughened by excessive cold shortening, the younger the animal the more tender the muscle will be. As an animal gets older the connective tissue gets stronger and more resistant to breakdown during cooking. This manifests itself as toughness. Because toughening due to connective tissue increases progressively with age, and there is no means of preventing this, it is important to recognize the age at which the level of this inherent toughness becomes undesirable. The CSIRO Division of Food Research Meat Research Laboratory has done extensive research into this. In a recent study (Bouton *et al.* 1978) (Table 1), 250 animals from Victoria ranging in age from two months to ten years, and weighing from 120-445 kg dressed weight, were slaughtered. One-half of the sides were protected from cold shortening (aitch bone hung) and all were subjected to conventional chilling.

TABLE 1

Effect of animal age on meat tenderness (cold shortening prevented)

Animal age (months)	Meat tenderness (*semimembranosus* muscle)
2	Very tender
9	Tender
16	"
27	"
42	Slightly tough
120	Tough

For cattle between 9 months and 42 months of age, there was no commercially significant increase in toughness of topside muscles that had not cold-shortened, indicating that the tenderness of muscles (non-shortened) with moderate levels of connective tissue is not adversely affected by animal age up to and including cattle with seven permanent incisor teeth (3½ years old). Some differences did occur outside this range (i.e. 2 and 120 months). Once an animal is full mouthed it is difficult to determine its age accurately. Such animals may be four years old or considerably older, so their tenderness could vary widely.

Whereas it is true that, in the absence of cold shortening, meat from a 42-month-old animal is acceptably tender, cold shortening may lead to young animals yielding meat that is much tougher than that of older animals. Research summarized in Table 2 has demonstrated the marked effect cold shortening can have on carcasses from young animals which are lighter, leaner, and therefore chill more rapidly, than heavier and fatter carcasses.

Older animals, as lean and light as the

TABLE 2

Effect of animal age and cold shortening on meat tenderness

Animal age (months)	Meat Tenderness: Cold shortening NOT prevented	Meat Tenderness: Cold shortening prevented
9	Tough	Tender
16	"	"
27	Slightly tough	"
42	Tender	"

younger animals referred to above, will be similarly toughened by cold shortening. Conversely, younger animals that are well finished with a good to heavy fat cover will not toughen to the same extent. Some degree of toughening will always occur, however, unless measures have been taken to prevent cold shortening.

Tenderstretch

Studies at the CSIRO Division of Food Research Meat Research Laboratory have shown that in most if not all abattoirs a measurable degree of shortening will occur with those muscles in a carcass that are free to contract. Prevention of this cold shortening by aitch bone hanging, for example, leads to a tenderness improvement in rump, thick flank, topside, silverside, striploin and cube roll (Bouton *et al.* 1973), which are valuable primal cuts. This improvement is equivalent to about three weeks' ageing of conventionally-hung meat held at 1°C.

Electrical stimulation

Electrical stimulation has been more widely accepted throughout the meat industry than has tenderstretch. Stimulated sides are hung conventionally and therefore cuts have a normal shape, whereas some cuts from tenderstretched carcasses have ususual shapes, e.g. rump and topside.

Electrical stimulation speeds up the reactions associated with the rigor process. Glycolysis, for example, is accelerated to a point where the lactic acid which would normally accumulate over about 20 hours, has done so in about three hours. This accelerated rigor virtually eliminates the capacity of the muscles to cold shorten under severe commercial chilling conditions. The muscle will therefore retain its inherent tenderness as determined by the age of the beast at slaughter.

For electrical stimulation to be effective, the rapid accumulation of lactic acid should yield a muscle pH of 6.0-6.3 one hour after slaughter. Unstimulated muscles would have a pH of about 0.6 pH units higher than that of stimulated muscles, one hour after slaughter.

Stimulated meat with an ultimate pH in the range of 5.9-6.2 (i.e. "dark cutting") can be quite tough. Such meat should not, theoretically, be identified as having enhanced tenderness. This is difficult to do in practice because carcasses are usually branded before ultimate pH can be estimated. The proportion of "dark cutters" amongst carcasses is very variable, and although this proportion is generally small, instances of up to 38% have been recorded.

Electrical stimulation can be applied using either high voltage (HV) or extra low voltage (ELV) equipment. Large plants generally employ automatic HV systems which can be used at any stage up to one hour post-slaughter. Small plants are more likely to adopt the cheaper, manually-operated ELV systems which must be used immediately post-slaughter. If ELV stimulation is carried out properly it is as effective as HV stimulation (Powell and Walker 1984) and tenderstretch (Powell *et al.* 1984).

Ageing

Ageing of conventionally-hung meat has become widely accepted throughout the industry as a means of tenderization. Vacuum-packing has enabled ageing to be undertaken on a large scale without excessive loss of product through premature spoilage and without loss from evaporation.

Unlike tenderstretch and electrical stimulation, the tenderizing role of ageing is achieved by enzymes attacking the myofibrillar structure of the muscle. Unfortunately, the muscle enzymes do not attack connective tissue (Bouton and Harris 1972), so meat from old animals, and cuts containing a high proportion of connective tissue do not respond well to ageing. It is also important to realize that muscles that have been severely cold shortened do not tenderize significantly upon ageing. Ageing of tenderstretched or electrically-stimulated meat produces further improvement in tenderness.

Conclusion

Meat from carcasses that have been tenderstretched or electrically stimulated and then conventionally chilled and boned will be more tender and of a more consistent quality than meat that has been treated similarly but neither tenderstretched nor stimulated. The improvement is comparable with about three weeks' ageing. However, age-induced toughness and connective tissue toughness are

not affected by tenderstretch or electrical stimulation, or, for that matter, by ageing.

Therefore, if we want to provide reliably tender meat we must ensure that the meat comes from carcasses that are either electrically stimulated or tenderstretched and/or aged, and from animals with seven permanent incisors or less.

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Food allergy – nature and study

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It has long been recognized that certain foods may produce abnormal reactions in susceptible individuals. The adage of Lucretius that "what is food to one may be poison to another" was, and still is, accepted as an expression of a widely held view. Hippocrates and Galen, the great physicians of the ancient world, both recorded the occurrence of untoward reactions following the ingestion of foods, particularly milk. It was not, however, until the introduction by Von Pirquet (1906) of the scientific concept of allergy as an abnormal reaction to a foreign protein that any systematic study of the phenomenon was undertaken. Even so, the development and use of powerful therapeutic agents during the early years of this century tended to overshadow any serious study of many dietary problems. More recently, the situation has begun to change with increasing recognition of some of the subtler effects of diet and more intensive study of the acute food reactions including allergies.

This article deals, particularly, with those food hypersensitivities which have been shown to be immunologically based and are thus amenable to study outside the immediate clinical environment.

Definition

It is important to define the term 'food allergy' in this context. Popular usage often generalizes the meaning to include effects resulting from unknown or uncertain mechanisms together with those which could more properly be said to have psychological causes. It has been proposed (Anon 1979) that 'food sensitivity' be used to include all short term food reactions and that under this general heading the non-immunologically based effects be grouped as 'intolerances', separate from the immune reaction allergies. The concept is illustrated in Table 1. A more complete discussion of the divisions which may be applied to food intolerances has been given by Taylor (1980) and the subject of specific problems caused by biochemical, metabolic abnormalities has been recently reviewed (Herman and Hagler 1979).

TABLE 1
Defining food allergy

Food sensitivity	
Intolerance (Non-immunologically based)	Allergy (Immunologically based)
Biochemical deficiencies e.g. lactose intolerance	Reaction type according to Coombs and Gell (1975)
Uncertain mechanism e.g. Chinese restaurant syndrome	Type I. Immediate hypersensitivity (minutes to 4 hours) IgE mediated (possibly also IgG_4 and IgD)
	[A]
	Type IV. Delayed hypersensitivity (6-24 hours delay) Cytotoxic reaction usually lymphocytic

[A]Types II and III are not represented in Food Allergy

Hypersensitivity

It is generally accepted that the symptoms of immediate (type I) hypersensitivity appear within a few minutes to hours after ingestion of the offending food. Delayed (type IV) reactions typically occur after 6 to 24 hours. This division, based on time interval, is somewhat arbitrary but usually reflects the distinction in immunological mechanism involved. Delayed symptoms are produced by cell-mediated reactions (lymphocytic) but the precise details are still under study and remain uncertain. For this reason most laboratory studies of food allergy are confined to type I reactions, involving immunoglobulin (IgE) mediated processes.

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Mechanism

Immediate hypersensitivity is most easily recognized because of its rapid onset; the mechanism has been studied intensively, particularly in relation to airborne allergens such as pollens, but the general principles also apply to ingested food allergens. The mechanism has been extensively reviewed (Haddad and Green 1983) so only a general outline will be given here. The processes involved in an allergic response may be illustrated in diagrammatic form (Fig. 1). It should be understood that IgE is a very minor serum constituent (< 450 ng/ml) when compared to the total immunoglobulin content of 15-20 mg/ml. However, a high proportion of the total IgE is not present in the blood but is bound to specific receptors on mediator cells,

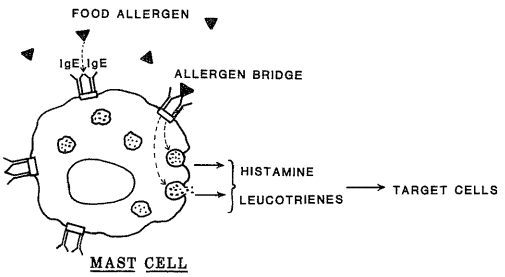

Fig. 1. Simplified outline of the events in the IgE-allergen interaction after Bellanti (1978). Food allergen crosses the gut wall barrier and interacts with the mast cell by bridging two adjacent IgE molecules. This signals, via cyclic-AMP, the release of mediators to affect target cells in smooth muscle and mucosa.

particularly mast cells, located throughout the body. At these sites the allergens/IgE interaction initiates its unpleasant and sometimes dangerous effects.

The range of allergenic foods containing specific substances, i.e. allergens, capable of interaction with IgE is very large. Comparatively little effort has been made to identify and characterize the allergens. Some notable exceptions are the study of codfish allergen M (Elsayed and Apold 1983), egg allergens (Langeland and Harbitz 1983; Hoffman 1983) and peanut (Barnett *et al.* 1983). Most individual allergens are believed to be proteins or glycoproteins which originate in the food. In some cases the offending substance may be a small molecule, such as an additive, which alone is incapable of eliciting an immune response. In such instances the small molecule may act as a hapten, interacting with serum protein, to become immunologically active.

It was long assumed that digestion of proteins in the gut to produce a mixture of the constituent amino-acids was necessary to allow assimilation of such food. However, it has been shown more recently (Williams 1981) that protein breakdown products of high molecular weight pass readily through the gut wall. These products may be the sensitizing agents for the development of ingested food allergy. Williams (1981) has also suggested that other subtle pharmacological effects may result from hormone-like peptides derived from foods. Such effects may contribute to those ill-defined food intolerances such as hyperactivity.

Allergenic foods and their effects

Although almost any food may provoke an allergic response in some sensitive individual, the range of foods to be seriously considered may be somewhat narrowed. Amongst the 10 most common offenders listed by Speer (1976) are a number of staple foods such as milk, wheat, barley, oats, eggs, corn and legumes. Citrus fruits, chocolate, tomatoes and shellfish may also affect significant numbers of people.

The precise number of persons affected by food sensitivities is uncertain, published figures having varied between 0.3 and 60% of the population. It is more generally agreed that the incidence of immediate hypersensitivity is probably confined to about 1 in 100 persons (Taylor 1980). Symptoms ascribed to type I food allergies include angioedema (contact swelling), urticaria (hives), gastro-intestinal distress (e.g. cramps), migraine headaches and respiratory effects, including asthma. A rarer but more serious effect may be anaphylactic shock, this condition being characterized by falling blood pressure and collapse, which if left untreated may be fatal. Foods which have been reported to induce anaphylaxis include milk, egg, peanut, sesame, sunflower seed and shellfish. Interestingly, all of these foods could be said to be relatively high in protein and thus may exert their serious effect by providing a large dose of offending agent in a small ingested sample.

Diagnosis of food allergy

Diagnosis by an allergy specialist is most effectively made by studying the detailed history of the patient, perhaps supplemented by a patient-maintained diary. Where reactions are immediate, diagnosis is generally straightforward. Sometimes the actual allergenic food may be 'hidden' as a minor ingredient of a composite such as a cake or soup. In many cases, avoidance of the suspected food followed by re-introduction will confirm the diagnosis if symptoms can be related to this regimen. For many foods, skin prick tests with an extract may provide useful information in a short time. In this test the mast cells of the

patient's skin will release mediators in response to the specific allergen (see Fig. 1). The mediators produce an inflammation reaction seen as a weal. A number of other *in vivo* tests, such as passive transfer, are available but more rarely carried out.

In vitro tests are useful in many circumstances with the radio-allergosorbent test (RAST) probably the most widely used and reliable. RAST was developed subsequent to the realization of the role of IgE in immediate reactions and is an immunoassay based on the measurement of allergen-specific IgE circulating in the serum (described in Fig. 2). Another *in vitro* method is the so-called cytotoxic test, in which microscopic examination of a blood sample mixed with food extract indicates cell damage by offending foods. Unfortunately, the technique is very operator dependent and has poor reproducibility. For these reasons this test is treated with considerable scepticism by many allergists. It should be understood that none of the present diagnostic tools are completely sound and data acquired from them require cautious interpretation. A major consideration with the tests is that a representative food extract is required, which is not always easy to define (see below). No reliable tests are available for delayed hypersensitivity reactions and diagnosis must be placed almost entirely on observation of dietary challenge and avoidance.

Treatment

The only sure method of treatment consists of complete avoidance of the offending food or foods. This can be difficult for staple items such as cereals and is a particular problem when multiple allergies have been diagnosed. Home-prepared foods of known composition are the safest, but manufactured foods present especial difficulties with the possibility of incomplete labelling and use of unspecified, premixed

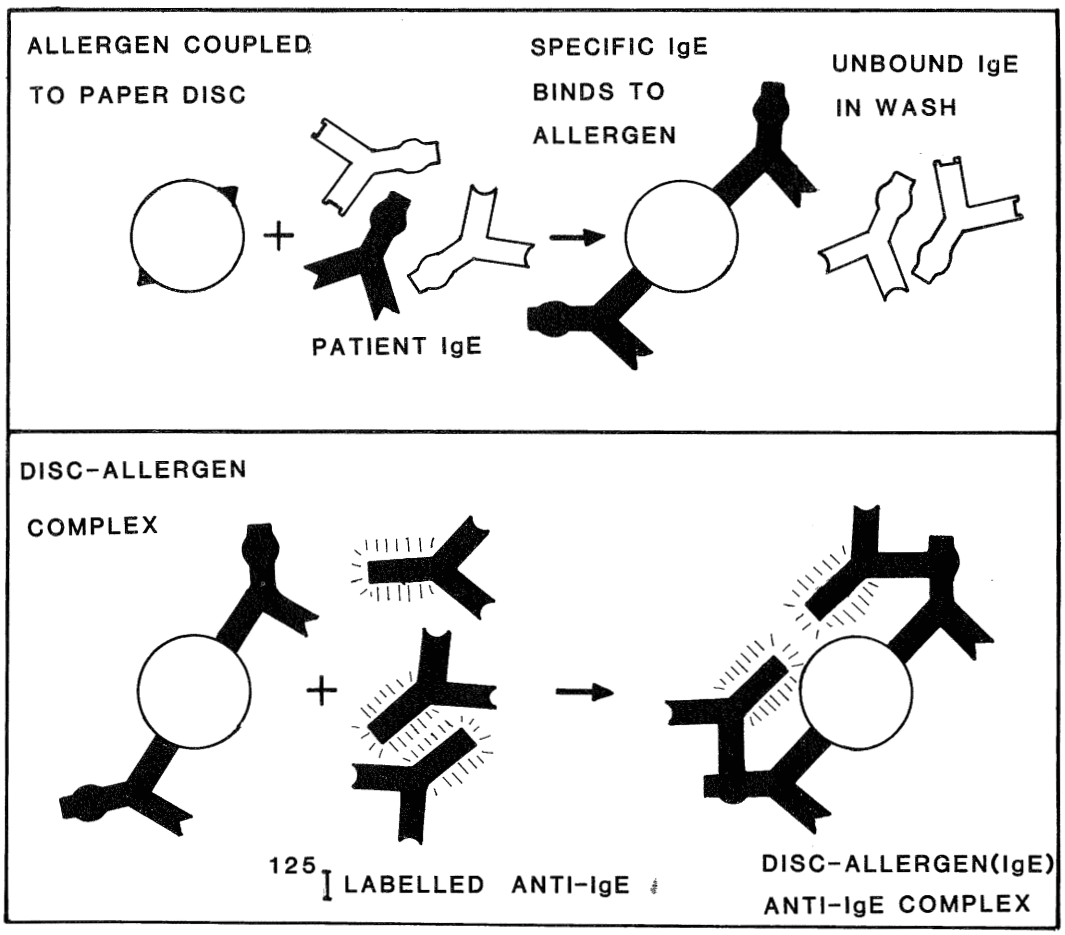

Fig. 2. Outline of the radio-allergosorbent test (RAST).

ingredients. The allergist may prescribe a number of drugs, including antihistamines, which may relieve the symptoms. A specific drug which has been used with considerable success is disodium cromoglycate which appears to act on the calcium-dependent cyclic AMP step in the mast cell release of inflammatory agents. Of course, some drugs may have undesirable side effects and are only treating the effect and not eliminating the cause.

A somewhat controversial treatment, which attempts to subvert the IgE reaction, is hyposensitization. This method involves stimulating the patient's immune system to produce specific antibodies, usually of the IgG class, in such amount as to defeat the IgE response by a competitive effect. The stimulation is commonly provided by a carefully graduated program of injections or oral doses of allergen extract in slowly increasing doses as immunization is achieved. There are a number of difficulties in such a procedure. Firstly, the causative allergens must be unequivocally identified and a representative extract be available – requirements often difficult to fulfil. Secondly, there is a danger of generating allergic responses to components of an extract not previously affecting the patient. Finally, in the case of those persons most severely affected, there is always a possibility of precipitating life-threatening anaphylaxis. Notwithstanding these disadvantages there are many instances of considerable alleviation of symptoms resulting from this treatment. Some of the claimed successes may be due to adventitious changes in the immune system. It is well known, for instance, that many children will lose some specific sensitivities as their immune system matures.

There is no doubt that the somewhat unsatisfactory situation with regard to allergy treatment, and diagnosis, could be improved considerably if more was known about those food proteins that appear to be commonly allergenic.

Laboratory study of immediate hypersensitivity

Although *in vivo* methods of study are probably the most reliable they require patients to be both constantly on hand and subjected to personal testing with possible unpleasant effects. It is fortunate therefore that, in many cases, the specific serum IgE can be well correlated to clinical sensitivity. This has been shown to be true for peanut (Kemp *et al.* 1984) and a number of other foods (Aas 1978a). Specific IgE may be measured by RAST (Fig. 2) in a semiquantitative manner using only a small serum sample (50μl) from a sensitive patient.

Food extracts

Successful diagnosis and study of food allergy presupposes that a truly representative food extract is available; this is a difficult criterion to meet. It is usually assumed that the allergens being sought are proteins and that they are soluble; commonly this is true but there are cases in which digestion, for instance, may expose new allergens or solubilize others (Spies 1974).

In practice it is usual to attempt to dissolve as much of the total protein as possible with a buffer which, it is hoped, produces no denaturation of the protein. Small contaminating molecules such as sugars may be dialysed away and the extract can then be used to obtain some answers to the following questions:

- How many allergens are present?
- What is their relative importance?
- Can the most important or significant allergen be discerned?
- What are the physico-chemical properties of the allergens?
- Is it feasible to separate the most significant allergen for closer detailed examination?

Answers are sought by use of a wide variety of biochemical methods. Selective methods, such as affinity chromatography, have been applied by some workers (Baldo *et al.* 1981). Most laboratory methods rely heavily for allergen detection on variations of the basic RAST system utilizing the radioactively labelled, specific anti-IgE.

Methods

Many experimental approaches may be used to obtain information on an allergenic food. Some of the methods used to detect particular allergens are presented here:

Initially, traditional biochemical separation techniques may be applied to the allergen extract which must be previously shown to be active. The techniques used could include fractionation by gel filtration or ion-exchange, thus distinguishing the allergens on the basis of size or charge respectively. Higher resolution methods may be used, including preparative iso-electric focusing and electrophoresis. There are certain disadvantages in the use of these techniques during initial screening of an extract; one difficulty is that each fraction from the separation requires separate testing and thus a large volume of serum can be used in the

procedure. A further problem arises in comparing the relative potency of the fractions because the buffers must be removed and the material lyophilized before testing in the RAST procedure. Two techniques that have been developed to overcome some of these problems and provide the required information will be described.

Crossed radioimmunoelectrophoresis (CRIE) – CRIE was developed to detect the allergens in Timothy grass pollen and has since found application in studies of many other allergen systems (Weeke and Lowenstein 1973). The system consists of an initial electrophoretic separation of the protein extract in a strip of agarose gel. Another gel, containing antibodies to the allergen extract raised in an experimental animal, is cast alongside the first strip. A second electrophoresis is carried out, perpendicular to the first. The gel is maintained at pH 8.6 to minimize migration of the antibodies. The migrating protein antigens form precipitin arcs with their corresponding antibodies. With careful adjustment of running conditions, and antibody/antigen ratios, patterns such as that shown in Fig. 3(a) may be obtained. The gel and precipitates are dried on their supporting plate, thus immobilizing the arcs, before overlaying the plate with diluted serum from a sensitive patient, containing specific IgE to the allergen extract being examined. The IgE will 'recognize' and bind to those of the precipitated antigens which are allergenic. Following a thorough wash, the plate is incubated with the specific anti-IgE, ^{125}I-labelled, which now attaches to the arcs specifically recognized by the serum IgE. Subsequent autoradiography will indicate the particular arcs that have been so recognized (Fig. 3(b)). CRIE will thus indicate the number of antigens that are allergenic, and a survey of patients may indicate the more significant amongst the allergens. However, the method suffers from several disadvantages, the most serious of which pertain to the raising of the precipitating antibodies in a laboratory animal. Firstly, the antiserum may be difficult to raise in a short time and, more importantly, it may be that some of the allergens present in the extract are non-immunogenic in the animal and, since no antibodies are raised, the particular allergens will thus remain undetected by the method. The separation is based only on the charge of the protein at pH 8.6 and little information is gained to enable subsequent preparation of the detected allergens.

Western immunoblotting – This technique was recently developed (Sutton *et al.* 1982) to enable allergens to be detected without recourse to the raising of antibodies. The technique was originally used for the examination of nucleic acid sequences and its adaptation to allergen detection produced a method with considerable advantage over CRIE.

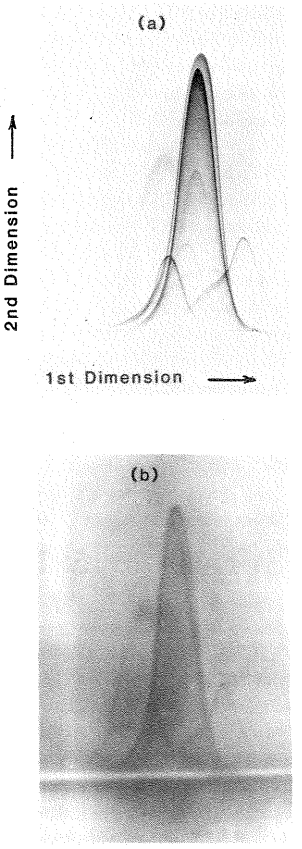

Fig. 3. Crossed radioimmunoelectrophoresis (CRIE) illustrated for peanut antigens migrating to the anode. (a) Twenty-two antigens can be discerned on this plate when stained for protein. (b) Autoradiograph of (a) to detect some sixteen allergens after IgE/anti-IgE labelling.

In this method the allergens are first separated by a high resolution gel technique such as iso-electric focusing or sodium dodecyl sulphate electrophoresis (SDS-PAGE), and then by transverse transfer, either by physical or electro-blotting, the proteins are immobilized on a nitrocellulose membrane. The membrane, with its protein array, may be cut into strips and treated with serum in an analogous manner to the RAST. The allergenic bands of the separation are subsequently detected by autoradiography as in CRIE. This method avoids the antibody difficulties of CRIE and may provide information about the molecular weight or isoelectric point of the allergens which could be used in preparative purification schemes. The autoradiograph shown in Fig. 4

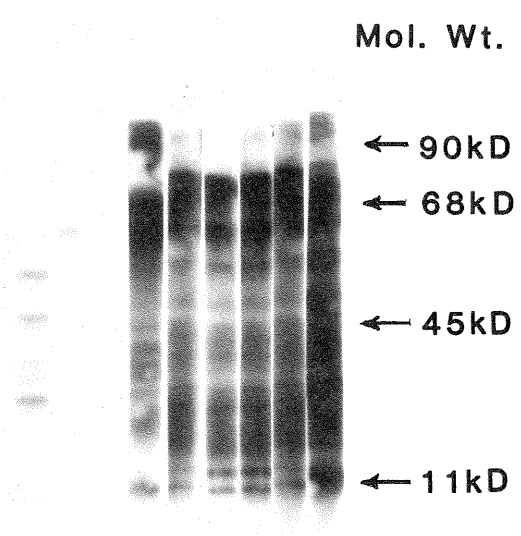

Fig. 4. Autoradiograph of nitrocellulose membranes after Western blotting from SDS-PAGE gels for the detection of peanut allergens. Patient sera A, B are controls, C to G from mildly affected persons and H to M are more severe cases.

illustrates the use of the method in a survey of sera from peanut-sensitive patients. With more extensive surveys, it may be possible to choose and isolate a particular allergen, which appears to be common to most of the patients, for closer study. One of the ultimate aims is to discover those features of structure which cause allergenicity.

Structure of food allergens

There is a lack of knowledge on this topic with the notable exception of the codfish allergen M (Elsayed and Apold 1983). The determinant, in that case, was found to consist of a hexadecapeptide which apparently formed a repeating sequence in the parvalbumin of the fish muscle. The determinant was able to mimic the whole protein in a variety of *in vivo* and *in vitro* tests. The sequence of the peptide was found to show homology between both terminal sequences of four amino-acids. This feature accords with the requirement for crosslinking in the allergenic interaction with cell-bound IgE (Ishizaka 1982). The pioneering work on the codfish allergen requires extension to other food allergens to establish whether the determinant characteristics are unique or are common to other allergenic proteins.

While the details of most allergens remain to be discovered there are some features that seem to be common (Aas 1978b). Heat stability is a frequently occurring property together with relative resistance to hydrolysis by proteolytic enzymes. As an example, a peanut glycoprotein allergen has been shown to be present in raw and roasted peanuts, peanut butter and a canned, vegetarian 'meat' loaf containing peanut. The allergen apparently survived a roasting treatment which consisted of a minimum of 150 °C for one hour (Barnett and Howden 1984). The same allergen also requires extended incubation with trypsin, up to 16 h at 37 °C, for complete digestion. The presence of such stability indicates that the allergenicity resides in a particular sequence of amino-acids rather than some topological feature of the whole protein which would be less easily retained. The property of resistance to proteolytic enzymes may also be important in the sensitizing process, resulting in the presence in the gut of large breakdown products that could be transported into the bloodstream. Legumes are known to be poorly digested and are also common and potent allergenic foods, facts that accord with the above hypothesis.

Future prospects and requirements

Many aspects of food allergy still remain a mystery and there is a pressing need for research to identify and characterize individual allergens. Also required are standardized food extracts, both for diagnosis and possible treatment. With standardized extracts,

treatments such as hyposensitization would be rendered safer and more effective. Basic research into food allergens should provide a more rational basis for the understanding of allergic phenomena.

With greater knowledge of allergen structure, better treatment strategies could be devised. It may be possible, for instance, to eliminate or reduce the allergenicity of some foods by chemical or processing techniques such as those currently applied to legumes in removing anti-nutritive factors. The use of peptides, which mimic the whole protein and are amenable to modification, opens up the possibility of developing synthetic vaccines such as are becoming currently available for certain infectious diseases.

The emphasis in this article has been on type I hypersensitivity but a great deal of personal distress is caused by type IV reactions. There exists, therefore, a need to research the mechanism and its causative agents. There is particular need for an *in vitro* method of detection similar to the RAST if laboratory studies are to proceed and diagnosis to be made more effective.

Finally, since the potentially helpful developments await scientific effort and resources, the most effective course for most allergic persons is to avoid the offending foods. With processed foods the position of such individuals would be considerably easier if ingredient labelling were made more comprehensive.

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The Monte Carlo Method and its use in food science

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Introduction

In attempting to understand and thus be able to control many biological processes, one is rapidly led to dealing with interactions at a microscopic, or even molecular, level. These interactions can be of many types, but can be classified into three general broad categories: physical (e.g. diffusion), non-specific (e.g. between charged particles), and specific (e.g. between large molecules where only specific sites interact).

The art in attempting to solve problems involving such interactions is to make the mental picture that you have of the way in which the system works, just sufficiently complex to account for the results of experiments. A model that is not sufficiently detailed will always fail to meet this requirement, while a model that is too complex will either have so many possible interpretations that it is of little help in understanding the problem, or it becomes so unwieldy that much time is wasted trying to use it.

Problems involving interactions in the first two categories mentioned above can often be handled using Monte Carlo techniques. This paper describes the principles, practice and limitations of this technique, and gives one example of the way in which it has been used at the Food Research Laboratory.

The name

The use of the name 'Monte Carlo' for this method of dealing with these problems is usually ascribed to Metropolis *et al.* (1953), although they make no particular claim of originality. The name arises from the well known casino whose reputation is based on the fact that the tables and games are not 'rigged' in any way. This means that in games such as roulette, each number/colour combination has an equal likelihood of occurring, and in games such as blackjack/pontoon, the probability of occurrence of each combination of cards can be calculated precisely.

So if we watch the games for a very long time and count up the number of times each particular number/colour combination occurs on the roulette wheel, and divide by the total number of spins of the wheel, for each combination the ratio should tend towards 1/37.

Similarly, the same procedure for the hands in pontoon should give ratios that tend towards the calculated probabilities for each hand. It is the fact that the results of a large number of trials tend towards the appropriate probabilities that gives the Monte Carlo method its name.

The problems

The Monte Carlo method may be applied to collections of molecules or small particles that can move about. The particles need not be identical although often they are, or for simplicity can be treated as if they are. Fig. 1 shows such a collection (in two dimensions). They could be vesicles in an emulsion, enzymes in a cell, proteins in a membrane, or indeed anything composed of mobile particles in suspension. The questions that can be asked about this collection of particles include: what happens if we increase the number or size of particles, will it set into a solid, will it aggregate in patches, what will be the density at which this starts, will the process snap from one state to the other or will it take place over a range of densities; what is the effect on the answers to these questions of adding some particles of a different size/shape, how does the effect depend on the number of these new particles that are added and so on? Many of these questions have already been answered for non-food systems using Monte Carlo techniques.

The method

The Monte Carlo method comes in two stages: 1. if the particles can be treated as 'hard', that is they do not know of each other's presence unless they actually come into contact with one another, and 2. if there is an energy of interaction between them even when they are

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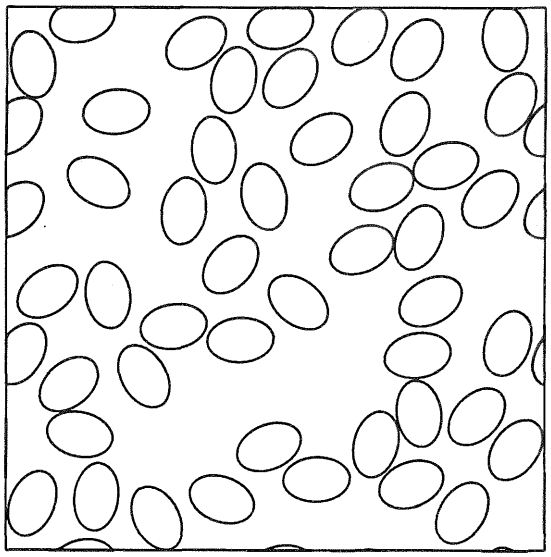

Fig. 1. A typical array of uniform particles (Ellipses, length : width = 3:2, density = 0.4)

not in contact (e.g. if they have a surface or bulk electric charge).

Hard particles

Fig. 1 can be considered as a snapshot of the collection of particles (ellipses in this case) as they move about. If we keep taking such snapshots, then we can estimate the probability of any particular arrangement of the particles occurring simply by counting the number of occurrences of that arrangement and dividing by the total number of snapshots. For example the number of times that a snapshot would show the particles spaced evenly on a square grid would be very low, but such a snapshot will occur eventually if we take enough pictures. With such complex systems it is not possible to calculate *ab initio* the precise probabilities of the various configurations, so the Monte Carlo method is used to obtain estimates of these probabilities.

Monte Carlo methods can only be used with the aid of a computer since millions of such snapshots are needed. The computer program is implemented as follows:

a. the chosen number of particles is placed within a square, either randomly or with their centres in a square or hexagonal pattern,

b. an attempt is made to move particle No. 1 by a small, randomly chosen distance in a randomly chosen direction and to rotate it through a small random angle. If this would cause particle No. 1 to overlap with any other particle, No. 1 is returned to its original position and a snapshot taken; if there is no overlap, No. 1 is moved to its new position and a snapshot taken,

c. the same procedure is carried out with each particle in turn,

d. steps b and c are repeated many times.

Left to themselves, the particles would simply move out of the square and 'evaporate'. Since the square is intended to be a small sample of a very large population of particles and since the size of computers is limited, it is usual to use between 20 and 200 particles. To prevent their 'evaporation' each edge of the square is assumed to be periodic, i.e. if a particle leaves the square through the left-hand edge, the same particle re-enters the square in a similar position through the right-hand edge. In effect the array of particles is surrounded by eight images of the array as shown in Fig. 2. This means that, when checking for overlaps near an edge, one has to test for overlaps not only with particles within the square but particles about to leave and re-enter the square.

Particles with interaction energy

For hard particles the acceptance of a trial move of a particle depends only on whether it overlaps with another particle. For particles that interact at a distance the acceptance criterion is more complicated. Since there is an energy of interaction it is possible to calculate the overall energy (E_1) of the system of particles. Now when an attempt is made to move particle No. 1 to a new position, the new energy (E_2) of the system is calculated. If E_2 is less than E_1 the move is accepted. If E_2 is greater

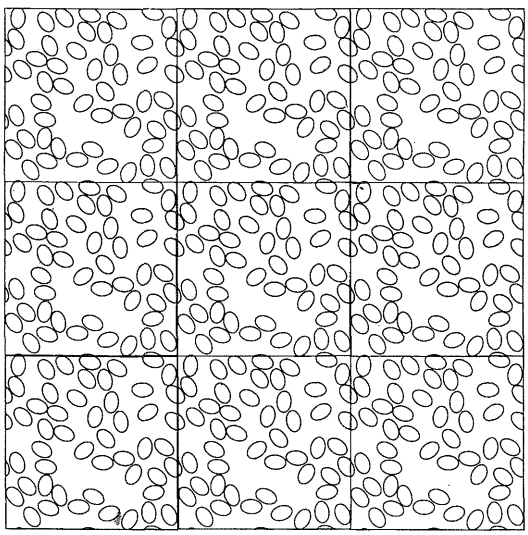

Fig. 2. The array with the eight surrounding images needed for periodic boundary conditions.

than E_1 then a random number between 0 and 1 is chosen. If this random number is less than $\exp((E_1 - E_2)/kT)$ the move is accepted. (k is Boltzmann's constant and T is the absolute temperature of the system.)

This rather odd-seeming procedure is the same as moving the particles randomly (as for hard particles) but giving each snapshot a probability proportional to $\exp(-E/kT)$ where E is the energy of the configuration of which the snapshot has been taken. In this way the system will come to equilibrium with a Boltzmann distribution of energies appropriate to its temperature, as required by thermodynamics.

It is important to stress that the Monte Carlo method does not simulate the actual motion of the particles. Successive snapshots do not represent successive positions of the actual particles being modelled, but simply depict different possible configurations into which they can be arranged.

Storing the information

The system of particles is allowed to come to 'equilibrium' by letting it run for, say, 100 000 snapshots before any data are recorded. This is to remove the effects of the original placement of the particles.

If we have 100 particles and take a million snapshots, we would have to store 100 000 000 pairs of numbers to store the locations of the centres of each of the particles. This represents a large amount of computer memory, so various schemes have been devised to reduce the storage requirements and the amount of data handling without significantly reducing the amount of useful information stored. The most successful scheme is to store the data as the pair density distribution function (PDDF). With this we do not store the locations of the particles but the distances between them. In effect we are answering the question "What is the probability of finding two such particles distance d apart?"

The PDDF is constructed as follows:

a. a number of boxes are chosen (say 100); these boxes represent different distances apart of the centres of the particles, e.g. for particles whose closest distance of approach (D) is 10 nm, No. 1 box might represent 10.0 to 10.5 nm No. 2 box 10.5 to 11.0 nm and so on. To improve accuracy the 100 boxes might only scan 3 or 4 times D. Particles further apart than this are skipped in the calculation, considerably speeding up the program,

b. the distance between the centres of particle No. 1 and particle No. 2 is calculated and 1 count is added to the box representing this distance,

c. this procedure is repeated for the distances between particle No. 1 and all other particles and any close images in the surrounding arrays,

d. particle No. 2 is now chosen and the distances from it to all other particles from No. 3 onwards are calculated and counts for each distance added to the appropriate boxes,

e. this procedure is continued for each particle in the snapshot,

f. a new snapshot is taken and the procedure started all over again at No. 1 particle,

g. after our million snapshots, we have the numbers in 100 boxes, i.e. in only 100 storage locations in the computer.

If we divide the number in each box by the number of snapshots, we get the average number of occurrences of each particular distance between the particle centres. In the same way as with the roulette wheel, these will enable us to estimate the probability of finding the particles at any given distance apart.

Fig. 3 shows the PDDF of the ellipses shown in Fig. 1. Information about the shape of the particles, their size, and the density are all contained in the shape of the leading edge of the PDDF and the location and heights of its peaks. This PDDF shows us that few particles are spaced at the closest possible distance (D) apart. Likewise the density is lower than average at about 2 D. However, the density of particles at spacings of 1.5 D and 3 D is higher than average. This means that the particles are tending to form a series of concentric circular shells about one another. Such apparent short range structure is typical of that found in liquids. As an illustration of the effect of changing the density, Fig. 4 shows the PDDF of the same particles but with a density of 0.6. It can be seen that this PDDF shows even more

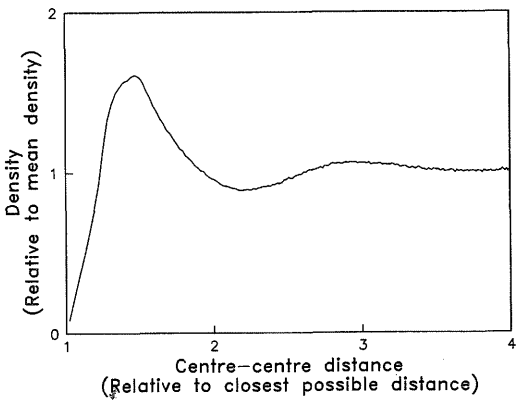

Fig. 3. Pair density distribution function for the array of Fig. 1.

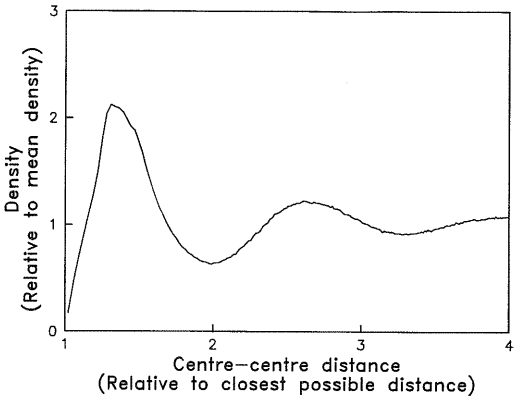

Fig. 4. Pair density distribution function for an array having 1.5 times the density of that in Fig. 1.

pronounced structure and that, at the higher density, the most probable spacing between the particles has been reduced to 1.4 D and more of them are likely to be at that spacing. As the density is further increased, the PDDF becomes a series of sharp peaks as the particles lock into a semi-crystalline solid.

The Monte Carlo Method in practice

The Monte Carlo method is essentially a system of modelling. What one can measure experimentally is the PDDF. One attempts first to define the density, particle shape, and interaction energy, then run the Monte Carlo program and see whether the PDDF produced matches the experimental one.

This is the way in which the modern theories of simple liquids were developed (e.g. see Hansen and McDonald 1976). The PDDF of a liquid can be derived from the results of X-ray and neutron scattering experiments. Much to everyone's surprise, it was found that using hard spheres to model the molecules produced PDDF that matched those of argon, for example, with remarkable accuracy. More refined models are now used that permit accurate prediction of various thermodynamic properties of the liquids, but the fact remains that, for most work, it is hardly ever necessary to go beyond hard particles if the choice of shape and density is correct.

The first Monte Carlo work of relevance to food science was published by Snook and van Megen (1976) when they reported Monte Carlo simulations of colloids that had been stabilized by electrostatic interactions alone. Their model was later refined by Dickinson *et al.* (1976) and Dickinson and Stainsby (1982) to include particles of different sizes.

The Monte Carlo approach has also been used in studying the problem of phase transitions (e.g. see Abraham 1981). This field, of great potential interest to food scientists, has been the scene of sporadic activity over the last 30 years. While it is relatively easy to measure PDDF above and below the phase transition of a liquid-solid transition, measurements made during the transition are often highly irreproducible, subject to hysteresis, and consequently uninterpretable. Modelling such unreliable data is clearly of little value. Unfortunately, there are not yet available sufficiently detailed theories of phase transitions that could give guidance about the way in which the various parameters interact. This is an area where the Monte Carlo technique is available in principle, but reliable experimental data are not.

In this laboratory, studies have concentrated on the movement of the molecules in cell membranes and similar structures (Cornell *et al.* 1981), as a large number of essential biological processes (e.g. photosynthesis in plants, heat resistance in bacterial spores, cell fusion, coalescence of certain colloids) are thought to occur at or in membranes.

Fig 5 is an electron micrograph of portion of the leaf of a wild barley mutant. Before being placed in the electron microscope, the membrane was rapidly frozen and then cleaved in the plane of the membrane. There has been considerable speculation about the nature of the 'particles' seen in this picture. It is now generally agreed that they represent large proteins or protein aggregates floating in a sea of lipid molecules. It is also believed that they are associated with photosynthesis within the membrane.

However, several difficult questions arise. Are the particles all the same? Some biologists have measured the apparent size distribution of the particles (Simpson 1978; Staehelin 1976) and drawn the conclusion that what we are seeing is a whole range of particles, each presumably roughly circular in section. This has led, in turn, to the suggestion that they are spheres cleaved at different levels and so can be treated by well known methods of statistical geometry (Blum 1965).

If the particles are indeed all identical, what is their shape? This is of considerable importance to biologists who are trying to understand the operation of the photosynthetic system since it is known that the physical movement of some of the particles is involved in its operation (Goodchild *et al.* 1983).

These questions can be answered by applying the Monte Carlo method to Fig. 5.

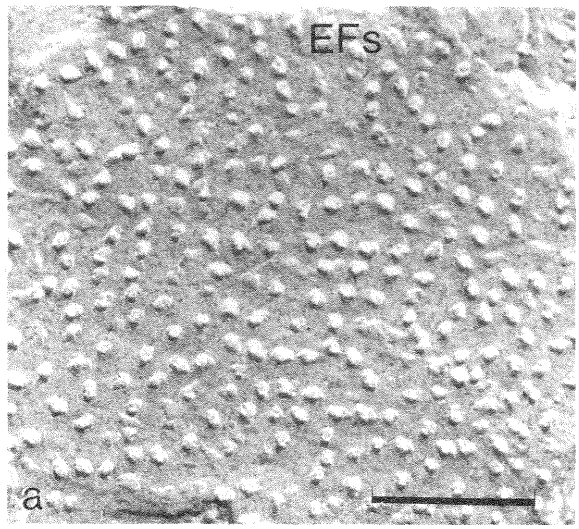

Fig. 5. Electron micrograph of freeze-fractured membrane of barley mutant. Bar = 125 nm. (Kindly supplied by Dr D. J. Simpson)

In doing this we are assuming that Fig. 5 is a snapshot of a system in equilibrium and that this snapshot has not been affected by the freeze-fracturing process. Fig. 6 shows the PDDF derived from Fig. 5 using the corrections devised by Bell (1968) to allow for the finite size of the measured area. Because of the small number of inter-particle distances to be measured, the PDDF is shown as a histogram. Typical of real systems, it is 'noisy'.

We now have to find a model that will generate a PDDF similar to the histogram shown in Fig. 6. The first one to try is an assembly of discs that has the distribution of sizes as measured by Simpson (1978). This gives the smooth PDDF also shown in Fig. 6. Since the two PDDF are quite dissimilar we can immediately assert that the particles are not discs with the claimed size distribution. Because of the location of the first peak in the histogram in Fig. 6, it is clear that the interaction between the particles is taking place at a distance greater than the apparent size of the particles depicted on the electron micrograph. In attempts to match this PDDF, various other possible size distributions have been tried, always without success.

We are left with the question: if they are the same size, what size and shape are they? When PDDF are generated for different shapes, e.g. circles, ellipses, triangles, squares, dumbbells etc., it soon becomes clear that each shape of particle generates a unique shape of PDDF. The shape of the histogram strongly suggests an ellipse for the shape of the particles. However, no combination of ellipticity and density (the only variables at our disposal) produces a PDDF that has the main features of the leading edge shown in Fig. 6 as well as the location and height of the major peak.

Two possibilities now present themselves: the particles have very complex shapes and/or the centres of the observed particles do not coincide with the centres of the interacting particles. Upon trying ellipses with the observed point lying at various distances along the long axis from the centre, the smooth PDDF of Fig. 7 is obtained as a best fit to the histogram. From this remarkably good match we are able to conclude that, below the surface, the particles are probably elliptical with dimension 21.4 nm

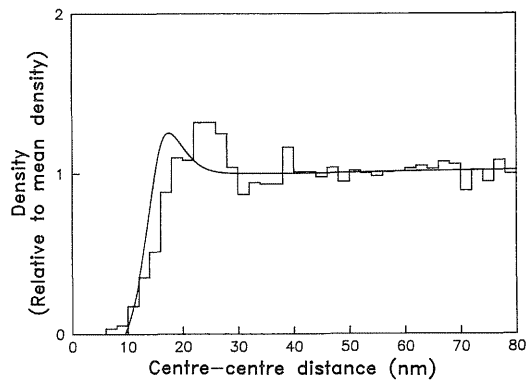

Fig. 6. Histogram: Pair density distribution function of particles shown in Fig. 5. Smooth curve: Pair density distribution function of an assembly of discs having the size distribution suggested by Simpson (1978).

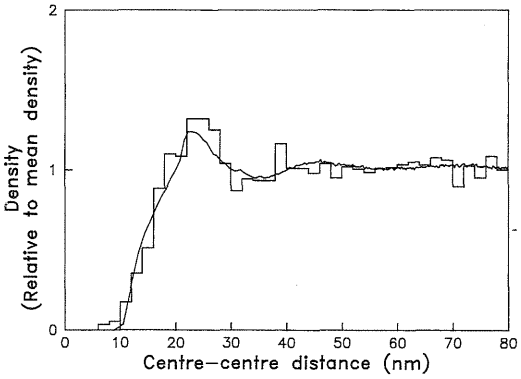

Fig. 7. Histogram: Pair density distribution function of particles shown in Fig. 5. Smooth curve: Pair density distribution function of ellipses 21.4 nm x 17.8 nm with the observed point offset 5.2 nm from the centre along the long axis.

by 17.8 nm and that the 'lumps' seen in the electron micrograph are raised parts situated 5.2 nm from the centre along the long axis.

Limitations

The Monte Carlo method can only be applied to systems that are in equilibrium. This does not mean that it cannot be applied to systems in which reactions are taking place, merely that the reaction rates must have reached a steady state. When studying phase transitions, it is necessary for the model to have reasonable numbers of particles in each of the phases, so the total number of particles that must be used becomes large. This makes it essential to have a large, fast computer for such work. Such a computer is also essential for any work involving three dimensions.

Because the method is basically a system of modelling, the PDDF obtained from it is only as good as the model chosen. The major limitation in this regard is the imagination of the user.

The most serious problem is that there is no proof that any model giving a desired PDDF is unique, i.e. that there is no other model that could give a similar PDDF. This means that when a model is proposed and is found not to give the required PDDF one can say categorically that the model is wrong. When a model does give the required PDDF all one can say is that, until other evidence proves otherwise, the model is acceptable.

Conclusion

The Monte Carlo method is being applied for the first time to problems in food science. It is a powerful tool for checking theories concerning the movement of particles in suspensions and indeed could be called the great demolisher of such theories. This makes it an excellent first check, since any theory producing the wrong PDDF can be disposed of immediately and no futher time wasted on it.

Once a satisfactory model is found, one can immediately get values for the dimensions of the interacting particles that often cannot be obtained in any other way. In particular we have shown that the dimensions measured from electron micrographs may not bear any obvious relationship to those of the particles portrayed.

The next few years will see a wide range of food research problems being attacked with this powerful method.

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A note on the energy content of meat

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In recent years nutritional organizations in several Western countries, including Australia, have recommended that people reduce their intake of fat (English 1983). This recommendation translates in practical terms to reducing the intake of "energy dense foods", including meat. Australians have a tradition of eating large quantities of meat and even in recent times (Fig. 1) annual consumption has been approximately 100 kg per capita.

Meat has been defined by Lawrie (1979) as the flesh of animals used as food. In this context flesh refers largely to skeletal muscle (lean) and adipose tissue (fat) but may include small quantities of other tissues, e.g. skin, blood and lymph vessels, and nerves. The organs of animals, e.g. liver, heart, kidneys, although eaten as food, are not usually considered as meat, but rather as edible animal products. World-wide there is a large range of animals killed for human consumption, but in Australia, cattle, sheep, pigs, and poultry are the major animals farmed and killed for meat production.

Tables on the composition of meat have been presented by Lawrie (1979) and Thomas and Corden (1977) but such tables have to be interpreted with caution as they often refer to lean raw meat rather than processed or cooked products which contain variable amounts of fat. An excess of fat is wasteful to all sections of the meat industry and if consumed in excess is possibly harmful to human health and well being. Some lipid is essential for both the normal structure and function of muscle, the physical and chemical characteristics of meat and the interaction of meat components. For instance, phospholipids and cholesterol are essential for muscle cell membrane and organelle function. Triacylglycerols, or storage lipids, are not essential for muscle function but provide a reserve of fatty acids for energy metabolism and contribute to meat characteristics such as flavour, colour (stability and contrast), texture, juiciness and interactions with protein. Thus, the meat we eat is a product dependent upon the physical and chemical interactions of the proteins of muscle and the lipids of adipose tissue.

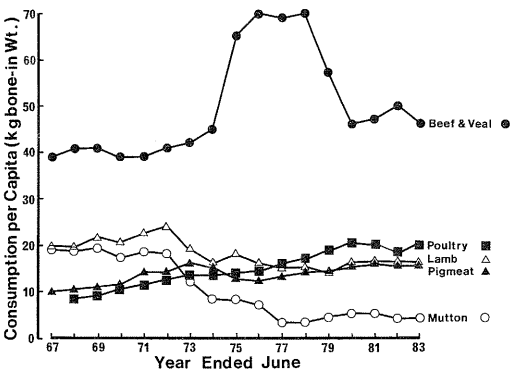

Fig. 1. Meat consumption by Australians in recent years. (Data from Australian Meat and Livestock Corporation Annual Reports.)

The broad composition of lean meat, i.e. adult mammalian skeletal muscle, is 75% water, 19% protein, 2.5% lipid, 1.2% carbohydrate and 2.3% soluble non-protein substances (Lawrie 1979). The lipid referred to by Lawrie is the functional lipid (largely unsaturated fatty acids) of muscle cells. However, the fat composition of meat destined to be eaten is largely determined by the amount of fat surrounding the muscles (subcutaneous), between the muscles (intermuscular) and between the muscle fibre bundles (intramuscular). These are the adipose tissue stores of lipid (chiefly saturated fatty acids in ruminant adipose tissue). For this reason lean meat from ruminant animals has a high ratio of unsaturated to saturated fatty acids and, conversely, fatty meat from these animals has a low ratio of unsaturated to saturated fatty acids. While the composition of lean meat is remarkably constant, the composition of mammalian adipose tissue varies enormously. For example, Vickery (1977) reported that the total lipid content of subcutaneous adipose tissue from cattle ranged from 2.7% (emaciated animals) to 80.4% (fat animals).

The energy content of meat that is eaten is chiefly dependent on its fat content (and upon

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cooking procedures). The equation ($y = a + bx$) describing the relationship between the energy content (EC) in kilojoules per 100 grams and the fat content (FC) in grams per 100 grams of fresh sheep meat is:

$EC = 343 + 33.4\ FC\ (\pm\ 0.12)$ (1)

(data from Thornton, Husband and Larsen (1981); n = 51, FC ranged from 1-50% and R^2 = 99.9%)

This equation is close to a theoretical model based on the assumptions that meat with no fat is 20% protein and the metabolizable energy values for protein and fat are 17 and 37 kJ/g respectively. From Eqn 1, 100 g of meat containing 20% fat has an energy content of 1011 kJ, while 100 g meat containing 50% fat has an energy content of 2013 kJ (Fig. 2). Using

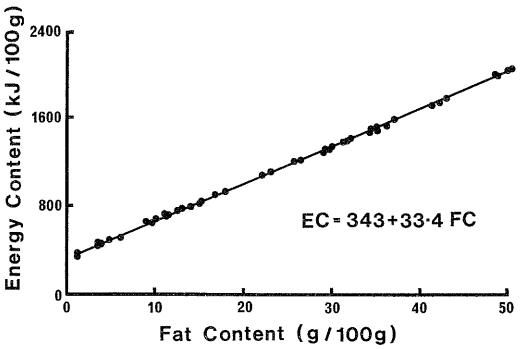

Fig. 2. Plot of energy content of fresh sheep meat (kJ/100 g) against fat content (g/100 g).

the data on cartoned boneless beef (FC ranged from 19-42%) presented by Thornton, Husband and Larsen (1981) the equation is:

$EC = 370 + 33.0\ FC\ (\pm\ 0.30)$ (2)

(n = 41 and R^2 = 99.7%)

Using the data of Thomas and Corden (1977) which covers a wide spectrum of meats (n = 69), the equation is:

$EC = 510 + 33.4\ FC\ (\pm\ 1.07)$ (3)

(FC ranged from 6-55%; R^2 = 93.6%)

The intercept (510) of Eqn 3 indicates that the protein content of lean meat is approximately 30% which is unreasonable. However, the slope at this relationship is not significantly different from those of Eqns 1 and 2.

It is clearly evident that fatty meat is an energy dense food. However, at least with traditional retail cuts of meat, most of the fat is visible and thus the consumer can select or adjust the fat content of the meat actually eaten. Furthermore, some cooking procedures remove considerable amounts of fat. In his note on the food value of meat, Plimmer (1921) commented, "the fat content is thus indeterminate for the individual, and it is not possible to give a figure for the energy value of meat as eaten".

From a nutritional science viewpoint meat is regarded as a good source of energy and of protein (as it contains a well-balanced complement of the essential amino-acids) and to a lesser extent of minerals [iron (readily available), potassium, zinc and copper] B vitamins (thiamin B_1, riboflavin B_2, niacin and B_{12}) and essential fatty acids. Furthermore, meat is a highly acceptable food in the general community; it is readily digestible, yet gives satiety, and people do not tire of eating it. Thus lean meat can be described as a highly desirable nutritious food.

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Aspects of the flavour of phenol, methylphenol and ethylphenol

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Introduction

Phenolic compounds are encountered frequently as components of flavours extracted from foodstuffs. They can be formed by chemical or biological breakdown of lignins or synthesized biologically from aromatic amino-acids. There is confusion about the role of compounds such as phenol, methylphenol or ethylphenol in the overall flavour and some doubt surrounding their occurrence as natural flavour compounds. Without attempting to be exhaustive, this review draws together some information concerning the occurrence in dairy products, the desirability of flavour and the threshold for human perception in various media of these compounds.

Occurrence in dairy products

Brewington *et al.* (1973, 1974) identified some 42 compounds present in cow's milk as conjugates (e.g. glucuronides or sulphates) and postulated that these conjugates existed as part of the detoxification mechanism of the animal. Among a number of phenolic conjugates were amounts of phenol, 4-methylphenol (*p*-cresol) and 4-ethylphenol which they assessed as medium, large and very large. Given the occurrence of these conjugates in milk and the availability of β-glucuronidase or aryl esterase activity in milk, it is not surprising that the free compounds have been found in dairy products. For example, in flavour extracts from:

Milk: phenol and methylphenol were found as minor components (Badings and Neeter 1980).

Butter: 0.016 ppm phenol, 0.0033 ppm 3-methylphenol, 0.0049 ppm 4-methylphenol were found by Urbach *et al.* (1972).

Hard cheese: McGugan and Howsam (1973) found 3- and 4-methylphenol in old Cheddar while McGugan (1975) noted the presence of phenol. Badings *et al.* (1968) found 4-methylphenol in defective Gouda and, later, 4-methylphenol and 4-ethylphenol were found in Cheddar cheese with a similar defect (CSIRO Division of Food Research 1980).

Soft cheese: phenol, 4-methylphenol and 4-ethylphenol were found as minor constituents of Roquefort or Camembert (Moinas *et al.* 1973) and Camembert (Dumont *et al.* 1974c). Phenol was a major component of the flavour extract of Vacherin and of the surface-ripened cheeses such as Livarot (Dumont *et al.* 1974a,b).

The flavour quality and desirability of phenol, methylphenol and ethylphenol

The sensory quality of phenolic compounds ranges from sharp, medicinal through sweet, aromatic to smoky, charred, caramel, unpleasant and sheepyard. The exact note depends on the concentration, the medium and the phenolic compound under examination. In some instances the smoky caramel character associated with very low concentrations of phenol or methylphenol is judged to be a desirable attribute – for example, in alcoholic beverages or smoked foods. Lehtonen (1982) concluded that the main difference between whiskies produced from peated malt and other whiskies lay in their contents of 2-, 3- and 4-methylphenol (below 0.1 ppm). In the opinion of the professional Australian butter graders small concentrations of phenol, 3- or 4-methylphenol added to a bland, synthetic butter medium imparted "fullness" and a desirable sweet note (Urbach *et al.* 1972). This study found that phenol and 4-methylphenol were present in butter above their threshold concentrations and concluded that they were part of the desirable flavour of the butter.

The importance of phenolic components in cheese flavour is emphasized by their inclusion in patented flavour formulations. Phenol in the range 1-10 ppm of cheese was added to Camembert, Roquefort or blue cheese flavours (Moinas *et al.* 1976) while phenol and

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methylphenol, at a level of 0.1 ppm of cheese, were included in a mix claimed to reproduce Cheddar flavour (Henning 1970).

On the other hand, phenols and methylphenols give to beer off-flavours detectable at about 0.03 ppm (Steiner 1968) and absorption of 0.2 ppm phenol and methylphenol into wine from atmospherically-contaminated grapes can cause off-flavours (Tanner 1972). Similarly a phenolic, musty, sheepy off-flavour in Gouda or Cheddar cheese was believed to be caused by levels of 0.5-5 ppm of 4-methylphenol. The flavour could be induced by manufacture of cheese using salt-resistant lactobacilli that had been introduced with the rennet (Badings *et al.* 1968; CSIRO Division of Food Research 1980).

Flavour threshold concentrations

The threshold concentration (TC) at which a flavour may be perceived is expressed in most instances as the value at which a proportion (often 50%) of the investigating panel can detect an additive. It is important to realize that the TC will depend on the composition, training and organoleptic acuity of the panel and that, by definition, some of the population will be able to detect a compound below its TC. Indeed, Zoeteman (1975) found that 5% of a panel could detect the odour of a number of chemical compounds at 1% of their TC in water. Furthermore, most TC are expressed with reference to detection and a higher concentration is required in many cases before the panel members can recognize the flavour. The chemical purity of the flavour compound is important in studies of its TC for a very minor amount of a more strongly flavoured impurity will have a major effect on the TC. Flavour is the overall impression of taste and odour and the medium has an important influence on both the value of the TC and on the quality of the flavour. For example, in aqueous media the concentration of ionic salts and the pH will play a major rôle in determining the flavour and the TC of ionizable components such as phenolic compounds. The physicochemical relationship between the compound in the medium and the compound in the headspace will determine the balance between taste and odour while the pH will determine the balance between the flavours of the ionized and non-iodized species of the compound.

The table shows some TC collected from the literature of the last twenty years for the taste and odour in several media of phenol, 4-methylphenol and 4-ethylphenol. The effect of most of the factors discussed above can be seen from the values listed in the table. With all these complicating factors it is remarkable that there is any agreement between the TC from different studies.

The flavour strength of the phenolic components appears to be in the order:

4-Methylphenol > 4-ethylphenol > > phenol

and the flavour strength in the medium appears to be in the order:

Water > lipid > > cheese

These relationships show clearly that the values of the TC in the table should be taken only as a guide to the contribution of a phenol to the flavour of a foodstuff. In particular cases of a known phenol in a known medium the best approach to assessing its contribution to the flavour is to compare the level of occurrence with the TC determined experimentally for the known medium.

Conclusions

- There is ample evidence that phenolic compounds such as phenol, methylphenol or ethylphenol occur in natural products.
- These phenolic compounds may be detected organoleptically in the range of ppm or lower.
- There is some evidence that they make a positive contribution to flavour at about the TC but tend towards an unpleasant note as their concentration rises.
- The desirable quality depends on the medium and a flavour that is desirable in smoked sausage is undesirable in a fresh cheese.

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Taste and odour threshold concentrations of phenolic compounds (ppm)

	Phenol	4-Methylphenol	4-Ethylphenol	Reference
Taste medium				
Water		0.018		Badings *et al.* 1968
	0.3	0.002	0.01	Dietz and Traud 1978
Paraffin oil		0.01		Badings *et al.* 1968
Butter (professional)	0.01	0.002		Urbach *et al.* 1972
(laboratory)	> 10	0.005		
Beer (detection)	20	0.02	0.05	Halcrow *et al.* 1966
(recognition)	40	0.08	0.10	
Cheese - Gouda		0.3		Badings *et al.* 1968
Cheddar		1	10	Ramshaw (unpublished)
Feta		0.5		,,
Sugar syrup	2	0.05		,,
Odour Medium				
Air (ppm by volume)	0.047	0.001		Leonardos *etal.* 1969
Water	4	0.2	0.6	Dietz and Traud 1978
10% ethanol in water	7.1	0.01	0.14	Jounela-Eriksson and Lehtonen 1981
20% ethanol in water	40	0.06	0.6	Swan and Burtles 1978

News from the Division

Technology transfer II: Spinning-cone distillation column

In *CSIRO Food Res. Q.* **44**, 3 (September 1984) we reported on the commercialization of the counter-current extraction process. Another process, also developed by Dr Don Casimir of FRL, came 'on-stream' recently. The San Bernadino Winery at Griffith in N.S.W. installed a spinning-cone distillation column in August 1984 to strip sulfur dioxide from grape juice. This is a convenient and cheap method of removing the preservative from a batch of juice before fermentation. The use of the SO_2 is necessary if the juice is to be stored without refrigeration or concentration until it is required.

The main advantage of the spinning-cone column is that it gives high rates of distillation whilst retaining compact dimensions. Further details are contained in CSIRO Industrial Research News No. 166 (September 1984) or may be obtained from the Division.

Review of the Division

In the last issue (December 1984) details were given of the membership and terms of reference of the committee appointed by the CSIRO Executive to review the Division of Food Research.

The Review Committee will be visiting all of the laboratories of the Division during March 1985 and we expect to be able to publish at least some of its findings before the end of the year.

Overseas Fellowship Scheme

Late in 1983 the CSIRO Executive introduced the CSIRO Overseas Fellowship Scheme with the objective of increasing the opportunities for CSIRO staff to work at overseas scientific research institutions to study relevant research activities as background to the foundation or future development of the Organization's research programs. Fellowships are awarded on a competitive basis and are offered for periods of between 6 and 12 months. They are funded by Divisions.

The first of the new Fellowships was recently awarded to Dr R. W. Sleigh of FRL's Food Structure Group.

He will spend most of his time at the Biomedical Centre, University of Uppsala, Sweden, where he will be working with Professor Porath. Professor Porath is well-known for his studies on the application of chromatographic methods to the separation of proteins. Dr Sleigh will be examining new techniques for the separation of food proteins. He will be particularly interested in high-pressure-liquid chromatographic methods (h.p.l.c.) with special reference to the use of improved column materials. Dr Sleigh will also be visiting several other laboratories in Japan, Europe and USA where advanced h.p.l.c. methods are in use.

Honour

Mr J. F. Kefford, Honorary Research Fellow and former Officer-in-Charge of FRL and Assistant Chief of the Division has been elected a Fellow of the Australian Academy of Technological Sciences.

FRL appointment

Mr A. C. Fogerty has succeeded Dr W. G. Murrell as Leader of the Food Safety and Nutritional Quality Group at FRL, in anticipation of Dr Murrell's retirement early in 1985.

Retirement — J. B. Davenport

James Bernard Davenport retired from the position of Principal Research Scientist at the Food Research Laboratory on 29 June 1984 after 32 years in the Division. He had been appointed on 3 March 1952 to work with F. E. Huelin at the Homebush laboratories on the chemical constitution of the oil in apple skin, thought to be implicated in the storage disorder, superficial scald, and his subsequent research career continued this involvement with the structure and function of biological lipids.

Davenport graduated in 1946 in organic chemistry at the University of Queensland and then worked on terpenes with M. D. Sutherland, gaining his M.Sc. in 1948. His initial interest was in fractionation and analysis of lipids and this led to the discovery in 1964, with Huelin and K. E. Murray, of farnesene in apple cuticle oil. His interest was extended to more complex biological structures, and in particular to the role of phospholipids in membrane structure, after a further period of study overseas. He spent 1959 with R. M. C. Dawson at the Institute for Animal Physiology, Babraham, and the next two years as Broodbank Fellow at the Low Temperature Research Station, Cambridge, subsequently

receiving a M.Sc. from the University of Cambridge for this work. On returning to the Division he was engaged in applying many of the new techniques to the fractionation of cell components and to analytical structural studies of membranes from muscle and bacterial cells. Much of his expertise was called on when he was an organizer of a summer school on 'Biochemistry and Methodology of Lipids' in 1969, and then edited (with A. R. Johnson) a text with the same title, contributing chapters on physical chemistry, column chromatography and infrared spectroscopy, and on nomenclature (with Johnson), and structure determination (with A. C. Fogerty).

James Davenport has always had a wide interest in Science outside his own speciality, and in the social and economic consequences of science and technology, so it is not surprising that he has often been involved in extramural activities in positions of authority. Thus, he helped found the Australian Biochemical Society and was Council member and State Representative 1967–8. He has been very active in ANZAAS in many roles, including Chairman of the Publications Committee 1968–9 when he conceived and founded 'Search' as the ANZAAS journal. He edited 'Search' 1970–6 and was then ANZAAS Chairman from 1976 to 1983. He was President of the Contemporary Arts Societies of N.S.W. and of Australia 1971 and 1972–4 and a member of the Visual Arts Board 1974–6. In 1973 he was seconded for three years to the Reserve Bank of Australia as Science Liaison Officer, where he administered the Rural Credits Development Fund, advised the Governor of energy matters and considered priorities and information retrieval in agricultural research.

Most scientists know it is only too easy not to see the wood for a particular tree in one's research field. We shall miss having James Davenport around to help us see the larger problems, and to advise us on the more esoteric aspects of lipid biochemistry.

MBS

Retirement – W. P. Rogers

Mr W. P. (Bill) Rogers retired in January, 1985 after 29 years service. Bill was a Senior Technical Officer at the Dairy Research Laboratory.

Major projects that Bill was involved with included developing techniques for the manufacture of recombined butter, including means of ensuring good initial quality and good keeping quality, and factors affecting the quality of butter stored in plastic tubs in retail trade. This was a relatively new packaging method for butter and assistance was requested by both butter and plastics manufacturers.

Bill was mainly responsible for the design of a series of experiments on the keeping quality of recombined butter. He also played a major role in designing experiments on processing factors affecting the quality and texture of recombined butter.

Working on the butter packaging trials he helped to solve complex technical problems involving the transparency of various plastics and of butter to light of different wavelengths, and the significance of these factors in the light-catalysed oxidation of butterfat. It was largely due to his initiative that several plastic manufacturers commenced research into new opaque films specially designed to prevent light penetration of butter tubs.